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Aims & Scope

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ABSTRACT

Plenary Lectures

PL01 | Multiplex imaging of neural activity and signaling dynamics

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A central goal of neuroscience is to elucidate how information is encoded and processed at the circuit, neuronal ensemble, single neuron, or subcellular structure resolution in vivo. Ca^{2+} imaging allows the tracking of population dynamics of neuronal activity at the cell soma levels as well as within neuronal compartments. We here rationally engineered a next-generation of linear and quadricolor, genetically encoded calcium indicator suite, XCaMPs. Using green XCaMP-Gf, single AP detection was achieved within 3–10 msec of spike onset, enabling measurements of fast-spike trains in parvalbumin-positive interneurons in the barrel cortex in vivo. A non-invasive, subcortical, imaging using red XCaMP-R uncovered somatosensation-evoked persistent activity in hippocampal CA1 neurons. A combinatorial use of XCaMP-Gf, XCaMP-R, and a blue XCaMP-B enabled fiber photometric recording of three distinct (two inhibitory and one excitatory) ensembles during pre-motion activity in freely moving mice. Finally, two-photon co-imaging of yellow XCaMP-Y and XCaMP-R allowed in vivo paired recording of pre- and postsynaptic firing in vivo, revealing spatiotemporal constraints of dendritic inhibition in layer 1 in vivo, between axons of somatostatin (SST)-positive interneurons and apical tufts dendrites of excitatory pyramidal neurons. Thus, XCaMPs represent new multiplexable GECIs, with previously unattained high SNR, linear property, and high-frequency spike resolution and offer a critical enhancement of solution space in studies of complex neuronal circuit dynamics. In combination with previous studies on activity-dependent synthetic promoters such as E-SARE, our findings collectively provide a novel toolkit to investigate key molecular, cellular, and circuit machineries that are essential for coordinating the formation and maintenance of long-term information processing and regulate cognitive behavior in vivo.

PL02 | Innate immunity in neurodegenerative disorders

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The accumulation of neurotoxic or misfolded amyloid beta peptides and/or neurofibrillary tangle formation represent key pathological hallmarks of neurodegenerative diseases including but not limited to Alzheimer's disease, frontotemporal dementia, and multi-system atrophy. The brain has been considered as an immune-privileged organ, however, evidence from preclinical, translational, genetic, and pathological studies suggests that activation of distinct innate immune pathways represent an important factor that contributes to disease progression and chronicity.

Microglia play a pivotal role in this immune response and are activated by binding of aggregated proteins or aberrant nucleic acids to pattern recognition receptors. While this microglial activation may be protective at the beginning, chronic immune activation leads to the sustained release of inflammatory mediators and distracts from physiological functions including debris clearance and trophic factor support. NLRP3 inflammasome activation and release of ASC specks contribute to spreading of pathology and impair microglia clearance mechanisms, and together contribute to neuronal spine loss, neuronal degeneration, and ultimately to the development of neurocognitive deficits such as spatial memory dysfunction.

In keeping with this immune hypothesis of neurodegeneration, modulation of innate immune mechanisms will require a deep understanding when and how to interfere with specific pathways in order to protect from or to delay ongoing neurodegeneration.

PL03 | Synapse degeneration in Alzheimer's disease

Tara Spires-Jones

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Deposition of fibrillar forms of tau in neurofibrillary tangles and neuropil threads follow a hierarchical progressive pattern across brain regions in Alzheimer's disease (AD) and this progression is associated with cognitive decline. Synapses loss occurs early in disease progression and correlates even more strongly with cognitive decline, but the links between tau pathology and synapse degeneration remain incompletely understood. In our group, we have used multiple high-resolution imaging techniques and observed that oligomeric, phosphorylated, and misfolded tau accumulate in a subset of synapses in human AD brain tissue. Synaptic tau is observed in areas without abundant tangles in human brain and tau pathology spreads through neural circuits via synaptic connections in animal models, indicating that tau pathology may spread through synapses. The amount of spreading is influenced by the risk gene *APOE4*, which is strongly expressed in astrocytes. We further observe astrocytes contain both pathological tau and synaptic proteins in AD, indicating that neuron-glia interactions may be an important modifier of synapse degeneration, tau pathology, and the propagation of pathology through the brain. Together, these data indicate that targeting synaptic tau or astrocytic ingestion of synapses and tau may prevent synapse degeneration and the spread of pathology through the brain.

PL04 | Improving ER proteostasis delays brain aging and age-related diseases

Claudio Hetz

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Aging is the main risk factor to develop neurodegenerative diseases. Alteration in the buffering capacity of the proteostasis network is proposed as one of the triggering steps leading to abnormal protein aggregation, which is also a central hallmark of brain aging. The endoplasmic reticulum (ER) is a major node of the proteostasis network altered in brain diseases and during aging. ER stress triggers a signaling reaction known as the unfolded protein response (UPR), which aims restoring proteostasis through the induction of adaptive programs or the activation of cell death programs when damage is chronic and cannot be repaired. Here we discuss our efforts to assess the significance of the UPR to brain aging and its contribution

to disease, in addition to develop gene therapy strategies to alleviate ER stress. A new concept is emerging where depending on the specific UPR component targeted and the disease model tested distinct and even opposite effects can be observed on the pathology.

PL05 | The activation mechanisms of PINK1 and Parkin during mitophagy

David Komander

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Early onset Parkinson's disease (EOPD) occurs early in life (onset <55 years) and can be linked to mutations in ~15 PARK genes. The E3 ubiquitin ligase Parkin/PARK2, and the ubiquitin kinase PINK1/PARK6 are amongst the most prevalent mutated genes. Cell biological studies in the past decade have linked PINK1 and Parkin to mitophagy, a cellular process responsible for the removal of damaged mitochondria by autophagy. A number of additional components have been added, and signalling mechanisms have been elucidated, highlighting a pervasive role for ubiquitination in mitophagy and EOPD.

My laboratory has contributed insights into mitophagy via mechanistic structural and biochemical studies. We have illuminated the activation process of Parkin, which undergoes significant conformational changes to first transition from an autoinhibited 'closed' state, to a fully active, 'open' state. A subset of Parkin mutations prevent Parkin activation through a variety of mechanisms, and these Parkin mutants can be rescued through additional activating mutants, or potentially through small molecule Parkin activators.

PINK1 is a protein kinase responsible for phosphorylating ubiquitin and recruiting and activating Parkin. PINK1 stabilisation is considered the most upstream event in mitophagy, and we recently delineated the PINK1 activation process in molecular detail. Our data illuminate how PINK1 is active in its unmodified state, but then dimerises to trans-autophosphorylate. A conformational change triggered by phosphorylation generates the ubiquitin binding site and resolves the PINK1 dimer. As for Parkin, a set of PINK1 patient mutations can be explained by defects in this activation cascade, and PINK1 activators could act via various points of engagement.

Overall, our studies have unlocked a complete picture of PINK1/Parkin mitophagy and boost our ambition to interfere with mitophagy in a meaningful way to eventually improve the life of patients with EOPD and potentially Parkinson's disease more generally.

ABSTRACT

Marthe Vogt Lecture

MVL01 | Non-coding RNA controllers of acetylcholine signaling as body-brain communicators

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Pursuing RNA regulators of coding transcripts ensuring balanced **acetylcholine (ACh) functioning in health and disease** revealed immense complexity in the cholinergic network and its changes under acute stress and nervous system diseases. The 3'-terminal regions in mRNAs respond to microRNAs (miRNAs) that interact with complementary recognition motifs of 7–8 nucleotides that enable suppression of those mRNAs and **operate as flags**. These short flags often exist in many coding mRNAs whose protein products together constitute entire biological networks, namely, one miRNA can often block many members of a specific pathway. The miRNAs targeting the acetylcholine-related genes, "**CholinomiRs**" such as miR-132 may block inflammation, impose metabolic impact, and regulate neuronal

functioning. However, recruiting CholinomiRs to solve a crisis situation may be too slow when blood cells urgently need to re-balance immune system activation to face this crisis, avoid brain penetration and prevent excessive inflammatory reactions. A "**changing of the guards**" process enables a solution, where blood cells CholinomiRs decline and cholinergic-targeted transfer RNA (tRNA) fragments (**CholinotRFs**) increase. Since tRNAs bring amino acids to the growing protein chains, they exist in all cells. Cutting each tRNA into pieces would only require a nuclease, and the fragments may rapidly operate like miRNAs. That non-coding regions bind to small RNA regulators further predicts diverse reactions to stressful events, individual heterogeneity in neurodegenerative diseases and new indications for disease prevention. Striking differences in the cortical miRNAs targeting cholinergic transcripts between **men and women** with schizophrenia and bipolar disorder reflects global differences in responses to therapeutics between men and women with mental and other diseases. The mRNA profiles should hence be viewed as a glimpse into a complex picture, which is consistently subjected to dynamic regulation in health and disease, in a sex- and age-related manner.

ABSTRACT

Young Scientist Lectureship

YSLA01 | Aberrant upregulation of glycolysis mediates CLN7 neuronal ceroid lipofuscinosis

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CLN7 neuronal ceroid lipofuscinosis is an inherited lysosomal storage neurodegenerative disease highly prevalent in children. CLN7/MFSD8 gene encodes a lysosomal membrane glycoprotein, but the biochemical processes affected by CLN7-loss of function are unexplored thus preventing development of potential treatments. Here, we found, in the Cln7^{Δex2} mouse model of CLN7 disease, that failure in the autophagy-lysosomal pathway causes accumulation of structurally and bioenergetically impaired neuronal mitochondria. In vivo genetic approach revealed elevated mitochondrial reactive oxygen species (mROS) in Cln7^{Δex2} neurons that mediates glycolysis activation and contributes to CLN7 pathogenesis. Mechanistically, mROS sustains a signaling cascade leading to protein stabilization of PFKFB3, a glycolytic-promoting enzyme normally unstable in healthy neurons. Pharmacological inhibition of PFKFB3 in Cln7^{Δex2} mouse brain in vivo and in CLN7 patients-derived cells rectified key disease hallmarks. Thus, aberrant upregulation of neuronal glycolysis contributes to CLN7 pathogenesis and targeting PFKFB3 may alleviate this and other lysosomal storage diseases.

YSLA02 | Defining mechanisms of blood-brain barrier dysfunction in dementia using advanced organ-on-a-chip models

Mootaz Salman

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Dementia is a multifactorial and heterogeneous condition and leading cause of morbidity and mortality. My work aims to answer the question: how does inflammation-mediated blood-brain barrier (BBB) dysfunction lead to the development of dementia? Increasing evidence supports the involvement of BBB dysfunction in neurodegenerative disorders including Parkinson's, Alzheimer's and small vessel disease (SVD); it is evident that this dysfunction happens even before the onset of dementia. In-depth understanding of the cell-cell interactions and signaling pathways between the core elements of the BBB will help in defining therapeutic targets for the prevention of dementia. In my previous work, I identified a number of molecular targets that contribute to barrier integrity function in astrocytes and pericytes and developed microfluidic BBB-on-a-chip models. Here, I am building on this by establishing advanced models using patient-derived iPSC lines in order to investigate the role of BBB dysfunction and glymphatic system under mechanobiological factors and determine how biophysical factors such as blood pressure, flow rate and heartbeat control brain waste clearance. My work will provide new tools to understand lifelong brain health, describe the basis of BBB dysfunction in the occurrence and development of dementia, and provide a platform to develop new treatments for neurodegeneration.

ABSTRACT

Symposia

S01-01 | A brief history of APSN, neurochemists of Asia-Pacific and their roles in ISN/APSN

Philip Beart

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Neurochemistry in the Asia-Pacific commenced much later than Europe owing its 1920s beginning to interests of Japanese psychiatrists in mechanisms underlying mental conditions. After these analyses of autopsied brain tissue, Japanese researchers applied Western approaches seeking mechanistic enlightenment. As neurochemical research advanced the Japanese Society for Neurochemistry was founded (1958), predating ISN which was formalized in 1967. Japan and India have neurochemical societies, but elsewhere neurochemistry is incorporated into neuroscience societies. The Asia Pacific Society for Neurochemistry (APSN, founded 1992), was established after an initiative by Japanese neurochemists at ISN Sydney 1991. APSN holds biennial meets and there have been 10 APSN Presidents from diverse countries. Neurochemists from the Asia-Pacific have made important contributions to ISN, Genkichi Takagaki being a member of its Provisional Organizing Committee, plus three Presidents: Peter Dunkley (2001–2003), Philip Beart (2011–2013) and Kazuhiro Ikenaka (2017–2019). 1st ISN President, Roger Rossiter was Australian, as is President-elect, Caroline Rae. ISN Meetings have been held in Tokyo (1973), Sydney (1991), Kyoto (1995), Busan (2009) and Cairns (2015), where Local Hosts were, respectively, Yasuzo Tsukada, Graham Johnston, Kinya Kuriyama, and John Rostas. Hong Kong 2003 Meeting (host Alfreda Stadlin) was cancelled. Advanced Schools of Neurochemistry have been held jointly with ISN meetings in Okazaki (1995), Gyeongju (2009) and Mission Beach (2015)—Local Organizers, respectively, Kazuhiro Ikenaka and Katshuhiko Mikoshiba, Eunjoon Kim and Elizabeth Coulson. Numerous neurochemists from the Asia-Pacific have served on ISN Council and on Editorial Board of *Journal of Neurochemistry*—surprisingly only current Chief Editor, Andrew Lawrence (2021–ongoing), has been based in the Asia-Pacific. Kunihiro Suzuki when Chief Editor (1978–81; ISN President 1993–95) was based in the USA and when ISN took possession of *Journal of Neurochemistry* (1979), he was amazed by the

greatly increased flow of monies to ISN allowing funding of diverse activities internationally.

S01-02 | Indian neurochemistry: Contribution of Neurochemists and journey

Phanithi Prakash Babu

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The genesis of Indian Neurochemistry was way back in the 1960s in different parts of the country. The first recorded neurochemistry research was initiated in the Christian Medical College (CMC), Vellore from southern India. Research had then begun among various groups in All India Institute of Medical Science (AIIMS), New Delhi, Indian Institute of Science (IISc), Bangalore, Kolkata and Baroda. In recent years this research is further expanded to NIMHANS, Bangalore, Jawaharlal Nehru University, New Delhi, University of Hyderabad, Hyderabad and National Brain Research Centre, Gurugram.

The Society for Neurochemistry, India (SNCI) specifically is a registered society with the Government of Telangana, India. The society was formed in the early 1980s and has been functioning in India. Its main objective is to promote neurochemistry teaching and research by organizing conferences and workshops/schools at different universities and institutes across India. The SNCI organizes annual meetings regularly that are compulsorily accompanied by a school or workshop. These workshops or schools are directed to provide hands-on training and teach young budding students the basic techniques in neurochemistry and neuroscience. The society has been organizing these jointly with ISN, APSN, and IBRO. Society inducts neuroscientists as life members and sends them the relevant information from time to time. During annual meetings, young students and faculty are given awards for best research presentations to encourage them. Further, the Society also felicitates doyens of the field with a lifetime achievement award.

This presentation builds on the history of Indian neurochemistry to current society activities, and the future plans the society holds.

S01-03 | A history of the origin and development of Japanese Society for Neurochemistry and international cooperation and contribution

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"Neurochemistry" in Japan has been established by intensive cooperation between psychiatrists and their collaborators, biochemists, who have sought to investigate the etiology of mental illness to establish treatments. It was a completely different direction from the flow of modern biochemistry that was born using microorganisms or eukaryotic cells as research materials. Neurochemists have aimed to elucidate the physiological or pathological functions of the brain through chemical analysis of the morphologically and functionally unique complexity and characteristics of a brain. I here describe some of the origin and history of neurochemistry in Japan how researchers were establishing Japanese Society for Neurochemistry in 1958 Yasuzo Tsukada as a president in collaboration with Isamu Sano, Genkichi Takagaki, Masanori Kurokawa. The formation of a research group with the support of the Ministry of Education, Science and Culture (MEXT) actually played a major role in promoting neurochemistry in Japan. Many international conferences held in Japan promoted the activity of neurochemistry: The International Society of Physiology in Tokyo in 1965, and the Japan-US Neurochemistry Conference at Oiso in 1965, and in 1967 the International Conference on Biochemistry. All these meetings offered an excitement to younger researchers by close interaction with the world top class researchers. The Asia-Pacific Society for Neurochemistry (APSN) was established in 1991 subsequent to an initiative by JSN. Three Presidents of APSN: Yasuzo Tsukada, Kazuhiro Ikenaka, and Akio Wanaka contributed to promote neurochemistry. The 4th ISN meeting was organized at Tokyo (Yasuzo Tsukada, as a president) in 1973 and the 15th ISN meeting at Kyoto (Kinya Kuriyama, as a president) in 1995. It is great to know that Kunihiro Suzuki and Kazuhiro Ikenaka who worked as ISN Presidents and greatly contributed in promoting the activity of ISN. These are the examples how JSN and ISN have kept a nice international collaboration.

S02-01 | Development and evolution of visual projections

Alain Chedotal

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In most animal species including humans, commissural axons connect neurons on the left and right side of the nervous system. This communication between the two sides of the brain and spinal cord is necessary for a series of complex function, including binocular vision, coordinated locomotor movements, and sound direction localization. In humans, abnormal axon midline crossing during development causes a whole range of neurological disorders ranging from congenital mirror

movements, horizontal gaze palsy, scoliosis or binocular vision deficits. The mechanisms which guide axons across the CNS midline were thought to be evolutionary conserved but our recent results suggest that they differ across vertebrates. I will discuss the evolution of visual projection laterality. In most vertebrates, camera-style eyes contain retinal ganglion cell (RGC) neurons projecting to visual centers on both sides of the brain. However, in fish, RGCs are thought to only innervate the contralateral side. This suggested that bilateral visual projections appeared in tetrapods as an adaptation to aerial vision. Using 3D imaging and tissue clearing we found that bilateral visual projections exist in non-teleost fishes. We also found that the developmental program specifying visual system laterality differs between fishes and mammals.

S02-02 | Molecular control of neuronal subtype specification and integration in the cerebral cortex

Carina Hanashima

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The functional integrity of the neocortical circuit relies on the precisely coordinated production of diverse neurons and their placement along the three-dimensional axis. Specifically in the cerebral cortex, progenitor cells produce distinct neuronal subtypes in a stereotypical order and establish a six-layer structure, which are further tangentially modified into functional areas. A prevailing view concerning the neurogenesis of the neocortex is that, neural stem cells undergo successive rounds of asymmetric cell divisions to produce the principal layer subtypes: preplate, deep-layer, and upper-layer neurons, through a progressive restriction in cell competence. Consistent with this view, we previously showed that transcription factors play a central role in establishing early gene network and switching neurogenesis from preplate cells to deep-layer neurons. However, our recent studies have also indicated that the specification and integration of neocortical neurons rely on communication between distinct cell types. Here, I would like to present our findings on the mechanisms by which neocortical subtype identities establish in the neocortex, by manipulating gene expression and number of neurogenesis in the developing mouse cortex. Our results indicate that neocortical progenitors integrate both intrinsic and extrinsic cues to generate distinct layer neurons, a system which ultimately balances the production of neocortical subtypes during development and possibly evolution.

S02-03 | Semaphorins regulate cortical layer-specific morphogenesis and function

Jeroen Pasterkamp

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Neuron positioning is essential for neural circuit development and defects in this process underly various brain disorders. However,



the molecular mechanisms that control neuron positioning, e.g. how neuron migration is terminated, remain incompletely understood. Semaphorins constitute a family of guidance proteins with roles in disparate developmental processes. Semaphorin-6A (SEMA6A) is a transmembrane semaphorin that signals through Plexin (PLXN)-A2 and -A4 receptors to control neuron migration. Intriguingly, SEMA6A can also act as a receptor with PLXN proteins functioning as ligands, a process termed reverse signaling. To elucidate the *in vivo* function of SEMA6A reverse signaling we generated a conditional *Sema6a-Δcyto* mutant mouse, in which the intracellular domain of *Sema6a* can be deleted. Loss of SEMA6A reverse signaling led to several neuroanatomical defects, including overmigration and ectopic cluster formation of upper layer (II/III) pyramidal neurons in the neocortex. Sparse *in utero* electroporation in *Sema6a-Δcyto* mice identified strongly altered morphology in ectopically located neurons and changes in dendritic spine properties linked to a decrease in the frequency of miniature excitatory post-synaptic currents (mEPSCs). Upper layer neurons form homophilic excitatory interactions with their contralateral counterparts and we therefore examined whether the decrease in excitatory inputs in *Sema6a-Δcyto* mutants resulted from decreased contralateral connectivity between upper layer neurons. While *Sema6a* is expressed by both neurons and glia in the neocortex, the observed neuron positioning defect was caused by loss of SEMA6A in radial glia. To dissect the regulatory mechanisms of SEMA6A/PLXN signaling between neurons and glia during cortical neuron migration, we devised an assay to target radial glial cells by *ex vivo* electroporation at the time of upper layer neuron migration termination, followed by their isolation and proteomic analysis. By comparing the (phospho-)proteome of radial glial cells from *Sema6a-Δcyto* mutants and control mice, we elucidated the molecular mechanisms downstream of SEMA6A/PLXN signaling in radial glia. In all, our work reveals a role for SEMA6A reverse signaling in radial glia cells in the positioning of layer II/III cortical neurons and shows that disrupted SEMA6A reverse signaling leads to defective neuron migration and neural circuitry.

S03-01 | Endoplasmic reticulum chaperone genes encode effectors of long-term memory

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Consolidation of newly acquired experiences into stable long-term memories relies on precisely timed transcriptional events in a

spatiotemporal fashion within the hippocampus. Fundamental studies have shown that a set of transcriptional regulatory proteins of the nuclear receptor 4a (Nr4a) family serve as molecular switches for long-term memory. We used state-of-art single nuclei and spatial transcriptomic approaches following a spatial learning paradigm to define the transcriptomic signature of Nr4a gene expression during critical time windows during memory consolidation. We found that the Nr4a family of transcription factors regulate the transcription of genes encoding chaperones that localize to the endoplasmic reticulum (ER). Nr4a-driven expression of these chaperones is critical for the trafficking of plasticity-related proteins to the cell surface, as well as for long lasting forms of synaptic plasticity and long-term memory. Analysis of human ADRD data reveals that pathological progression correlates with downregulation of Nr4a transcription factors in the hippocampus. Further, in a tau-based mouse model of ADRD, hippocampal levels of both the Nr4a family members and the ER chaperone effector genes are reduced. Importantly, overexpressing Nr4a1 or ER chaperone Hspa5 ameliorates the long-term memory deficits in this mouse model, pointing towards novel therapeutic approaches for treating memory loss. Our findings establish a unique molecular concept underlying long-term memory and provide insights into the mechanistic basis of cognitive deficits in dementia.

S03-02 | Generation of multi-input synapses enables memory storage in old age

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Although it is a widely held view that LTP mediates memory storage, LTP impairments do not always preclude memory storage. To assess which synaptic changes underlie memory storage in aged mice when LTP is impaired, we have performed a 3D EM analysis in the hippocampus after contextual fear conditioning. The power of this analysis is that all synapse types can be assessed, including multi-synapses. We found that multi-excitatory-input synapses are generated and are required for contextual fear memory in aged, but not in young-adult mice. This finding establishes that the synaptic basis of memory storage changes with ageing.

S04-01 | Regulation of microglia morphology and surveillance in health and disease

Lorena Arancibia Carcamo

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Genetic studies have strongly implicated a role for microglia in the development of Alzheimer's disease, and transcriptomic studies have revealed profound changes in the cell states of microglia during disease progression. However, how these changes in gene expression relate to

microglia function remains unknown. Microglia are critically involved in the maintenance of a healthy central nervous system, with the ability to both protect against and potentiate neurological disease. In order to be able to carry out their various functions, microglia constantly extend and retract their processes to survey the brain, and in response to injury they can send out targeted processes to envelop sites of tissue damage. Using 2-photon imaging in combination with pharmacological and genetic manipulation approaches we can begin to investigate the different mechanisms that regulate these processes. Here we describe how microglia surveillance and morphology is modulated by both cell-autonomous and non-cell-autonomous mechanisms in normal brain function and neurological conditions.

S04-02 | Disparate phenotypes of microglia in Alzheimer's disease in vitro

Lezanne Ooi

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Previously microglia were considered to play a role in Alzheimer's disease only in promoting or even causing neuronal death but evidence has shifted to suggest their role is much more complex including neuroprotective removal of toxic protein aggregates. Recent developments using induced pluripotent stem cells to model Alzheimer's disease have provided novel information that contribute to our understanding of microglia in Alzheimer's disease. Generating microglia-like cells from patient-derived stem cells has permitted the opportunity to study specific effects of genotype on phenotype and identified disparate phenotypes of human iPSC-derived microglia driven by genotype and functional effects of microglia in co-cultures and cortical organoids. This presentation will discuss AD microglial phenotypic differences, the potential mechanisms underlying altered microglial function and the impact of those changes on neuronal function and degeneration.

S04-03 | Genetic and proteomic analyses of TREM2 identify new genes and proteins implicated on TREM2 biology and Alzheimer disease

Carlos Cruchaga

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CSF soluble TREM2 is associated with Alzheimer disease (AD). Recent studies by our group found that *MS4A4* and/or *MS4A6A* locus that plays a role in modulating sTREM2 and is associated with AD risk. The goal of this study is to extend those findings using a larger cohort, identify functional genes, and identify proteomic signatures of TREM2. We measured sTREM2 levels in 3000 CSF and 3000 plasma samples. High-throughput proteomics was also generated on these samples including more than 50 TREM2-risk variant carriers. Genetic

analyses were performed using an additive model including rare and common variants. Proteomic signatures of TREM2 carriers were identified by performing differential levels analyses. Protein enrichment analyses was performed using the proteins associated with TREM2 risk variant status.

Genetic analyses validated the previously discovered *MS4A* gene region on chr11 ($p = 7.00e-58$), as well as a three additional signals on Chr3 ($p = 2.227e-09$), TREM gene region and APOE. The signal on chr11 is located on the *MS4A* region, colocalize with AD risk, and includes several independent signals with missense variants in *MS4A6A*, *MS4A4A*, *MS4A4E*, and *MS4A2*. The signal of APOE is independent of the APOE2/4 and the signal of chr 3 includes two genes: *RBMS3* and *TGFBR2* both expressed in microglia. Proteomic analyses of TREM2 risk variant carriers identified 38 and 21 proteins in CSF and plasma, respectively. These proteins were able to differentiate TREM2 carriers from AD cases and controls and were part of the autophagy and autopsy pathways.

This is the largest study to date aiming at identifying genetic modifiers of CSF sTREM2, as well as proteomic signatures of TREM2 risk variant carriers. The findings validated discoveries from previous studies. Two independent signals were identified at *MS4A* locus as well as a novel signal on chr3, APOE and TREM region, indicating that sTREM2 levels are highly regulated by several genes. Proteomic analyses also indicate that TREM2 leads to disease through different mechanism to those than for sporadic AD.

S04-04 | A multi-pronged human microglia response to Alzheimer's disease A β pathology

Renzo Mancuso

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Microglial activation and neuroinflammation are initial steps in the pathogenesis of Alzheimer's disease (AD). However, studies in mouse models and human postmortem samples have yielded divergent results regarding microglia cell states relevant to AD. Here, we investigate 127000 single-cell expression profiles of human microglia isolated freshly from a xenotransplantation model for early AD. While human microglia adopt a disease-associated (DAM) profile, they display a much more pronounced HLA-cell state related to antigen presentation in response to amyloid plaques. In parallel, a distinctive pro-inflammatory cytokine and chemokine CRM response is mounted against oligomeric amyloid- β . TREM2 and, to a lesser extent, APOE polymorphisms, modulate the response of microglia to amyloid- β plaques, in contrast with the response to oligomeric A β . Specific polygenic risk genes are enriched in each branch of these multi-pronged response of human microglia to amyloid pathology (ARM). ARM responses can be captured in post-mortem studies when reanalyzed in light of this novel, comprehensive data set. In conclusion, therapeutic strategies targeting microglia in AD need to carefully assess how they affect the different cell states, as the overall balance between distinct microglial profiles might determine a protective or damaging outcome.

**S06-01 | Recent progress on genetically encoded sensors for calcium, neurotransmitters, neuromodulators, and drugs**Abhi Aggarwal², Kaspar Podgorski², Alison Tebo¹¹Janelia Research Campus, Howard Hughes Medical Institute, HHMI, Ashburn, VA, United States²Allen Institute for Neural Dynamics, AIND, Seattle, WA, United States

Genetically encoded fluorescent indicators for calcium, neurotransmitters, and neuromodulators (GECIs, GENIs, SnFRs) that modulate their fluorescent intensity in response to changes in ligand concentrations are powerful optogenetic tools for the understanding of neuronal signaling. The most widely used set of neurotransmitter-based indicators are the intensity-based glutamate-sensing fluorescent reporter (iGluSnFR) and its variants, that are engineered by fusing the glutamate binding protein GltI from *Escherichia coli* to a green fluorescent protein (GFP) or its derivatives. Variants of iGluSnFR have been used to image synaptic glutamate release, including spontaneous and evoked quantal release, as well as extra-synaptic signaling and glutamate clearance. Following the presentation of the second generation of iGluSnFR, SF-iGluSnFR-A184V, we have used an optimized multi-assay screen in bacteria, soluble protein, and cultured neurons, to develop an improved third generation, termed iGluSnFR3, with improved kinetics and signal-to-noise ratio. Furthermore, we have developed surface display constructs that improve iGluSnFR's nanoscopic localization to post-synapses. I will discuss our work on the development of iGluSnFR3, as well as recent, unpublished advances in our efforts towards developing new tools that enable multiplex imaging of glutamate with GABA and calcium.

S06-02 | Imaging spatial and temporal dynamics of neurochemical release in living brain with genetically encoded indicatorsAlessio Andreoni, Nikki Tjahjono, Ruqiang Liang, Lin Tian
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The toolbox of genetically encoded fluorescent sensors for neurochemicals has expanded at an increasing pace to target the vast spectrum of molecules essential for synaptic transmission and neuromodulation. Sensors based on G-protein coupled receptors or periplasmic binding proteins for major neurotransmitters and modulators are now available to cover a range of applications. However, there is not a "one-size-fits-all" solution for all applications and iterative optimization and evolution is paramount to tailor the intrinsic properties of these sensors to the specific biological questions being pursued. Here, we will present our effort in protein engineering to expand the type of neurochemicals that can be measured optically and to enhance the sensitivity of existing suites of sensors for serotonin, dopamine and glutamate for microscopy and fiber photometry. We also engineered red-shifted sensors, based on red fluorescent

proteins and new reporter scaffolds (e.g., HaloTag), for multiplexed imaging and enhanced depth of penetration in tissue. Combination of these new sensors with existing ones will empower us to broaden the scope of all-optical investigations of neuronal communication.

S06-03 | Dissection of hippocampal NMDA receptor co-agonist signalling assisted by new optical sensorsChristian Henneberger^{1,2}¹University of Bonn, Institute of Cellular Neurosciences, Bonn, Germany²German Center for Neurodegenerative Diseases (DZNE), N/A, Bonn, Germany.

Glutamate receptors of the NMDA subtype (NMDARs) require the binding of glutamate and of a co-agonist, D-serine or glycine, and depolarization to open. Astrocytes can control the supply of D-serine in an activity-dependent manner and in turn NMDAR-dependent synaptic long-term potentiation. This implies that NMDAR-dependent supra-linear integration is controlled by astrocytes too. We found that exogenous D-serine reduces the threshold of dendritic spikes, a hallmark of supra-linear dendritic integration, and increases their amplitude at CA1 pyramidal cells in acute slices. This was also triggered by pyramidal cell population activity, which involved astrocytic endocannabinoid receptors (CB1Rs), astrocytic Ca²⁺ signaling and an increase in extracellular D-serine levels. Interestingly, pyramidal cell activity in the theta range was particularly effective in activating this signaling cascade. Thus, theta activity engages a positive feedback loop via astrocytes that promotes dendritic spiking. Importantly, disrupting this feedback loop by conditional deletion of CB1Rs from astrocytes impaired object location memory and reversal learning. These observations raised the question if supply of the co-agonist glycine is also controlled in an activity-dependent manner. A novel optical glycine sensor (GlyFS) enabled us to optically probe the mechanisms that control extracellular glycine concentrations in the CA1 region and dentate gyrus (DG) of the hippocampus. Interestingly, we found that stimuli that are widely used to induce long-term potentiation and depression of synaptic transmission also increased the extracellular glycine concentration in CA1, which involved glycine transporters, but not in DG. Overall, these results demonstrate that supply of both NMDAR co-agonists is controlled by distinct patterns of neuronal activity.

S06-04 | Probing mechanisms of neurotransmitter release and inter-synaptic cross-talk with multiplexed imaging in vivo

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Memory formation in neural networks is thought to involve changes in synaptic connectivity and in cell intrinsic excitability, but how this process unfolds in the living brain has remained poorly understood.

We advanced a multiplexed imaging method involving optical indicators of glutamate and Ca^{2+} , to monitor changes in the reliability of individual excitatory synapses in vivo. In the mouse barrel cortex, we thus detected increased fidelity coupled with reduced excitation of thalamocortical connections that undergo whisker-stimulation induced LTP. High-resolution imaging also revealed that whisker stimuli trigger synaptic activity that generates extrasynaptic glutamate transients reaching the bulk of synapses in the target cortical area. Our findings help understand basic plasticity features of the synaptic connectome while revealing that a significant component of glutamatergic signalling among cells in the intact brain could be volume-transmitted.

S07-01 | GABAergic plasticity directs developmental assembly of vestibular circuitry for behavior

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Activity-dependent neuronal plasticity allows refinement of brain circuits. We ask how synaptic efficacy of vestibular neurons undergo postnatal tuning to sharpen spatial coding of sensorimotor circuits for presentation of relevant behaviors. Using the central vestibular circuit as a model, we show that early/neonatal regulation of plasticity in GABAergic transmission is crucial for functional maturation of such circuits. Early GABAergic transmission governed connectivity of interneuron circuits that gate the output of the vestibular nucleus, with derangements in gating leading to deficits in navigation. We further found that the neuromodulator endocannabinoid was instrumental in regulating the plasticity of GABAergic transmission in neonates. Disruption to the endocannabinoid system not only delayed maturation of vestibular reflexes, but also caused long-lasting deficits in navigation in adulthood. Our results provide direct evidence that well-regulated GABAergic transmission is critical for developmental assembly of sensorimotor circuits. Activity-dependent neuronal plasticity allows refinement of brain circuits. We ask how synaptic efficacy of vestibular neurons undergo postnatal tuning to sharpen spatial coding of sensorimotor circuits for presentation of relevant behaviors. Using the central vestibular circuit as a model, we show that neonatal regulation of plasticity in GABAergic transmission is crucial for functional maturation of such circuits. Early GABAergic transmission governed connectivity of interneuron circuits that gate the output of the vestibular nucleus. We further showed that derangements in gating led to deficits in navigation. The endocannabinoid system was revealed as the key regulatory mechanism for plasticity of GABAergic transmission in early postnatal circuits. Disruption to the endocannabinoid system not only delayed maturation of vestibular reflexes, but also caused long-lasting deficits in navigational behavior in adulthood. Our results provide direct evidence that well-regulated GABAergic transmission is critical for developmental assembly of sensorimotor circuits. [HKRGC-GRF 17113717; HMRF 06172866]

S07-02 | Astrocytes shape the variability of basal synaptic strengths in the hippocampus

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N-Methyl-D-Aspartate receptors (NMDARs) play a major role in experience-dependent plasticity from developmental circuit refinement to learning and memory. Like neurons, astrocytes also express NMDARs although their exact function has remained controversial. We have identified a role for GluN2C NMDARs in layer-specific tuning of synaptic strengths in mouse hippocampal CA1 pyramidal neurons. Interfering with astrocyte GluN2C NMDAR activity results in narrowing of the presynaptic strength distribution specifically in the *stratum radiatum* inputs without an appreciable change in the mean presynaptic strength. Mathematical modeling demonstrates that such reduction in the width of presynaptic strength distribution compromises long-term synaptic plasticity. Our findings suggest a novel, feedback signaling system in which astrocyte GluN2C NMDARs adjust the basal synaptic weight distribution of Schaffer collateral inputs, which in turn influences computations performed by CA1 pyramidal neurons.

S07-03 | The ins and outs of neurexins in homeostatic plasticity and learning

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Our research aims to understand how experience shapes our synaptic properties and influences our ability to learn and to form memory. In this study, we focus on the neurexin (*Nrxn*) trans-synaptic adhesion molecule family. *Nrxns* are subjected to alternative splicing at six canonical and highly homologous sites. Among these sites, alternative splicing at splice site 4 (SS4) produces SS4+ and SS4- isoforms, which are known to affect synapse formation, synaptic properties, and plasticity. We systematically investigated the role of individual members of the *Nrxn* family in homeostatic synaptic plasticity using the *Nrxn1-3* conditional knockout mice. Additionally, we investigated the effect of inclusion or exclusion of splice site 4 (SS4) of *Nrxn* genes on homeostatic plasticity using the conditional SS4+/- knockin mice. We found that although none of the presynaptic neurexins are obligatory components of a trans-synaptic adhesion complex required for homeostatic plasticity, inclusion of SS4 in *Nrxn1* or exclusion of SS4 in *Nrxn2* completely blocks homeostatic synaptic plasticity. Thus, although *Nrxns* are not required for



homeostatic plasticity, gene-dependent SS4 inclusion/exclusion acts as a gating switch that determines whether homeostatic plasticity may occur at impacted synapses. We further explored Nrnx SS4 alternative splicing patterns in the FXS mouse, and found that mis-expression of non-permissive Nrnx splicing isoforms may contribute to impaired homeostatic synaptic plasticity in the FXS hippocampal circuit. In summary, our results suggest that there exists a synaptic “neurexin code” for homeostatic synaptic plasticity, and that the ability to undergo homeostatic plasticity is not a default state of all synapses, but is subjected to regulation by behavioral experience. This work is supported by NIH (NS115660, HD104458, MH086403).

S07-04 | Role of chandelier interneurons in network oscillations in the amygdala

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Synchronized activity in neural circuits, seen as oscillations in the extracellular field potential, have been associated with learning and memory. The basolateral amygdala (BLA), a mid-temporal lobe structure, generates oscillations in specific frequency bands to mediate emotional memory functions. Sharp wave oscillations (SWs) containing a low frequency (<20Hz) envelope, often coupled with ripples (100–300Hz) have been associated with memory consolidation. We show that SWs in the BLA are generated by local circuits. We demonstrate that a single action potential in a subset of interneurons, chandelier interneurons, is sufficient to initiate SWs through local circuits. Using a physiologically constrained model, we show that microcircuits organized as chandelier interneuron-driven modules reproduce SWs and associated cellular events, revealing a functional role for chandelier interneurons and microcircuits for SW generation.

S08-01 | Local apoptosis promotes complement tagging of synapses for microglial phagocytosis

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Synapses are constantly formed and eliminated throughout life. Microglia refine neural circuits through complement-dependent synaptic phagocytosis. However, it has not been determined whether and how complements ‘tag’ only synapses, or specific synapses. Here, we found that elevated neuronal activity induces synaptic caspase expression, which promotes complement-dependent synaptic phagocytosis by microglia. Live imaging of a neuron–glia co-culture system revealed that complement tags synapses selectively, allowing microglia to

phagocytose only synapses without snipping off axons. The induction of complement tagging at a synapse required coincident timing of up-regulation of cleaved caspase-3 at that synapse, which was regulated by elevated neuronal activity. Finally, experimental febrile seizures induced activity-dependent local apoptosis at inhibitory synapses and temporally linked complement cascade activation in microglia, resulting in phagocytosis of inhibitory synapses and increased seizure sensitivity. Thus, we propose that activity-induced synaptic apoptosis promotes complement tagging of synapses for microglial phagocytosis and modulates brain functions.

S09-01 | Molecular and structural mechanisms of PTPRD-mediated synapse formation

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Synapse formation is triggered by trans-synaptic interactions of cell adhesion molecules termed synaptic organizers. Protein tyrosine phosphatase δ (PTPRD), one of presynaptic organizers, exists in several isoforms generated by alternative microexons' splicing and interacts with various postsynaptic ligands in a microexons' splicing-dependent manner. However, structural and molecular mechanisms for PTPRD microexon-derived peptides to regulate synaptic differentiation and physiological significance of the alternative splicing regulation of the microexons remained elusive. Crystal structural analyses of PTPRD in complex with postsynaptic binding partners, IL1RAPL1, IL-1RAcP, Slitrk2, SALM5, and NLGN3 revealed that microexon-derived peptides form binding interfaces in themselves or function as adjustable linkers for optimal interactions and suggested that ligand-induced dimerization of PTPRD is required for induction of presynaptic differentiation. The mutant mice designed to decrease splicing rates of one of the *Ptprd* microexons without changing the total amount of PTPRD protein showed severe behavioral abnormalities including hypoesthesia, increased anxiety, lowered locomotor activity, and impaired motor coordination. In contrast, heterozygous *Ptprd* knockout mice, showing ~50% decrease in the total PTPRD protein with the ratios of each isoform unaltered, caused minimal behavioral alterations. These results suggest the physiological importance of the microexons' splicing regulation of *Ptprd* gene in brain development and function.

S09-02 | Bridge over troubled synapses with C1q family proteins

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Synaptic organizers, which mediate these processes, are classified into secreted factors, such as Wnt and FGF, and cell adhesion molecules, such as neurexins and neuroligins. Recently, a new class of synaptic organizers, secreted extracellular scaffolding proteins (ESPs), such as

C1q family proteins, LGI1, neuronal pentraxins and glial thrombospondins, have been discovered. They serve as a scaffold for pre- and postsynaptic membrane proteins at the synaptic extracellular matrix. Cbln1, a prototype of the C1q family, is unique in that it is secreted from presynaptic neurons in an activity-dependent manner, and rapidly induces synapse formation in the adult brain. In contrast, Cbln2, a subfamily member of Cbln1, is reported to be secreted from dendrites of pyramidal neurons in the hippocampus and controls glutamate receptor activity without affecting synapse numbers. Furthermore, Cbln4 is reported to regulate inhibitory synapse formation between somatostatin-positive interneurons and pyramidal neurons in the cortex. In this talk, I would like to summarize what is known so far about the C1q family and discuss how we could develop new therapeutic reagents against neuropsychiatric and neurological disorders based on the known structures of known ESPs.

S09-03 | Dissecting the molecular connectome in developing circuits

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Neural circuits are composed of distinct neuronal cell types connected in highly specific patterns. Unraveling how neurons form appropriate synaptic connections during development is a key challenge in neuroscience and is essential to understand brain function and disease. Neural circuit formation critically relies on cell-cell recognition and communication mediated by cell-surface ligands and receptors. The complexity of the cell-surface interactions that pattern precise synaptic connectivity is only beginning to emerge. In this talk, I will discuss our recent work dissecting the cell-surface interaction networks that shape connectivity in hippocampal and cortical circuits.

S10-1 | Expression and function of nicotinic acetylcholine receptors expressed by striatal interneurons

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Nicotinic acetylcholine receptors (nAChRs) are broadly expressed in the brain where they are involved in the control of neural activity and

behavior. Although the nicotinic expression is widespread, it is also specific as different neuronal types express various levels of different subtypes of nAChRs. In the mouse striatum, projection neurons representing approximately 95% of all local neurons do not express nAChRs or in very low levels and nAChRs are primarily located on striatal interneurons. It has not been known which of several types of striatal interneurons express nAChRs and what is the functional significance of these receptors. In the present study, we used double-probe FISH to evaluate the expression of beta2-containing nAChRs, the relatively most common type of striatal nAChRs, by individual types of interneurons. Our analysis revealed that striatal cholinergic interneurons express approximately 80% of all beta2-containing nAChRs expressed by striatal neurons. Then we used the Cre/loxP approach to induce a deletion of beta2 nicotinic subunit in dorsal striatal neurons and to examine their role in behavior. Mice with the deletion showed specific behavioral alterations including decreased sociability, increased anxiety-like behavior, and increased locomotor response to the stimulant drug amphetamine. In addition, the acute administration of amphetamine caused an overall increase of c-Fos expression in the dorsal striatum of mutant mice compared to control animals. The c-Fos expression was increased in both DARPP32-positive and -negative cells, which indicates that both striatal projection neurons and interneurons were affected. In contrast, the c-Fos expression was decreased in cholinergic interneurons, likely reflecting their decreased activity after the nAChRs deletion. In conclusion, the expression of beta2-containing nAChRs by striatal neurons is mostly limited to cholinergic interneurons. Despite their relatively low numbers, deletion of these receptors has a significant effect on striatal activity and striatal-based behavior.

S10-2 | Cholinergic mechanisms in the basolateral amygdala underlying cue-reward learning

Marina Picciotto

Marina Picciotto, Psychiatry, Neuroscience and Pharmacology, New Haven, United States

The basolateral amygdala (BLA) is densely innervated by cholinergic fibers from the nucleus basalis of Meynert (NBM). It is increasingly clear that the BLA is important not only for behaviors related to fear learning, but also for learning cue-reward outcomes. Acetylcholine (ACh) is important for BLA plasticity and multiple types of learning. We have used fiber photometry to measure real-time ACh release in the BLA using a GRABACH sensor as mice learn a cue-reward association in an operant task. We found that reward-related events initially were associated with a peak of ACh release that shifted toward the cue as animals learned the task. We then used an optogenetic strategy to stimulate ACh terminals in BLA and found that it improved cue-reward learning. Surprisingly, stimulation did not have to be contingent with the reward outcome to improve performance, suggesting that the ACh release was not signaling a reward prediction error. Pharmacological studies have identified a role for muscarinic acetylcholine receptors



in both performance of the task and memory consolidation after each session. This study demonstrates that BLA ACh signaling is important for cue-reward learning, and suggests that ACh release induces plasticity in the structure that does not require release timed to a rewarding event.

S10-3 | Cholinergic striatal interneurons use parallel and convergent neurochemical signals in the service of behavior

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Striatum plays important roles in controlling motor functions, goal-directed, habitual and reward-related behaviors. Cholinergic interneurons (CINs) in the striatum are thought to play major regulatory functions in motor behaviors, cognition and reward. These neurons express two vesicular transporters that can load either acetylcholine (ACh, VACHT) or glutamate (Glu, VGLUT3) into synaptic vesicles. Consequently, CINs can release both neurotransmitters, providing numerous mechanisms to regulate striatal outputs with consequences for behavior and cognition. However, most experimental manipulations do not separate the effects of these two neurotransmitters secreted by CINs, creating a gap in the understanding of their function. By using VACHT and VGLUT3-striatum selective KOs, we find that unbalanced co-transmission, by eliminating VACHT in the dorsal striatum, increases habitual behavior, decreases behavior flexibility and triggers stress-induced mal-adaptive eating. In contrast, deletion of VGLUT3 favors goal-directed behavior, without affecting behavioral flexibility or mal-adaptive eating habits. Interestingly, whether motivation was not affected by deletion of either vesicular transporter by itself, deletion of both VACHT and VGLUT3 affected motivation and impaired sequence learning using a novel touchscreen test, suggesting that these two neurotransmitters may synergize together in the service of certain behaviors. Given the critical roles of Pavlovian association for most of these behavioral tasks, we examined the role of ACh/Glu co-transmission using an AutoShaping touchscreen task in which we measured approaches to a conditional or unconditional stimuli. Simultaneously, we recorded with fibre photometry dopamine, ACh or Ca signalling in medium spiny neurons, the major output gateway from the striatum. We find that nucleus accumbens acetylcholine tone provides instantaneous, but also flexible, updating of dopamine signals during this simple behavior, suggesting that ACh-dopamine balance is required for striatal regulation. Indeed, behavioral deficits in VACHT KO mice could be rescued by either treatment with cholinesterase inhibitors, increase of dopaminergic tone with L-Dopa or restoring VACHT expression using an AAV-VACHT virus. These experiments reveal that neurons able to secrete more than one neurotransmitter evolved multiple mechanisms to regulate the activity of local circuits, providing flexible ways to control behavioral outputs. Cholinergic neurons secrete

acetylcholine and glutamate that modulate dopamine signalling, but also directly influence medium spiny neuronal activity. We suggest that the balance of CINs-dopaminergic activity has critical roles in the service of behavior outputs.

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S11-01 | Microtubule basis of hereditary spastic paraplegia

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Hereditary spastic paraplegia (HSP) is a disease in which dieback degeneration of corticospinal tracts, accompanied by axonal swellings, leads to gait deficiencies. SPG4-HSP, the most common form of the disease, results from mutations of SPAST, which is the gene that encodes spastin, a microtubule-severing protein. However, other genes whose mutations cause the disease are not so directly linked to microtubules, prompting the question of whether the corticospinal degeneration is actually due to microtubule abnormalities. In recognition of potential loss-of-function and gain-of-function contributions to the disease, we are using three different mouse models in addition to wildtype. One of these is a heterozygous knockout, another knocks in a human mutant spastin, and the third is the cross of these two. The first shows axonal swellings but no dieback degeneration, the second shows dieback degeneration but no axonal swellings, and the third shows dieback degeneration with axonal swellings. The first shows no gait deficiencies, the second shows gait deficiencies, and the third shows worse gait deficiencies. Our mechanistic studies reveal changes in casein kinase 2 (CK2) and HDAC6 activities, axonal transport defects, autophagy defects and alterations in the composition of microtubules. In particular, we found changes in the acetylation status of the microtubules as well as their content of the microtubule-related proteins tau and MAP6. We propose a mechanistic etiology for SPG4-HSP in which loss-of-function and gain-of-function pathways converge to produce the disease through pathological changes in the microtubule arrays of corticospinal axons.

S11-02 | Depletion of Fidgetin promotes axon growth via both local mTOR activation and labile microtubule augment

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Fidgetin, an ATP-dependent microtubule (MT)-severing enzyme, whose functions are associated with neurite outgrowth, axon regeneration

and cell migration, is indeed a multifunctional protein. Although most of the previous studies have indicated that fidgetin be involved in those biological activities by modulating labile MT mass, it remains unknown whether it is the sole attributed mechanism. Here, we showed that RNAi based depletion of fidgetin promoted axon outgrowth in cultured neurons and enhanced axon regeneration after spinal cord injury (SCI), whose underlying mechanisms was firstly attributed to local activation of the mTOR pathway, as well as increased MT dynamicity. Furthermore, we identified that fidgetin was associated with end binding protein-3 (EB3), a protein that avidly binds to the plus end of MTs and promotes their dynamics. This interaction may potentially facilitate fidgetin to preferably sever the dynamic MTs. Last but not the least, treatment of L-leucine, one of the mTOR activators, together with fidgetin depletion, appeared to synergistically augment axon regeneration after SCI. In all, our results indicated a novel pathway for fidgetin to elicit its impact on axon growth, in addition to its MT related biological roles.

S11-04 | Microtubule mechanisms in dendrite pruning of nociceptive sensory neurons in *Drosophila*

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Selective removal of unnecessary or exuberant neuronal processes without loss of parent neurons, referred to as neurite pruning, is a crucial step of neurite remodeling during animal development. Developmental dendrite pruning shares a number of histological features with pathological neurite degeneration occurring in age-dependent neurological disorders and therefore also serves as an important model for understanding molecular and cellular mechanisms of neurodegeneration.

Drosophila nociceptive sensory neuron, ddaC, is an excellent model to study dendrite-specific pruning, as ddaC neurons selectively prune their larval dendrites in response to a late-larval pulse of the molting steroid hormone ecdysone. We have identified a genetic pathway composed of the transcription factor Sox14 and the important cytoskeletal regulator Mical that act downstream of the ecdysone signaling to regulate neurite pruning (Kirilly D., et al., *Nat Neurosci.* 2009). We also identified two epigenetic factor, namely a Brahma (Brm)-containing chromatin remodeler, a histone acetyltransferase CREB-binding protein (CBP) that binds to the sox14 locus in an ecdysone-dependent manner to induce Sox14 expression (Kirilly D. et al., *Neuron* 2011). This RNAi screen also revealed a conserved E3 ligase that inactivates the insulin signaling pathway to regulate dendrite pruning (Wong J.L. et al., *PLoS Biol.* 2013). Moreover, we also identified Rab5/ESCRT-dependent endocytic pathways which play crucial roles in dendrite pruning of ddaC neurons. We identified a highly conserved L1-type cell adhesion molecule (CAM) Neuroglian (Nrg), which is degraded by the endolysosomal pathway prior to dendrite pruning (Zhang H., et al., *Dev Cell* 2014; Wang Y., et al., *Development* 2017; Zong W., et al., *PLoS Biology* 2018). More

recently, we have identified several microtubule-binding protein, for their crucial roles in dendrite pruning of ddaC neurons. Our study demonstrates that minus-end-out MT arrays is required for dendrite-specific pruning (Wang, et al., *Elife* 2019; Tang et al., *EMBO J* 2000). On the other hand, our lab is also establishing fruit fly as a model to understand the pharmacological effects of cannabinoids in nociception, ethanol addiction and epilepsy.

S12-01 | Synaptotagmin 1 mediates botulinum neurotoxin type A toxicity

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We recently discovered a novel tripartite endocytic sorting mechanism that controls the neuroparalysis caused by Botulinum neurotoxin type A (BoNT/A). BoNT/A is the most potent neurotoxin found in nature and, paradoxically, one of the most versatile therapeutics in modern medicine. According to the long-standing dual receptor model, the extreme neurotoxicity of BoNT/A is mediated by its direct binding to two plasma membrane receptors: polysialogangliosides (PSGs) and synaptic vesicle glycoprotein 2 (SV2). We



discovered that this model is incomplete, as the targeted endocytosis of BoNT/A into synaptic vesicles (SVs), the subsequent neuro-intoxication, also depends on synaptotagmin 1 (Syt1). Syt1 forms a tripartite PSG-Syt-SV2 endocytic sorting complex on the neuronal plasma membrane, controlling targeted endocytosis of the toxin into synaptic vesicles, which is a key step in its intoxication. Our findings suggest that the tripartite endocytosis mechanism is a common intoxication pathway shared by other BoNT serotypes. In my talk, I will discuss these findings and the use of super-resolution imaging to study the molecular steps leading to BoNT/A intoxication.

S12-02 | Distinct functions of phospholipase D1-derived phosphatidic acid species during regulated exocytosis in neuroendocrine cells

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Phosphatidic acid (PA) synthesized by phospholipase D1 (PLD1) has been proposed to play numerous important cellular roles ranging from cell signaling to vesicular trafficking, but the detailed function of PA in these processes remains elusive. Using a combination of pharmacological and molecular approaches associated with genetic mouse models, we have shown that PA produced by PLD1 regulates the last key steps of secretory granule exocytosis in neuroendocrine chromaffin cells. Interestingly, these observations revealed that mono-unsaturated PA control the number of exocytotic events most likely by contributing to granule recruitment/docking, whereas polyunsaturated PA regulate fusion pore stability and expansion. Extending these studies, we found that PA also modulates secretory granule biogenesis, transport, and recycling, revealing a very complex regulation of the entire life cycle of secretory vesicles by PA. We will also present novel tools to study in more detail these pleiotropic functions of PA in vesicular trafficking. Altogether, this work opens novel insights into the different roles in a given cellular function that subspecies of the same phospholipid may play based on their fatty acyl chain composition and suggests a possible contribution of PUFA in cognitive functions.

S12-03 | Rescue of a lysosomal storage disorder caused by Grn loss-of-function with a brain penetrant progranulin biologic

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GRN mutations cause frontotemporal dementia (GRN-FTD) due to deficiency in progranulin (PGRN), a lysosomal and secreted protein with unclear function. Here, we found that Grn^{-/-} mice exhibit a global deficiency in bis(monoacylglycerol)phosphate (BMP), an endolysosomal phospholipid we identified as a pH-dependent PGRN interactor as well as a redox-sensitive enhancer of lysosomal proteolysis and lipolysis. Grn^{-/-} brains also showed an age-dependent, secondary storage of glucocerebrosidase substrate glucosylsphingosine. We investigated a protein replacement strategy by engineering protein transport vehicle (PTV):PGRN—a recombinant protein linking PGRN to a modified Fc domain that binds human transferrin receptor for enhanced CNS biodistribution. PTV:PGRN rescued various Grn^{-/-} phenotypes in primary murine macrophages and human iPSC-derived microglia, including oxidative stress, lysosomal dysfunction and endomembrane damage. Peripherally delivered PTV:PGRN corrected levels of BMP, glucosylsphingosine and disease pathology in Grn^{-/-} CNS, including microgliosis, lipofuscinosis and neuronal damage. PTV:PGRN thus represents a potential bio-therapeutic for GRN-FTD.

S13-01 | Histone acetyltransferase KAT2A is a critical epigenetic regulator of cocaine responses in the nucleus accumbens

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Substance use disorder is characterized by cycles of drug-use, abstinence, drug-seeking, and relapse. The neural basis of these long-lasting drug-associated behaviors have been linked to neural circuit function through changes in neurotransmission and receptor-based changes across the reward circuitry of the brain. However, the underlying molecular mechanisms which contribute the persistence of these drug-induced changes to neural function remain relatively poorly understood. Previous work from our lab and others has identified cocaine-induced changes to transcriptional and proteomic profiles within the nucleus accumbens (NAc). To understand how drugs of abuse, such as cocaine, generate long-lasting behavioral changes, it is critical to link between neuronal activity and changes in gene expression. One potential avenue are epigenetic adaptations, where DNA-protein interactions are modified to alter accessibility and likelihood of targeted gene expression. We identified temporally specific changes in histone H3 post-translational modifications and identify a key regulator in these changes—KAT2A. KAT2A is a histone acetyltransferase known to regulate activity-dependent transcription. We find that KAT2A-regulated phosphoacetylation of H3 is increased following chronic cocaine self-administration. Moreover, we demonstrate that loss of KAT2A function in D1-MSNs alters sensitivity and motivation for cocaine. Lastly, we generate a cocaine self-administration activity profile of D1-MSNs that is subsequently altered by alterations in KAT2A function. The results of these studies contribute evidence for persistent cocaine-induced

epigenetic adaptations and are the first step in generating a mechanistic link between epigenetic adaptations and changes in neuronal firing. In addition, we provide data linking these changes in epigenetic state to cocaine-seeking behavior.

S13-02 | Cell-type-specific epigenetic priming of gene expression by cocaine

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A major focus of research into the molecular pathology of drug addiction is the mechanism by which altered patterns of gene regulation are preserved in a central brain region of reward, the nucleus accumbens (NAc). Stable changes in chromatin are thought to underlie the altered transcriptional states in this brain region, which persist despite long-term drug withdrawal. However, there is no direct link between drug-induced epigenetic marks and aberrant gene expression driving relapse. A fundamental challenge is determining which neuronal subtypes are responsible: the NAc is primarily composed of two opposing types of medium spiny neurons (MSNs), the D1 and D2 dopamine receptor-expressing subtypes, which exhibit dramatic differences in activity and effects on drug reward. In these distinct subtypes, we investigated how chronic cocaine modifies chromatin structure genome-wide and characterized immediate versus persistent changes in gene regulation. We found that prolonged withdrawal from cocaine is associated with the dramatic depletion of the histone variant H2A.Z, increased genome accessibility, and latent priming of gene expression in D1 MSNs. These changes are linked to aberrant gene expression upon drug relapse. The histone chaperone ANP32E removes H2A.Z from chromatin, and we demonstrate that D1 MSN-selective *Anp32e* knockdown prevents cocaine-induced H2A.Z depletion and blocks cocaine's rewarding actions. By contrast, very different effects of cocaine, withdrawal, and relapse were found for D2-MSNs. These findings provide new insight into circuit-specific epigenetic priming as a critical mechanism and promising clinical target employed by drugs of abuse to modify brain function and behavior in lasting ways.

S13-03 | An emerging role of histone H2A variants in behavioural regulation

Iva Zovkic

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Epigenetic modifications have an established role in learning and memory, but a role for histone variants in behavioral regulation was only recently uncovered. I will discuss emerging evidence for opposing actions of individual histone variants in memory, whereby

histone H2A.Z suppresses and histone macroH2A1 promotes memory formation. Their effects on memory will be discussed in the context of transcriptional changes in the mouse hippocampus and related to sex differences in Alzheimer's disease and age-related memory decline.

S14-01 | Metabotropic NMDA receptor signaling, synaptic depression and Alzheimer's disease

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Until recently, NMDA receptor (NMDAR) functions have been attributed to its ability to conduct calcium ions. However, growing evidence demonstrates that glutamate binding alone can induce synaptic depression, suggesting that the NMDAR has a metabotropic function. Using fluorescence lifetime imaging and electrophysiology, we demonstrated that NMDARs are capable of ion-flux independent signaling through conformational change in the NMDAR intracellular domain resulting in long-term depression of synaptic transmission (LTD). We also found that overexpression of PSD-95, a major scaffolding protein at the synapse interacting with NMDARs, blocks agonist induced conformational movement in the NMDAR intracellular domain as well as LTD that is NMDAR-dependent and ion-flux independent. Moreover, PSD-95 is significantly depleted in neurons exposed to beta-amyloid as well as in brain tissue of patients with Alzheimer's disease. Our results show that increasing synaptic PSD-95 by blocking its depalmitoylation can reverse beta-amyloid induced synaptic depression as well as other effects of beta-amyloid on dendritic spines such as reduced spine density. This suggests that ion-flux independent LTD probably contributes to synaptic dysfunction in neurons affected by beta-amyloid. To study this in more detail, we are examining ion-flux dependent and independent LTD in WT and APP/PS1 mice, an Alzheimer's disease mouse model. Overall, we find that ion-flux independent LTD is predominant in synapses with low amounts of PSD-95, occurring mostly during development and the progression of Alzheimer's disease. Finally, blockade of NMDAR metabotropic function by increased synaptic PSD-95 could be involved in the protection of important synapses and could lead to novel therapies for Alzheimer's disease.

S14-02 | Presynaptic NMDA receptors signal metabotroically in neocortical plasticity

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Previously, we reported that presynaptic NMDA receptors (preNMDARs) at visual cortex layer-5 (L5) pyramidal cell (PC) synapses depend on Mg^{2+} and RIM1 $\alpha\beta$ to regulate high-frequency evoked



release, but signal metabotroically via JNK2 to regulate spontaneous release independently of frequency. Similarly, at L5 PC-PC connections, timing-dependent long-term depression (tLTD) depends on preNMDARs but not on frequency. We therefore tested if this tLTD relies on metabotropic preNMDAR signaling.

We elicited tLTD at L5 PC-PC synapses using quadruple patch in P11-18 acute visual cortex slices. After homozygous RIM1 α β deletion, tLTD was unaffected (deletion $65\pm 7\%$, $n = 10$ vs. tLTD $62\pm 4\%$, $n = 15$, $p = 0.74$). The JNK2 inhibitor SP600125, however, abolished tLTD ($96\pm 2\%$, $n = 9$ vs. tLTD, $p < 0.001$). Moreover, while postsynaptic dialysis of a JNK2-blocking peptide did not affect tLTD (post peptide $61\pm 5\%$, $n = 12$ vs. tLTD $52\pm 6\%$, $n = 7$, $p = 0.30$), presynaptic dialysis abolished tLTD (pre peptide $96\pm 4\%$, $n = 10$ vs. tLTD, $p < 0.001$), suggesting metabotropic preNMDAR signaling. In agreement blocking NMDAR ionotropic signaling with 7-CK did not abolish tLTD ($73\pm 5\%$, $n = 9$, $p < 0.001$).

To summarize, we find that JNK2 mediates metabotropic preNMDAR signaling in neocortical tLTD. These findings show that the textbook view of NMDARs as ionotropic coincidence detectors in synaptic plasticity may need to be reassessed.

S15-01 | Cell-type specific transcriptional networks related to autism

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Mutations in *FOXP1* and *FOXP2* are among the most significant recurrent de novo mutations associated with autism spectrum disorder (ASD). Our previous work demonstrated a cell autonomous role for *Foxp1* in mouse striatum related to its role as a transcription factor in regulating molecular pathways at risk in ASD. The striatum is an important brain region for speech and language, and its function may be disrupted in ASD. There are distinct cell-type specific roles for major neuronal classes in the striatum, in particular, the dopamine receptor 1 (D1) and dopamine receptor 2 (D2) expressing spiny projection neurons (SPNs). Therefore, understanding how ASD-relevant genes facilitate striatal function at cellular resolution should bring key insights into many types of ASD with striatal features. Our data from genomic, electrophysiological, and behavioral experiments suggest that *Foxp1* has an important role in the identity and function of D2 SPNs. In contrast, D1 SPNs have fewer altered features with loss of *Foxp1*. *Foxp2*, on the other hand, is primarily expressed in D1 SPNs where its expression increases further with the loss of *Foxp1*. Since *Foxp1* and *Foxp2* can heterodimerize to regulate gene expression, they may have compensatory roles in D1 SPNs. Specific loss of *Foxp2* in D1 SPNs results in minimally altered features in line with *Foxp1* loss of function studies. However, the combined loss of *Foxp1* and *Foxp2* in D1 SPNs results in significantly impaired behavioral, electrophysiological, and cell-type-specific gene expression changes. These data provide important new insights about the functional contribution of both individual and combined ASD risk genes in specific cell types in the striatum,

and how they relate to ASD features. These results should facilitate the development of novel strategies for normalizing striatal physiology in the treatment of certain forms of ASD.

S15-02 | Cadherin-13 is a critical regulator of GABAergic modulation in human stem-cell-derived neuronal networks

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Cadherin-13 (CDH13) has been associated with autism and attention-deficit/hyperactivity disorder. CDH13 localizes at inhibitory presynapses, specifically of parvalbumin (PV) and somatostatin (SST) expressing GABAergic neurons. However, the mechanism by which CDH13 regulates the function of inhibitory synapses in human neurons remains unknown. Starting from human-induced pluripotent stem cells, we established a robust method to generate a homogenous population of SST and MEF2C (PV-precursor marker protein) expressing GABAergic neurons (iGABA) in vitro, and co-cultured these with glutamatergic neurons at defined E/I ratios on micro-electrode arrays. We identified functional network parameters that are most reliably affected by GABAergic modulation as such, and through alterations of E/I balance by reduced expression of CDH13 in iGABAs. We found that CDH13 deficiency in iGABAs decreased E/I balance by means of increased of increased inhibition. Moreover, CDH13 interacts with Integrin- β 1 and Integrin- β 3, which play opposite roles in the regulation of inhibitory synaptic strength via this interaction. Taken together, this model allows for standardized investigation of the E/I balance in a human neuronal background and can be deployed to dissect the cell-type-specific contribution of disease genes to the E/I balance.

S15-03 | Synapse imbalance, cognitive dysfunction and neurodevelopmental disorders

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The remarkable capacity and plasticity of the brain, which underlies behaviour and how we adaptively navigate complex environments, hinges on the organization and dynamics of synapses—specialised machinery at the connections between cells that form the most fundamental information-processing units in the nervous system. Disruptions in synapse function and plasticity are central to cognitive dysfunction. Moreover, human genetic studies continue to highlight perturbations in synapse biology as a converging mechanism shared across the spectrum of neurodevelopmental disorders.



Excitatory (glutamatergic) and inhibitory (GABAergic) synapses are essential for regulating the balance of excitation/inhibition in brain circuits, thus optimal information processing and plasticity. Our work has recently focused on *neuroligins*, a family of postsynaptic cell-adhesion molecules essential for organising activity-dependent stabilisation of excitatory and/or inhibitory synapses, thus playing central roles in balancing synaptic signalling and plasticity. But whether mutations in the neuroligin gene family members differentially disrupts selective components of cognitive behaviour is not well understood. I will discuss our recent work dissecting cognitive behaviour in mouse models with neuroligin (NLgn 1–4) gene deletions. Combining rodent touchscreen cognitive testing with systems neuroscience approaches, our work shows that neuroligins divergently regulate adaptive behaviour, decision-making and learning. Our work contributes to the growing advancement in approaches for how we measure complex cognitive constructs in preclinical animal models, enabling deeper understandings into the neurobiological basis of transdiagnostic measures of cognitive function and dysfunction in neurodevelopmental disorders.

S15-04 | Towards understanding the pathophysiology of autism using disease models

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Autism spectrum disorder (ASD) is a complex psychiatric illness that has received considerable attention as a developmental brain disorder. Substantial evidence suggests that chromosomal abnormalities, including copy number variations (CNV), contribute to autism risk. The duplication of human chromosome 15q11-13 is the most frequent cytogenetic abnormality in ASD. We modeled this genetic change in mice using chromosome engineering to generate a 6.3-Mb duplication of the conserved linkage group on mouse chromosome 7. Mice with a paternal duplication display autistic-like behavioral features such as poor social interaction, behavioral inflexibility, and abnormal ultrasonic vocalizations. This chromosome-engineered mouse model for ASD (*15q dup* mouse) seems to replicate various aspects of human autistic phenotypes and validates the relevance of the human CNV. This *15q dup* mouse is the first CNV model of ASD and a founder mouse for forward genetics of a developmental brain disorder. Our multi-dimensional approach reveals that *15q dup* mice show impaired spine phenotypes, abnormality in serotonin, and excitatory/inhibitory imbalance. The rescue experiment during the developmental stage suggests the significance of serotonin on neural and behavioral development. Screening the essential gene in the duplicated region using spine phenotypes identified *Necdin* as a driver gene. I will talk about our recent analyses on the *15 dup* mice and add our new direction towards understanding the pathophysiology of ASD.

S16-01 | Mechanism of action of LSD in mental disorders

Danilo De Gregorio

Danilo De Gregorio, Division of Neuroscience, Vita-Salute San Raffaele University, MILANO, Italy

Combining behavioral, neurophysiological, optogenetic and biomolecular data, this talk will give an overview about the mechanism of action of lysergic acid diethylamide (LSD) and its ability to modulate social behavior and to treat anxiety. In particular, we will focus on the impact of LSD on glutamatergic, dopaminergic and serotonergic neurotransmission.

S16-02 | From plant synergy to neuroplasticity: 30 years of translational ayahuasca research in Brazil

Rafael Guimarães dos Santos

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Ayahuasca is a plant-based hallucinogen traditionally used by indigenous and syncretic groups for healing and ritual/religious purposes. It contains the tryptamine hallucinogen dimethyltryptamine (DMT) and beta-carbolines (harmine, tetrahydroharmine, and harmaline), which render DMT active after peripheral monoamine oxidase (MAO) inhibition. Although some authors suggest that the begging of the so-called Psychedelic Renaissance was in 2006, research groups in Switzerland, Germany, Spain, the US, and Brazil were conducting human hallucinogen research with psilocybin, mescaline and ayahuasca since the early 1990s. Indeed, in the specific case of ayahuasca, pre-clinical and human research with was being developed in Brazil at least since 1992. This presentation will resume the often not mentioned history of this early ayahuasca research and the expansion of the field that followed in the next 30 years. I will briefly present pre-clinical, observational, experimental, and clinical results of studies conducted mostly in Brazil and Spain, the leading countries in ayahuasca research. I will also comment on the cultural and pharmacological different between ayahuasca and other hallucinogens, and the challenges associated with its possible use in mental health. In resume, positive and promising results were observed both in animal models of substance use disorders and mood and anxiety disorders, as well as in observational studies and in preliminary, proof-of-concept, randomized-controlled trials for this same psychiatric disorders. However, research is still in its infancy, and positive results need to be replicated in larger trials.



S16-03 | Psychedelics and related plasticity-promoting neurotherapeutics

Lee Dunlap, David Olson

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Psychedelics have received increasing recognition for their potential to treat a wide range of neuropsychiatric disorders, including depression, post-traumatic stress disorder (PTSD), and substance use disorder. However, safety concerns and their legal status has limited their clinical development. Therefore, our work has focused on the development of analogs of psychedelics that retain their therapeutic properties with reduced hallucinogenic potential. To guide our drug discovery efforts, we developed psychLight, a genetically encoded fluorescent sensor based on the 5-HT_{2A}R, capable of predicting the hallucinogenic effects 5-HT_{2A}R ligands. Using psychLight we were able to identify analogs of the psychedelics *N,N*-dimethyltryptamine (DMT) and lysergic acid diethylamide (LSD) possessing anti-depressant and anti-psychotic activity with diminished hallucinogenic side effects.

S16-04 | Psilocybin mitigates the cognitive deficits observed in a rat model of fragile X syndrome

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Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability (ID) and the leading monogenic cause of autism spectrum disorder (ASD). Serotonergic neurotransmission has a key role in the modulation of neuronal activity during development and therefore it has been hypothesized to be involved in ASD and co-occurring conditions including FXS. As serotonin is involved in synaptic remodeling and maturation, serotonergic insufficiency during childhood may have a compounding effect on brain patterning in neurodevelopmental disorders, manifesting as behavioral and emotional symptoms. Thus, compounds that stimulate serotonergic signaling such as psilocybin may offer promise as effective early interventions for developmental disorders such as ASD and FXS. The aim of the present study was to test whether different protocols of psilocybin administration mitigate cognitive deficits displayed by the recently validated *Fmr1*^{Δ_{exon 8}} rat model of ASD, which is also a model of FXS. Our results revealed that systemic and oral administration of psilocybin microdoses normalizes the aberrant cognitive performances displayed by adolescent *Fmr1*^{Δ_{exon 8}} rats in the novel object recognition test—a measure of exploratory behavior, perception, and recognition. These data support the hypothesis that serotonin modulating drugs such as psilocybin may be useful to ameliorate ASD-related

cognitive deficits. Overall, this study provides evidence of the beneficial effects of different schedules of psilocybin treatment in mitigating the cognitive deficit observed in a rat model of FXS.

S17-01 | Dendritic spine initiation, stabilization and depletion in synaptic plasticity

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The majority of the postsynaptic terminals of excitatory synapses in the central nervous system exist on small bulbous structures on dendrites known as dendritic spines. The actin cytoskeleton is a structural element underlying the proper development and morphology of dendritic spines. Synaptic activity patterns rapidly change actin dynamics, leading to morphological changes in dendritic spines. Synaptic activity regulates the formation of new spines, stabilization of existing ones as well as depletion of spines. However, how synaptic activity is linked to these morphological changes is relatively poorly understood.

In my talk, I will present our recent findings on molecular mechanisms which link synaptic activity to dendritic spine initiation, stabilization and depletion. For spine initiation, I will present our data on F-BAR domain containing Gas7 protein, which clusters on the plasmamembrane upon neuronal activation, simultaneously increasing actin clustering and inducing formation of new spines. For synaptic activity regulated spine stabilization, I will present our earlier findings how synaptic plasticity changes the state of actin phosphorylation. Long-term potentiation (LTP) first increases actin phosphorylation to ease re-organization of actin filaments. After re-organization, actin filaments are dephosphorylated. Dephosphorylation stabilizes actin filaments. For synaptic activity induced spine depletion, I will present our data on actin severing protein gelsolin, which is involved in long-term depression (LTD) induced spine depletion. We showed that gelsolin can distinguish between LTD and LTP induced longer and shorter Ca²⁺ signaling. I present our model in which LTD-induced modest—but relatively long-lasting—elevation of Ca²⁺ concentration increases the affinity of gelsolin to F-actin leading to actin filament severing and depletion of a spine.

S17-02 | Oligodendrocyte dysfunctions underlie autism-related behaviors in a mouse model of the neurodevelopmental disorder ANKS1B syndrome

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Our understanding of oligodendrocytes in brain function and disease is changing rapidly. We recently reported that heterozygous deletions in the *ANKS1B* gene lead to *ANKS1B* syndrome, a neurodevelopmental

disorder presenting with attention deficit hyperactivity disorder, speech and motor deficits, and autism spectrum disorder (ASD). ASD is present in greater than 60% of patients. The *ANKS1B* gene encodes for AIDA-1, a brain-specific protein that is highly enriched at postsynaptic densities of neuronal synapses where it regulates NMDA receptor function and synaptic plasticity. Therefore, synaptopathies are expected to contribute to *ANKS1B* syndrome. Unexpectedly, we find that social deficits and other behavioral correlates of ASD observed in mouse models of *ANKS1B* syndrome originate from a novel role for AIDA-1 in oligodendrocytes. We report that a CNS-wide *Anks1b* haploinsufficiency mouse model displays structural and white matter abnormalities in the corpus callosum that are reminiscent of clinical findings in patients. Immunohistochemical and histological analyses show that reduced oligodendrocyte abundance, maturation, and myelination likely underlie these callosal structural abnormalities. We find that AIDA-1 protein is highly expressed in oligodendrocytes and oligodendrocyte precursor cells (OPCs), in addition to neurons. AIDA-1 is highly localized at sites of neurite extension and is essential for OPC migration in callosal regions. Moreover, punctate staining of AIDA-1 throughout cell bodies of oligodendrocytes colocalizes with PSD95, suggesting expression at neuron-OPC synaptic junctions. Surprisingly, selective loss of *Anks1b* from the oligodendrocyte lineage, but not from neuronal populations that highly express AIDA-1, leads to deficits in social preference and sensory reactivity. We find that these social deficits are rescued by treatment with clemastine, an FDA-approved antihistamine used for seasonal allergies that was recently and unexpectedly found to increase OPC maturation and CNS myelination. These results show that induction of new myelin formation can normalize deficits in social behaviors in an *ANKS1B* syndrome mouse model, even at 7-months of age. They also suggest that a synaptopathy may indeed underlie *ANKS1B* syndrome, but one of unique neuron-OPC synapses rather than one of canonical neuron-neuron synapses.

S17-03 | Control of neuronal cytoskeleton and intracellular transport machinery by arginine methylation

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Neurons receive most excitatory synaptic inputs at dendritic spines, which are actin-enriched compartments that undergo dynamic changes during development and synaptic plasticity. Mushroom spines are crucial for memory storage, and their formation requires specific RNA-binding proteins (RBPs) that carry mRNAs along the microtubule from soma to dendrite and regulate their local translation. Deficiency of these RBPs may lead to over-production of immature filopodia that are associated with neurodevelopmental disorders. Arginine methylation is a well-known post-translational modification on histone and other nuclear proteins. Many RBPs in dendrite also contain RGG motifs which are hot spots of arginine methylation, suggesting that the importance of this post-translational modification may extend beyond its conventional function in gene transcription. Here I will describe the regulation of synaptic actin cytoskeleton by

the arginine methyltransferase PRMT8, an unusual methyltransferase that is localized at neuronal synapses and up-regulated in the hippocampus during dendritic spine maturation. PRMT8 induces arginine methylation of G3BP1, a dendritic RBP, within the RGG motif and limits filopodia formation via the control of Rac1-PAK signaling, actin dynamics and mRNA translation. Interestingly, recent findings from us and others have revealed that besides RBPs, other transport proteins such as kinesin and huntingtin also undergo arginine methylation. Intracellular transport and cytoskeleton are therefore major targets modulated by arginine methylation during synapse development.

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S17-04 | The role of RNA methylation reader protein YTHDF1 in postmitotic neuron development

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Epitranscriptomics has recently emerged as a versatile and powerful post-transcriptional regulatory pathway in the central nervous system. RNA modification N6-methyl-adenosine (m6A) has been found in axonal and synaptically localized mRNAs whose local translation is required for axon growth, synaptogenesis, and synaptic plasticity. However, the molecular mechanisms responsible for m6A-mediated regulation of postmitotic neuronal development are not completely understood. Here, we report that cytoplasmic m6A reader YTHDF1, which binds m6A-modified mRNAs, is widely expressed in the mouse brain and enriched in neurons compared to the other brain cell types such as astrocytes, oligodendrocytes or microglia. Time-sensitive downregulation of YTHDF1 in cultured hippocampal neurons indicates that YTHDF1 is required for the proper development of neurons in culture, from early polarization to spine maturation. In addition, using an inducible knockdown system in vivo, we demonstrate that YTHDF1 is required for in vivo development and positioning of hippocampal neurons to dentate gyrus. To better understand the molecular mechanisms underlying the neuronal development defects, we focus on the growth cone structure, which is crucial for the guidance and elongation of the growing axon and dendrites, and we show that YTHDF1 localizes to a subset of the plus-ends of dynamic microtubules in the growth cone and deregulation of YTHDF1 leads to stalled growth cone dynamics. Moreover, we show that YTHDF1 interacts with the scaffold protein Adenomatous Polyposis Coli (APC), which is a known regulator of the microtubule-actin cytoskeleton in the growth cone, and that reducing YTHDF1 expression results in a decrease in APC immunostaining in the axon



and growth cone. Given that APC has been identified as a risk gene for autism spectrum disorder and intellectual disabilities, our findings suggest that deregulation of the YTHDF1/m6a/APC axis pathway could contribute to the physiopathology of these neurological disorders.

S18-01 | Role of FGF receptor signaling in oligodendrocytes regulates synaptic plasticity, learning and memory in the adult brain

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Memory decline is one of the most feared consequences in our aging society. Despite extensive efforts, the lack of effective treatments for neurodegenerative disorders, accounts for increasing numbers of people suffering from memory impairment. Multiple Sclerosis (MS) is classically a demyelinating and inflammatory white matter disease, but over 50% of MS patients show cognitive impairment. This memory impairment is believed to be somehow associated with inflammation and myelin/oligodendrocyte (OL) loss in the hippocampus, potentially disrupting the maintenance of excitatory synapses, as observed in the hippocampi from MS patients and in a mouse model of MS. However, the mechanisms involved are not well understood. Here we show that the loss of Fibroblast Growth Factor Receptor-1 & -2 (FGFR1/2) signaling, specifically within OL-lineage cells, results in memory impairment and significant deficits in synaptic plasticity, reflected by the impairment of long-term potentiation in the hippocampus. Further, electron microscopy showed a dramatic reduction in the numbers of docked synaptic vesicles in the presynaptic terminals. However, these changes in neuronal function occurred without obvious change in the numbers of OL-progenitors or OLs, demyelination, or inflammation, suggesting that the loss of FGFR1/2-signaling in OL-lineage cells, rather than the loss of these cells altogether, is sufficient to lead to neuronal dysfunction in the hippocampus, with adverse implications for memory and learning. Thus, these observations bring up the clinically relevant possibility that FGFR-signaling in OL-lineage cells may play a potentially novel, previously unrecognized role in OL-neuron communication for the maintenance of synaptic plasticity and memory functions in the normal adult/aging brain and that its perturbation may contribute to cognitive dysfunction in neurodegenerative diseases. Supported by NIH Grant to RB: R37NS038878.

S18-02 | Experience-dependent myelin plasticity in the encoding and recall of fear memories

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The formation of long-lasting, enduring memories requires anatomical substrates that are both plastic, to represent new experiences, as well as perseverant, to preserve long-term representations of those experiences can modulate oligodendrogenesis in the adult brain, and that.

S18-03 | Role of oligodendrocytes and myelin in motor skills learning and working memory training

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Generation of new myelinating oligodendrocytes (OLs) from their precursor cells is stimulated by—and required for—motor skill learning in mice. Involvement of myelin in learning and memory is also suggested by MRI studies that reveal microstructural changes in white matter tracts of people who learn a new visuomotor skill, or engage in a cognitive task such as learning a second language or training to improve working memory. We asked whether new OL production is required for training-dependent memory improvement in mice, using T-maze and 8-arm radial maze tasks designed to exercise and evaluate spatial working memory. Blocking OL genesis by conditional knockout of the transcription factor Myrf in OL precursors impaired the ability of adult mice to improve their performance in these tasks over 8 days of training, relative to control littermates. In wild type mice, maze training stimulated production of additional OLs and myelin in the anterior cingulate cortex and underlying corpus callosum—regions known to be involved in working memory processes. Strikingly, in individual mice there was a close correlation ($R^2 > 0.7$) between the number of new OLs generated in those brain regions during training and ultimate performance score in the radial maze. These results suggest an essential and rather direct requirement for OLs and myelin in working memory performance, which is known to underpin all kinds of cognitive abilities and correlates strongly with measures of “fluid intelligence” in humans. Since both motor skill learning and working memory improvement

depend on practice over days, we propose that, in general, OL generation is required for learning and memory processes that depend on reiterative training.

S18-04 | Activity-dependent remodeling of myelinated axons and oligodendrocyte differentiation is stimulated by environmental enrichment

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Oligodendrocyte production and central nervous system (CNS) myelination is a protracted process, extending into adulthood. While stimulation of neuronal circuits has been shown to enhance oligodendrocyte production and myelination during development, the extent to which physiological stimuli induces activity-dependent plasticity within oligodendrocytes and myelin is unclear, particularly in the adult CNS. Here, we find that using environmental enrichment (EE) to physiologically stimulate neuronal activity for 6-weeks during young adulthood in C57Bl/6 mice results in an enlargement of callosal axon diameters, with a corresponding increase in thickness of pre-existing myelin sheaths. Additionally, EE uniformly promotes the direct differentiation of pre-existing oligodendroglia in both the corpus callosum and somatosensory cortex, while differentially impeding OPC homeostasis in these regions. Furthermore, results of this study indicate that physiologically relevant stimulation in young adulthood exerts little influence upon the de novo generation of new myelin sheaths on previously unmyelinated segments and does not enhance OPC proliferation. Rather in this context, activity-dependent plasticity involves the coincident structural remodeling of axons and pre-existing myelin sheaths and increases the direct differentiation of pre-existing oligodendroglia, implying constraints on maximal de novo production in the adult CNS. Together, our findings of myelinated axon remodeling and increased pre-existing oligodendroglial differentiation constitute a previously undescribed form of adaptive myelination that likely contributes to neuronal circuit maturation and the maintenance of optimum cognitive function in the young adult CNS.

S19-01 | Regulation of presynaptic function and plasticity through mitochondrial dynamics and calcium buffering

Josef Kittler

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The tight regulation of mitochondrial distribution and function in brain cells is essential for providing ATP at the correct spatial location to power neural function and computation, and for providing calcium buffering at sites of calcium entry or release. Mitochondrial dysfunction is also increasingly associated with neuronal pathology in neurological and neurodegenerative disease. Mitochondrial transport and positioning is regulated by Miro family proteins which link mitochondria to kinesin, dynein and myosin motors. Mitochondria positioned at presynapses can efficiently take up local Ca^{2+} via the mitochondrial calcium uniporter (MCU) to regulate presynaptic Ca^{2+} homeostasis. Here I will discuss our work combining mouse transgenic, optical and electrophysiological techniques to establish the importance of mitochondrial regulation by Miro and MCU, for the operation of axons and their synapses. My talk will highlight, in particular, the importance of presynaptic mitochondria for regulating neurotransmission and presynaptic plasticity of a major hippocampal excitatory pathway.

S19-02 | A mitophagy mechanism underlying synaptic defects in Alzheimer's disease

Qian Cai

Rutgers, The State University of New Jersey, Cell Biology and Neuroscience, Piscataway, USA

Synaptic mitochondrial deficit is a hallmark of Alzheimer's disease (AD). Mitophagy comprises a key pathway in the quality control of mitochondria by which damaged mitochondria are targeted for autophagy. However, how mitophagy ensures synaptic mitochondrial integrity is poorly understood and very few details are known regarding the intersection of mitophagy and pathologies in AD. Our studies revealed that Rheb-dependent mitophagy in coordination with retrograde transport plays a critical role in mitochondrial maintenance at synapses. Defects in such a mechanism lead to synaptic mitophagy stress and induce synaptic damage in AD neurons. Moreover, we recently demonstrated that broad activation of Parkin-mediated mitophagy triggers an unexpected effect—depletion of mitochondria from synaptic terminals, a characteristic feature of early tauopathy in AD. Excessive Parkin activation consequently halts the mitochondrial anterograde movement, disrupting mitochondrial supply to tauopathy synapses and impairing synaptic function. Taken together, our work provides new mechanistic insights into how mitophagy is involved in maintaining synaptic mitochondrial homeostasis and how altered mitophagy participates in AD-linked synaptic pathogenesis, and establishes a foundation



for future investigations into new therapeutic strategies to protect against mitochondrial dysfunction and synaptic degeneration in AD.

S19-03 | Energy matters: Reprogramming axonal mitochondrial transport and energy metabolism in CNS regeneration

Zu-Hang Sheng

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Mitochondria are cellular power plants that generate energy in the form of ATP to power neuronal growth, survival, and regeneration. In contrast to chronic neurodegeneration, brain injury triggers acute mitochondrial damage leading to local energy crisis. Axonal survival and regeneration require high levels of energy consumption, and acute energy crisis contributes to regeneration failure leading to permanent neurological impairments. Thus, replacing damaged mitochondria with healthy ones will accelerate bioenergetic recovery, and thus meet increased energy demand for neuron survival and axon repair. Investigations into the regulation of mitochondrial trafficking and energy metabolism represent an important emerging area. We previously identified syntaphilin (SNPH) *Cell*. In the presentation, I will discuss how mitochondrial transport progressively declines with neuron maturation, how traumatic brain injury, spinal cord injury, and ischemia trigger acute mitochondrial damage leading to local energy crisis, and how enhancing mitochondrial transport rescues energy deficits in injured axons, thus facilitating CNS regeneration and functional recovery (Zhou et al., *JCB* 2016; Han et al., *Cell Metabolism* 2020). We also reveal intrinsic signaling pathways in mature neurons and adult brains that remobilize damaged mitochondria by turning off the SNPH anchoring switch in response to local axonal injury and ischemia and thus replenish healthy mitochondria to support axon regeneration (Huang et al., *Current Biology* 2021; Cheng et al., *Neuron* 2022). We further reveal transcellular signaling pathways between glial cells and neurons that boost axonal energetic metabolism (Chamberlain et al., *Neuron* 2021). Our studies elucidate important mechanisms for reversing energy crises, protecting neuron survival, and facilitating CNS regeneration after injury and ischemia by reprogramming axonal mitochondrial transport and energy metabolism (Supported by the Intramural Research Program of NINDS, NIH).

S20-01 | Astrocytes in higher brain function

Tirzah Kreisel, Adi Doron, Adi Kol, Adar Adamsky, Inbal Goshen
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In recent years, groundbreaking research revealed many surprising roles for astrocytes, in addition to their well characterized supportive roles, in modulating neuronal activity and even behavior. First, to directly and specifically modulate astrocytic activity we employed a chemogenetic approach: We expressed the Gq-coupled

designer receptor hM3Dq or the Gi-coupled designer receptor hM4Di in astrocytes, which allowed their time-restricted manipulation by the application of the designer drug clozapine-N-oxide (CNO).

We discovered that astrocytic activation is not only necessary for synaptic plasticity, but also sufficient to induce NMDA-dependent *de-novo* long term potentiation. In-vivo, astrocytic activation enhanced memory allocation, i.e. it increased neuronal activity in a *task-specific* way, only when coupled with learning but not in home-caged mice. Furthermore, astrocytic activation using chemogenetic or optogenetic tools during acquisition resulted in memory recall enhancement on the following day. Gi activation in astrocytes during memory acquisition impairs remote, but not recent, recall. We show that this effect is mediated by a specific disrupting of the projection from the hippocampus to the anterior cingulate cortex by astrocytes.

We showed that modulations of astrocytic calcium dynamics impact memory processes, but their real-time calcium activity in awake mice has not been investigated yet. We chronically imaged CA1 astrocytes using 2-photon microscopy when head-fixed mice performed a spatial task. We found that astrocytic activity persistently ramps towards the reward location in a familiar environment. When the reward location was changed in the same environment or when mice were introduced to a novel context, the ramping was not apparent. Accordingly, using linear decoders we accurately reconstructed mice location trajectories in a familiar environment from astrocyte activity alone, but could not do the same in the novel environment. Following additional training, as the mice were familiarized with the new reward location or novel context, the ramping was reestablished, suggesting that spatial modulation of astrocytic activity is experience dependent. This is the first indication that astrocytes can encode position related information in learnt spatial contexts, thus broadening their known computational abilities and their role in cognitive functions.

S20-02 | Do astrocytes encode behaviorally relevant information?

Axel Nimmerjahn

Salk Institute for Biological Studies, Biophotonics, La Jolla, United States

While it is generally accepted that neurons control complex behavior and brain computation, the role of non-neuronal cells in this context remains unclear. Astrocytes, glial cells of the central nervous system, exhibit complex forms of chemical excitation, most prominently calcium transients, evoked by local and projection neuron activity. This presentation will discuss new data linking astrocytes' spatiotemporally complex calcium activity patterns, neuronal molecular signaling, and animal behavior. The results—obtained using a visual detection task, in vivo calcium imaging, statistical analyses, and machine learning approaches—suggest that cortical astrocytes encode the animal's decision, reward, performance level, and sensory properties. Behavioral context and motor activity-related parameters strongly impact astrocyte responses, and cell-intrinsic mechanisms curb astrocyte calcium activity. Error



analysis further suggests that astrocytes carry behaviorally relevant information, supporting their complementary role to neuronal coding beyond their established homeostatic and metabolic functions.

S20-03 | The involvement of astrocyte calcium-dependent signaling in behavior

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Astrocytes are critical players in the regulation of brain development and function. They sense and respond to neuronal activity by elevating intracellular calcium levels derived from different sources and displaying complex spatiotemporal properties. Calcium elevations appear spatially distributed in global (soma and main processes) and focal regions (microdomains). This astrocytic calcium activity is thought to underlie the astrocyte involvement in synaptic transmission, metabolism, and brain homeostasis. In this work, we studied the IP3 receptor type 2 knockout (IP3R2 KO) mouse model that lacks global calcium elevations in astrocytes to disclose its implications for circuit structure and function. We found an influence of global astrocyte calcium on long-term memory performance. Thus, we performed a structural and molecular analysis of cortico-limbic regions that revealed a shift to immature spines in pyramidal neurons of the dorsal hippocampus that could support the changes in synaptic plasticity underlying our behavioral observations. The characterization of the IP3R2 KO mouse model provided new insights into the importance of astrocytic calcium-dependent signaling in the modulating neural circuits. These findings broaden the scope of astrocytic modulation of circuit function and behavior.

S20-04 | Norepinephrine links astrocytic activity to regulation of cortical state

Kira Poskanzer

University of California, San Francisco, Biochemistry & Biophysics, San Francisco, United States

Cortical state, defined by population-level neuronal activity patterns, determines sensory perception. While arousal-associated neuromodulators—including norepinephrine (NE)—reduce cortical synchrony, how the cortex resynchronizes remains unknown. Furthermore, general mechanisms regulating cortical synchrony in the wake state are poorly understood. Using in vivo imaging and electrophysiology in mouse visual cortex, we describe a critical role for cortical astrocytes in circuit resynchronization. We characterize astrocytes' calcium responses to changes in behavioral arousal and NE, and show that astrocytes signal when arousal-driven neuronal activity is reduced and bi-hemispheric cortical synchrony is increased. Using in vivo pharmacology, we uncover a paradoxical, synchronizing response to Adra1a

receptor stimulation. We reconcile these results by demonstrating that astrocyte-specific deletion of Adra1a enhances arousal-driven neuronal activity, while impairing arousal-related cortical synchrony. Our findings demonstrate that astrocytic NE signaling acts as a distinct neuromodulatory pathway, regulating cortical state and linking arousal-associated desynchrony to cortical circuit resynchronization.

S21-01 | Brain metabolism probed by hyperpolarized ¹³C magnetic resonance

Arnaud Comment

Cancer Research UK, Cambridge Institute, Cambridge, United Kingdom

The advent of hyperpolarization by dynamic nuclear polarization (DNP) to enhance the polarization of carbon-13 (¹³C) nuclear spins led to the emergence of a new kind of magnetic resonance (MR) measurements providing the opportunity to probe metabolism in vivo in real time. It has been shown that, following the injection of hyperpolarized ¹³C-labeled substrates prepared using DNP, specific metabolic probes that can be used to differentiate between healthy and pathological tissue in preclinical and clinical studies can be readily detected by MR thanks to the tremendous ¹³C signal enhancement. Recent studies of cerebral function and metabolism based on the use of hyperpolarized ¹³C MR will be presented in this talk. The constraints and future opportunities that this technology could offer will be discussed.

S21-02 | Functional metabolism investigated by fMRS: An overview on methods and outcome

Nathalie Just

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During the past decade, increasing efforts within the neuroscientific community have led to a better understanding of the normal and diseased brain metabolism. Non-invasive techniques such as magnetic resonance imaging (MRI) and spectroscopy (MRS) have, in particular, contributed to crucial steps for unraveling the underpinnings of neurovascular and neurometabolic coupling mechanisms leading to the birth of *functional MRS (fMRS)*.

fMRS allows the measurement of changes in the concentration of metabolites due to brain activation. In the human brain, proton fMRS at high magnetic field strength (>7 T) reproducibly demonstrated that visual stimulation of the visual cortex led to increased levels of glutamate (+ 3–4%) and increased levels of lactate (+ 20%)^{1,2} while glucose levels decreased significantly. In addition, GABA level changes during brain activation have also been measured³. Gradually these neurochemical measurements become complementary to blood oxygen level dependent (BOLD) fMRI measurements and show great potential for the assessment of metabolic changes in various diseases and disorders such as pain and schizophrenia or cognitive impairments⁴ at clinical fields. fMRS was also developed at the preclinical level^{5,6}



demonstrating translational outcome despite important sensitivity issues. These difficulties can be alleviated by the possibility to combine fMRS with various other modalities. Notably, the feasibility of combining fMRS with optogenetics⁷ and chemogenetics provides an unprecedented manner to selectively stimulate specific populations of cells or modulate and map specific pathways in the rodent brain. fMRS can therefore bring novel insights into brain circuitry and represents a unique non-invasive tool for causal circuit interrogation.

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S21-03 | Advanced ¹H MRS in clinical applications

Gulin Oz

University of Minnesota, Radiology, Minneapolis, USA

Magnetic Resonance Spectroscopy (MRS) allows non-invasive quantification of numerous neurochemicals, including markers of neurons and glia, neurotransmitters (glutamate, g-aminobutyric acid), antioxidants (glutathione, vitamin C) and energy metabolites. Regional quantification of these metabolites provides access to multiple aspects of pathology in neurological disorders. This presentation will share our experience in utilizing MRS for detection of early neurochemical changes and for monitoring treatments in movement disorders at high-field MR scanners. The talk will also address the limitations of standard, commercial clinical MRS tools, which are overcome with advanced MRS software developed in the research setting. Finally, I will demonstrate that advanced MRS methodology can be harmonized across sites and vendors and share our experience in using the advanced technology in multi-site settings.

S21-04 | Combining MRS and PET to elucidate the pathophysiology of aging brain

Yuhei Takado

National Institutes for Quantum Science and Technology, Institute for Quantum Medical Science, Chiba, Japan

As the global population ages, it is critical to elucidate aging mechanisms and develop therapeutic strategies against aging-associated neurodegenerative diseases such as Alzheimer's disease. MRS is a tool for non-invasive evaluation of brain function and pathology in humans and mice, and has recently found increasing application in neuroscience and clinical practice. PET has been used clinically to evaluate abnormal proteins in Alzheimer's disease, such as tau and amyloid proteins. Combining these imaging techniques will advance

our understanding of the pathophysiology of aging and Alzheimer's disease. We have been studying the pathophysiology of Alzheimer's disease using the combination of MRS and PET; Advanced MRS and MRSI for humans, ultra-high field MRS and awake MRS for mice, as well as a PET ligand 18F PM-PBB3 (florolotau[¹⁸F]) for mice and human to detect tau proteins. In this presentation, we will introduce our efforts to elucidate the pathomechanism of Alzheimer's disease using those multi-imaging modalities in patients and mouse models.

S23-01 | Glial Ca²⁺ signaling and neuron–microglia interaction

Yuki Fujita

Shimane University, Department of Developmental Biology, Faculty of Medicine, Izumo-shi, Shimane, Japan

Accumulating studies have shown bidirectional communication between glial cells and neurons. Microglia are the resident immune cells of the central nervous system and are important for cellular processes. In addition to their classical roles in pathophysiological conditions, these immune cells also dynamically interact with neurons and influence their structure and function in physiological conditions. We reported the neuroprotective function of microglia in the developing brain. Microglia accumulate along subcerebral projection axons in the white matter and support neuronal survival during the early postnatal period. Inactivation or ablation of microglia increased apoptosis in layer V subcerebral and callosal projection neurons and increased degeneration of their axons. We assessed candidate molecules for the neuroprotective role of microglia and identified that microglia-derived insulin-like growth factor 1 (IGF1) supported neuronal survival. Thus, microglia support the survival of neurons and axons in the postnatal brain.

The next question is how microglia accumulate towards subcerebral projecting axon. Although it is well established that neuron-derived fractalkine (CX3CL1) mediates the interactions between neurons and CX3CR1-expressing microglia, the number of microglia did not decrease in the brain of Cx3cr1-deficient mice. This suggests that fractalkine-CX3CR1 signaling is not required for the migration of microglia. To assess the molecular mechanism of their interaction, we investigated whether microglia distribute along postnatal axons depending on the axon-derived factor. These results will help to understand how neuron–microglia communication determines axonal survival and degeneration in the developing brain.

S23-02 | Striatal astrocyte–neuron interactions in health and disease

Baljit S Khakh

Department of Physiology UCLA, Los Angeles, USA

Astrocytes tile the central nervous system, but their functions in neural circuits and their roles in mammalian behaviour and disease are incompletely defined. I will report data from my laboratory



whereby we used state-of-the-art methods and new genetic approaches to evaluate, reduce and activate striatal astrocyte signaling in vivo. Our data show that brain area specific astrocytes regulate neural circuits to shape behaviour and also contribute to phenotypes associated with psychiatric and neurodegenerative diseases. I will present a mixture of recently published and unpublished data concerning these topics for Huntington's disease and obsessive-compulsive disorder phenotypes and their molecular basis in mice.

S23-03 | Neuronal hyper-excitability driven by astrocytic P2Y1 receptor signaling

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Astrocytes alter their phenotype in response to brain insult and disease to become reactive astrocytes. Reactive astrocytes exhibit aberrant Ca^{2+} signals, which are generally augmented Ca^{2+} signals in terms of amplitude and frequency. Gq-protein coupled receptors (Gq-GPCRs) play a crucial role in aberrant Ca^{2+} signals. P2Y1 receptor, one of Gq-GPCRs, is upregulated in astrocytes in neurological disorders, such as epilepsy and Alzheimer's disease. However, its pathophysiological significance is not fully elucidated. To reveal the pathophysiological significance of P2Y1 receptor upregulation in astrocytes, we used transgenic mice in which astrocytes specifically overexpress P2Y1 receptor using Tet-Off system (P2Y1OE). We found that P2Y1OE mice were more susceptible to drug-induced seizure and showed more frequent abnormal spikes in EEG recordings, suggesting P2Y1OE induced neuronal hyper-excitability. To understand the cellular mechanisms underlying the neuronal hyper-excitability, we performed dual-color Ca^{2+} imaging of both astrocytes and neurons in the hippocampal slices using GCaMP6f, a green genetically encoded Ca^{2+} indicator (GECI) and jRGECO1a, a red GECI, respectively. Then, we found that electrical stimulation of the Schaffer collateral resulted in fast Ca^{2+} rise in dendrites of neurons followed by slow-onset Ca^{2+} rise in astrocytes. Slow-onset Ca^{2+} rise was mediated by P2Y1 receptor activation by ATP or ADP released from neurons, while fast Ca^{2+} rise in dendrites was augmented in P2Y1OE and mediated by ionotropic glutamate receptor. Extracellular glutamate imaging showed that P2Y1OE enhanced evoked glutamate release. However, the dynamics of glutamate was closely matched with neuronal but not astrocytic Ca^{2+} signals,

indicating that the glutamate was derived from presynaptic terminals but not from astrocytes. Transcriptome analysis of isolated astrocytes from P2Y1OE revealed a novel candidate molecule X as an astrocyte-derived excitatory signals. Treatment of antibodies against molecule X decreased neuronal Ca^{2+} signals in P2Y1OE without affecting astrocytic Ca^{2+} signals, suggesting that molecule X could contribute to augmented fast Ca^{2+} signal. Overall, the data suggest that astrocyte P2Y1 receptor-mediated signals enhanced glutamatergic synaptic transmission through a novel candidate molecule which could contribute to hyper-excitability in neurological diseases.

S24-01 | LRRK2 is important to maintain nuclear envelope integrity: Implications to Parkinson's disease

Simone Engelen

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

Parkinson's disease (PD) is characterized by degeneration of dopaminergic neurons in the substantia nigra and accumulation of α -synuclein into Lewy bodies. Mutations in the LRRK2 gene cause dominant and sporadic forms of PD, with the most common mutation resulting in substitution G2019S. Notably, inducible expression of LRRK2 G2019S enhances α -synuclein pathology in mice.

LRRK2 is a protein kinase, and disease mutations increase its kinase activity. Disruption of kinase activity attenuates the toxic effects of LRRK2 disease mutants. However, there are also kinase-independent mechanisms that contribute to LRRK2 toxicity, including altered interaction with chaperone 14-3-3 and aggregation.

We recently discovered that dopaminergic nigral and cortical neurons from PD patients (idiopathic and with G2019S mutation) exhibit abnormalities of the nuclear lamina. Accordingly, we found that dopaminergic neurons from LRRK2 G2019S transgenic mice exhibit decreased nuclear lamina circularity and leakage of the nuclear protein 53BP1 into the cytosol. We also observed that LRRK2 translocates to the nucleus by binding to SIAH1, and in the nucleus, LRRK2 directly interacts with lamin A/C. LRRK2 disease mutations, on the other hand, do not interact with lamin A/C and prevent the LRRK2 wild-type from interacting with lamin A/C, resulting in nuclear lamina disorganization and nuclear protein leakage. Moreover, LRRK2 knockdown and LRRK2 $-/-$ mice develop nuclear lamina abnormalities and nuclear disruption.

Our data suggest that nuclear lamina disruption may play a role in PD and that LRRK2 plays an essential role in maintaining nuclear envelope integrity. Impairment of this function by disease mutations suggests a novel phosphorylation-independent mechanism that may contribute to LRRK2 toxicity in PD.



S25-01 | Human spinal cord organoids with neural tube morphogenesis

Woong Sun

Korea University College of Medicine, Department of Anatomy, Seoul, South Korea

Spinal cord is generated by the folding of the neural plate along anterior–posterior axis via an embryonic process called neurulation. Perturbation of this process often leads to a common congenital malformation, neural tube defects, highlighting the importance to develop in vitro model recapitulating human neurulation. The advent of organoid technology, which produces 3D structure resembling parts of organs from ESCs/iPSCs, has provided ways to study human organogenesis and to model human diseases. Recently, we developed a novel organoid model that exhibits specific morphogenetic features of spinal cord development, such as neural tube formation. The human spinal cord organoids (hSCOs) exhibited tube-forming morphogenesis, and differentiation into the major types of caudal spinal-cord cells including motoneurons and glial cells. Furthermore, they exhibited the maturation of synaptic contacts and synchronized neural activities after long-term culture. In this talk, I will present an example that the hSCOs were used to highly quantifiable toxicology screen for drugs that might cause neural-tube defects. Collectively, hSCOs are useful for studying the mystery of human spinal cord development, the modelling of diseases associated with neural-tube defects.

S25-02 | Modeling Alzheimer's disease and tauopathy using iPSCs-derived brain organoids

Hideyuki Okano

Keio University School of Medicine, Department of Physiology, Tokyo, Japan

We have introduced iPSCs and brain organoid technologies for disease modeling and drug discovery for cardinal neurodegenerative diseases

including amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and Alzheimer's disease (AD). Historically, disease modeling and drug discovery research have been conducted using model animals, primarily rodents. However, it is now recognized that these model animals do not always reproduce human disease pathology and that drugs developed using model animals often fail to show efficacy in clinical trials. Especially for neurological diseases, the differences in epigenomic information between humans and other species, differences in the types of cells that make up the brain, and differences in the neural networks within the brain, all limit the use of such animal models for disease and condition research. In order to solve these problems of animal experimental approaches, pathological analysis of diseases and drug discovery have been conducted using human disease-specific iPSCs, and clinical trial results have begun to appear for some diseases. Particularly, the iPSCs technology is a promising approach for creating in vitro human AD models, and brain organoids are expected to be applied as an alternative disease model to animal models in the future because they consist of multiple cell types and have high neuronal maturity. In addition, A β pathology could be observed in brain organoids generated from iPSCs derived from AD patients. We are currently applying the established methods and models to create a new AD-like model mimicking the Tau pathology by injecting Tau seeds into brain organoids and transplanting brain organoids into mouse brains.

ABSTRACT

Young Members Symposia

YMS01-01 | Involvement of nucleus accumbens neurons in cue-reward associative learning

Carina Soares-Cunha^{1,2}, Ana Verónica Domingues^{1,2}, Raquel Correia^{1,2}, Bárbara Coimbra^{1,2}, Natacha Vieitas-Gaspar^{1,2}, Luísa Pinto^{1,2}, Nuno Sousa^{1,2}, Ana João Rodrigues^{1,2}

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In an ever-changing environment, organisms evolve to perceive their surroundings and adapt behaviour to increases chance of survival and propagation. Organisms use neutral environmental cues to perceive availability of rewards and learn these associations to optimize behaviour and obtain desired outcomes. This process is highly mediated by the nucleus accumbens (NAc). The NAc is mostly comprised by GABAergic medium spiny neurons (MSNs), segregated into those that express dopamine receptor D1 (D1-MSNs), and those that express dopamine receptor D2 (D2-MSNs). MSNs project directly to the ventral tegmental area (VTA) through the direct pathway, mediated exclusively by D1-MSNs or indirectly to the ventral pallidum (VP) through both D1- and D2-MSNs. While MSNs have been classically described as having a dichotomous function in reward, recent data has shown that this opposing functional assumption is too simplistic, and does not reflect the complex role of these neurons in cue-reward associative learning.

Thus, to better understand the involvement of D1- and D2-MSNs in cue-reward associative learning we performed calcium imaging recordings and show that these neuronal populations are required for distinct phases and events of learning. In addition, we performed optical manipulation of MSNs during different segments of behavior and show that cue-paired optogenetic activation of D2-MSN is sufficient to increase motivation. Optical inhibition results in the opposite behavioral effect. Interestingly, optical activation of D2-MSNs during reward delivery significantly decreases motivation; the opposite is observed for optical inhibition. In agreement, in a free choice instrumental task, animals prefer the lever that originates one pellet in opposition to pellet plus D2-MSN-VP optogenetic activation, and vice versa for optogenetic inhibition.

In summary, we show that NAc MSNs bidirectionally modulate cue-outcome associative learning, revealing a more complex role of these neurons than previously anticipated.

YMS01-02 | Medial prefrontal serotonergic input regulates cognitive flexibility behavior in mice

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The medial prefrontal cortex (mPFC) regulates cognitive flexibility and emotional behavior. Furthermore, neurons that release serotonin (5-HT) project to the mPFC, and drugs targeting the 5-HT system influence emotional regulation and cognitive flexibility. Yet, the specific role of endogenous 5-HT release in the mPFC on neurophysiology and behavior is unknown. Here we selectively mapped, monitored, and manipulated 5-HT input into the mPFC to gain insight into the functional roles of this pathway. Using in vitro optogenetics paired with whole-cell slice electrophysiology we observed strong and dominant 5-HT_{1a} receptor-mediated inhibition of mPFC pyramidal neurons. In vivo fiber photometry recordings revealed task-specific activity signatures in 5-HTergic neurons projecting from the dorsal raphe to the mPFC during a cognitive flexibility task but not in the open field test. Furthermore, in vivo optogenetic activation of the 5-HTergic dorsal raphe-to-mPFC pathway selectively improved extradimensional rule shift performance while inhibition impaired it, demonstrating sufficiency and necessity for mPFC 5-HT release in cognitive flexibility. Locomotor activity or anxiety-like behavior in the open field test was not affected by either optogenetic manipulation. Collectively, our data reveal a powerful and specific modulatory role of endogenous 5-HT release from dorsal raphe-to-mPFC projecting neurons in cognitive flexibility.

YMS01-03 | Laterodorsal tegmentum-Nucleus accumbens projections underlie cocaine-induced preference

Bárbara Coimbra, Ana Verónica Domingues, Ricardo Gonçalves, Carina Soares-Cunha, Nuno Sousa, Ana João Rodrigues
University of Minho, ICVS/3B's - Associate Laboratory, BRAGA, Portugal

The laterodorsal tegmentum (LDT) has attracted attention due to its involvement in reward information processing and reinforcement learning, and thus, in the development of addictive behaviors



induced by drugs of abuse. Interestingly, evidence from our group revealed a key role for LDT excitatory projections to nucleus accumbens (NAc) (cholinergic and glutamatergic) to incentive salience—by shifting preference, enhancing motivation, and driving positive reinforcement in natural reward-related behavioral tests. However, it is still unclear what is the contribution of these novel direct projections from the LDT to the NAc and their functional relevance when exposure to either natural rewards or drugs of abuse occurs is still unknown. Here, we propose to understand how the psychostimulant cocaine alters LDT-NAc neurons, and if/how these neuroplasticity events are critical for the development of natural reward deficits. Our hypothesis is that LDT signals induce functional changes of neurons within the NAc to facilitate neuronal and behavioral plasticity associated with cocaine exposure, and that cocaine-induced activation of LDT increases value encoding in the NAc of this drug over natural rewards.

Here we report that the inhibition LDT cholinergic projection to NAc blunts the rewarding stimuli and preference for cocaine as activation is rewarding alone, without further increasing cocaine-induced CPP. In contrast, activation of glutamatergic LDT projections to NAc reduce the rewarding preference for cocaine in the CPP. Thus, the LDT, mainly cholinergic projections, are an active participant in modulating cocaine-relevant neuronal microcircuits in the NAc, that ultimately result in changes in behavior to promote an addictive-like phenotype.

YMS02-01 | TIMP-1 released early in Alzheimer's disease from reactive astrocytes enhances memory and synaptic plasticity in 5xFAD mice

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Astrocytes play a key role in the pathophysiology of several neurodegenerative disorders including Alzheimer's disease (AD). Astrocytes' response to any pathological insult to the brain is termed as reactive astrogliosis which is increasingly being recognized as a defense mechanism rather than an offensive one. They undergo changes at the molecular, morphological and functional levels, secreting an array of cytokines, early in AD. The identity and the function/s of these cytokines remain poorly investigated. We show that amyloid- β (A β) induces a state of reactivity and elicits a cytokine pool from primary astrocytes as early as six hours. We identified tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) as a major neuroprotective candidate in this secretome through neuron-astrocyte co-culture studies. In primary neurons, TIMP-1 inhibited both A β -induced - FOXO3a-mediated apoptosis and impaired autophagy flux - by increasing Akt^{S473} phosphorylation. Furthermore, we show that CD63 expression is essential for TIMP-1's attachment on neurons and for mediating downstream Akt phosphorylation through

knockdown studies. Interestingly, intracerebroventricular TIMP-1 injection in transgenic 5xFAD mice not only reduced apoptosis and prevented autophagic dysregulation in the hippocampus but also decreased A β plaque burden. Notably, 5xFAD mice performed better following treatment with TIMP-1 in cognitive behavioral tests especially in fear-conditioning tests assessing amygdala-hippocampal communication. We found that synaptic protein expressions, spine density and spine shape were improved in whole tissue and in pure synaptosomal isolations/preparations from hippocampi and cortices of TIMP-1-treated 5xFAD mice. TIMP-1 increased long-term potentiation (LTP) at the Schaffer collateral-CA1 synapses in acute hippocampal slices from 5xFAD in electrophysiology experiments. Biochemically, Akt/GSK3 β signaling was detected as an essential mechanism for TIMP-1-mediated LTP induction. In conclusion, TIMP-1 emerges as a major neuroprotective cytokine secreted by reactive astrocytes early in AD that can ameliorate memory deficits and synaptic plasticity and therefore has an exciting prospect in AD therapeutic research.

YMS02-02 | Pathogenesis of white matter microglial dystrophy in human microvascular brain injury and Alzheimer's disease

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Dystrophic microglia (DM) are enriched in the gray matter of aging brains, particularly in AD where they have been proposed to be either senescent or disease-associated microglia. Here, we defined mechanisms of microglial dystrophy in the cerebral white matter of human autopsy brains diagnosed with microvascular brain injury (mVBI) with or without Alzheimer's disease (AD). We hypothesized that DM accumulate myelin debris, which disrupts myelin regeneration and repair and pre-disposes to iron-mediated microglial injury. The frontal periventricular white matter was analyzed in 40 human autopsy brains, which included 22 cases with mVBI and 18 with only AD. We employed a novel photobleaching protocol to quench endogenous lipofuscin autofluorescence, which permitted fluorescent immunohistochemical visualization by 3D confocal microscopy of myelin debris in dystrophic microglia identified with light chain ferritin (FTL). We employed design-based stereology to quantify a perilipin family lipid storage protein, (PLIN-2), 4-hydroxynonenal 4-HNE), and mitochondrial dysfunction (TOM20). There was significant correlation between DM and total microglia labeled with Iba1 ($r^2 = 0.8355$, $p = 0.0015$) in mVBI in contrast to AD ($r^2 = 0.4868$, $p = 0.0814$). DM in mVBI were significantly enriched in myelin debris (MBPd) ($r^2 = 0.9982$; $p < 0.0001$) unlike AD ($r^2 = 0.3573$; $p = 0.0891$). The density of cells with myelin debris was significantly lower in



non-dystrophic microglia vs. DM ($p < 0.0001$). PLIN-2 was enriched in FTL+ DM. FTL+Plin2+ cells were significantly elevated in mVBI ($r^2 = 0.6315$; $p < 0.0105$), but not in AD ($r^2 = 0.3769$; $p = 0.1948$). The percentage of DM with myelin debris inclusions was significantly higher (~50% of DM; $p < 0.0001$) unlike non-DM (~15%). DM also displayed highly significant elevations in both 4HNE and TOM20, a mitochondrial outer membrane translocase, in mVBI ($p < 0.0001$). DM comprise a novel population of oxidative stress-associated microglia (OSAMs) that are enriched in the aging white matter of VCID and AD cases. DM are identified with FTL, PLIN2, 4HNE, TOM20, and myelin debris. Excessive iron accumulation and failure to digest myelin debris in DM may contribute to failed remyelination after white matter injury.

YMS02-03 | Correction of mTORC1-mediated protein synthesis rescues memory in mouse models of Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by synapse failure and cognitive decline. Brain mRNA translation is central to synaptic plasticity and cognition, and converging evidence indicates it is impaired in AD. In particular, the mammalian target of the rapamycin complex 1 (mTORC1) pathway plays a key role in regulating protein synthesis. Not surprisingly, the mTORC1 signaling has received considerable attention in recent AD research. However, results from such studies remain controversial. In this work, we analyzed the mTORC1 signaling proteins in hippocampi from mice infused intracerebroventricular (i.c.v.) with amyloid- β oligomers (A β O), the major neurotoxins in AD. We found a decrease in the levels of mTORC1 proteins in mice, 7 days after A β O infusion. Further, we tested whether enhancing mRNA translation could rescue defective translation and memory in mouse models of AD. Results show that haploinsufficiency for the translational repressors' eukaryotic initiation factor 4E binding protein 2 (4E-BP2) or fragile X mental retardation protein (FMRP) prevented the inhibition

of brain protein synthesis and memory impairment induced by A β O. These findings establish that targeting mRNA translation initiation corrects translational and memory deficits in AD models, and suggest a potential target to combat cognitive decline in AD.

YMS03-01 | Protein kinase signalling cascades associated with neuronal death in excitotoxicity

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

**YMS03-02 | The role of activity-dependent phosphorylation in the presynaptic function of α -synuclein**

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Repeated rounds of synaptic vesicle exocytosis and endocytosis play a critical role in the maintenance of neurotransmission. α -Synuclein, a protein extensively studied for its role in Parkinson's disease, is a known modulator of this synaptic vesicle cycle. However, its exact function remains unclear. This can be elucidated by examining post-translational modifications as a regulatory mechanism of the protein's presynaptic function. Through a phosphoproteomic screen of primary hippocampal neuron lysates we identified two residues in α -synuclein that become more highly phosphorylated with neuronal activity. These two novel activity-dependent phosphorylation sites were studied by transfecting α -synuclein knockout hippocampal neuron cultures with variants of α -synuclein that mimicked or abolished phosphorylation at these residues. Neurons were subjected to live cell fluorescence pHluorin imaging and changes in fluorescence intensity were used to quantify synaptic vesicle and protein trafficking in real time. The presence of different α -synuclein phosphomutant variants impacted the distribution between plasma membrane and synaptic vesicles of the SNARE protein synaptobrevin-2, a known binding partner of α -synuclein crucial for efficient synaptic vesicle fusion. Furthermore, these phosphomutants had no effect on various parameters of synaptic vesicle dynamics including evoked exocytic trafficking of synaptobrevin-2, endocytic retrieval after stimulation or the size of the recycling pool of synaptic vesicles. Instead, we found that the phosphorylation state of one of the identified residues specifically impacted the endocytic retrieval of synaptobrevin-2 during neuronal activity. This constitutes the first evidence that dynamic, activity-dependent phosphorylation of α -synuclein modulates synaptic vesicle protein trafficking. Loss of this form of synaptic vesicle cycle regulation in disease may interfere with efficient neurotransmission, especially considering that α -synuclein is sequestered into Lewy bodies and neurites in Parkinson's disease and Lewy body dementia. As such, disruption of activity-dependent α -synuclein phosphorylation has the potential to contribute to the underlying pathogenesis of these synucleinopathies.

YMS03-03 | Repression of EIF2-mediated BRAIN protein synthesis in the pathophysiology of major depressive disorder

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Major depressive disorders (MDD) are one of the most significant causes of disability worldwide. The number of depressed adults has notably risen in the past decade, bringing up considerable social and economic impacts worldwide. However, the underlying causes and mechanisms of MDD have not been fully understood and many patients are refractory to available treatments. Impaired control of brain protein synthesis has been shown to underlie several neurodevelopmental and neurodegenerative conditions. Nonetheless, a potential role for impaired translational control in depressive disorders remains elusive. The control of translation initiation relies on the phosphorylation of the α subunit of the eukaryotic initiation factor 2 (eIF2 α -P) which, in turn, blocks the activity of eIF2B, thereby reducing translation rates. Here we investigated whether attenuation of brain protein synthesis associates with human depression and drives depressive-like states in mouse models. Preliminary results using *postmortem* tissue from dorso-lateral prefrontal cortex and hippocampus of patients diagnosed with MDD or control patients demonstrated that gene expression of EIF2B5 (the catalytic subunit of eIF2B) is reduced in MDD, suggesting alterations in translational control in these individuals. We further showed that pharmacological repression of protein synthesis by salubrinal, an inhibitor of the eIF2 α phosphatase GADD34 results in depressive-like behavior in adult C57BL/6J mice, as assessed by the tail suspension test and Porsolt forced swim test. This behavior was blocked by ISRIB, a small-molecule compound that directly activates eIF2B under high eIF2 α -P levels, suggesting that eIF2 α -P-dependent effects over translation promote the despair-associated components of depressive-like states. Taken together, our initial results suggest that alterations in translational control are related to the pathophysiology of MDD. Targeting protein synthesis may potentially offer novel therapeutic targets for the treatment of mood disorders.

YMS04-01 | Chemogenetic regulation of microglial activity in an Alzheimer's disease model

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Microglia, the immune cells of the central nervous system, play complex roles in Alzheimer's disease (AD). Microglial phagocytosis of toxic amyloid peptides may be beneficial; however, their excessive release of inflammatory mediators can exacerbate AD progression. Gq protein-coupled receptors expressed by microglia may regulate these actions. We generated mice expressing the hM3Dq Designer Receptor Exclusively Activated by Designer Drugs (DREADD) selectively in microglia to study the role of Gq signaling in these cells. Activation of hM3Dq in primary microglia derived from these mice increased phagocytosis and uptake of amyloid-beta oligomers. In mice, chronic hM3Dq activation by repeated intraperitoneal injection of clozapine N-oxide (CNO) decreased the expression of pro-inflammatory cytokines in the brains of LPS-injected mice, likely desensitizing microglia to inflammatory stimuli. To determine whether microglial Gq signaling promotes amyloid clearance and tolerance towards amyloid-induced inflammation, we crossed hM3Dq DREADD mice with APP^{NL-G-F} mice to create a model of AD in which we can activate microglial Gq signaling. Chronic activation of hM3Dq, by two- or eight-week treatment with CNO, did not affect cortical amyloid-beta levels nor plaque deposition in 4- or 6-month-old hM3Dq-APP^{NL-G-F} mice. Surprisingly, the percentage of microglia expressing hM3Dq in APP^{NL-G-F} mice was significantly decreased compared to controls, although the remaining DREADD-positive microglia associate closely with amyloid plaques. Furthermore, chronic CNO treatment had no effect on the expression of pro-inflammatory cytokines in the cortex of hM3Dq-APP^{NL-G-F} mice. Taken together, our results suggest that activation of Gq signaling in microglia can promote their uptake of amyloid-beta in vitro and increase tolerance for LPS-induced neuroinflammation in vivo, however activation of hM3Dq in the APP^{NL-G-F} mouse model of AD did not ameliorate plaque burden nor neuroinflammation. This chemogenetic approach to modulate Alzheimer's-like pathology may be more effective on a less aggressive model of AD, in which hM3Dq expression may not be affected.

YMS04-02 | Schizophrenic patients hiPSC-derived astrocytes impair synaptic engulfment by induced microglial-like cells (iMGs) in vitro

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Schizophrenia (SCZ) is a neuropsychiatric disorder caused by the interaction between genetic and environmental factors. SCZ individuals exhibit cognitive deficits, positive (psychosis, hallucinations and delusions) and negative symptoms (depression, avolition and anhedonia), as well as reduced gray matter volume. Microanatomical analyses suggest this phenomenon occurs due to diminished dendritic spine density, which likely happens as a result of exaggerated synaptic pruning. It has already been shown that the classical complement cascade and CX3CL1/CX3CR1 pathway play a direct role in this process, triggering synaptic engulfment by microglia. Taking into account that pre-natal infection acts as an environmental risk factor for SCZ, hiPSCs-derived astrocytes from both healthy control (HC) and SCZ individuals after stimulation with TNF- α . Using proteomics of Astrocyte Conditioned Media (ACM), we initially found that CX3CL1 is upregulated in SCZ ACM after TNF- α stimulation, compared to HC ACM, a result later confirmed by qPCR and ELISA in both astrocytes transcripts and ACM, respectively, while the classical complement components (C1q, C3 and C4) display similar expression levels. In order to verify whether SCZ astrocyte secreted CX3CL1 stimulates higher synaptic engulfment by microglia, HC induced microglial-like cells (iMGs) were incubated with ACM from both diagnostic groups, under non-stimulated (N.S.) or TNF- α stimulated conditions and in the presence or absence of the CX3CR1 antagonist (AZD8797) with CM-Dil-labeled synaptoneuroosomes. Surprisingly, TNF- α -stimulated HC ACM, N.S. SCZ ACM and TNF- α -stimulated SCZ ACM exposure leads to lower synaptoneurosome phagocytic engulfment by iMGs relative to N.S. HC ACM. In addition, pre-treatment with AZD8797 enhances synaptic phagocytosis only in iMGs exposed to N.S. HC ACM. Cell surface biotinylation data suggests that this data is due to reduced membrane CX3CR1 in iMGs in all treatment groups, but N.S. HC ACM. Since secreted cytokines can impact microglial phagocytic capacity, we analyzed cytokine expression in HC and SCZ astrocytes stimulated with TNF- α . Interestingly, TNF- α and IL-6 mRNA levels are elevated in stimulated SCZ astrocytes relative to HC astrocytes. Altogether, these results indicate that SCZ astrocytes secreted factors impair HC iMG synaptic engulfment.

**YMS04-03 | Ketogenic diet promotes resilience to social stress and modifies microglial morphology and ultrastructure in male mice**

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Ketogenic diet (KD) is high-fat, low-carbohydrate diet which has emerged as a lifestyle change that may promote stress resilience. Microglia are the resident immune cells of the brain; besides coordinating brain immune responses, they react to psychological stress and mediate stress-related brain changes. This study focused on the effect of KD, its relationship to stress resilience and its effect on microglia. Two-month-old adult male C57BL/6 mice received KD versus normal diet (ND) starting 4 weeks prior to the experiment. The consequences of the diet on the response to chronic stress were investigated by comparing non-stressed controls (CTRL) with animals undergoing 10 days of repeated social defeat (RSD). After RSD, mice were classified as resilient (RES) or susceptible (SUS) to stress based on their performance in a social interaction test. Our results show that upon RSD, KD increased the proportion of RES animals, 57.14% of KD mice ($n = 28$) versus 36.36% of ND ($n = 22$). We studied the underlying mechanisms by focusing on microglia in the ventral hippocampus, a region affected by chronic stress. Using TMEM119/IBA1 double staining, we found that KD does not affect microglial number or distribution. However, changes in their soma and arborization area were linked to the effect of KD, stress and their interaction. Ultrastructural analysis of microglia by electron microscopy showed that KD reduced the number and proportion of Golgi apparatus and endoplasmic reticulum with dystrophy, which is a marker of cellular stress and a feature associated to neuropathological contexts. Microglia also displayed general effects of stress in the number of contacts with synaptic elements. Finally, preliminary lipidomic analysis of hippocampus showed distinct lipidomic signatures: a basal effect of diet in CTRL animals. This study provides valuable results regarding the dynamic relationship of diet-induced energetic shifts, psychological stress, and microglia.

YMS05-01 | Frontotemporal dementia caused by progranulin mutations is characterised by disrupted myelin lipid metabolism

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Frontotemporal dementia (FTD) is a common cause of younger-onset dementia. Heterozygous mutations in the progranulin (GRN) or C9orf72 genes are the two most common causes of inherited FTD. Evidence suggests that GRN mutations disrupt lysosomal catabolism of lipids; however, there is no lipidomic data from human brain to support this hypothesis.

To address this knowledge gap, we combined comprehensive lipidomic analysis of post-mortem tissue with in-vivo MRI assessment of white matter integrity. Lipidomic analysis (824 lipids from 27 structural classes) was performed on post-mortem frontal and parietal lobe grey and white matter tissue from FTD cases with GRN ($n = 7$; FTD-GRN) or C9orf72 ($n = 11$; FTD-C9orf72) mutations, and age-matched neuropathologically normal controls ($n = 11$). Substantial loss of myelin-enriched sphingolipids sulfatide, galactosylceramide and sphingomyelin was observed in frontal white matter of FTD-GRN cases, and a less-pronounced, statistically significant loss of these lipids in FTD-C9orf72 cases. Similarly, western blotting showed significant reduction of myelin-specific proteins. Decreased white matter tract integrity by MRI was inversely correlated with sulfatide levels in frontal white matter. Partial least squares-discriminant analysis distinguished FTD-GRN from FTD-C9orf72 and control cases through pronounced accumulation of cholesterol esters in both frontal and parietal white matter, suggesting impaired lysosomal catabolism of cholesterol released from myelin in FTD-GRN cases. Significantly increased lysosomal and phagocytic protein markers, and decreased activity of the lysosomal enzyme glucocerebrosidase, were evident in both FTD-GRN and FTD-C9orf72, however galactocerebrosidase activity (required for lysosomal catabolism of galactosylceramide and sulfatide) was selectively increased in FTD-GRN cases. Our findings indicate that both C9orf72 and GRN mutations disrupt lysosomal homeostasis, but GRN mutations have a pronounced impact on myelin lipid metabolism and myelin integrity. Hyperactive myelin catabolism is probably a major driver of aggressive gliosis and neurodegeneration in FTD-GRN cases, and myelin degeneration may be a valuable biomarker of disease onset and progression.

YMS05-02 | Lipid-mediated impairment of axonal lysosome transport contributing to autophagic stress in Niemann–Pick disease type C

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Efficient degradation of autophagic vacuoles (AVs) generated at axon terminals by mature lysosomes enriched in the cell body represents an exceptional challenge that neurons face in maintaining cellular homeostasis. Here, we reveal a lipid-mediated impairment of lysosome transport to distal axons contributing to axonal AV accumulation in the neurodegenerative lysosomal storage disorder Niemann–Pick disease type C (NPC). Using transmission electron microscopy, we demonstrate that organelles of the endocytic and autophagic pathways accumulate in NPC dystrophic axons, indicating defects in the clearance of organelles destined for lysosomal degradation. Using STED super-resolution and live-neuron imaging, we further reveal that elevated cholesterol on NPC lysosome membranes abnormally sequesters motor-adaptors of axonal lysosome delivery, resulting in impaired anterograde lysosome transport into distal axons that disrupts maturation of axonal AVs during their retrograde transport route. Pharmacologic reduction of lysosomal membrane cholesterol with 2-hydroxypropyl- β -cyclodextrin or elevated expression of anterograde motor-adaptor Arl8b rescues lysosome transport into axons and reduces axonal AV accumulation in presymptomatic NPC neurons. Together, these findings demonstrate a pathological mechanism by which altered membrane lipid composition compromises axonal lysosome trafficking and positioning and shows that lowering lysosomal lipid levels rescues lysosome transport into NPC axons, thus reducing axonal autophagic stress at early stages of NPC disease. This work was supported by the Intramural Research Program of NINDS, NIH.

YMS05-03 | Impacted brain perfusion upon genetic deletion of hormone-sensitive lipase in mice

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The hormone-sensitive lipase (HSL) is mainly present in white adipose tissue, where it hydrolyses mainly diacylglycerol (DAG) under regulation by catecholamines and insulin. Recently, we have demonstrated that HSL is enriched in neuronal synapses, show activity throughout the brain and is important for brain function, namely

memory performance. HSL was further suggested to regulate brain availability of bioactive lipids, namely eicosanoids that modulate cerebral blood flow (CBF). Thus, we set to test that genetic HSL deletion increases the susceptibility for impaired brain perfusion in diet-induced obesity. HSL^{-/-}, HSL^{+/-} and wild-type mice were fed a high-fat diet (HFD) or control diet (CD) for 2 months, which induces obesity and metabolic stress, and were characterized in behaviour tests (novel object recognition (NOR) and open field (OF)) and metabolic tests (insulin- and glucose tolerance test, metabolic cages). CBF was determined by arterial spin labelling (ASL) magnetic resonance imaging (MRI) under isoflurane anaesthesia. We show that upon genetic deletion of HSL, CBF is decreased by genetic HSL deletion. CBF was decreased in cortex and hippocampus in ^{-/-} mice independent of diet and in decreased in ^{+/-} mice in hippocampus. This follows a similar pattern of reduced time in the centre of the OF arena, indicating increased anxiety. NOR show a diet but not genotype effect throughout the groups, that is, memory impairment was observed in all mice exposed to HFD, relative to CD. This study points specific roles of HSL in the brain and shows that deletion of HSL impacts CBF and triggers increased anxiety-related behaviour, which is not further aggravated by HFD exposure. On the other hand, object recognition memory was affected by HFD but not impacted by HSL deletion.

YMS05-04 | Differential brain lipid composition in the *Octodon degus*, a matter of age and sex

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Modification of the human brain lipid composition have been related to physiological aging and to pathological conditions. However, how these changes contribute to the neurodegenerative processes is not fully understood and few studies have been conducted on natural models of neurodegeneration. The diurnal rodent *O. degus* spontaneously develops different neurodegeneration-associated traits, such as cognitive decline, neuroinflammation and proteins (e.g. β -amyloid) accumulation in the brain. However, the lipidic profile in this species has not been explored yet. We studied in detail the lipid composition on different brain areas along aging paying special attention to sex-related differences. In particular, Prefrontal cortex (PFC) and cerebellum from a total of 44 young, adult, old and senile (6 months old to 7 years old) male and female animals, were analyzed in this study. In the PFC, we found a significant increase in the total lipid content during aging. When younger and older animals were compared, a

significant increase in some specific lipids was found: ganglioside GT1b, galactosylceramide, sulfatide and cholesterol. We found no sex-associated differences in the lipid profile in the PFC. In the cerebellum, both sex and age influence the lipid composition. Sex differences were particularly found in ganglioside GM1, sulfatide, galactosylceramide and cholesterol. A statistically significant accumulation of sulfatide, phosphatidylcholine, and cholesterol was found during aging. However, the levels of other lipids, in particular galactosylceramide, were significantly reduced in the aged animals. These results evidence the brain lipid changes occur in a region-specific manner and depending on sex and age in this model and offer a first piece of information for its lipidic characterization. Future research could contribute to understand the role of specific lipidic species on pathological neurodegeneration.

YMS06-01 | Therapeutic effects with lesion-specific delivery of the NgR-Fc peptide in an experimental model of multiple sclerosis

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Nogo receptor 1 (NgR1) is a cognate receptor for a number of myelin-associated inhibitory factors (MAIFs), which are deposited throughout the extracellular milieu during inflammatory demyelination. Signaling through the NgR1 complex has been associated with axonal degeneration in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS), while deletion of this receptor homologue is associated with preserving overall axonal integrity and myelination status. Since MS pathogenesis displays a plethora of inflammatory cell infiltrates within central nervous system (CNS) demyelinating plaques, we utilized transplantable hematopoietic stem cells (HSCs) to target these disseminated lesions, delivering an NgR(310)ecto-Fc fusion protein in situ. Briefly, we modified the HSCs with LV-NgR(310)ecto-Fc on immune cell lineages and successfully identified CNS infiltrating macrophages as the immune-positive cell type in EAE-induced recipient female mice. These differentiated, tagged phagocytes were prominent at the peak of the disease and expedited the engulfment of NgR-Fc protein-MAIF complexes, but most importantly, these recipient mice recovered from the neurological decline in EAE. Moreover, axonal regeneration (GAP43+ axons) and remyelination (mobilization of

oligodendrocyte precursors, recovered diffusion weighted MRI and reestablished g ratios) were demonstrated in the white matter tracts of EAE-induced mice transplanted with the HSCs, expressing the NgR-Fc fusion protein. Our data suggest that genetically modified HSCs can be utilized as carriers of the therapeutic NgR-Fc peptide for targeted delivery into CNS demyelinating lesions, potentiating neurological recovery, following neuroinflammatory degeneration.

YMS06-02 | Cannabidiol and neural stem cells, a new hope for Rett syndrome?

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Rett Syndrome (RTT) is a rare progressive neurodevelopmental X-linked disorder, caused mostly by mutations in MECP2. This transcriptional and epigenetic regulator has been proposed to modulate neurogenesis and neuronal maturation, processes known to be affected in both RTT patients and mouse models. Cannabidiol (CBDV) a non-psychotomimetic cannabinoid, currently undergoing phase 2 clinical trials for medical use in humans, has been reported to bind to TRPV1, with unknown effects on adult neurogenesis. Using the neurosphere assay, cells were subjected to pharmacological treatments for 2 or 7 days according to the experimental protocol. CBDV-treated [1 µM] cells for 2 days in vitro (DIV2) promoted an increase in cell survival (PI+cells) and cell proliferation (BrdU+ cells). While at DIV7, CBDV [1 µM] promoted an increase in neuronal differentiation (NeuN+ cells) and inhibited oligodendroglial maturation (MBP+ cells). Importantly, TRPV1 antagonist 5'-Iodoresiniferatoxin [300 nM] blocked the effects on cell death and cell proliferation mediated by CBDV, further suggesting TRPV1-dependency. In vivo, using a female mouse model of RTT, animals were subjected to a chronic treatment with CBDV (3 mg/kg/day) on the pre-symptomatic stage, followed by a battery of behaviour tests, used to gauge the putative therapeutic effects of CBDV administration. The Novel Object Recognition Test suggested that CBDV ameliorates cognitive impairments in these animals. No odour and anxiety impairments were found in RTT animals. Locomotion deficits were not recovered by treatment with CBDV. However, the motor coordination impairments present in RTT mice were rescued by CBDV. Additionally, RTT animals show an increased abnormal hippocampal neurogenesis that tends to be attenuated in CBDV-treated animals. Taken together, this project explores the novel neurogenic potential of CBDV via TRPV1. These findings will provide new insights for future research aiming to repurpose CBDV as a viable drug to treat RTT.

YMS06-03 | CoREST3 is involved in neuronal differentiation and HDAC2 expression in cortical neurons derived from human pluripotent stem cells

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Repressor element-1 silencing transcription factor (REST) is a transcriptional repressor involved in neurodevelopment and neuroprotection that forms a complex with the corepressor of REST1 (CoREST1), CoREST2 and CoREST3 (encoded by *RCOR1*, *RCOR2* and *RCOR3*, respectively). Emerging evidence suggests the CoREST family have the ability to target unique genes, in a REST-independent manner, in various neural and glial cell types at different stages of development. There is limited knowledge on expression and function of CoREST3 in neurodevelopment and neurodegeneration, particularly in humans. This study used 2D and 3D human pluripotent stem cell (hPSC) models to interrogate *RCOR3* gene expression levels in human neurons using RT-qPCR. *RCOR3* expression was shown to significantly increase in glutamatergic cortical and GABAergic ventral forebrain neurons, mature functional NGN2 induced neurons and a trend for an increase in 3D cerebral organoids. CoREST3 was shown to have an age-related and region-specific expression pattern in the aging rat brain, with expression in the prefrontal cortex increasing in adult rats (post-natal day 96) and in the caudate putamen in aged rats (~14 months) via Western blotting. CoREST3 expression was analysed in an Alzheimer's disease cohort using *post-mortem* tissue via western blot analysis. The long isoform of CoREST3 was significantly decreased by ~30%. *RCOR3* shRNA knockdown in cortical neurons derived from hPSCs resulted in an increase in the cortical neural progenitor marker, *PAX6*, and neuronal markers *TUBB3* (encodes β -III-tubulin) and *MAP2*, in addition to a trend for increased neurite length. A significant increase in *HDAC2* expression was also observed. In summary, our findings are the first to show an increase of *RCOR3* expression with human embryonic neural differentiation, CoREST3 expression is disrupted in Alzheimer's disease and a potential role for CoREST3 in the regulation of neuronal differentiation and *HDAC2* expression.

YMS06-04 | Characterizing the role of mitochondrial dynamics in postnatal neural stem cell fate

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Neural stem cells (NSCs) are found in discrete regions of the adult mammalian brain. During adulthood, NSCs can differentiate into neurons, astrocytes and oligodendrocytes, making them a powerful tool to treat disease-related neural loss. Several studies suggest that mitochondria have an important role in regulating NSC differentiation and lineage determination. A major aspect that remains unclear is whether mitochondrial dynamics and metabolism have a role in directing NSC fate. Hence, our work aims to dissect how mitochondria biogenesis, morphology and bioenergetics can modulate NSC differentiation. For this, NSCs were obtained by isolating subventricular zone (SVZ) and dentate gyrus (DG) cells from P1-3 C57Bl6 mice. The isolated cells were grown in neurospheres, and consequently passaged to guarantee higher yields of NSCs. Thereafter, neurospheres were plated under specific differentiation conditions giving rise to neurons, astrocytes and oligodendrocytes. Additionally, expression of proteins involved in mitochondrial biogenesis and fusion/fission was determined. Overall, expression of mitochondrial biogenesis-related proteins did not significantly change with NSC differentiation, in both neurogenic niches. Importantly, the levels of proteins involved in mitochondrial fusion (*Mfn1*/*Mfn2*) significantly increased while proteins involved in fission (*DRP1*) significantly decreased along differentiation, only in SVZ cells. Furthermore, mitochondrial number, length and area differed in the different cell types (NSCs and differentiated cells). Mitochondrial number significantly increased during astroglial and neuronal differentiation. Moreover, both NSCs and oligodendrocyte precursor cells were the cells with more elongated mitochondria. Interestingly, mitochondrial area did not change in neuronal cells, while there was a significant reduction along oligodendroglial maturation. These results will pave the road towards novel findings concerning the role of mitochondrial dynamics in NSC fate.

**YMS07-01 | Trafficking of the glutamate transporter is impaired in LRRK2-related Parkinson's disease**

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The excitatory amino acid transporter 2 (EAAT2) accounts for 80% of brain glutamate clearance and is mainly expressed in astrocytic perisynaptic processes. EAAT2 function is finely regulated by endocytic events, recycling to the plasma membrane and degradation. Noteworthy, deficits in EAAT2 have been associated with neuronal excitotoxicity and neurodegeneration. In this study, we show that EAAT2 trafficking is impaired by the leucine-rich repeat kinase 2 (LRRK2) pathogenic variant G2019S, a common cause of late-onset familial Parkinson's disease (PD). In LRRK2 G2019S human brains and experimental animal models, EAAT2 protein levels are significantly decreased, which is associated with elevated gliosis. The decreased expression of the transporter correlates with its reduced functionality in mouse LRRK2 G2019S purified astrocytic terminals and in *Xenopus laevis* oocytes expressing human LRRK2 G2019S. In LRRK2 G2019S knock-in mouse brain, the correct surface localization of the endogenous transporter is impaired, resulting in its interaction with a plethora of endo-vesicular proteins. Mechanistically, we report that pathogenic LRRK2 kinase activity delays the recycling of the transporter to the plasma membrane via Rab inactivation, causing its intracellular relocalization and degradation. Taken together, our results demonstrate that pathogenic LRRK2 interferes with the physiology of EAAT2, pointing to extracellular glutamate overload as a possible contributor to neurodegeneration in PD.

YMS07-02 | It takes two kinases to tango: Regulation of LRRK2 by PAK6 in health and disease

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Starting from an unbiased screen, we discovered that the Parkinson's disease (PD) kinase LRRK2 interacts with PAK6 through its GTPase/ROC domain. Mechanistically, PAK6 requires LRRK2 to promote neurite outgrowth in vivo and its kinase activity rescues LRRK2 G2019S-associated neurite shortening phenotype through a mechanism involving LRRK2 dephosphorylation at S935 and reduction of LRRK2 activity. Of note, PAK6 fails to lower LRRK2 pathological activity in the presence of GTPase/ROC mutant, likely due to the intrinsically lower phosphorylation at S935 of this mutant, as observed in mouse tissues. In line with a putative protective role of PAK6, we found reduced PAK6 levels in the plasma of PD patients pointing to its potential as a disease biomarker. Moreover, another unbiased screen revealed the ciliary protein CLUAP1 as a novel PAK6 interactor. Accordingly, a recent set of data indicate that PAK6 promotes ciliogenesis via the regulation of LRRK2 kinase activity, highlighting novel functions for PAK6 in both physiological and pathological contexts.

YMS07-03 | Metabolic and plasma membrane alterations in β -glucocerebrosidase-related pathologies: Steps to understand neurodegeneration

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β -Glucocerebrosidase is a lysosomal glycohydrolase responsible for the catabolism of the sphingolipid glucosylceramide. Deficiency of this enzyme causes accumulation of glucosylceramide and the onset of so called β -glucocerebrosidase-related pathologies, ranging from Gaucher Disease, when mutations are homozygous, to Parkinson's disease, where monoallelic mutations become the major genetic risk factor for the onset of Parkinson's disease. To understand the link among β -glucocerebrosidase loss of function, glucosylceramide accumulation and neurodegeneration, we developed an in vitro model of the neuronal form of Gaucher Disease represented by iPSCs-derived dopaminergic neurons obtained from healthy subjects' fibroblasts and treated them for 30 days with

conduiritol B epoxide, a specific inhibitor of β -glucocerebrosidase. Conduiritol B epoxide-treated neurons present neurodegenerative features and are characterized by the accumulation of glucosylceramide not only at lysosomal level but also at the plasma membrane where they affect the structure of signalling domains, called 'lipids rafts'.

Considering the central role of lysosomes in the recycling of metabolites, we investigate the effect of the lysosomal impairment, due to glucosylceramide accumulation, on the metabolic flow, through targeted metabolomics analysis by LC-MS/MS. Lysosomal impairment is paralleled by alterations in the glucose metabolism and in the content of aminoacids, used as an alternative energy source.

The obtained data demonstrate that β -glucocerebrosidase loss function induces an impairment of the lysosomal compartment, that is responsible for two distinct events: (i) the establishment of an aberrant lysosome-plasma membrane axis which alters the plasma membrane architecture with consequences on the intracellular signalling pathways; (ii) alterations in the metabolic homeostasis of the neurons.

YMS08-01 | Sensory processing dysregulations as reliable translational biomarkers in SYNGAP1 haploinsufficiency

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Amongst the numerous genes associated with intellectual disability, SYNGAP1 stands out for its frequency and penetrance of loss-of-function variants found in patients, as well as the wide range of co-morbid disorders associated with its mutation. Most studies exploring the pathophysiological alterations caused by Syngap1 haploinsufficiency in mouse models have focused on cognitive problems and epilepsy; however, whether and to what extent sensory perception and processing are altered by Syngap1 haploinsufficiency is less clear. By performing EEG recordings in awake mice, we identified specific alterations in multiple aspects of auditory and visual processing, including increased baseline gamma oscillation power, increased theta/gamma phase amplitude coupling following stimulus presentation and abnormal neural entrainment in response to different sensory modality-specific frequencies. We also report lack of habituation to repetitive auditory stimuli and abnormal deviant sound detection. Interestingly, we found that most of these alterations are present in human patients as well, thus making them strong candidates as translational biomarkers of sensory-processing alterations associated with SYNGAP1/Syngap1 haploinsufficiency.

YMS08-02 | *Withania somnifera* (L.) Dunal helps regain earlier muscle function restoration after a compression injury to sciatic nerve in mice

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Withania somnifera (L.) Dunal, also known as Ashwagandha and commonly referred to as Indian ginseng, is traditionally used as a medicine. Its medicinal properties include anxiolytic activity, cognition promoting effects, neuroprotective effects, anti-inflammatory, effective anti-stress, and anti-aging medicinal herb. The root extract of this plant augments superoxide dismutase (SOD) and catalase activities and lowers lipid peroxidation. Based on the available data on its neuroprotective activities, it appears to be an interesting candidate in accelerating the functions reclamation after a traumatic peripheral nerve injury. The present study was designed to explore the possible effect of the *Withania somnifera* (L.) Dunal (roots) powder on the rate of muscle function regain in a mouse model subjected to an induced mechanical insult to the sciatic nerve. A dose of 25 mg/kg of body weight was orally administered from the day of nerve lesion until the termination of the experiment. The motor functions were assessed by measuring hind limbs muscle grip strength, muscle masses and sciatic functional index, while the restoration of sensory functions was estimated by performing a hotplate test. The hematological and serological analyses were done to evaluate the impact of treatment on oxidative stress and other systemic indexes. We found that the motor functions regain, measured by muscle grip strength test and sciatic functional index, was significantly improved and was earlier in the treatment group. Similarly, sensory response to thermal stimulus was also noticeable in the treated group at an early time point. The restoration of muscle mass and attenuated oxidative stress were remarkable in the treatment group. Moreover, an elevated hemoglobin level and anti-glycemic effects were also noted in response to the treatment. Statistically, both motor and sensory functions were significantly improved and prompted in the treatment group. The present findings highlight that *Withania somnifera* (L.) Dunal (roots) is helpful in accelerating functional recovery after an insult to the peripheral nerve in a rodent model. It can be a novel remedial agent for traumatic nerve injuries, particularly peripheral nerve injuries.



YMS08-03 | Furan neurotoxicity in Wistar rats involves behavioural defects, microgliosis, astrogliosis and oxidative damage

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Evaluation of health risks poses by the consumption of furan, a widespread food contaminant found in coffee and infant's ready-to-eat meals, is increasing. Except liver, previous studies in rodents exposed to 8 mg/kg found no alteration in peripheral organs. Implications of furan in the CNS are ill-defined. We assessed brain responses and alterations in Wistar rats treated with 0.0, 2.5, 5 and 10 mg/kg of furan after 28 days. Furan-treated rats displayed hyperactivity characterized by heightened covered distance, speed rotations, head turn angles and intermittent freezing, relative to control. They were easily distracted on hole-board, climbed staircase rapidly and fall from metal-rope easily. They elicited poor natural spontaneous alternation in Y-Maze but located escape holes spontaneously under aversive stimuli in Barnes Maze. The highest deficit was at 5 mg/kg. No Evan-blue foci across doses, and lipid staining was uniform. Across doses, IBA1 (not GFAP) staining showed nodules at PFC thalamus and hypothalamus but parenchyma-wide and cerebellum-only at 10 mg/kg. Density of PFC's IBA1+ cells increased while intercell spacing decreased dose dependently. 2.5 mg/kg cells have round soma with thinner hyper-ramified processes; 5 mg/kg showed larger soma, with retracted/thicker processes, and 10 mg/kg were rod-shaped with polarized processes. GFAP and phagocytic MERTK intensity increased dose-dependently. Oxidative balance, dependent on SOD and GST activities, were least perturbed in cerebellum and hippocampus and highest in striatum>hypothalamus>PFC. In latter regions, only 2.5 mg/kg elicited oxidative damage with marked reduction in SOD activity. Overall, furan at all resulted in hyperactivity. It is unlikely that the behavioural dysfunction is associated with gliosis because 10 mg/kg, which elicited reactive gliosis the most, caused declined behavioural deficit and limited oxidative damage. However, reactive microglia may be involved in clean-up of yet-to-be deciphered materials. Given the most severe oxidative stress in

striatum, a region which controls movement, oxidative damage may be involved possibly through direct infiltration of furan or its reactive metabolite. Since the lowest dose of furan elicited continuous oxidative damage, long-term exposure to low furan doses may have unpleasant implications in the central nervous system.

YMS08-04 | Reboxetine treatment reduces neuroinflammation and glial activation in the P301S mouse model

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Noradrenaline (NA) is a major modulatory neurotransmitter of the central nervous system (CNS) and besides its actions as a neurotransmitter, it presents a potent anti-inflammatory and neuroprotective effect mainly due to its ability to reduce the activation of glial cells. The main source of NA in the CNS is the locus coeruleus (LC), which is the brain region where neurofibrillary tangles of tau first start to accumulate in Alzheimer's disease (AD) patients, being this the earliest detectable AD-like neuropathology. These toxic tau aggregates lead to the death of noradrenergic neurons and a subsequent decrease in NA levels that facilitates the progression of AD. Therefore, treatments to increase NA levels could slow down the progression of AD. Based on this, our main aim was to analyze the effects of a NA elevating drug in a mouse model of tauopathy. We treated WT and P301S mice with vehicle or reboxetine, a NA reuptake inhibitor, with mini-osmotic pumps for 28 days. After the treatment, we collected brain samples for biochemical analysis. Our data indicate that reboxetine treatment is able to reduce the neuroinflammation present in the hippocampus of P301S mice through the reduction of astrocytes and microglia activation. In conclusion, reboxetine and other NA-elevating drugs could be an interesting therapeutic approach to reduce the neuroinflammation and glial cells over-activation that contribute to the progression of tauopathies such as AD.

ABSTRACT

Workshops

W01-01 | Workshop "Scientific Publishing: From study design to post-acceptance marketing"

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Getting a paper published can be a long process, from sound experimental design to conduct, data analysis including statistics, then submission to a suitable journal, peer review process, and eventual acceptance with all follow-up steps such as license agreements and post-publication marketing. In this workshop, we will try to close the gap between the author's view on the process—how to summarize the study in an appropriate and appealing manner—and a referee's perspective on what a comprehensive manuscript should be like. Furthermore, we will cover aspects of post-acceptance, from changes in the publication landscape towards data sharing and Open Access repositories, and broader dissemination via social media

W02-01 | Overcoming molecular brakes for promoting myelin repair

Richard Lu

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The repair and functional recovery of the nervous system is a highly regulated process that requires the coordination of many different components including the proper myelination of regenerated axons. Dysmyelination or remyelination failures after injury result in defective nerve conduction, impairing normal nervous system functions. In recent years, epigenetic chromatin modifications have been implicated in CNS myelination and functional nerve restoration. The pro-regenerative transcriptional program is likely silenced or repressed in adult neural cells including neurons and myelinating cells in the central and peripheral nervous systems limiting the capacity for repair after injury. In this talk, I will discuss the roles of epigenetic chromatin modifications in the establishment and maintenance of the myelination program during normal development and regeneration. We also discuss how to harness the epigenetic mechanisms to improve functional nerve regeneration in neurodegenerative diseases or injury.

W02-02 | Engineering organoid models for understanding human neurodevelopment and neural repair

Guo-li Ming

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Human induced pluripotent stem cells (hiPSCs) have the intrinsic potential to generate all cell types of a human body under 2D culture conditions. hiPSCs can also be directed to form organ like structures—organoids under 3D culture conditions, including brain organoids resembling the developing brain. Human brain organoids offer unique advantages in understanding molecular and cellular mechanisms governing embryonic neural development and in understanding the neural regenerative potential. I will discuss our recent work in developing protocols for generating brain-region specific organoids by first specifying hiPSCs into brain region specific neural stem cells, which are capable of self-organizing into distinct brain structures. I will also discuss our recent work on using these organoid models to understand molecular and cellular mechanisms underlying neurodevelopment and neural repair.

W02-03 | Evolutionarily conserved mechanisms of astrocyte morphogenesis

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Astrocytes are the most numerous glial cells in the human brain, and they possess an intricate morphology with highly ramified cellular processes interacting closely with individual synapses, neuronal cell bodies, and other brain cells. Astrocytes perform a range of essential developmental and homeostatic functions in neural circuits, and it is thought that these functions depend on their remarkable morphological complexity and close association with neural elements. However, we know surprisingly little about the molecular and cellular mechanisms that underlie astrocyte morphogenesis or how astrocytes modulate brain circuits in vivo. By leveraging a combination of molecular-genetic tools and live-imaging techniques in *Drosophila* and zebrafish, we sought to identify evolutionarily conserved pathways that regulate astrocyte development and function



in vivo. Using *Drosophila*, we found that RNAi-mediated depletion of *trapped in endoderm 1* (*tre1*) GPCR in astrocytes resulted in severely reduced astrocyte complexity across all brain regions. We showed that endogenous *tre1* is highly enriched in astrocytes and validated the astrocyte morphology phenotypes in *tre1* genetic mutants. We demonstrated that Tre1 acts cell autonomously to direct the elaboration of fine astrocyte processes into the synaptic neuropil, by genetically interacting with small GTPase Rac1 to regulate the actin cytoskeleton. Furthermore, we found this is a conserved signaling modality across species, as loss of the functional vertebrate GPCR analog *sphingosine-1-phosphate receptor 1* (*s1pr1*) in zebrafish led to disrupted astrocyte morphology similar to that observed in *Drosophila tre1* mutants. We used a combination of mutants and pharmacology, while live-imaging astrocyte morphogenesis in zebrafish, and found that S1pr1 is required throughout astrocyte development to control astrocyte process outgrowth and dynamics. Together, our data reveal a crucial and conserved Tre1/S1pr1-signaling in governing astrocyte morphogenesis in vivo. Future directions of our work aim to define activating ligands for Tre1/S1pr1 and to use these mutants as tools to understand how impaired astrocyte morphology affects neural circuit connectivity and function.

W02-04 | Molecular landscape of immature neurons in the human hippocampus across the lifespan and in Alzheimer's disease

Hongjun Song

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Immature dentate granule cells (imGCs) arising from adult hippocampal neurogenesis contribute to plasticity and unique brain functions in rodents and they are dysregulated in multiple human neurological disorders. Little is known about molecular characteristics of adult human hippocampal imGCs and even their existence is under debate. We performed single-nucleus RNA-sequencing to identify imGCs and quantify their abundance in the postnatal human hippocampus during infant, child, adolescent, adult, and aging stages. We identified common molecular hallmarks of imGCs across the lifespan and discovered age-dependent transcriptional dynamics unique to human imGCs that suggest changes in cellular functionality, niche interactions, and disease relevance. We also found a decreased number of imGCs with altered gene expression in Alzheimer's disease (AD). Finally, we demonstrated the capacity for neurogenesis in the adult human hippocampus with the presence of dentate granule cell fate-specific proliferating neural progenitors and with cultured surgical specimens. Together, our findings suggest the presence of a significant number of imGCs in the adult human hippocampus and our study reveals their molecular properties across the lifespan and in AD.

W03-01 | Neural Stem Cell Lineage Progression in Health and Disease

Simon Hippenmeyer

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

W03-02 | Neural circuits for multisensory integration in health and psychiatric disorders

Seung-Hee Lee

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Multisensory integration is a fundamental process underlying optimal decision-making in mammals. However, the state-dependent flexibility in multisensory decisions remains elusive. Here, I present our recent findings on the flexibility of the multisensory integration process in the posterior parietal cortex (PPC) and how this process is disrupted in the animal model of autism, Shank3 knock-out mice. First, we found that the posterior parietal cortex (PPC) represents audiovisual information flexibly in mice making perceptual decisions under audiovisual conflicts. Population activity of the PPC neurons represented visual sensory information, which was suppressed by the auditory inputs only when mice made auditory-dominant decisions in response to the conflicting audiovisual stimuli. Notably, prolonged locomotion diminished auditory cortical neurons projecting to the PPC (ACppc) but not the neurons projecting to the striatum (ACstr) and decreased auditory-dominant decisions without impairing audition-only decisions. The weakness of ACppc neurons was due to their inhibition by the secondary motor cortex (M2). Next, we found that the ACppc inputs were significantly reduced in the Shank3 knock-out (−/−) mice, an animal model of autism. We further found that a weakening of the auditory

and the frontoparietal inputs to the PPC was responsible for the alteration in multisensory behaviors in Shank3^{-/-} mice. Collectively, our data demonstrate cortico-parietal circuits are critical for multisensory behaviors, and anatomical and functional deficits in these circuits cause multisensory dysfunctions in Shank3^{-/-} mice.

W03-03 | Molecular link among cortical development and psychiatric disorders: Insight from Negr1 and FGFR2 biology

Giovanni Piccoli

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Autism spectrum disorders (ASD) are neurodevelopmental conditions with diverse etiologies, all characterized by common core symptoms such as impaired social skills and communication, as well as repetitive behavior. Cell-adhesion molecules (CAMs), receptor tyrosine kinase (RTK) and associated downstream signaling have been strongly implicated in both neurodevelopment and ASD. We found that downregulation of the CAM Negr1 or RTK FGFR2 similarly affect neuronal migration and spine density during mouse cortical development in vivo and results in impaired core behaviors related to ASD. Mechanistically, Negr1 physically interacts with FGFR2 and modulates FGFR2-dependent ERK and AKT signaling by decreasing FGFR2 degradation from the plasma membrane. Accordingly, FGFR2 overexpression rescues all defects by Negr1 knock-down in vivo. Negr1 KO mice presented phenotypes similar to Negr1-downregulated animals. These data indicate that Negr1 and FGFR2 cooperatively regulate cortical development and suggest a role for defective Negr1-FGFR2 complex and converging downstream ERK and AKT signaling in ASD.

W03-04 | RNA on the brain: Dynamic control of cortical development and disease

Debra Silver

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The cerebral cortex is a highly organized structure, and its proper development is critical for cognition and learning. Cortical development is orchestrated by neural progenitors which give rise to neurons and glia. Aberrant cortical development can result in devastating neurodevelopmental diseases, including microcephaly, autism spectrum disorder (ASD), and intellectual disability. Thus, understanding how the cortex is built during development has enormous clinical implications. Recent studies from our lab using genetics, genomics and imaging highlight new roles for RNA regulation in cortical development and disease. This includes discovery of several RNA binding proteins which function in progenitors and neurons to control cortical development and contribute to neurodevelopmental disease. We will also describe how diverse layers of RNA regulation, including subcellular localization and translation, influence progenitor fate and function. This talk will thus emphasize how

studying post-transcriptional regulation has helped us to understand new intricacies controlling progenitors and corticogenesis.

W03-05 | Neuronal polarization and germinal zone exit are inhibited by a Hif1 α pathway and oxygen tension-dependent developmental switch

David Solecki

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Postnatal brain circuit assembly is driven by temporally regulated intrinsic and cell-extrinsic cues that organize neurogenesis, migration, and axo-dendritic specification in post-mitotic neurons. While cell polarity is an intrinsic organizer of morphogenic events, environmental cues in the germinal zone (GZ) instructing neuron polarization and their coupling during postnatal development are unclear. We report that oxygen tension, which rises at birth, and the von Hippel-Lindau (VHL)-hypoxia-inducible factor 1 α (Hif1 α) pathway regulate polarization and maturation of post-mitotic cerebellar granule neurons (CGNs). At early postnatal stages with low GZ vascularization, Hif1 α restrains CGN-progenitor cell-cycle exit. Unexpectedly, cell-intrinsic VHL-Hif1 α pathway activation also delays the timing of CGN differentiation, germinal zone exit, and migration initiation through transcriptional repression of the partitioning-defective (Pard) complex. As vascularization proceeds, these inhibitory mechanisms are downregulated, implicating increasing oxygen tension as a critical switch for neuronal polarization and cerebellar GZ exit.

W03-06 | Regulation of inhibitory interneuron wiring by the Cadherin superfamily

Julie Lefebvre

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Brain circuits require the precise wiring of GABAergic interneurons. Within any brain region, there is an array of structurally and functionally diverse interneurons that provide specific forms of synaptic inhibition and shape network activities. To establish the inhibitory circuitry, developing interneurons migrate to target locations, and extend dendrites and axons locally that connect to select targets and subcellular domains among a myriad of potential partners. The mechanisms that specify interneuron wiring remain largely unknown. Focusing on interneuron populations in the cerebral and cerebellar cortex, we have identified roles for members of the Cadherin superfamily in regulating interneuron number and synapse specificity. In this talk, I will present our work on the clustered Protocadherins (cPCDHs) in controlling interneuron survival during developmentally regulated cell death, where more than a third of GABAergic cells are eliminated during the first postnatal week. I will also present our discovery of a novel role for an atypical Cadherin in synapse specificity. In the cerebellum, excitatory mossy fibers (MF) synapse onto



the excitatory granule cells as well as onto the inhibitory interneuron population of Golgi cells. We find that Cadherin-23 is expressed in a complementary pattern in partnering Golgi cells and the pre-cerebellar mossy fiber afferents. Cadherin-23 misexpression in vivo drives mossy fiber targeting to ectopic locations, while loss-of-function causes reduced mossy fiber terminals at Golgi cells. Together, these findings suggest that Cadherin-23 controls synapse targeting at the MF-Golgi excitatory-inhibitory synapse. Although Cadherin-23 has well-known roles in sensory transduction with human mutations leading to deafness and blindness, our findings identify a novel role in brain circuit development. Together, these studies reveal Cadherin superfamily members as critical regulators of inhibitory interneuron assembly.

W04-04 | How to find a mentor and move on after your PhD

Felipe Ribeiro

Federal University of Rio de Janeiro, Institute of Biophysics Carlos Chagas Filho, Rio de Janeiro, Brazil

Completing a PhD opens several professional opportunities, making it very challenging to decide which pathway to take. From

non-academic to academic positions, networking is a key part of the job search. Attending schools, conferences and workshops is a unique opportunity to interact with potential mentors, ask questions about the research and the workplace environment and meet team members. In this talk, I will discuss opportunities for transitioning to the next step in academia and propose tips to find a good mentor. I will further discuss funding opportunities for future postdocs and for transitioning to an independent research position.

W04-05 | Mental health and well-being in academia

Loretta White

Dr Loretta White, Starship Hospital, Auckland, New Zealand

I will discuss the important topic of mental health and well-being in academia and factors which influence these. I will speak about topics such as imposter syndrome, perfectionism and procrastination and tips to overcome these. My talk will be based on my 14 years post doctoral experience as a Clinical Psychologist in healthcare, including working as a Clinical Psychologist for a University Well-being Service.

ABSTRACT

Poster Sessions Monday/Tuesday

MTU01-01 | Transcriptional and epigenetic influences in neurodegenerative disease

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Chronic phase of type 2 diabetic conditions have been associated with certain cerebral complications, which we term "diabetic encephalopathy," which includes neurobehavioral dysfunctional patterns and morphological alterations of neurons, especially within the hippocampus. Epigenetic alterations in the brain are well known to affect age-associated disorders, however, its association with the evolving diabetes-induced damage in the brain is still not fully understood. DNA hypermethylation within the neurons tends to silence the gene expression of several regulatory proteins. Molecular chaperones and synaptic proteins are important functional regulators for maintaining the synaptic transmission in the hippocampus. The findings in the study have shown an increase in global DNA methylation within the HFD/STZ-induced diabetic mice brain. Inhibiting DNA methylation, restored the levels of hsf1, the master regulator for molecular chaperones as well as an integral protein for maintaining synaptic fidelity, and also the chaperones hsp40, 60, and 70 in the hippocampus of the diabetic mice. Rescue in synaptic proteins PSD95 and synaptophysin, important for synaptic transmission, further confirmed positive reinforcements. Astrocyte activation (GFAP) was further significantly decreased in the 5-azadeoxycytidine group (DNMT inhibitor), suggesting an attempt in restoring neuroinflammation, further evidenced by the decrease in proinflammatory cytokines TNF α , IL-6, and mediators iNOS and phospho-NF κ B. Our results suggest that changes in DNA methylation advocate epigenetic dysregulation and its involvement in disrupting the synaptic exactitude in the hippocampus of diabetic mice model, providing an insight into the pathophysiology of diabetic encephalopathy.

MTU01-02 | Histone acetyltransferase KAT2A is a critical epigenetic regulator of cocaine responses in the nucleus accumbens

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Substance use disorder is characterized by cycles of drug-use, abstinence, drug-seeking, and relapse. The neural basis of these long-lasting drug-associated behaviors have been linked to neural circuit function through changes in neurotransmission and receptor-based changes across the reward circuitry of the brain. However, the underlying molecular mechanisms which contribute the persistence of these drug-induced changes to neural function remain relatively poorly understood. Previous work from our lab and others has identified cocaine-induced changes to transcriptional and proteomic profiles within the nucleus accumbens (NAc). To understand how drugs of abuse, such as cocaine, generate long-lasting behavioral changes, it is critical to link between neuronal activity and changes in gene expression. One potential avenue are epigenetic adaptations, where DNA-protein interactions are modified to alter accessibility and likelihood of targeted gene expression. We identified temporally specific changes in histone H3 post-translational modifications and identify a key regulator in these changes—KAT2A. KAT2A is a histone acetyltransferase known to regulate activity-dependent transcription. We find that KAT2A-regulated phosphoacetylation of H3 is increased following chronic cocaine self-administration. Moreover, we demonstrate that loss of KAT2A function in D1-MSNs alters sensitivity and motivation for cocaine. Lastly, we generate a cocaine self-administration activity profile of D1-MSNs that is subsequently altered by alterations in KAT2A function. The results of these studies contribute evidence for persistent cocaine-induced epigenetic adaptations and are the first step in generating a mechanistic link between epigenetic adaptations and changes in neuronal firing. In addition, we provide data linking these changes in epigenetic state to cocaine-seeking behavior.

**MTU02-01 | Targeting neuroinflammatory signaling cascade: A Possible Therapeutic Strategy in Halting Epilepsy**

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Background: Despite extensive research on epileptogenesis, current medications only provide symptomatic control of seizures. Thus, there is high demand in the investigation of new targets and approaches for the development of treatments for 30% of epilepsy cases that prove drug resistant. Inflammation, neural loss, plasticity, mossy fibre sprouting and blood-brain barrier dysfunction are the most common causes of epileptogenesis.

Objective: With advancement in the research of epilepsy, neuroinflammation has been shown as the core features that contribute to etiopathogenesis of epilepsy. Increasing evidence supports that the transforming growth factor (TGF- β)/non-SMAD pathway is of utmost importance in neuroinflammatory-mediated epileptogenesis. Therefore, exploring TGF- β /non-SMAD pathway could provide an interesting opportunity to discover and validate targets for novel therapeutics for controlling pharmacoresistant epilepsies.

Methodology: Male Balb/c mice were categorized into five groups, that is, normal-control, pentylenetetrazole-control, drug control (diazepam and valproic acid) and test groups, that is, Isoxylitones (E/Z-2-propanone-1,3,5,5-trimethyl-2-cyclohexen-1-ylidene) abbreviated as (ISOX). Kindling was induced by administering sub-convulsive dose of 40 mg/kg PTZ on every alternate day until seizure score 5 develops in the PTZ-control group. Treatments were given to the respective groups 30 min prior to PTZ dosing. When animals acquired consistent score 5 for at least 3 days, experiments were terminated and brain samples were isolated for gene expression studies of SMAD-independent TGF- β signalling cascade in cortex and hippocampus samples.

Result: The experimental findings revealed that ISOX (30 mg/kg) not only significantly suppressed the PTZ-induced seizures but also halted the epileptogenesis by altering the non-SMAD associated TGF- β genes. ISOX pre-treatment significantly upregulated the RhoA, ROCK2, and AKT expressions and downregulated the ROCK1, MAPK14, and NFkB expressions as compared to PTZ-control group in hippocampus region, suggesting the disease modifying effect of ISOX and other treatments in epileptogenesis

Conclusion: Our findings suggest that non-SMAD/ TGF- β signaling pathway act as critical target in epilepsy, and also rationalizes the ISOX as a promising neuroprotective, anti-inflammatory, and disease-modifying agent in forestalling the epileptogenesis.

MTU02-02 | Alterations of extracellular ATP-dependent signal transduction in rodent model of environmentally triggered autism

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Autism spectrum disorders (ASD) include social, communication, and behavioral challenges. The elusive ASD etiology may include multiple environmental stressors interacting with genetic susceptibility factors. Stress-induced abnormal release of ATP from brain cells into the extracellular compartment and subsequent activation of purinergic receptors may alter energy metabolism, leading to long-term synaptic disturbances and behavioral problems. In these studies, we have examined alterations of extracellular ATP-dependent signal transduction in rodent model of environmentally triggered autism. Wistar female rats were subjected to a single intraperitoneal injection of valproic acid (VPA) at a dose of 450 mg/kg of body weight on gestational day 12.5. The experiments were conducted on cerebrospinal fluid (CSF) and the brain of the young-adult (52-day-old) male offspring and on primary neuronal cultures isolated from VPA pups at 19th embryonic day. An increase in the level of ATP and its metabolites, ADP and AMP in the CSF of VPA offspring indicates activation of purinergic signaling. Additionally, we observed the hyperactivity of purinergic receptors in primary neurons isolated from VPA animals. Stimulation of these cells with 1 μ M ATP induced 4-fold increase in intracellular calcium level compared to control. We also identified the receptors that expression is altered during development of VPA-affected offspring. At an early stage of embryonic development, the level of P1 receptors for adenosine increases, while in the brain of young-adult animals the level of the ionotropic P2X1 and P2X3 receptors for ATP is higher. Excessive stimulation of nucleotide receptors and an increased influx of calcium ions could be responsible for severe energy disturbances and mitochondria failure observed in the brain of VPA offspring. Thus, alterations in the ATP-signaling pathway could be a potential mechanism associated with synaptic disturbances and behavioral problems in autism.

MTU02-03 | Counteracting organophosphate-induced toxicity by improved bioscavenging capacity of BChE and damage prevention at cell level

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The phosphorylation of acetylcholinesterase (AChE), a pivotal enzyme in hydrolysis of the neurotransmitter acetylcholine, by nerve agents (NAs) still has no adequate or fully efficient medical countermeasures. Furthermore, oxime antidotes known for their nucleophilic properties have limited potency in the reactivation



of phosphorylated AChE in the central nervous system (CNS), so even after survival, long-term effects of poisoning could occur. However, the AChE-related enzyme butyrylcholinesterase (BChE), typically present in the blood, serves as bioscavenger of OPs, before they reach the crucial AChE in CNS. In our study, we wanted to investigate with a combination of *in silico*, *in vitro*, and *ex vivo* methods, the feasibility of an approach to develop a safe bioscavenger of NAs, based on the oxime-assisted reactivation of BChE. Firstly, we identified a promising reactivator of cyclosarin-inhibited BChE as a model system with no impact on the viability of neuroblastoma cells upon 4-hour treatment. Herein, the efficient oxime-assisted catalytic BChE degradation of cyclosarin was demonstrated *ex vivo*. This pair was then applied to OP-treated cells and, as the result shows, it acted by, protecting cells' homeostasis by preserving from 50% to nearly 100% of neural cells. The most significant result was obtained if the oxime and BChE pair were applied to cells before exposure to cyclosarin or in other words, as pretreatment. This result is in accordance with *ex vivo* effects determined in the whole blood showing up to 80% of restored phosphorylated cholinesterase activity within two minutes. In conclusion, our findings showed that an antidote should act on two levels—on cholinesterase reactivity and prevention of the long-term effects of poisoning.

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MTU02-04 | EGF-dependent H-Ras activation and lipid raft mobility is controlled by the RasGAP neurofibromin in cortical neurons

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The epidermal growth factor receptor (EGFR), an archetype receptor-tyrosine kinase, is widely expressed in the CNS, with established roles in progenitor cell proliferation and general neurotrophic effects on postmitotic neurons. EGFR exerts its effects through activation of the Raf/MEK/ERK kinase cascade, yet, the precise mechanism remains obscure. The binary on/off switch of ERK activation is Ras activation, to which a liganded EGFR gains access through interactions with RasGEFs; the other side of Ras activation involves recruitment of RasGAPs, which stimulate the GTPase activity of Ras for its de-activation. The most abundant RasGAP in CNS is neurofibromin, the product of the *NF1* gene, mutations of which cause neurofibromatosis 1 (NF-1), a disease that presents with a great range of symptoms, including learning or autism spectrum disorders. Using chick embryo tissues and primary neurons, we show that both neurofibromin abundance (Westerns) and switches in the *NF1* gene alternative splicing (qPCR)—and thus neurofibromin isoforms of different RasGAP potencies—occur when neurons become

postmitotic. Moreover, as we have shown that EGFR agonism phosphorylates neurofibromin via PKC ϵ , a modification that recruits neurofibromin to lipid rafts (LRs), we examined H-Ras mobility in detergent-free, cortical neuron membrane preparations, along with affinity precipitation of H-RasGTP in sister cultures. We found that [50 nM]EGF acutely activated H-Ras (45"), which started to flow out of LR; at 90", H-Ras remained activated and, while still enriched in the caveolin-rich fractions, it also reappeared in the highest buoyancy fraction of LR, a pattern that persisted for 3 min, declining thereafter. As assessments were made in the presence of cycloheximide, this "treadmilling" mobility of H-Ras suggests that neurofibromin filters out the plethora of activated H-Ras molecules to prevent a strong ERK activation. Indeed, EGF elicited a low and sustained ERK activation in primary neurons, consistent with signalling for differentiation.

MTU02-05 | Signaling pathway in neovascularization of glioblastoma: Role of metabotropic glutamate receptors

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The most common primary brain tumor with worst prognosis in adults is glioblastoma. It is characterized by its high aggressive progression, high vascularization and exceptional invasive ability to normal brain, often along blood vessels. Glioblastoma invariably recur despite of radical surgical resection and radiochemotherapy. The key events in the severe progression and recurrence of glioblastoma is invasion and neovascularization. The process of neovascularization involves increase of vascular permeability, endothelial cells migration and proliferation. Several factors play role in neovascularization of glioma cells in the tumour microenvironment. Since glioblastoma tumors secrete significant amount of glutamate into tumour microenvironment, we evaluated role of glutamate receptors in neovascularization. Our earlier studies have shown significant role of NMDA and AMPA type of glutamate receptors in proliferation, migration and invasion of glioblastoma. The research objective of the current study was to analyse the role of metabotropic glutamate receptors in neovascularization in glioblastoma and their signaling. This was analysed in patient derived tumour tissue samples and U87MG, U251MG and LN18 glioma cell lines were used for evaluation of signaling. We observed that pro-angiogenic factors and glutamate receptors were highly expressed in these tumour tissue samples. Further, experiments in glioma cell lines indicated that activation of metabotropic glutamate receptors enhanced the expression/activity of matrix metalloproteinases and *in vitro* neovascularization process. The neovascularization process was inhibited by PF-04691502. In summary, these results indicate that in glioma tumour microenvironment, mTOR inhibitor probably play significant role in the signaling pathway of metabotropic glutamate receptor-mediated neovascularization.

MTU 02-06 | Measuring amyloid- β dynamics in response to selective excitation and inhibition of glutamatergic and GABAergic signaling

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Amyloid- β (A β), the primary component of AD plaques, is known to be released into the ISF and increases during periods of increased synaptic activity. It is currently unclear which types of neurons are producing A β in vivo. The Cirrito Lab has developed a microimmuno-electrode (MIE), which is an antibody-based implantable electrode that can be used in awake and behaving mice to detect changes in protein levels (depending on the antibody) every minute for several hours. The MIE can detect A β 40 and A β 42 with at least 8000-fold selectivity over mouse A β in vivo. To parse out the neuronal source of amyloid generation under the endogenous amyloid precursor protein promoter, the APP-NL/F/NL/F knock-in mouse model was used. The model produces human A β with two mutations known to accelerate pathology in familial AD. Manipulating the activity of cells expressing transporters for either GABAergic or glutamatergic signaling was achieved by crossing the APP-NL/F/NL/F model with either a Vgat-Cre or Vglut2-Cre mouse; this provided the ability to specifically target inhibitory and excitatory signaling, respectively. To modulate signaling in vivo, Cre-dependent DREADD (Designer Receptors Exclusively Activated by Designer Drugs) G-protein receptors were expressed selectively in the hippocampus using a viral (AAV) vector. These receptors could activate (hM3Dq) or inhibit (hM4Di) the cell type selected by the Cre driver upon systemic administration of the inert compound clozapine-N-oxide. Our preliminary results demonstrate that A β 40 generation is potentiated by inhibition of VGAT expressing cells in the hippocampus. Activation of VGAT and VGlut2 neurons did not result in increased A β 40 release. A future direction will be to measure EEG activity under similar conditions to verify how network neuronal activity is modulated.

@RDHendrix on Twitter

MTU03-01 | MICE LACKING 5-LIPOXYGENASE DISPLAY MOTOR DEFICITS ASSOCIATED WITH CORTICAL AND HIPPOCAMPAL SYNAPSE ABNORMALITIES

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5-Lipoxygenase (5-LOX) is an enzyme essential for the synthesis of lipid inflammatory mediators. It is widely expressed in immune system cells. However, reports suggest that central nervous system (CNS) neurons and glial cells also express high levels of 5-LOX, outside the context of inflammation, but the physiological role for 5-LOX in the CNS remains unclear. Our aim was to explore the role of 5-LOX in microglial activation, synaptic density and behavior. We used adult male 5-LOX knockout mice (5-LO^{-/-}) and wild-type (WT). We found that 5-LO^{-/-} mice present increased repetitive behavior (evaluated with the marble burying test) but no alterations in memory function (measured by the novel object recognition/passive avoidance tasks) or anxiety-like behavior (evaluated with the elevated plus maze task), as compared with WT. Moreover, 5-LO^{-/-} mice presented motor deficits (assessed by the Rotarod test), but no differences were observed in sensorial tests (Von Frey hair and hot plate test). Associated with these results we observed increased levels of the synaptic proteins PSD95 and synaptophysin (assessed by Western Blot) both in the motor cortex and hippocampus but found that BDNF expression was unchanged in the same brain areas. This increase in synaptic proteins led us to hypothesize a deficit in synaptic pruning. We used qPCR to analyze targets known to be involved in this process and found an increase in CX3CR1, along with a decrease in its ligand, CX3CL1, in the motor cortex of 5-LO^{-/-} mice. Despite this disturbance in the CX3CR1-CX3CL1 axis, microglial morphology and density (as evaluated by IBA-1 immunostaining) were the same between the groups. Together, our results suggest that 5-LOX expression may be related to fine motor performance, repetitive behavior and changes in synaptic density. The absence of 5-LOX products may lead to altered microglia-neuron interactions, through the CX3CR1/CX3CL1 signaling axis, affecting microglial pruning.

MTU03-02 | Neuroprotective effect of thymoquinone on lipopolysaccharides (LPS)-induced neurodegeneration and cognitive impairment in mice

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Background: The hallmark of neurodegenerative diseases, such as Alzheimer's disease, is chronic neuroinflammation. Lipopolysaccharide (LPS) is an essential component of the gram-negative bacterial cell wall and acts as a potent stimulator of neuroinflammation that mediates neurodegeneration. Thymoquinone (TQ) is a compound known to possess multiple forms of desirable biological activity including anti-inflammatory and antioxidant properties. However, its neuroprotective effect against neuroinflammation-induced neurodegeneration and memory dysfunction is still not fully explored. We thus evaluated its neuroprotective on LPS-induced neurodegeneration and cognitive dysfunction in mice.

Methods: Thirty-five mice were assigned into five groups ($n = 7$): group 1 (vehicle), group 2 (TQ/15 mg/kg), group 3 (TQ/30 mg/kg), group 4 (Donepezil/5 mg/kg), group 5 (vehicle). Animals in different groups were administered with vehicle, thymoquinone and donepezil for fourteen days. Starting from day 8, sixty minutes after administration of vehicle/thymoquinone/donepezil, animals in groups 2, 3, 4, and 5 received LPS (500 µg/kg, i.p.) consecutively for seven days. Twenty-four hour after treatment on day fourteen, Novel Object Recognition, Y-maze, were used to assess cognitive functions; elevated plus maze, open field and light and dark box tests were used to assess anxiety. Tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) were assessed using ELISA techniques, microglial, iNOS were quantified using immunohistochemistry, while β -secretase, IL-4, and IL-10 were measured with qPCR. Cox Golgi staining technique was used to evaluate hippocampal damage.

Results: LPS significantly impaired performance in the Y-maze and NORT and induced behavioural abnormalities, compared to control. There were all ameliorated by treatment with TQ. The TQ also significantly ($p < 0.05$) reduced LPS-induced pro-inflammatory cytokines, microglial, β -secretase/mRNA in the hippocampus and prefrontal cortex. However, TQ increased IL-4/mRNA, IL-10/mRNA, in hippocampus and PFC. TQ significantly ($p < 0.05$) decreased neurodegeneration of dendrite and neuronal cells in the hippocampus of LPS-treated mice.

Conclusion: Our results suggest that TQ may possess neuroprotective effect against LPS-induced neurodegeneration and cognitive impairment via mechanisms involving its anti-inflammatory properties.

MTU03-04 | Analysis of cytokines in patients with major depressive disorder

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Major depressive disorder (MDD) is a complex neuropsychiatric disorder which etiopathophysiology is not clearly understood. Depression is one of the diseases with the greatest global burden and is expected to be the second leading cause of disability. The search for biological markers of severe MDD could be important for improving patient care and developing more effective treatments. Cytokines play many key roles in the body and have pleiotropic effects. Epidermal growth factor (EGF) participates in the regulation of cell proliferation and differentiation. Monocyte chemoattractant protein-1 (MCP1) can affect innate and acquired immunity by regulating monocytes and Th systems 1 and 2. Vascular endothelial growth factor (VEGF) is important for vasculogenesis, angiogenesis and vascular permeability. EGF, MCP1 and VEGF also play an

important role in the brain and could participate in the development and development of MDD. In patients diagnosed with MDD, we observed a statistically significant increase in the concentration of all three cytokines. The concentration of EGF in patients was 13.17 ± 3.21 pg/ml. Compared to healthy individuals, the EGF concentration was 287.35 % higher ($p = 0.0048$). The concentration of MCP1 in depressed patients was 155.05 ± 17.73 pg/ml which represents an increase of 58.32 % compared to the concentration in healthy individuals ($p = 0.004$). VEGF concentration in patients was 29.55 ± 3.88 pg/ml. Compared to healthy individuals, the VEGF concentration was 44.92 % higher ($p = 0.046$). Nowadays, still more attention is paid to the personalized therapy and individual approach to each patient. Changes in cytokine synthesis could contribute to a certain disease phenotype. After a more detailed study, their usefulness as diagnostic or prognostic biomarkers of MDD could be evaluated. The project was supported: Ministry of Education, Science, Research and Sport of the Slovak Republic VEGA 1/0266/18.

MTU03-05 | IL-6: A putative link between neuroinflammation and disturbed magnesium homeostasis?

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Aim: The aim of our study was to elucidate the potential role of neuroinflammation, modeled by presence of interleukin-6 (IL-6), in process of impaired magnesium (Mg) homeostasis (IMH). Our hypothesis was substantiated by the involvement of IL-6 and IMH in neurodegeneration.

Background: Neuroinflammation is a critical component in pathogenesis of neurodegeneration. IMH is another important factor contributing to development of neurodegenerative ailments. The Mg^{2+} extrusion system SLC41A1 is a part of the PARK16 locus associated with Parkinson's disease. IL-6 is a cytokine with both anti-inflammatory and pro-inflammatory effects. One subunit of its heteromeric receptor is ubiquitously present on the cell surface, whilst the other crucial subunit is expressed only by the specific cell types. IL-6 bound to this receptor triggers JAK-STAT3 signaling pathway which results in activation of STAT3 transcription factor. A putative STAT3-binding site was predicted to be localized in SLC41A1 promoter region.

Methods: A specific cellular signaling triggered by IL-6 application was verified by western blot using antibodies specific for particular post-translational modifications of STAT3. Four responsive cell lines (HEK-BlueTM IL-6, HepG2, U-266 and PANC-1) were subsequently treated with two different concentrations of IL-6 and possible changes in the expression of SLC41A1 and other magnesiumotropic genes (SLC41A2, SLC41A3, TRPM7, MAGT1, NIPA1, N33, CNNM2, TRPM6) were analyzed by RT-qPCR. Potential changes in the activity of Mg^{2+} -transport systems were monitored by using mag-fura-2 Mg^{2+} -sensor.



Results: Our results indicate that neither 1 nor 24 hours-long IL-6 treatment does significantly alter the expression of *SLC41A1* or any other studied magnesiotropic gene. Preliminary analysis also shows the absence of any changes in Mg^{2+} net transport capacity. In conclusion the IL-6-JAK-STAT3 pathway does not alter the expression of magnesiotropic genes, but this does not rule out another mechanism by which IL-6 may affect Mg homeostasis.

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MTU03-06 | A role for inflammation on lesion-induced neuroplasticity during critical periods of brain development

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Early CNS lesions are followed by rapid structural reorganization of circuits, related to functional brain recovery, whereas in adults, recovery is slow, with anomalous plasticity. In parallel, focal lesions induce neuroinflammation that encourages the study of the dynamics between neuroplasticity mechanisms and immune activation in CNS injury context. Here we investigate differences in the neuro-immune interactions after monocular enucleation (ME) during and after critical period of development (CP) and, evaluate a role for TNF- α as mediator in microglia-dependent retinocollicular-induced plasticity. Lister Hooded rats submitted to ME at PND10 (within CP)/PND21 (after CP) were analyzed at different survival times. Other group, both enucleated at PND10, received systemic treatment with minocycline 3h after lesion or local treatment (ELVAX) with TNF- α neutralizing antibody before. Cytokines were analyzed by RT-PCR. Structural plasticity was mapped using neuroanatomical tracer. Immunofluorescence or western blot were used to study microglial and astrocytes reactivity, and, BDNF content. At P10, ME induces robust sprout of intact axons through visual layers of the superior colliculus (SC), with the greatest intensity just 24h after lesion while injury in P21 induces discrete sprouting after 7 d. Regarding glial reactivity, there is progressive increase of amoeboid Iba1+ cells in the contralateral SC 24h after ME, that peak at 72h, when GFAP is expressed beyond the border of SC. In 7 d, glial reactivity returns to non-lesion levels. An apparent peak in BDNF content and TNF- α mRNA is also observed 24h after lesion in P10. Other cytokines such as IL-6 and TGF- β are also modulated. In P21, microglia slow reactivity pairs with axonal sprouting and reactivity peaks only in 7 d with a layer-specific distribution, corresponding with intralaminar plasticity. GFAP and BDNF also respond late,

peaking 14d after ME. Both minocycline and ELVAX anti-TNF- α neutralizing antibody treatment reduce microglial reactivity and the sprout of intact axons. Together, data support that inflammation plays an important role during critical periods of development and a differentiated neuroimmune dynamics may justify rapid and slow neuroplastic changes during and after CP via TNF- α regulatory signaling.

MTU03-07 | Mechanistic considerations of nickel in neurodegenerative diseases: an updated systematic review

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The environment has been implicated to be a strong determinant of brain health with a higher risk of disruption to neurodevelopment. The drastic rise in the prevalence of neurodevelopmental disorders supports the idea that environmental factors may play a major role in these disorders. This paper reported available studies on the nickel levels in NDD covering both animal and human studies. PubMed and Google Scholar databases were searched for original articles reporting the quantity of nickel in various neurodegenerative disorders. Searches were not limited to year of study but limited to studies published in the English Language. Data were extracted and synthesized by ensuring the articles were related to nickel and neuro disorders. Thirty-five papers fulfilled the inclusion criteria. Neurodegenerative disorder (NDD) is an umbrella word for different conditions resulting from aberrant brain development which includes Alzheimer's disease, Parkinson's disease, Autism spectrum disorder, Huntington's disease and multiple sclerosis autism spectrum disorder (ASD). Nickel (Ni) is one of the listed metals reported to pose a serious threat to human health. Ni is found in the body through the biological system (food, water, skin contact and air). This leads to the production of free radicals (which results in oxidative stress) as well as behavioural deficits in later life. Reduction in the exposure to Ni contaminants may hold a promise in reducing the incidence of neurodegenerative diseases

MTU03-08 | The role of methylglyoxal accumulation in the induction of diabetic neuropathic pain

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Neuropathic pain is a common complication of both type 1 and type 2 diabetes. Although neuropathy debase the quality of life, no effective treatment is available at the moment indicating the complexity

of the pathophysiology of the disease. Chronic diabetic pain can induce changes in the expression and distribution of various inflammatory cytokines in sensory neurons and resulting in a subsequent peripheral sensitization. However, both peripheral sensitization and maladaptive central changes may equally be involved in the induction and maintenance of neuropathy. Incidentally, disturbances of glucose metabolism in diabetes lead to the accumulation of methylglyoxal (MGO), a well-known cytotoxic metabolite which may induce centrally restricted effects. Here we investigated the effect of MGO on glial cell cultures and in the spinal dorsal horn, where primary pain processing takes place.

MGO increased calcium signaling and the expression of COX-2 and TNF α in primary cultured astrocytes. However, no alteration in IL-1 β or IL-6 expression has been observed in these cells. Intrathecal administration of MGO decreased paw withdrawal threshold in adult rats. At the same time, MGO increased the immunoreactivity for GFAP, and the amount of COX-2 and TNF α associated with astroglial processes in the spinal dorsal horn.

Our results indicate that MGO directly induces the polarization of spinal astrocytes into A1 neurotoxic phenotype. A1 astrocytes release TNF α and possibly prostaglandins resulting in MGO-induced reactive gliosis which can lead to neuroinflammation in the spinal dorsal horn. We assume, that MGO-dependent neuroinflammation may be sufficient to induce central sensitization independent of peripheral neuropathy, leading to allodynia and spontaneous pain in diabetic patients.

MTU03-09 | Tramadol modulates cognition and lipid-immune signalling in the gut-liver-brain axis of sleep-deprived periadolescent rats

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Introduction and Aim: Brain development and behavioural maturity peak at adolescence, therefore highly sensitive to stressors such as sleep deprivation, and environmental influences including drug abuse. Though sleep deprivation and tramadol abuse distinctively dysregulate cognitive processes, the mechanisms underlying their combined effects are largely unknown. Thus, this study

investigated the role of tramadol hydrochloride on cognition and lipid-immune signalling in the gut-liver-brain axis of sleep-deprived periadolescent rats.

Materials and Methods: Periadolescent female rats were either concomitantly deprived of rapid eye movement (REM) sleep using the Platform Over Water protocol (8 h/day) and treated orally with 50 mg/kg b.w. tramadol hydrochloride for 45 days; or sleep-deprived for 45 days with tramadol treatment discontinued on the 31st day. Thereafter, cognitive behaviours were evaluated. Following euthanasia, blood plasma, hippocampal, intestinal, and liver tissues were collected and processed for molecular, biochemical and immunohistochemical assessments.

Results: Our results showed that treatment with tramadol (in the tramadol discontinued group) improved hippocampal-dependent spatial navigation and novel recognition memory but not social recognition memory in the sleep-deprived rats. This was associated with altered mRNA expression of the clock gene *Brain and muscle ARNT-Like 1 (BMAL1)* in the hippocampus. We also demonstrate a dysregulation in ApoE levels, lipid profile, low-density lipoprotein receptor-related protein 1 (LDLR1) expression, and lipid peroxidation in the liver, intestine, and hippocampus following concomitant exposure to sleep deprivation and tramadol. Furthermore, we observed altered NF- κ B activity, plasma c-reactive protein levels, as well as in the expression of the macrophage marker, Iba1 in the liver-intestinal-hippocampal axis, highlighting the contributory role of the gut-liver-dependent lipid-immune signalling in the hippocampal-dependent cognitive processing observed following sleep deprivation and tramadol treatment.

Conclusion: Our findings suggest that the tramadol-driven lipid-immune changes in both periphery and brain are modulated by sleep deprivation, suggesting its potential role in the alteration of the gut-liver-brain signalling that occurs during metabolic disorders and psychiatric co-morbidity.

MTU03-10 | Minocycline Attenuates Impaired Hypothalamic-Ovarian Antioxidant Capacity by Modulating NLRP3 in a Rat Model of Cerebral Ischemia

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Introduction and Aim: Stroke is the second leading cause of death worldwide, and a driver of infertility indices, especially among women. Minocycline, a semisynthetic antibiotic with anti-inflammatory role as been explored in experimental models of stroke; however, its impact on the hypothalamic-gonadal axis has not been examined following cerebral ischemia injury. Therefore, the present study aimed at investigating the role of minocycline therapy on oxido-inflammatory responses in the hypothalamic-ovarian axis of rats following cerebral ischemia injury.



Materials and Methods: Cerebral ischemia injury was induced in female adult Wistar rats by occluding the common carotid artery (CCA). Animals were treated with 45 mg/kg bw i.p. of minocycline, 1 and 4 hours on the first day, then 22.5 mg/kg bw i.p., 24 and 36 hours on the second day after occlusion. Following animal sacrifice, the hypothalamic and ovarian tissues were extracted and processed for biochemical, histological and immunohistochemical analyses.

Results: Our findings revealed upregulation of inflammasome expression, reduction in neuronal cell count, as well as significant depletion of catalase and glutathione levels in the hypothalamus and ovaries of the CCA-occluded rats. However, treatment with minocycline significantly increased hypothalamic and ovarian catalase and glutathione levels, as well as a significant downregulation of inflammasome expression in the hypothalamus and ovaries compared to the untreated CCA occluded rats. This was associated with rescue of hypothalamic neurons with a marked increase in NeuN-positive cells.

Conclusion: Treatment with minocycline attenuates impaired hypothalamic-ovarian antioxidant capacity by modulating NLRP3 inflammasome.

MTU03-11 | Chronic glial activation causes cholinergic cell loss in the basal forebrain during pathological aging

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Aims: Neuroinflammation leads to gradual neurodegeneration during pathological aging, contributing to disorders like Alzheimer's disease (AD). Basal forebrain cholinergic neuronal (BFCN) loss is associated with AD; however, the underlying mechanism is still unknown. This study investigated the effects of both aging and chronic neuroinflammation on the BFCN, focusing on the medial septum (MS).

Method: Aged, 12- and 24-month WT and GFAP-IL6 mice (overexpressing IL-6 under the GFAP promoter) were used. Immunohistochemistry combined with unbiased stereology and 3D morphology analysis was performed to estimate the number and morphology of Iba1+ microglia and ChAT+ in the MS, combined with transcriptomic analysis using qPCR.

Results: Stereological estimation of microglia number displayed a significant increase both at 12 and 24 months in GFAP-IL6 compared to WT. We found a significant, 28% and 38% decrease, in microglial 3D surface area, and volume between WT and GFAP-IL6 respectively, at both 12 and 24 months, indicating increased glial reactivity. Meanwhile, BFCN in the MS displayed a significant, 31% and 30%, decrease in GFAP-IL6 compared to WT, respectively, at both 12 and 24 months. A significant 58% and 67% reduction in the 3D

dendritic field surface area, and volume, respectively, between WT and GFAP-IL6 at 12 months; and a significant 41% and 46% decrease between WT at 12 and 24 months, respectively, revealing neuronal degeneration.

Conclusion: We report evidence of the significant impact of both aging and chronic glial activation on the microglial and BFCN numbers and morphology in the MS, resulting in neurodegeneration, as seen in AD.

MTU03-12 | SLN targeting for treatment of neurocysticercosis

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Neurocysticercosis (NCC) is a primary infection of brain, spinal cord or peri-meningeal structures with the larval forms of *Taenia solium* and associated inflammation, which is aggravated by debris of parasites by anthelmintics. The study aimed to develop Lactic acid (LA)-conjugated solid lipid nanoparticles (SLN-LA) bearing albendazole (ALB) and dexamethasone (DEX) for effective management of NCC. SLN were prepared using modified solvent injection method with slight modifications. LA was coupled to SLN by post-insertion technique. SLNs were characterized for particle size, size distribution, shape, and %drug entrapment efficiency. In vitro drug release kinetics, and transendothelial transport studies were carried out to predict the fullest drug delivery potential. In vivo studies included fluorescence study and hematological toxicity studies.

The SLNs were found to be spherical with high drug entrapments. In vitro release showed initial quick release, followed by sustained release for more than 48 hr. Fluorescence study and in vitro transendothelial transport depicted selective brain uptake of SLN-LA compared to SLN attributed to carrier-mediated transport via monocarboxylic acid transporters. Pharmacokinetic parameters such as AUC_{0-t}, AUMC_{0-t} and C_{last} showed good drugs withholding capacity of SLNs. Organ distribution studies reflected high accumulation of drugs in the brain after 24 hr in case of SLN-LA as compared to plain drugs solution. SLN-LA in hematological studies revealed insignificant toxicity to blood cells.

On basis of above findings, developed lactic acid conjugated SLN could be a promising tool to treat NCC effectively by dual drug delivery to brain. Since, NCC being chronic in nature requires long-term therapy, SLN-LA not only targeted brain but also offered sustained and prolonged release of these synergistic drugs in which albendazole kills the parasites and dexamethasone alleviates inflammation due to deceased parasites in the brain. Hence, this targeted brain delivery was found to be safe and efficacious for the management of NCC.

MTU03-13 | Neuroprotective and anti-inflammatory effects of evodiamine in Parkinson's disease allied depressive-like behavior

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Evidence from preclinical and clinical studies proposed the devastating impact of stress on progressive and accelerating damaging effects on the nigrostriatal pathway in PD. However, the underpinning mechanism elaborating stress and MPTP-induced redox dysregulation, SIRT1/AMPK, and ASK1-related apoptosis signaling in cellular and mice models are not validated and explained to date. Furthermore, EVD neuroprotective roles were evaluated for in vitro and in vivo models. Firstly, we established and validated impact of prolonged restraint stress (168h: 7h/day; 1d–14d, 22d–28d, 3h/day; 15d–21d) on subacute MPTP doses (23.6 mg/kg, i.p. for 7 consecutive days, 15d–21d) injected in mice. Severe behavioral changes were noticed in the RS+MPTP group compared to RS and MPTP grouped mice for lowering brain vascularity, disrupted redox balance, and highly activated apoptotic signaling in the SNpc region of mice brain. Secondly, in vitro studies were conducted for determining the neuroprotective effects of EVD on LPS-stimulated N9 microglial cells and MPP⁺-induced neurotoxicity in N2a cells. Anti-inflammatory effect was found for EVD via repressing active NF- κ B signaling while activating Nrf2 regulated peroxiredoxin-5 (Prx-5) antioxidant axis in N9 cells. Moreover, pre-treatment with EVD suppresses inflammation and degenerated N2a cells by LPS conditioned media stimulated N9 cells and further EVD inhibited MPP⁺-mediated neurotoxicity through its anti-apoptotic, antioxidant, and activation of neuronal survival (SIRT1/AMPK) mechanism in N2a cells. Lastly, neuroprotective benefits of EVD (50 mg/kg and 100 mg/kg) against the RS+MPTP-induced neurobehavioral and neurochemical anomalies. Moreover, we utilized 3H-1,2-dithiole-3-thione (D3T); Nrf2 activator alone and combination with EVD (100mg/kg) to conclude its Nrf2 pathway activation. EVD, a higher dose ameliorates blood flow to the brain on the 28th day, increased the level of monoamines, lowered corticosterone level, mitigated the raised oxido-nitrosative stress, and restored the SOD, catalase, reduced NAD level in SNpc of mice. EVD potentially downregulated the NF- κ B-mediated inflammatory, apoptotic signaling mediators and activated SIRT1/AMPK/Nrf2 provoked anti-inflammatory signaling in the SNpc of mice. Results suggest that EVD may be a good therapeutic approach as an adjunct or nutraceutical in subsiding the motor defects and depressive-like behavior in Parkinson's disease.

MTU03-14 | Highly active antiretroviral therapy-AgNPs conjugate interacts with neuronal & glial cells and alleviates anxiety in diabetic rats

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The inception of highly active antiretroviral therapy (HAART) has improved life expectancy of people living with HIV. However, neurological complications associated with chronic HAART administration has not been fully addressed. Therefore, this study evaluated the potential benefits of silver nanoparticles (AgNPs)-conjugated HAART and its interaction with neuronal and glial cells in type 2 diabetic rats. Forty-two adult male Sprague–Dawley rats (250 $\pm$ 13 g) were divided into non-diabetic and diabetic groups. After induction of diabetes, animals were administered either with de-ionized water, HAART (98.2 mg/kg, p.o) or AgNPs + HAART (24.5 mg/kg, i. p) for 8 weeks. After that, metabolic biomarkers, oxidative injury, tissue inflammation, and animal behavioral changes, prefrontal cortex immunohistochemical and ultrastructural were analyzed. The HAART-treated diabetic rats showed a significant increase in blood glucose level, malondialdehyde (MDA), and pro-inflammatory cytokines (TNF- α , IL-1 β) while locomotion, reduced glutathione (GSH), superoxide dismutase (SOD) activity, and PFC-GFAP positive cells were significantly reduced compared with diabetic control. However, administration of AgNPs + HAART to diabetic rats significantly improved the blood glucose level, metabolic activities, SOD, GSH, PFC-GFAP positive cells, protecting neuronal injury while reducing MDA and anxiety-like behaviour. Administration of HAART aggravates anxiety-like behaviours and promotes neurotoxic effects in the PFC of diabetic rats. However, AgNPs + HAART alleviates the anxiogenic effects of HAART, and preserves PFC GFAP-positive cells and neuronal cytoarchitecture by reducing oxidative and neuroinflammatory injury

MTU03-15 | Astrocytic calcium signaling as a neuroprotective mechanism against α -synuclein-induced inflammation

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α -synuclein aggregation has been linked with sustained neuroinflammation in Parkinson's disease (PD) aggravating neuronal degeneration. Even though α -synuclein can be phagocytosed by microglia and/or astrocytes, the molecular pathways that trigger and prolong inflammation in the PD brain remain unclear. Aberrant astrocytic calcium signaling has been linked with the pathogenesis of several neurodegenerative diseases and could contribute to the initiation or sustainance of neuroinflammation. In the present study, we investigated the role of calcium signalling in α -synuclein-induced inflammation in vivo, using the human α -synuclein A53T transgenic mouse model, where the presence of α -synuclein oligomers is correlated with sustained inflammatory responses as indicated by significant elevations in the levels of endogenous antibodies and pro-inflammatory cytokines (TNF α , IFN γ and IL1 β). Similar results were obtained in post-mortem human brain samples of PD patients. 3D cell reconstruction and morphometric analysis of the GFAP+ astrocytes in the striatum revealed increased number and distinct morphological alterations in the A53T mice compared to wild type mice. qPCR experiments verified that a subset of A53T animals expressed high levels of genes specific to reactive astrocytes. Further analysis of the striatum of A53T mice using immunofluorescence and immunoblotting revealed an activation of the p38/MAPK pathway in microglia that stimulated the NF κ B pathway in astrocytes. Such activation resulted in the upregulation of astrocytic T-type Cav3.2 voltage-gated Ca²⁺ channel (VGCC). Secretomic analysis in quiescent astrocytes overexpressing the Cav3.2 VGCC highlighted the secretion of the chemoattractant CXCL10 and insulin-like growth factor-binding protein-like 1 (IBPL1), a potent regulator of axonal growth. The proteomics data along with the absence of neuronal death in A53T mice lead us to the assumption that the elevation of astrocytic Cav3.2 levels acts as a compensatory mechanism that protect neurons from the inflammation induced by α -synuclein oligomers.

MTU03-16 | Neurodevelopmental toxicity of embryonic exposure of Acetamidrid in the animal model

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In recent years, reports have shown pesticides would potentially affect not only insects but also humans, especially in the fragile developing brain. We investigate the effects of acetamidrid (ACE) on the structures and functions of the fetal cerebellum using rats. ACE, an insecticide having a nicotine-like structure, easily binds to nAChR, and ACE bound to the receptor is hard to be degraded by acetylcholinesterase.

We administered 20, 40, and 60 mg/kg body weight of ACE administration (ACE20, ACE40 and ACE60) to Wistar rats on an

embryonic day 16 (E16), and their cerebellum were observed at postnatal day 7(P7), 14(P14), and 18(P18). ACE exposure decreased developed granule cells expressing neuronal nuclei (NeuN). No PCs showed abnormalities in ACE20 pups; however, in the cerebellum of ACE40 and ACE60 pups, PCs misalignment was observed at P7 and P14. This misalignment was also observed in nicotine-administrated pups. This data suggests that ACE exposure causes GCs decrement resulting in misalignment of PCs via suppression of neural differentiation. At P14 and P18, the number of activated microglia (MGs) was increased in the ACE-exposed pups than in controls. Also, at P18, ACE-exposed pups contained fewer PCs compared to control pups. Our data shows that ACE40 and above caused the activation of M1-phenotype MGs. Therefore, we observed just phagocytosed-PCs images by M1 phenotype MGs at P18. The number of immune-stained GFAP fibers of Bergmann glial cells at P18 in ACE-exposed pups was significantly decreased compared to those in control pups. At P14 and P18, PCs in the ACE-exposed pups had shorter dendrite lengths, except for ACE40. We suggest that the decrease in Bergmann glial fibers would affect the length of PC dendrites.

In conclusion, exposure to ACE40 and above shows signs of developmental neurotoxicity.

MTU04-01 | Aqueous lyophilisate of *Malvaviscus arboreus* leaves exerts antiepileptogenic properties

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Epilepsy remains one of the most challenging neurological disorders worldwide and particularly in sub-Saharan Africa, where the epilepsy treatment gap remains very high. The present study thus aimed to evaluate the antiepileptogenic properties of *Malvaviscus arboreus* in rats. Apart from the vehicle, animals were challenged with PTZ (70 mg/kg). All the challenged animals were later divided into the treatment groups (negative control, valproate 300 mg/kg, *M. arboreus* 122.5, 245 and 490 mg/kg). Except the vehicle, all the other groups were subjected to kindling PTZ, consisting of repeated administration of PTZ at a dose of 35 mg/kg every other day. The percentage of protection against seizure and the seizure progression were assessed. TNF α and TGF β 1 levels were assessed using ELISA. GABA, GABA transaminase and oxidative stress were assessed in the hippocampus using spectrophotometric assay and histological analysis using cresyl-violet staining. Results showed that the aqueous extract of *M. arboreus* leaves at a dose of 245 mg/kg significantly increased the number of injections needed to reach the kindled state ($p < 0.001$), protected animals against the development of epilepsy ($p < 0.001$). The lyophilisate significantly decreased the activity of GABA transaminase ($p < 0.01$) the

levels of TNF α ($p < 0.001$), TGF β 1 ($p < 0.001$) and malondialdehyde ($p < 0.05$), and increased the concentration of GABA ($p < 0.05$), Reduced glutathione ($p < 0.001$) and Catalase ($p < 0.001$), and preserved the architecture of the hippocampus and prevented necrosis of neurons. The aqueous lyophilisate of the leaves of *M. arboreus* thus has antiepileptogenic properties and could therefore be of great benefit in the alternative and complementary therapy of epilepsy.

MTU04-03 | Modulation of multidrug resistance protein 1-mediated export processes by the antiviral drug ritonavir

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The multidrug resistance protein 1 (Mrp1) is an ATP-dependent efflux transporter that is one of the major facilitators of drug resistance in mammalian cells during cancer and HIV therapy. Elucidating the underlying mechanisms of drug-induced modulation of Mrp1-mediated transport processes remains a major challenge. To address such questions, we investigated the consequences of an application of the antiviral drug ritonavir to cultured rat astrocytes. Ritonavir strongly stimulates the Mrp1-mediated export of glutathione (GSH) by decreasing the K_m value from 200 nmol/mg to 28 nmol/mg, while ritonavir lowered the export of the other Mrp1 substrates glutathione disulfide (GSSG) and glutathione-bimane. To unveil the potential mechanism explaining these apparently contradictory observations, we performed *in silico* docking analysis and molecular dynamics simulations using a homology model of rat Mrp1 to predict binding of ritonavir, GSH and GSSG by the substrate binding site of Mrp1. The results obtained suggest that ritonavir binds to the hydrophilic part of the bipartite binding site of Mrp1 and thereby differently affects binding and transport of the other Mrp1 substrates. The presented combination of experimental data with molecular dynamic simulations reveals new insights into the drug-dependent modulation of Mrp1-mediated export processes, which provides a platform for the development of new chemotherapeutic strategies.

MTU04-04 | Neuronal loss, Oxidative Stress, inflammatory and Junctional Perturbations in Hippocampus of Diabetic Rats Treated With Alcohol

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Diabetes is associated with immunologic perturbations and angiopathies with attendant hippocampal deficits. These diabetes-induced conditions are further burdened by the lifestyle factors such as alcohol use and abuse. Therefore, this study investigated the effects of sub-chronic alcohol consumption in type 2 diabetes on oxidative stress, pro-inflammatory cytokines, histomorphology, and apoptotic mRNA levels in the hippocampus. Male Sprague–Dawley rats were divided into four groups of six rats each; NC (untreated groups), AL (normal rats treated with 10% v/v ethanol in distilled water, DB (diabetic rats), and DAL (diabetic rats treated with ethanol). After 90 days of treatment, the rats were terminated, brains were excised for immunoassay of proinflammatory cytokines (IL-1 α , IL-6, and TNF α) and oxidative stress (MDA and GPx), histomorphology analysis, qPCR analysis of junctional (CLDN5 and OCLDN) and apoptotic (BAX, BCL and caspase 3) markers. Our results show that alcohol alone (AL), significantly elevated levels of cytokines, insignificantly upregulates BAX, BCL, caspase 3 and downregulates CLDN5, OCLDN mRNA levels but induces oxidative stress and reduces hippocampal neurons at CA1, CA3, and DG regions. While in DB and DAL oxidative stress was significantly increased, with upregulation of BCL, and insignificant downregulation of CLDN5 mRNA levels. However, some deferential effects are noted such as cytokine levels (significantly decreased in DB, insignificantly increased in DAL), upregulation of BAX, and caspase 3 (significant in DAL but not in DB), downregulation of OCLDN (significant in DB, but not in DAL). In addition to these perturbations in cytokines and junctional proteins, alcohol in diabetes (DAL) intensifies the reduction of neurons in the CA1 (compared to control, AL, and DB), CA3 (compared to control, and AL), DG (compared to control). These results indicate that alcohol consumption in diabetes may worsen alcohol toxicity on the hippocampal neurons and apoptosis in the absence of sufficient neuroinflammatory response.



MTU04-05 | Aberrant expression and activity of neuronal nitric oxide synthase alters calcium signaling in Alzheimer's disease neurons

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Nitric oxide (NO), produced by the calcium (Ca²⁺)-activated enzyme neuronal nitric oxide synthase (nNOS), functions as a second messenger and modulates glutamatergic Ca²⁺ signaling in neurons. Aberrant Ca²⁺ signaling occurs in Alzheimer's disease, including in sporadic AD (sAD), however, the contribution of nNOS activity and NO to this phenotype is not well understood. The aim of this study was to first quantify nNOS expression and NO levels in sAD and then assess the impact of inhibiting nNOS activity or scavenging NO on neuronal Ca²⁺ signaling. Quantification of nNOS expression using immunostaining and western blotting showed a significant increase in nNOS amount in both human post-mortem tissue ($p = 0.004$) and induced pluripotent stem cell (iPSC) derived neurons ($p = 0.005$), from sAD patients compared to healthy donors. This was observed in conjunction with significantly elevated levels of nitrite in sAD iPSC-derived neurons ($p < 0.001$), quantified using the Griess assay as a downstream marker of NO. Live cell Ca²⁺ imaging demonstrated that inhibition of nNOS activity, or scavenging of endogenous NO, significantly decreased the glutamatergic Ca²⁺ response in healthy iPSC-derived neurons ($p < 0.001$). In contrast, this modulatory effect was lost in the sAD neurons, although there was a decrease in the proportion of spontaneously signaling neurons, suggesting pathogenic modification of signaling receptors. In conclusion, the results of this study show that NO increases the Ca²⁺ response to glutamate in healthy neurons, however, it contributes to spontaneous Ca²⁺ signaling during sAD, highlighting the contribution of this second messenger to Ca²⁺ signaling breakdown during AD.

MTU04-06 | Mitochondrial respiration and vulnerability to oxidative stress in the hippocampus of insulin-resistant Goto-Kakizaki rats

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Brain insulin resistance in type 2 diabetes (T2D) has been associated to cognitive decline predisposition. Increased oxidative stress, either as a cause or consequence of mitochondrial

dysfunction, is thought to be closely linked to the cellular damage leading neuronal dysfunction in T2D. In contrast, previous studies have reported significant increased levels of the antioxidant ascorbic acid, and we have found reduced lipid peroxidation and tyrosine nitration in the hippocampus of 6-month-old insulin-resistant Goto-Kakizaki (GK) rats. While it appears that young GK rats have a compensatory antioxidant protection in the hippocampus, it is unknown whether it is present at older age. In this study, we evaluated respiratory control ratio (RCR) indexes, proton leakage, maximum respiratory rate, coupling efficiency and spare respiratory capacity of hippocampus from male and female Wistar and GK rats of four different ages (6, 12, 18, and 24 months). Real-time PCR was used to determine mRNA expression of glutathione synthetase (GSS), glutamate-cysteine ligase catalytic subunit (GCLC), nuclear factor-erythroid factor 2-related factor 2 (Nrf2), catalase, superoxide dismutase (SOD) 1 and 2, and glutathione peroxidase (GPx) 1 and 4. The antioxidant profile further included activity measurements for catalase, SOD and GPx. GK rats of either gender showed a significant increase in maximum respiratory capacity as well as in the antioxidant profile compared to age-matched controls. Complex I-II capacity was higher in both 6 and 24 months-old GK rats. However, oxygen consumption associated to leakage or oxidative phosphorylation remained unaltered in the hippocampus of GK rats, when compared to age-matched controls. Altogether, these results suggest that aging is associated to global increased respiration capacity of hippocampal mitochondria in insulin resistance, accompanied by increased antioxidant protection. However, it remains to be identified the cellular compartment (neurons, astrocytes, or other cells) in which these alterations take place.

MTU04-09 | Elucidating the role of Tau isoform expression in human iPSC-derived Tauopathy models

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As our society ages, it is faced with an increasing number of patients suffering from dementias, with the most prevalent being Alzheimer's disease (AD) and Frontotemporal dementia (FTD). Many dementias, including AD and FTD are characterized by malfunction of the protein Tau with Tau mutations causing familial FTD. Tau expression and splicing are highly regulated and mis-regulation of the two classes of splice isoforms, 3R and 4R Tau, leads to FTD. Despite its importance in physiology and disease, currently available Tauopathy models largely do not recapitulate adult human Tau isoform expression at a 3R to 4R ratio of 1:1.

To overcome this drawback, we developed a novel CRISPR/Cas9 genome editing strategy to alter endogenous Tau isoform expression

from the physiological genomic locus in induced pluripotent stem cell (iPSC)-derived cortical neurons. As a result, our edited cell lines express a 1:1 ratio of 3R and 4R Tau isoforms soon after neuronal differentiation and thus much earlier than unedited WT neurons. Currently, we use these edited neurons in 2D as well as 3D cortical brain tissue-like spheroid cultures to investigate the effects of pathogenic Tau mutations in a background of adult human 3R:4R Tau isoform expression. First results demonstrate accelerated phenotype formation when combining adult human Tau isoform expression and FTD mutations. In conclusion, we can achieve expression of 4R Tau already in young iPSC-derived neurons using CRISPR/Cas9 genome editing. Our model increases the utility of iPSC-derived neurons to study adult-onset neurodegenerative Tauopathies and provides a novel platform to investigate 3R/4R splice ratio-mediated differences in Tau biology and pathology.

MTU04-10 | New interaction between Gβγ protein and human Monoamine Transporters (hMATs), insights from in silico studies

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The human monoamine transporters (hMATs) play a crucial role in the reuptake of monoamines such as dopamine (DA), serotonin (5HT), and norepinephrine (NE), terminating the action of these neurotransmitters. Different drugs approved for the treatment of neuropsychiatric disorders interact with MATs, blocking the reuptake, increasing the extracellular concentration of DA, 5HT, and NE, or even producing efflux through the MATs. Another regulation of MATs occurs by proteins such as protein kinase A and C (PKA/PKC), syntaxin 1, and newly described Gβγ. The interaction of human dopamine transporter (hDAT) and Gβγ were described to be at the C-terminus of hDAT, and due to this physical contact, efflux of DA is produced through hDAT. However, no description of this interaction with human serotonin Transporter (hSERT) or NOREPINEPHRINE transporter (hNET) exists. Using in silico simulations, we proposed a common binding site between MATs and Gβγ, producing conformational changes related to the efflux of neurotransmitters in a physiological state.

MTU04-11 | Glycine receptor autoantibodies bind to the glycine receptor β subunit representing a novel target of stiff person syndrome

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Glycine receptors (GlyRs) enable fast inhibitory neurotransmission in the mammalian spinal cord and brain stem. They form pentameric α homomers located pre- and extrasynaptically, or postsynaptic αβ heteromers with a 4α:1β stoichiometry. Loss of glycinergic function causes enhanced excitability leading to symptoms like muscle stiffness, spasms and exaggerated startle, typical for the rare autoimmune diseases Stiff Person syndrome (SPS) and progressive encephalomyelitis with rigidity and myoclonus (PERM). Half of PERM patients and 20 % of SPS patients are associated with autoantibodies (aAb) against GlyRs. At the molecular level, GlyR aAb binding impairs receptor function by direct blocking effects and leads to less surface receptors due to enhanced receptor internalisation. So far, GlyR aAbs have been found to bind to different GlyR α subunits. Sequence homology in the extracellular domain, which is targeted by aAbs, suggests that GlyR aAbs might also bind the GlyR β subunit. In this study, we investigated serum samples from 56 patients with SPS-like symptoms and found half of them positive for GlyR α1 aAbs. Whereas, none of the GlyR α1 negative patient samples showed binding to GlyR β, we could identify two patients with aAbs not only binding to the GlyR α1 but additionally the GlyR β subunit. GlyR specificity was verified in primary spinal cord neuronal cultures and spinal cord tissue of mice lacking the α1 subunit by binding to GlyR β localized at synaptic sites. Quantitative analysis revealed that aAbs bound with the same efficacy to GlyR β colocalizing with its scaffold protein gephyrin in spinal cord neurons with and without expression of α1. Ongoing electrophysiological measurements in the presence and absence of GlyR aAbs will unravel functional differences between GlyRs targeted by patient sera positive for GlyR α1 only and sera with aAbs against both α1 and β.

MTU04-12 | GSK-3 in Huntington's disease

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Aims: In Huntington's disease (HD), the mutant huntingtin (mHtt) accumulates as toxic aggregates in the brain, with deleterious effects on motor-coordination and cognitive functions. Reducing the levels of mHtt is therefore a promising therapeutic strategy. Previously we showed that selective inhibition of GSK-3 enhances mHtt clearance and improves motor and coordination abilities in the HD model of R6/2 mice. However, how GSK-3 impacts mHtt dynamics is not fully understood.

Methods: We established cell systems and primary neurons expressing mHtt, and conducted genetic and pharmacological manipulations to identify GSK-3-mediated down targets/pathways responsible for its deleterious activity toward accumulation of mHtt amounts and aggregates.

Results: GSK-3 alpha isozyme has a prominent effect in accelerating mHtt aggregates as compared to that achieved with GSK-3b. GSK-3a reduced autophagosome biogenesis and inhibited autophagic flux.



The use of deleted constructs of GSK-3 alpha indicated that both N- and C-terminals are important for mHtt upregulation. Proteomic analysis further indicated that overexpression of GSK-3 alpha altered cellular traffic, membrane transport, and mitochondria activity, while the use of GSK-3 inhibitor recovered expression levels of cellular structure- cell motility- and membrane transport- proteins that were sharply decrease by mHtt.

Conclusions: GSK-3 alpha is a major player in accelerating the formation and abundance of mHtt aggregates. This involves impairment in autophagy, cellular traffic, and membrane transport. The importance of both N/C terminals of GSK-3 alpha in this process suggests that unique protein-protein interactions are required for producing its maximal deleterious effect in boosting mHtt aggregates.

MTU04-13 | Systematic study of extracellular vesicles and their integrative analysis with Parkinson's organoids MAP

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Synaptopathy, improper establishment of synapse communication, emerged as central mechanism in Parkinson's disease (PD) and redefined our current understanding by focusing on dysfunctional cell interconnectivity, with extracellular vesicles (EV) implication. To overcome the major known challenges and limitation associated with the use of primary human neuronal culture and ensure non-neuronal midbrain cells contribution, we propose a robust 3D iPSC-based comprehensive co-culture for PD development and progression studies. Our complex model is cultured on microfluidic microphysiological analysis platform (MAP) that is coupled to a novel high-yield EVs isolation module, both recently developed in our lab. Our novel platform ensures proper sourcing of EVs from natural neuronal microenvironment, assures high-yield retaining of these EVs for further analysis of circular/microRNA cargo and enables RNA profiling at previously inaccessible quality. Outcome from our studies addresses PD neuropathology, role of exosomes' RNA cargo and promises new alternatives in repairing of RNA-mediated PD's synaptopathy.

MTU04-14 | Identifying prenatal molecular origins of neurodevelopmental disturbances

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Infection during pregnancy increases the risk for offspring to develop a neurodevelopmental disorder later in life. The impact of maternal SARS-CoV2 infection on offspring neurodevelopment is unknown. Rodent modelling has shown it is the maternal immune response that causes adverse brain and behavioural outcomes in

offspring. We aimed to identify the molecular origins of brain and behaviour dysfunction caused by in utero exposure to maternal immune activation (MIA). We hypothesised gene pathways critical to neurodevelopment may be altered by MIA.

An Illumina EPIC BeadChip was used to analyse 850,000 methylation sites in the human genome in buccal swab DNA from infants exposed in utero to SARS-CoV2 infection as well as age and sociodemographic matched controls (n = 4/group). MIA was modelled in mice using the viral mimetic, poly-I:C (5 mg/kg, i.p.), at two gestational stages; gestational day (GD 9-11), or GD13-15. Fetal brain tissue was collected 24-hour post MIA and gene expression assessed using Biomark Fluidigm array (n = 7/group). In another cohort, adult offspring were assessed in a battery of behavioural and cognitive paradigms (n = 12/group).

Over 1000 differentially methylated sites were identified in SARS-CoV2 exposed infant DNA compared to controls. Gene ontology enrichment analysis revealed differential methylation of genes corresponding to neurodevelopmental pathways, including epidermal growth factor (EGF) signalling and dendrite morphogenesis. In the mouse model Egf and downstream molecules, Mapk8, Akt and Stat1 were up-regulated 24-hours post MIA in fetal brains. Adult offspring showed alterations in working memory, cognitive flexibility and sensorimotor gating.

Both clinical and preclinical data suggest altered EGF pathway signalling following in utero exposure to maternal infection/immune activation. In mice these were associated with long-term behavioural disturbances. Repetition of the clinical study with a larger sample and longitudinal clinical assessments on child neurodevelopment are underway to further substantiate these findings and potentially uncover novel biomarkers of neurodevelopmental disturbances.

MTU04-16 | Ribosomal S6 Kinase confers hippocampal cell layer-specific resistance to excitotoxic cell death

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A major unanswered question in the field of neurodegeneration relates to the differential vulnerability to cell death within distinct regions of the brain. A prime example of this phenomenon is within the hippocampus, a critical brain region for learning and memory, where the CA1 subfield is highly vulnerable to damage, resulting in both acute and delayed neuronal death. In contrast, the dentate gyrus granule cell layer (GCL) is relatively resistant to injury. This differential vulnerability of the CA1 and GCL provides us with a powerful model system to examine the molecular signaling mechanisms that confer resistance, and vulnerability, to cell death. Given that the profound neurological deficits resulting from neurodegenerative diseases (e.g. stroke, epilepsy and Alzheimer's disease) are often the result of selective cell loss, the identification of cell-type

specific neuroprotective signaling events would be an exciting step towards the development of new pharmacological approaches designed to limit cell damage, and, in turn the extent of the neurological insult. Along these lines, recent work has implicated the p44/42 mitogen-activated protein kinase (MAPK) pathway as a regulator of neuronal cell death. Robust activation of this pathway is observed in the GCL following excitotoxic insults, but limited activation has been reported in the CA1 region of the hippocampus. Accordingly, this observation raises the possibility that the lack of MAPK pathway activation within the CA1 cell layer could contribute to its enhanced relative level of vulnerability to neuronal cell death. Here, we describe the development of a brain slice culture model that will allow us to induce and measure selective vulnerability within hippocampal subfields. Further, our preliminary data reveal that the MAPK pathway effector, ribosomal S6 kinase (RSK), functions as a key signaling intermediate that confers neuroprotection to the GCL. Ultimately, this knowledge could contribute to the design of new neuroprotective therapeutic strategies.

MTU04-18 | Molecular mechanisms of intermittent fasting-induced ischemic tolerance

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Lifestyle factors such as exercise, exogenous supplements, or calorie restriction are known to be involved in the pathophysiological consequences of neurological diseases, including stroke. Although dietary modifications are proven to be beneficial in multiple cellular and molecular aspects of metabolic disorders, their role in modulating post-stroke functional recovery remains to be studied. We currently investigated potential mechanisms of ischemic tolerance induced by intermittent fasting (IF) using an experimental stroke model. When a cohort of adult male mice were subjected to 6 weeks of IF before inducing transient focal ischemia, there was a significant reduction in infarct volume compared with an ad libitum (AL)-fed group. Furthermore, post-stroke motor as well as cognitive impairments were significantly lower in the IF group compared with the AL group. Notably, RNA sequencing data showed differential expression of transcripts related to synaptic plasticity between AL and IF groups. Collectively, these results indicate that IF promotes post-stroke synaptic plasticity, thereby ramping up functional recovery.

MTU04-19 | The modulatory role of curcumin and quercetin on *Drosophila* GSK-3: a potential therapeutic intervention in Parkinson's disease

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Parkinson's disease (PD), It is estimated that 6–10 million individuals worldwide suffer from PD. The key signalling pathway component, glycogen synthase kinase-3 (GSK-3) is a serine/threonine protein kinase implicated in the progression of PD. It has been reported that increases in GSK-3 β contribute to several cellular processes that promote the pathology of neurodegenerative diseases, including PD. Recently the Russell lab have used genome engineering approaches to established *Drosophila* models of GSK-3 (shaggy, sgg) isoform specific functions. In brief, they have demonstrated nervous system specific phenotypes, including reduced longevity and disrupted locomotor activity, associated with a new set of isoform-specific sgg loss of function alleles. Curcumin, and has been shown to exhibit protective antioxidant activity in a number of disease contexts, including PD and, more recently, has been implicated in the downregulation of GSK-3; quercetin can act synergistically with curcumin but there is some debate as to this activity in a neural context.

Hypothesis: The antioxidant properties of curcumin with quercetin may mediate an anti

inflammatory response, ameliorating oxidative stress-induced mitochondria imbalance in the brain of PD patients. We hypothesise that excess GSK-3 accumulation in the substantia nigra is driven by oxidative stress and aim to test the effects of these compounds on the localisation and activity of GSK-3 in the well-established model organism *Drosophila melanogaster*.

Specific aims: We will explore the mechanistic interactions and potential therapeutic benefits of curcumin and quercetin co-administration with a specific focus on GSK-3 activity and mitochondrial dynamics in the *Drosophila* nervous system.

MTU04-20 | Non-canonical role of PINK1 in modulating PKA function and dendrite outgrowth

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Over more than 40 loss of function mutations in PTEN-induced Kinase 1 (PINK1) cause early-onset Parkinson's disease (PD) in humans. In the mitochondrion, full-length PINK1 is a serine-threonine kinase with a predominant role in enhancing mitophagy in oxidatively stressed neurons. However, emerging evidence suggests an extra-mitochondrial role of post-translationally processed, cleaved PINK1 (c-PINK1) in promoting neuronal development and plasticity.



Like c-PINK1, Protein Kinase A (PKA) activity is vital for neuronal physiology, and in vitro and in vivo models of PD have shown that a deficiency in PKA function contributes to PD pathology. Our previous studies have shown that c-PINK1 and PKA interact to modulate neuronal plasticity by stabilizing dendritic networks through the extracellular release of the BDNF. The molecular mechanism(s) and spatiotemporal dynamics by which PINK1 promotes PKA-mediated dendrite outgrowth remain to be determined. Primary cortical neurons (PCNs), prepared from 14- to 15-day C57BL/6 and PINK1-KO mouse embryos, were used as an experimental model and kinetin (50 μ M) was used to activate endogenous PINK1 pharmacologically. Western blotting and ELISA were used to measure PKA subunit expression and phosphorylation patterns. A significant reduction in the endogenous level and activation status of various subunits of the PKA holoenzyme was observed in PINK1-KO primary cortical neurons (PCNs) compared to WT-PCNs, suggesting that a loss of PINK1 function leads to reduced global neuroprotective PKA activity. In addition, in vitro PKA activity assays suggest that PINK1-mediated activation of PKA occurs within 8 hrs. and plateaus by 12 hours following treatment of PCNs with kinetin. Mechanistically, c-PINK1 enhances the autocatalytic-mediated activation of PKA and reduces the activation threshold by phosphorylating Type II regulatory subunits of PKA. To further elucidate the correlation between PINK1 activity and PKA function in dendrites, FRET-based PKA sensors were employed to analyze the spatio-temporal dynamics of PKA activation by PINK1 in dendrites of PCNs. Overall, our study demonstrates that PINK1 modulates PKA function and highlights the extra-mitochondrial and neuroprotective roles of PINK1 in neurons.

MTU04-21 | Human stem-cell-derived cortical tissue models to investigate Alzheimer's disease

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Alzheimer's disease (AD) is the most common cause of dementia, but despite decades of research, there are still no treatments available to stop or cure the disease. Both the development of disease-modifying drugs and underlying basic research heavily depend on model systems, especially transgenic mice. It is therefore conceivable that drawbacks of these models, such as species differences, contribute to the failures of drugs in human patients. Induced pluripotent stem cell (iPSC)-based AD models enable investigation of pathomechanisms in human, disease-relevant brain cell

types and thus offer great potential for mechanistic and translational studies. However, current iPSC-AD models often show low reproducibility and cell type diversity and lack physiological human cell-cell and cell-matrix contacts. In addition, they typically only enable investigation of early pathologies including endosomal abnormalities and A β accumulation but lack hallmarks such as neuroinflammation and widespread protein aggregation.

Therefore, we aim to develop novel, more reproducible iPSC-AD models made of multiple brain cell types that enable investigation of pathomechanisms in a 3D cortical tissue-like environment.

To create these models, we generated iPSCs with synergistic AD mutations by genome editing, and optimized protocols to differentiate these iPSCs into disease-relevant brain cell types, including cortical neurons, astrocytes and microglia. By 3D co-culturing all cell types we established highly controllable and reproducible human cortical tissue models. Our cultures are stable and postmitotic, display a dense network of neurites and astrocytic processes that is tiled by ramified microglia, without necrotic core formation. Embedded neurons form functional synapses over time, and incorporated microglia are phagocytically active and show typical surveillance of the environment and reaction to focal injuries in live cell imaging. Using our AD lines, we observed central phenotypes such as increased A β secretion and pTau levels, accumulation of extracellular and insoluble A β , and potential microglial activation. Currently, we are optimizing the model to elicit later-stage pathology such as A β plaques and further investigate microglial states and reactivity.

Our model will form the basis for studies elucidating novel, potentially human-specific pathomechanisms and provide a framework for therapeutic screening approaches.

MTU04-22 | CDKL5 deficiency slows synaptic vesicle endocytosis at central nerve terminals

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At the presynaptic terminal, neurotransmitters are packed into synaptic vesicles, which are released and retrieved from the plasma membrane thus maintaining neuronal communication. Deficits in synaptic vesicle recycling have been linked to a range of neurodevelopmental and epileptic disorders. Cyclin-dependent kinase-like 5 (CDKL5) deficiency disorder (CDD) is an X-linked monogenic disorder characterised by early-onset epilepsy and severe neurodevelopmental impairment. CDD is widely considered to result from *de novo* mutations in the CDKL5 gene, which encodes for a neuronal serine-threonine kinase. CDKL5 has been implicated in various activities,

such as axon formation, microtubule remodelling, and cargo trafficking; however, whether CDKL5 has any presynaptic role remains elusive. Using a novel CDKL5 knockout rat model, we discovered that CDKL5 is required for synaptic vesicle endocytosis at central nerve terminals, with no effect on synaptic vesicle exocytosis. This defect was due to the loss of its enzyme activity since kinase-inactive mutations were unable to correct endocytosis in CDKL5-null neurons. Importantly, expression of the isolated CDKL5 kinase domain restored synaptic vesicle endocytosis kinetics, suggesting that CDKL5 kinase activity is necessary and sufficient for the process. We also found that the only previously identified substrate of CDKL5 at the presynaptic terminal, amphiphysin 1, is not the CDKL5 kinase target controlling endocytosis. Overall, this study offers the first evidence of a presynaptic role of CDKL5 that is mediated through its kinase activity and creates the basis for future research on presynaptic CDKL5 with potential translational significance for CDD.

MTU04-23 | LRRK2 and α -synuclein fibrils reciprocally regulate to enhance insoluble α -synuclein release under lysosomal overload stress

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α -Synuclein and LRRK2 are two genes associated with both familial and idiopathic Parkinson's disease (PD), although the mechanistic link between them remains elusive. It has been shown that α -synuclein aggregates cause lysosomal stress upon entry into cells, and LRRK2, a Rab kinase, is upregulated when lysosomes are stressed. We have previously shown that treating cells with lysosomotropic drugs causes the recruitment of LRRK2 and its substrate Rab10 onto overloaded lysosomes, leading to the extracellular release of lysosomal contents via Rab10 phosphorylation. Here we report that lysosomal overload causes extracellular release of insoluble α -synuclein from macrophages and microglial cells loaded with α -synuclein fibrils. This release was mostly dependent on Rab10 phosphorylation by LRRK2 and was not observed in neuronal cells. On the other hand, lysosomal uptake of α -synuclein fibrils enhanced LRRK2 phosphorylation of Rab10, which was accompanied by an increased recruitment of LRRK2/Rab10 to lysosomal surface, as caused by lysosomal overload. These data suggest that lysosomal α -synuclein aggregates activate the LRRK2-Rab10 pathway, which in turn upregulates the release of α -synuclein aggregates, leading to a vicious cycle enhancing α -synuclein propagation.

MTU04-24 | The role of ubiquitin-proteasome system in psychiatric disorders using chronic restraint stress model of depression in mice

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While chronic restraint stress results in depression-like behaviors possibly through oxidative stress in the brain, its molecular etiology and the development of therapeutic strategies remain elusive. Since oxidized proteins can be targeted by the ubiquitin-proteasome system, we investigated whether increased proteasome activity might affect the stress response in mice. Transgenic mice, expressing the N-terminally deleted version of α 3 subunit of the proteasome, which has been shown to generate open-gated mutant proteasomes, in the forebrain were viable and fertile, but showed higher proteasome activity. After being challenged with chronic restraint stress for 14 d, the mutant mice with hyperactive proteasomes showed significantly less immobility time in the forced swimming test compared with their wild-type littermates, suggesting that the transgenic mice are resistant to chronic restraint stress. The accumulation of ER stress markers, such as polyubiquitin conjugates and phospho-IRE1 α , was also significantly delayed in the hippocampus of the mutants. Notably, the transgenic mice exhibited little deficits in other behavioral tasks, suggesting that stress resilience is likely due to the degradation of misfolded proteins by the open-gated proteasomes. These data strongly indicate that not only is the proteasome a critical modulator of stress response in vivo but also a possible therapeutic target for reducing chronic stress.

MTU05-01 | Relationship between reelin and morphological features in the human infant hippocampal dentate gyrus (DG)

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Morphological changes within the hippocampal dentate gyrus (DG) have been identified in a proportion of infants who have died sudden and unexpectedly (SUDI) and tend to mirror those seen in temporal lobe epilepsy, thus leading to the hypothesis that a subset of these SUDI may have had unrecognized seizures prior to death. The protein reelin is critical in neuronal migration and cell positioning within the DG. The aim of this study was to qualitatively assess whether morphological changes within the granule cell layer (GCL) of the DG correlate with changes in reelin expression. Immunohistochemistry was applied on formalin fixed and paraffin embedded hippocampal tissue from 38 SUDI cases. Analysing reelin positive cells within the granule



cell layer (GCL) of the DG, we found it to be present in every section however its presence was affected by morphological changes. These included: loss of reelin expression where the DG showed bilamination, focal split, and thinning. Reelin expression was inconsistent in those sections with granule cell dispersion and shows that expression is dependent on the level of dispersion. Only when thick-walled capillaries protruded through the GCL, separating the cells, reelin was not expressed. Thin-walled capillaries within the GCL had no effect on reelin expression and nor did hyperconvolution. This study shows for the first time, that the location of reelin containing cells does correlate with DG changes and suggests that these changes are due to altered migrational processes. Whether these are normal during infant development, remains to be determined.

MTU05-02 | KCC2 Manipulation alters features of migrating interneurons in ferret neocortex

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KCC2 is a brain-specific chloride-potassium cotransporter affecting neuronal development including migration and cellular maturation. It modulates chloride homeostasis influencing the switch of GABA from depolarizing to hyperpolarizing, which contributes to the cues that influence the termination of neuronal migration. The expression of KCC2 during migration of interneurons, therefore, correlates with the ability of these cells to respond to GABA as a stop signal. Manipulation of KCC2 in development can affect various aspects of migrating neurons, including the speed. We describe the effect of KCC2 downregulation and inhibition on features of migrating interneurons of normal ferret kits and those treated with methylazoxymethanol acetate, which increases KCC2. Treatment of organotypic cultures with Bisphenol A, an environmental toxin that alters gene expression, also downregulates KCC2 protein. In organotypic slices treated with the KCC2 antagonist VU0240551, chloride imaging shows inhibition of KCC2 via blockade of chloride flux. Time-lapse video imaging of organotypic cultures treated with either drugs, shows a significant increase in the average speed, step size, and number of turns made by migrating neurons leaving the ganglionic eminence. Our findings demonstrate the harmful effect of environmental toxins on brain development and potential consequences in the pathogenesis of neurodevelopmental disorders.

MTU05-03 | Vanadium improves memory and spatial learning and protects the pyramidal cells of the hippocampus in juvenile hydrocephalic mice

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Hydrocephalus causes learning and memory disabilities due to its damaging effect on the hippocampal pyramidal neurons. Pediatric hydrocephalus develops worldwide with greatest burden on the African, Latin American, and Southeast Asian regions. Vanadium at low doses has been observed to improve learning and memory abilities in neurological disorders. We aimed at investigating the morphological and functional changes of the pyramidal neurons of the hippocampus of juvenile hydrocephalic mice treated with vanadium. Hydrocephalus was induced by intra-cisternal injection of sterile-kaolin into juvenile mice which were then divided into four groups ($n = 10$) with one group serving as a positive control while others were treated with 0.15, 0.3 and 3 mg/kg i.p. of vanadium, respectively, for 7 days post-induction. The controls ($n = 10$) were sham operated without any treatment. Mice were weighed before dosing and sacrifice. Y-maze, Morris Water Maze and Novel Object Recognition tests were carried out before the sacrifice. The brains were harvested, and processed for Hematoxylin and Eosin, and Cresyl Violet staining with pyramidal neurons of the CA1 and CA3 regions of the hippocampus assessed qualitatively and quantitatively. Data were analyzed using GraphPad prism 8.

There was a significant difference in mean % alternation ($p = 0.0241$), escape latency ($p = 0.0296$), time spent in correct quadrant ($p = 0.0066$), number of platform crossing ($p = <0.0001$) and % novel object recognition time ($p = 0.0431$) between the treated and control groups. The treated groups showed a dose-dependent recovery of the disarray in the pyramidal layers. The pyknotic index in the pyramidal layer in CA1 in the low and high dose vanadium groups was lower than in untreated hydrocephalic and control groups.

Vanadium protects the pyramidal cells of the hippocampus and improves cognition in juvenile hydrocephalic mice.

MTU05-04 | Two phosphorylated sites of GAP-43 are the markers of the growing axons in both rodents and primates

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The growth cone is an important structure that is involved in both processes, and GAP-43 (growth associated protein-43 kDa) is believed to be the classical molecular marker. Previously, we used growth cone

phosphoproteomics to demonstrate that S96 GAP-43 in rodents is a highly phosphorylated site that is phosphorylated by c-jun N-terminal protein kinase (JNK) [1]. We also revealed that phosphorylated pS96 antibody recognize growing axons in the developing brain and regenerating axons in adult peripheral nerves. In rodents, T172 and S142 are the additional putative JNK-dependent phosphorylation site that is modified at a lower frequency than S96. Here, we characterized this site using pT172- and pS142-specific antibodies. We confirmed that these two sites were detected by co-expressing mouse GAP-43 and JNK1. These antibodies labeled growth cones and growing axons in developing mouse neurons. Comparison of amino acid sequences indicated that rodent S142 and T172 correspond to human S151 and T181, we confirmed that these antibodies recognized human phospho-GAP-43 using activated JNK1, and also that its immunostaining patterns in neurons differentiated from human induced pluripotent cells (hiPSCs) were similar to those observed in mice. These results indicate that S142 and T172 residue is phosphorylated by JNK1 and that pS142 and pT172 antibodies are new potential molecular markers for axonal growth in both rodents and human [2, 3]. Ref.: 1) iScience 4: 190 [18]; 2) Mol Brain 14: 66 [21]; 3) Neurochem Res (in press)

MTU05-05 | Glial fibrillary acidic protein (GFAP) distribution in brains of juvenile, subadult and adult Japanese quail (*Curtonix japonica*)

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The present study investigated the distribution of glial fibrillary acidic protein (GFAP)-immunopositive elements in the juvenile, subadult and adult male Japanese quail brain. The animals were perfused transcardially with 0.9% cold saline solution followed by 4% paraformaldehyde in a 0.1M phosphate buffer solution. Immunohistochemistry was performed with a mouse monoclonal anti-GFAP (Millipore Sigma-Aldrich, MAB 360, clone G-A.5). GFAP immunoreactive cell bodies and processes were observed within the brain of the three age groups with three morphological subtypes identified at different regional location. The first type of astrocytes, composed of intensely stained unipolar cells, traversed the inner surface of the pia mater, the large blood vessels and the hippocampus. A second type presented as multipolar astrocytes of variable size, with an irregular cell body and found in the ventricular wall and in large fibre tracts. The third astrocyte type showed an immuno-negative cell body that could be identified only by converging processes scattered within the white and grey matter. These three types of astrocytes with several isolated processes were differentially distributed within both the grey and white matter with a highest decline in GFAP positivity in the 12-week adult quail. GFAP immunoreactive cells was absent in the

brainstem. These results indicates that GFAP is a good marker for identification of the normal differentiation during astrogliogenesis population in quail brain which could also become reactive during inflammation.

MTU05-06 | Self-patterning of brain organoids by adhesion-based cell assembly

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Recent advances in cell culture technology have made it possible to recapitulate organogenesis in a dish. Brain organoids from pluripotent stem cells are a good example for this in vitro recapitulation, and they can establish in vivo-like brain structures. In particular, the brain is subdivided into distinct regions, and this regionalization is spontaneously organized within brain organoids. In this study, we examine how this self-patterning takes place during the development of organoids from a homogeneous population of stem cells. We found that morphogen-secreting cells were initially randomly distributed, but eventually localized to a single place, giving rise to stable organoid-wide morphogen gradient and regional patterning. This self-localization of morphogen-secreting cells was regulated by differential expression of intercellular adhesion molecules. This study highlights robust mechanisms of self-organized patterning in the brain organoids.

MTU05-08 | nsSNPs within the extracellular loops of M6a impair its neuroplastic function

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Neuronal glycoprotein M6a expression levels or polymorphisms within the GPM6A gene are associated with neuropsychiatric disorders such as schizophrenia, depression and claustrophobia. Membrane glycoprotein M6a promotes neurite outgrowth, filopodia formation and dendritic spines and synapses maintenance in vitro. Even though strong evidence suggests that the extracellular loops of M6a (ECs) are responsible for its function, the molecular mechanisms linking M6a to the onset of such diseases remain unknown. To gain knowledge on this mechanisms, we aim to characterize new non-synonymous polymorphisms (nsSNPs) in the coding region of ECs of GPM6A. We identified six nsSNPs (T71P, T76I, M15FV, F156Y, R163Q and T210N) that impair M6a-induced plasticity in neuronal cultures; even though the protein's expression, subcellular localization and folding are not affected by the presence of these nsSNPs. Previous reports showed that M6a dimerization is necessary to induce filopodia and synapse formation. M6a's ECs are involved in homo- and heterotypic protein-protein interactions and might lead to the formation of M6a oligomers at the plasma membrane. Thus, we are currently evaluating whether the nsSNPs might disturb the protein's

oligomerization through number and brightness analysis (N&B), which allows us to monitor RFP-tagged M6a oligomer distribution in live cells. Our preliminary results suggest that the damaging effect of these nsS-NPs could be related to a decrease in protein oligomerization. Our results highlight the importance of the reverse genetics approaches to gain knowledge of M6a's mechanisms of action and genetic susceptibility of certain GPM6A variants. Also, it will offer new routes to improve diagnosis and develop more effective treatments.

MTU05-09 | Ameliorative properties of kolaviron on aluminium chloride-induced degeneration in the hippocampus of fetal Wistar rats in-utero

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Aim: This study investigated the role of kolaviron on aluminium chloride-induced neurodegeneration in the hippocampus of fetal Wistar rats in utero.

Materials and Methods: Female Wistar rats (25) were randomly selected, mated, and then assigned into 5 groups of 5 ($n = 5$) once mating was confirmed through vaginal smear. Group A received distilled water, group B; 0.6mls of corn oil, group C; 200 mg/kg of Kolaviron, group D; 100 mg/kg of aluminium chloride, and group E; 100 mg/kg of aluminium chloride + 200 mg/kg of kolaviron. The administration was done orally in the 2nd week of gestation from days 8-10. Pregnant animals were sacrificed on day 20 of gestation; fetuses, their brains, and hippocampi were excised, respectively. Hippocampal tissues of fetuses were homogenized in 0.25M of sucrose solution for biochemical assay which included acetylcholinesterase, cytochrome-c oxidase, glucose-6-phosphate dehydrogenase while some were fixed in 4 % paraformaldehyde for immunohistochemical studies.

Results: The group treated with kolaviron showed a significant decrease in acetylcholinesterase levels while cyt-c oxidase and glucose-6-phosphate dehydrogenase were high compared to the aluminium chloride group. A reduction in glial fibrillary acidic protein-positive cells and neuron-specific enolase expression was also observed in the kolaviron-treated group.

Conclusion: This study demonstrated that kolaviron could serve as a therapeutic tool in conscious or accidental exposure to aluminium chloride during the 2nd week of gestation.

MTU06-01 | N-Acetyl Cysteine ameliorates mitochondrial dysfunction in ischemic injury via attenuating Drp-1-mediated mitochondrial autophagy

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Background and Purpose: Ischemic reperfusion (I/R) injury causes a wide array of functional and structure alternations of mitochondria, associated with oxidative stress and increased the severity of injury. Despite the previous evidence for N-acetyl L-cysteine (NAC) provide neuroprotection after I/R injury, it is unknown to evaluate the effect of NAC on altered mitochondrial autophagy forms an essential axis to impaired mitochondrial quality control in cerebral I/R injury.

Methods: Male wistar rats subjected to I/R injury were used as transient middle cerebral artery

occlusion (tMCAO) model. After I/R injury, the degree of cerebral tissue injury was detected by infarct volume, H&E staining and behavioral assessment. We also performed mitochondrial reactive oxygen species and mitochondrial membrane potential by flow cytometry and mitochondrial respiratory complexes to evaluate the mitochondrial dysfunction. Finally, we performed the western blotting analysis to measure the apoptotic and autophagic marker.

Results: We found that NAC administration significantly ameliorates brain injury, improves neurobehavioral outcome, decreases neuroinflammation and mitochondrial-mediated oxidative stress. We evaluated the neuroprotective effect of NAC against neuronal apoptosis by assessing its ability to sustained mitochondrial integrity and function. Further studies revealed that beneficial effects of NAC is through targeting the mitochondrial autophagy via regulating the GSK-3 β /Drp1-mediated mitochondrial fission and inhibiting the expression of beclin-1 and conversion of LC3, as well as activating the p-Akt pro-survival pathway.

Conclusion: Our results suggest that NAC exerts neuroprotective effects to inhibit the altered mitochondrial changes and cell death in I/R injury via regulation of p-GSK-3 β -mediated Drp-1 translocation to the mitochondria.

MTU06-02 | ASTROCYTIC INSULIN SIGNALING AND INFLAMMATION IN EXPERIMENTAL ALZHEIMER'S DISEASE

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Insulin resistance (IR) and chronic inflammation are associated with the development of cognitive disorders and neurodegenerative diseases such as Alzheimer's disease (AD). However, it is not clear whether there



is a causal link between these factors, which one appears earlier in the pathology or if either one of them is triggered by the increasing circulating levels of A β or amyloid deposits in early AD. Our objective was to study the metabolic and inflammatory status of a model of AD, the PDAPP-J20 mouse at the age of 8 months. We also treated a WT group with a high-fat diet (HFD) as a positive control for IR. Our hypothesis was that in early stages of AD, the brain develops IR with astrocytes showing reactivity and impaired insulin signaling. Final body weight, glycemia and insulinemia were not affected by genotype or HFD. The open-field test showed an anxious-like behavior in transgenic and in HFD-fed mice. Insulin signaling measured by pAkt/Akt ratio was decreased in the hippocampus of AD mice ($p < 0.05$) but not in the hypothalamus or the liver. Pancreatic IL1 β and COX2 levels were unchanged. Insulin receptor puncta colocalizing with GFAP+ cells in the hippocampus by fluorescent immunolabeling showed a decreasing tendency in transgenic animals while astrocytic reactivity markers GFAP and S100b were increased ($p < 0.05$). Finally, we evaluated the effect of fibrillar A β or palmitate on astrocytes in vitro. Astrocytes exposed to A β showed increased nuclear translocation of NF κ B and decreased AKT phosphorylation ($p < 0.05$), suggesting inflammatory activation and impaired insulin signaling, respectively. Our results show that inflammation and insulin signaling impairment in the hippocampus are found in an early stage of experimental AD. The inflammatory context triggered by increased circulating A β or amyloid deposits in the brain could affect astrocytic insulin receptors, hence decreasing insulin signaling and affecting their neuroprotective capacity.

MTU06-03 | Metabolic impact of regulatory volume change

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Maintenance of cell volume is of key importance in brain where changes in cell volume are restricted according to the Monroe–Kellie doctrine. Perturbations in cell volume (swelling or shrinkage) threaten cellular integrity and may lead to cell death, either through membrane rupture and necrosis (via hypotonic swelling) or through metabolic dysregulation and apoptosis (via hypertonic shrinkage). Cells subject to osmotic shock respond by attempting to restore volume through regulatory volume processes. These processes have metabolic costs as do interventions designed to enhance volume restoration. Here, we examined hyponatremia and its osmotic restoration with mannitol; hypernatremia and hyperosmotic stress induced by mannitol. We used the guinea pig cortical tissue slice along with [1-¹³C]glucose and [1,2-¹³C]acetate to study the impact of the above osmotic conditions on the metabolism of these substrates. Slices were sampled four times with results considered significant if $P < 0.05$.

Hypo-osmotic stress resulted in decreased Krebs cycle activity, increased aerobic glycolysis and a loss of organic osmolytes. This loss was not prevented by restoration of osmolarity with mannitol but Krebs cycle activity was restored and glycolytic activity reduced. Mannitol Hypernatremia, on

the other hand, resulted in increased metabolite pools, increased net flux into Krebs cycle intermediates and decreased levels and labelling of lactate, Ala, citrate and glutamine. Media made hyperosmotic with mannitol mostly reduced pools and decreased fluxes but had the opposite effect to high sodium on glutamine levels and fluxes which were increased by mannitol, particularly glutamine derived from acetate (i.e. glial glutamine). This suggests that restoration of hypoosmotic milieu with mannitol largely restores normal metabolism but that hypernatremia has less impact on metabolism than hyperosmotic mannitol. The significantly different impact of mannitol on astrocyte glutamine is a point of difference to consider in treating osmolarity.

MTU06-04 | Pyruvate release and consumption in cultured primary rat astrocytes

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Glycolysis produces pyruvate which is either oxidized in mitochondria to CO₂ or can be metabolized in the cytosol, mainly to lactate or alanine. To investigate the pyruvate metabolism of brain astrocytes, primary rat astrocyte cultures were incubated with either glucose or pyruvate. During incubation with millimolar concentrations of glucose, viable astrocytes released lactate that accumulated to millimolar extracellular concentrations. However, during such incubations astrocytes also exported pyruvate that reached extracellular concentrations of up to 150 μ M. After the applied glucose had been metabolized, the released pyruvate was more rapidly consumed by the astrocytes than released lactate, despite the large excess of extracellular lactate. In glucose-free medium, astrocytes consumed pyruvate almost proportional to time in a concentration-dependent manner with apparent Michaelis-Menten kinetics ($K_m = 0.6 \pm 0.1$ mM, $V_{max} = 5.1 \pm 0.8$ nmol/(min $\times$ mg protein)). Cellular pyruvate consumption was strongly inhibited in the presence of the monocarboxylate transporter 1 (MCT1) inhibitor AR-C155858 or by application of a 10 times excess of the MCT1 substrates lactate and beta-hydroxybutyrate. Pyruvate consumption by viable astrocytes was impaired in the presence of the respiratory chain inhibitor antimycin A, while the mitochondrial uncoupler BAM15 strongly accelerated cellular pyruvate consumption and lowered extracellular lactate accumulation. In conclusion, cultured astrocytes release pyruvate during glucose metabolism, in addition to lactate, and the released pyruvate is more quickly consumed than the released lactate after the glucose had been removed from the medium by the cells. In addition, consumption of extracellular pyruvate by astrocytes involves uptake via MCT1 and is strongly affected by the activity of the mitochondrial respiration.



MTU06-05 | Aging promotes an increase in mitochondrial fragmentation of astrocytes

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Aging is an inevitable event in the life cycle of all organisms, characterized by progressive physiological deterioration and greater susceptibility to death. Aging is the main risk factor for the development of dementia. In this scenario, cerebral mitochondrial dysfunction emerges as a central mechanism for synaptic and cognitive deficits and neuronal death in neurodegenerative diseases. Considering that astrocytic mitochondrial activity has a direct impact on neuronal function, the aim of this proposal is to investigate the effects of aging on astrocytic mitochondrial changes in the cerebral cortex. For this purpose, we used two experimental models: young (3-4 months) and older (over 18 months) mice, and cultures of senescent and control mouse astrocytes. Confirming previous data from the literature, we demonstrated by immunohistochemistry that older mice exhibited increased astrocyte reactivity, as represented by an increase in GFAP levels. Surprisingly, we detected an increase in mitochondrial density of astrocytes, represented by increased colocalization of GFAP/TOMM20. Using fluorescence microscopy, we demonstrated that cultured senescent astrocytes had the same mitochondrial membrane potential compared with control astrocytes. Furthermore, we demonstrated by high-resolution respirometry that cultures of senescent astrocytes exhibited similar basal respiration and coupled oxidative phosphorylation as control cells. Surprisingly, senescent astrocytes showed higher maximal respiration and greater reserve capacity of mitochondrial respiration. In addition, we demonstrated that senescent astrocytes showed an increase in the density and a decrease in the size of their mitochondria, suggesting an increase in mitochondrial fragmentation. To confirm these data, we used qPCR and immunocytochemistry to detect an increase in DRP-1, a central protein in the mitochondrial fragmentation pathway. In summary, these data suggest that astrocytic mitochondrial fragmentation increases with brain aging and shed light on the mechanisms of astrocytic metabolic disorders associated with brain aging.

MTU06-06 | Aminolevulinic acid and iron rescued mitochondrial activity and antioxidant defenses in DARS2-deficient fibroblasts

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Background: The leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL) is a mitochondrial disorder

caused by mutations in the mitochondrial aspartyl-tRNA synthetase gene *DARS2*. Clinical presentation varies from severe infantile to chronic, slowly progressive neuronal deterioration in adolescents or adults. There is no cure or effective treatment for LBSL.

Patients: We have recently reported a family affected with LBSL. A c.492+2T>C mutation and a novel c.228-17C>G variant in the intron-2 hotspot were found in 2 affected siblings (patients 1 and 2) who presented with headaches and MRI consistent with demyelination. Reduced *DARS2* expression and protein content, increased lactate levels and reactive oxygen species, impaired oxygen consumption rate (OCR) and abnormal mitochondrial morphology were observed in patients' fibroblasts.

Methods: A skin biopsy from both patients and a non-carrier asymptomatic sibling, were used to establish fibroblast cultures. Due to its capacity in increasing iron-containing proteins, aminolevulinic acid 100 μ M and iron 50 μ M (ALA/ Fe^{++}) were added to the cell culture media for 14 days. Glutathione (GSH), oxidized glutathione (GSSG), respiratory-chain complexes activities and OCR were assessed as previously established by our group.

Results: Fibroblasts from patient 1 showed reduced GSH and GSH/GSSG ratio, reduced citrate synthase (CS), complexes I (CI) and IV (CIV) activities compared to the control. ALA/ Fe^{++} exposure also rescued all the above abnormalities in patient 1 and improved CS and CIV activities in patient 2 and control. Additionally, OCR was enhanced after the treatment in patient 1.

Conclusion: The ALA/ Fe^{++} treatment was able to increase mitochondrial activity and restored the antioxidant levels in *DARS2* affected fibroblasts. In agreement with previous reports for other mitochondrial disorders, our data suggest a potential role for ALA/ Fe^{++} as a treatment for LBSL disease.

MTU06-07 | Brain dysfunction in obesity-associated insulin resistance: Neuroprotection by taurine and N-acetylcysteine

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Obesity constitutes a risk factor for dementia development. Long-term exposure to obesogenic diets leads to hippocampal taurine accumulation in rodents. Since taurine has putative cytoprotective effects, hippocampal taurine accumulation in obese and diabetic models might constitute a counteracting response to metabolic stress. We tested the hypothesis that treatment with taurine or with N-acetylcysteine (NAC), which provides cysteine for the synthesis of taurine and glutathione, prevent obesity-associated hippocampal alterations and memory impairment. Female mice were fed either a regular diet or a high-fat diet (HFD). Some mice had access to 3%(w/v) taurine or 3%(w/v) NAC in the drinking water. After 2 months, magnetic resonance spectroscopy (MRS) was used to measure metabolite profiles. Memory was assessed in novel object and novel location recognition

tests. HFD feeding caused memory impairment in both tests, and reduced concentration of lactate, phosphocreatine-to-creatine ratio, and the neuronal marker N-acetylaspartate in the hippocampus. Taurine and NAC prevented HFD-induced memory impairment and N-acetylaspartate reduction. NAC, but not taurine, prevented the reduction of lactate and phosphocreatine-to-creatine ratio. MRS revealed NAC/taurine-induced increase of hippocampal glutamate and GABA levels. We conclude that NAC and taurine prevent of neuronal dysfunction, while only NAC prevents energy metabolism dysregulation in HFD-induced obesity in female mice.

MTU07-01 | Behavioral effect of selective deletion of beta2* nicotinic acetylcholine receptors in cortical GABAergic neurons

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Cholinergic receptors are differentially distributed across the cortex and the activation of nicotinic acetylcholine receptors (nAChRs), located on principal neurons and GABAergic interneurons (GABA_{IN}s), modulates synaptic plasticity and behavior. Despite their undisputed importance, functional consequences of selective activation or inhibition of nAChRs expressed by the different types of cortical neurons is not well understood. By using a CRISPR/Cas9-based approach, we aimed to delete the most abundant beta2-nicotinic subunit expressed by several different types of cortical GABA_{IN}s in a cell-type-specific manner and investigate behavioral consequences. First, we used fluorescent in situ hybridization to characterize the expression of beta2* nAChRs in neuropeptideY- (NPY) and serotonin receptor 5HT3A-expressing cortical neurons. Then we crossed a mouse line expressing Cas9-GFP in a Cre-dependent manner with lines expressing Cre in NPY or 5HT3A-positive neurons. The resulting mice were injected with AAV vector carrying sgRNA targeting CHRNA2, gene coding for beta2-nicotinic subunit, in the prefrontal cortex (PFC). Mutant mice and littermate controls, injected with scrambled gRNA, were tested in a battery of behavioral tasks exploring different behavioral domains, followed by immunohistochemical evaluation of Fos, a marker of neuronal activity. The CRISPR-induced mutations in CHRNA2 gene in the PFC of either NPY- or 5HT3A-expressing neurons led to an increase in social interactions in accordance with data known from literature. In contrast, beta2-mutations in NPY- but not the 5HT3A-expressing neurons decreased anxiety-like behavior during the elevated plus maze task. Finally, to quantify the efficiency of CRISPR-induced mutations, we isolated AAV-transduced and Cre-expressing cells by FACS sorting and analyzed the isolated DNA by next generation sequencing. In conclusion, selective deletion of nAChRs in specific neuronal types can be achieved by CRISPR/Cas9-based approach with differential effects on behavior depending on the type of targeted neurons.

MTU07-02 | Optogenetics-mediated local cerebral blood flow affects mouse behavior and neural activity

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It is known that cerebral blood flow (CBF) increases when neural activity occurs. Conversely, what happens to neural activity and mouse behavior when cerebral blood flow increases? To answer these questions, we developed a technique using optogenetics to artificially increase or decrease local CBF. We generated mice that express channelrhodopsin 2 (ChR2) or photoactivated adenylyl cyclase (PAC) in the cells of blood vessels; photostimulation of ChR2 decreases CBF, while photostimulation of PAC increases CBF. Mice expressing opsin in blood vessels have already been reported. However, there is no information on the spatiotemporal dynamics of the resulting CBF changes, making interpretation of in vivo experiments difficult. Therefore, we first clarified the spatiotemporal dynamics of CBF using a Doppler technique and functional MRI. Next, we measured the neural activity associated with CBF manipulation. We found that a decrease in CBF reduced neural activity. Conversely, transient increases in CBF did not alter neural activity. Furthermore, decreasing CBF in the striatum under freely moving conditions reduced locomotor activity in mice. These experiments indicated that it is possible to manipulate CBF in a target brain region under freely moving conditions and examine the resulting changes in neural activity and behavior.

MTU07-03 | Motivation behavior in mice under restricted feeding conditions: Involvement of clock proteins and dopaminergic proteins

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In mammals, the circadian system modulates several behavioral and physiological processes, including the response to natural rewards such as food. On the other hand, when food is temporally restricted, animals display an anticipatory food activity (FAA) that is controlled by a food-entrainable oscillator (FEO).

We have previously shown that mice exhibit a diurnal rhythm in motivation for food reward, becoming more motivated during the night. In this work, we present evidence that motivation for food reward it is involved in FAA. Mice in a restricted feeding (RF) protocol were allowed to consume food only 3 hours during daytime or nighttime. Then, motivation behavior was assayed through the progressive ratio (PR) schedule. Our results show that mice are highly motivated to work for food reward when FAA is present regardless of the time of day. We also investigated the local rhythmic expression profile—through IHQ—of circadian proteins (BMAL1), dopaminergic proteins (DRD2 and TH) and neuronal activation markers (cFOS) within reward-related



areas (NAc, striatum and VTA) and other cerebral areas relevant for FAA behavior. Our results suggest that, during FAA, different components related to reward pathways might be activated and consequently generate an increase in motivation levels.

MTU07-04 | Alterations of behavior, faecal bacteria and fatty acids, brain and intestinal morphology in rats exposed to Cobalt chloride

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Metal ions including cobalt (Co) ions have been reported to possess antibacterial activities. We hypothesized that oral exposure to Co may have implications for gut-dysbiosis and hence, alterations of microbiota-gut-brain signaling in the host. In this preliminary study, we sought to examine whether exposure of male Wistar rats to cobalt chloride (CoCl₂) at 0, 25, 50 and 100 mg/kg for two weeks affects cultivable groups of bacteria in rat faeces, faecal fatty acids, selected behavioural parameters, as well as the morphology of the intestines and brain. In the elevated plus maze (EPM) test, CoCl₂-exposed rats showed a dosage-dependent increase in the frequency of entry into closed arms, compared to controls. Also, there was a dose-dependent reduction in hanging latency in the hanging wire (HW) test. These changes correlated directly with dose-dependent reductions in total heterotrophic count, *coliforms*, *E. coli*, *enterococcal* and *lactobacilli* counts in the faeces. Administration of CoCl₂ at 100 mg/kg evoked the appearance of unsaturated fatty acids including palmitoleic, oleic and linoleic acids in the faeces as detected by gas chromatography-flame ion detection (GD-FID) analysis using fatty acid methyl esters (FAME) standards. The major histopathological lesion in the brain was the presence of chromatolysis in the purkinje cell layer of the cerebellum. In the intestines, there was moderate to severe infiltration of inflammatory cells into different parts of the mucosa of the duodenum, ileum, jejunum and colon while villi erosions were seen prominently in the ileum. These initial findings suggest that short-term exposure to Co can lead to gut microbiome changes that may underlie neurotoxicity and alterations in behavior induced by Co.

MTU07-05 | Sex-dependent behavioural and brain effects of young rats after alcohol binge drinking and methamphetamine self-administration

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The overconsumption of alcohol by adolescents may encourage co-administration with other drugs, and the prevalence of alcohol and methamphetamine (METH) co-abuse is high. The main aim of this study was to evaluate the consequences of prior alcohol binge drinking history in adolescents on METH self-administration in adult rats. Adolescent male ($n = 60$) and female ($n = 54$) Sprague-Dawley rats were trained to lever press for alcohol (Alcopop or Ethanol) or control solutions (Water or Sucrose) in 1-hour oral self-administration sessions. After jugular catheter implantation while adults, they were trained to nose-poke for intravenous METH in 2-hour sessions. Rats then underwent to sequential alcohol and METH access, where alcohol or control solutions were available for the first hour, followed by 1-hour of intravenous METH access. Relapse to oral- and METH-cues were performed after 1-month withdrawal. RNAscope assay was applied in the brains targeting the expression of vGlut-1, Gad-1 and GFAP in the amygdala. Male and female Alcopop rats had greater ethanol intake compared to Ethanol rats with both alcohol concentrations. At the low METH dose, female rats had more infusions compared to males, but no difference across conditions at the high METH dose. During sequential access, METH intake was not affected by alcohol intake. On METH-cue relapse testing, except for the ethanol group, female rats relapsed more than male rats. Brain analyses will also be discussed at the meeting. Sweetened alcoholic beverages can stimulate higher ethanol intake in both male and female rats. Adolescent alcohol exposure partially affects only female rats as they took more low dose METH. Alcopop females had greater ethanol intake than males but had a similar pattern of METH intake. However, Alcopop females exhibited greater METH-seeking behaviour than males on relapse. Overall, it seems that sugar may be affecting the drinking behaviour and METH relapse only in female rats.

MTU07-07 | The underlying mechanisms related to frustration—early adversities and programming of emotional behavior

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Maternal separation (MS) is a model of early stress that modifies long-term neurobiological parameters leading to significant

differences in emotional states; one of them is frustration, a negative emotional state which may be involved with susceptibility to psychiatric disorders. The aim of this research was to evaluate the effects of MS on frustration in adult rats, considering possible sex-specific effects. In addition, we verified modifications in synaptic plasticity, which could be related to the behavioral alterations observed. Litters of Wistar rats, 139 animals (93 females and 46 males) were divided into control (no intervention) and subjected to MS (separated from the dam 3h per day at 32° C from post-natal day (PND) 1 to PND10. From PND80, animals were subjected to the unexpected reduction of reward. Twenty-four h after the last behavioral session, animals were killed and dorsal hippocampus (dHC) and basolateral amygdala (BLA) were dissected for neurochemical evaluation using western blot. The results showed that males that went through MS had higher vulnerability to the unexpected reduction of reward (they showed higher frustration) when compared to controls [$F(3.942.327.191) = 2.789, p = 0.027$], while females did not present this effect. Possibly related to this finding, we also observed alterations on NMDA subunits GluN2A (reduced immunocontent) [$F(1.24) = 8.710, p = 0.007$] and GluN2B (increased immunocontent) [$F(1.24) = 9.686, p = 0.005$] in the dHC of male animals. No significant modifications were observed on synaptophysin, PSD95, SNAP25 or CRHR1 in dHC or BLA. No significant differences were observed in any one of these proteins in females. We concluded that MS affects the response to unexpected reduction in reward, more particularly in males. Alterations in the amount of NMDA receptor subunits GluN2A and GluN2B in dHC suggest a change in the ratio of these subunits, possibly making stronger the aversive memory related to frustration. In agreement with the resilience profile of the separated females, no alterations were observed in the immunocontent of the proteins analyzed in dHC or BLA of these animals.

MTU07-08 | Neuroplasticity in geriatric brain after hearing use: An electrophysiological measure

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The hearing aid (HA) use reorganizes auditory system and can improve speech understanding over the period of time suggesting existence of neural plasticity in aging auditory pathways. This study aims to examine electrophysiological changes in geriatric auditory brainstem after HA usage. 16 geriatrics with HA usage were examined with click-evoked auditory brainstem response (ABR) at threshold level and 20dBSL. The ears aided with hearing aid were reexamined with ABR after 3 months. The wave V mean amplitude at threshold level and at 20dBSL was larger approximately 70µV and 100µV, respectively, in the ears. The findings suggest HA use can encourage physiological changes at auditory brainstem level and may enhance the acoustic processing in brain which in turn may improve the speech understanding geriatric population

MTU07-09 | Changes in biochemical and memory profile of hippocampus of Sprague-Dawley rat pups exposed intrauterine to kolanut extracts

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Kolanut, commonly consumed by pregnant women to suppress symptoms of morning sickness. Effects of kolanut on biochemistry of hippocampus and dependent memory was investigated in rat pups. Kolanut extract (FHI 109605) at 400mg/kg body weight of dams was infused into gelatine cubes, fed to pregnant dams from first day of pregnancy till parturition. Behavioral function tests conducted includes surface righting (SR), cliff avoidance (CA) (post-natal day (PND) 4, 5, 6, and 7), open field, novel object recognition and location, and radial-arm maze (PND 21 and 56). Also, levels of brain derived neurotrophic factor (BDNF), acetylcholine (ACh), and malondialdehyde (MDA) of matched hippocampal tissues were done in pups. Kolanut-treated pups had significant reduction in behavioral indices compared to control. Latency of postural imbalance was significantly higher on PND 5 in treated, also higher in risk avoidance memory but not significant as compared to control. Frequency of pivoting (PND 7), rearing (PND 56) was significantly higher in control than treated pups, but frequency of urine and faecal bolus were significantly lower in control than in treated pups at PND 21 and 56. Discrimination ratio of control in NOR and NOL were significantly higher than treated in both PND 21 and 56. Time taken by the treated pups to complete RAM was significantly higher with more errors than the control pups. There were increased levels of ACh and BDNF in treated pups compared to control pups. A positive correlation was found between MDA and SR ($r = 0.7207; p = 0.0437$), grooming ($r = 0.7707; p = 0.0252$) and faecal bolus ($r = 0.7606; p = 0.0284$) as well with BDNF level in treated with grooming ($r = 0.7570; p = 0.0297$). But a negative correlation between ACh and rearing ($r = -0.8261; p = 0.0115$), faecal bolus ($r = -0.8066; p = 0.0156$) and a positive correlation with NOL ($r = 0.8358; p = 0.0098$) were observed. Kolanut affected both biochemical and hippocampal memory profiles.

MTU07-11 | Adenosine A2A receptors control the extinction of fear memories

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Psychological trauma-related disorders are characterized by pathological fear responses and impaired fear extinction. Adenosine A_{2A}



receptors ($A_{2A}R$) control the acquisition and recall of fear memories, through modulation of information processing in the amygdala and ventral hippocampus (VH) (Simões et al., 2016, *Neuropsychopharmacol* 41:2862; Wei et al., 2014, *Biol Psychiatry* 75:855). However, it is not known if $A_{2A}R$ also control fear extinction, which was now tested. Adult male mice were contextual fear conditioned (FC) and some were then re-exposed repeatedly without paired shocks (extinction-EXT); their freezing behavior was evaluated and, after sacrifice, field excitatory postsynaptic potentials (fEPSP) were recorded in Schaffer fibers-CA1 synapses of VH slices with long-term potentiation (LTP) being triggered by high-frequency stimulation (HFS; 100Hz, 1s). The role of $A_{2A}R$ was investigated by injecting a selective antagonist, SCH58261 (0.1 mg/kg, intraperitoneal), 1 hour before each extinction trial. To test the specific contribution of $A_{2A}R$ in the VH, guide cannulas were implanted and SCH58261 (50 nM) was bilaterally injected in the CA1 region of the VH, 1 hour before each extinction trial. FC caused a stable increase of LTP ($87.0 \pm 11.3\%$, $n = 9$) in the VH compared to naïve ($49.2 \pm 9.4\%$, $n = 6$) and EXT animals ($56.6 \pm 12.0\%$, $n = 6$). *Ex vivo* blockade of $A_{2A}R$ prevented the fear-induced exacerbation of LTP ($49.2 \pm 9.4\%$, $n = 9$) in slices from FC mice. Global $A_{2A}R$ blockade accelerated the extinction of fear 24h after the first injection of SCH58261 (saline: $30.4 \pm 4.6\%$ of freezing vs. SCH: $17.1 \pm 2.5\%$, $n = 10$) as did the local blockade of $A_{2A}R$ in the VH ($34.4 \pm 5.8\%$, $n = 5$ vs. SCH: $14.6 \pm 3.9\%$, $n = 6$). These results evidence a role for $A_{2A}R$ on fear extinction, particularly in the VH, reinforcing the interest in targeting $A_{2A}R$ to manage fear-related disorders.

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MTU07-12 | Extracellular vesicles from palmitate-exposed microglia trigger brain dysfunction in mice

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Obesity is associated to brain dysfunction, and saturated fat has been suggested to trigger neuroinflammation and contribute to neurodegeneration. However, proposed mechanisms of microgliosis and neuroinflammation in obesity are controversial, and might not involve the typical overexpression of cytokines. We propose that extracellular vesicles (EVs) from fat-exposed microglia impact the hippocampus leading to memory impairment, and hypothalamus resulting in systemic dysregulation of metabolism. Thus, we aimed at characterizing the cargo of microglia-derived EVs, and at evaluating the effects of their intracerebroventricular injection in mice. BV-2 microglia cells were treated with either palmitate (0.2 mmol/L) or vehicle (0.25% BSA) for 24 hours. Palmitate increased cell proliferation but not expression of pro-inflammatory cytokines. Respirometry and ¹³C metabolic flux

analysis showed that BV-2 cells exposed to palmitate dampens the tricarboxylic acid cycle rate, ATP production, maximal respiration and mitochondrial spare capacity, while exacerbates glycolysis. Further, EVs were isolated and analyzed in the Nanosight and mass spectrometry. Palmitate-exposed microglia showed unaltered number of released EVs, but tended to produce larger rather than small-sized EVs (exosomes). Major EV proteome changes were observed upon palmitate overload, including proteins involved in energy metabolism. Finally, microglia-derived EVs were injected into the lateral ventricle of Swiss mice. After 7 days, novel object recognition (NOR) and location (NOL), and sucrose splash tests were performed to evaluate memory and mood, respectively. Fasting glucose and insulin, and glucose tolerance were determined. Mice injected with EVs derived from palmitate-exposed microglia developed glucose intolerance suggesting alterations in central regulation of metabolism, showed lower capacity to recognize the novelty in NOR and NOL tests, and increased latency to grooming in the splash test. We conclude that activated microglia can mediate deleterious effects of saturated fat via released EVs rather than pro-inflammatory cytokines.

MTU07-13 | Cannabis extract alone and in combination with cannabidiol reduces relapse to methamphetamine and locomotor sensitization

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Methamphetamine (METH) use disorder is associated with severe health problems, with poor available treatments. Cannabidiol (CBD) reduces relapse to METH seeking behaviour and METH-induced sensitization. Here we tested the ability of Goo (cannabis extract), and its combination with CBD, to reduce METH/sucrose relapse and METH induced sensitization, and verified the neural activation related to these effects. In Experiment (Exp.) 1, twelve adult male Sprague-Dawley (SD) rats underwent modified behavioural sensitization where they received 7 daily METH injections (5 mg/kg; i.p.). After withdrawal, locomotor changes were examined over 4 challenge days where Vehicle (VEH), CBD (80mg/kg), Goo (43.5 mg/kg), and CBD+Goo were given 30 mins before METH challenge (1mg/kg i.p.), then locomotor activity was measured for 60 mins. In Exp. 2, twenty adult male SD rats were trained to intravenously self-administer METH via lever press during 2 hr sessions on a fixed ratio 1 schedule of reinforcement, then progressed to extinction. Relapse-reducing effects of the treatments were tested during METH-primed reinstatement sessions and administered as per Exp. 1. In Exp. 3, sixteen male SD rats underwent the same procedures as per Exp. 2, but they lever pressed for oral sucrose instead, in 30 min sessions. Cannabinoids were tested as mentioned above. In Exp. 4, c-Fos, using DAB immunohistochemistry, was visualised in the brain tissues from Exp.2. CBD and CBD+Goo treatment

decreased METH-induced locomotor sensitization. All the treatments reduced METH relapse, but the combined treatment was more effective than CBD alone. Treatments had no effect on sucrose relapse. The results from Exp.4 are still under analysis and will be discussed at the meeting. This is the first study to analyse the effect of a cannabis extract alone and supplemented with CBD, suggesting that combining cannabis extract constituents offers treatment potential for METH use disorder better than those provided by CBD alone.

MTU07-14 | Alteration on effort decision making in a model of prenatal stress: NAC and ACC functional changes

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Individuals select actions based on cost-benefit to allocate resources into goal-directed actions. Different brain regions coordinate this complex decision, including the nucleus accumbens (NAc) and anterior cingulate cortex (ACC). In utero exposure to glucocorticoids (iuGC) triggers prominent motivation deficits in adulthood, but the impact of this exposure in the ACC-NAc circuit is unknown.

In this work, we tested adult iuGC-exposed animals on a classical motivation task and on an effort-based decision-making task. We also evaluated basal and evoked neuronal activity of the ACC and NAc.

We show that iuGC exposure causes decreased motivation for food in a progressive ratio task and impaired effort-based decision-making in adulthood. These behavioral deficits were associated with reduced neuronal activation of the NAc and ACC, as evaluated through the number of c-fos+ neurons after task performance. Interestingly, iuGC treatment led to increased NAc and ACC basal neuronal activity. Optogenetic activation of ACC terminals in the NAc triggered a different response in iuGC animals in comparison to control group.

In sum, these data suggest that iuGC animals present motivational and effort-based decision-making deficits that occur in parallel with ACC-NAc dysfunction.

MTU07-15 | Physical Exercise Restores the Neuroepigenetic Aberrations in the Hippocampus of Diabetic Brain

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Epigenetic changes are inaudible signatures of several pathological processes in the brain. This study shows the effect of

treadmill-mediated physical exercise on elevated levels of DNA methylation, a major epigenetic modification, in the hippocampal neurons and its direct link on restoring the cellular chaperones and synaptic proteins. Chronic high-fat diet and streptozotocin-induced diabetic C57BL/6J mice showed significantly hypermethylated DNA within the hippocampal neurons, subsequently leading to cognitive dysfunction. Three months of continuous treadmill exercise restored back the global DNA hypermethylation and DNMTs (DNA methyl transferase), enzyme responsible for methylating the DNA, within the hippocampus. Our prior studies suggested DNMT dependent-decrease in the chaperones and synaptic vesicles, which were restored back to normal levels in the exercise-group. Further, the mature BDNF levels were also slightly increased in the exercise group, suggesting increased neurogenesis in the hippocampus. Neuroinflammation, a very critical and an initial sign of neuronal stress was also studied. We found that there were significant number of native astrocyte forms in the exercise group, rather than the reactive form seen in the diseased diabetic hippocampus. Along with it, the proinflammatory cytokines, TNF- α and IL-6 were decreased and anti-inflammatory cytokine IL-10, were increased in the treadmill exercise group. Taken together, our findings suggest the protective effects of physical exercise on restoring the aberrantly amplified DNA hypermethylation, and also significantly normalizing the methylation-mediated changes on proteostasis and synaptic fidelity within the hippocampus of diabetic rodent brain.

MTU07-16 | Reactivation of leptin receptor alleviate the deleterious consequences of absence of leptin signaling in obese mice brain

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Obesity is a metabolic disorder associated with deleterious effects in the brain, including brain atrophy and cognitive impairment. Leptin regulates energy balance and has neurotrophic effects during development. Here, we used Lep^{Ob} mice and mice null for the leptin receptor (LepR) gene (LepRNull), which are mouse models of obesity, to investigate alterations in brain volume, cognition, and neurogenesis. Using in vivo magnetic resonance imaging, we showed decreases in brain volume in obese mice. We further observed impaired cognition and neurogenesis in the hippocampus and lateral ventricle in Lep^{Ob} and LepRNull mice. Additionally, in order to investigate the importance of leptin in the brain in early



life, we restored leptin signaling at the tenth week of age in mice LepRNull. Reactivation of leptin receptors alleviated alterations in brain volume, cognition, and neurogenesis in LepRNull. Our findings revealed that early defects in leptin signaling cause deleterious effects in brain volume, cognition, and neurogenesis and that reactivation of leptin receptors alleviate the deleterious consequences of absence of leptin signaling in the brain.

MTU07-17 | Integration of behavior and neuronal physiology in a mouse model of Rett Syndrome: a focus on serotonergic system modulation

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Rett syndrome (RTT) is an X-linked neurodevelopmental disorder, caused by mutations in the gene encoding the methyl CpG-binding protein 2, a broad gene transcriptional regulator. Although this disease affects mainly girls—leading to a marked developmental regression, with impairment of motor, social and cognitive functions, breathing abnormalities and dysregulation of the sleep/activity patterns—the mouse studies have disregarded the phenotypic characterization of the females, whose behaviour and neuronal pathology remains poorly explored. Here, we have performed an extensive behavioral characterization of a heterozygous female RTT mouse model (MeCP2^{tm1.1Bird}) followed by a neuropathological and electrophysiological characterization of key brain areas known to regulate the behavioural outputs found to be altered in the model, including motor, cognitive and social systems, with the deficits being detected as soon as 7 weeks of age and progressing over time. Additionally, and taking advantage of a recently developed automated cage system that monitors animals' activity 24/7, we have also uncovered alterations in the circadian pattern of activity of these female mice, as previously described for RTT patients. At the electrophysiological level, we observed an hyperexcitation of the VTA dopaminergic cells in MeCP2 females, a brain region highly involved in social behaviour. Thus, we are currently using fibre photometry techniques in freely behaving animals to pair activity dynamics in the VTA and projecting regions with key social behavioral events, shedding light on the neural correlates that underlie these behavioural impairments. Finally, considering the contribution of serotonergic circuitry to RTT pathology and its involvement in the abovementioned behavioural impairments, we are currently testing the therapeutic efficacy of a novel specific 5HT1A agonist, NLX-101, on the behavioural and neuropathological perturbations previously described. Taken together, this study provides valuable information on the pathogenesis of Rett

Syndrome, while searching for novel therapeutic approaches for RTT patients.

MTU07-18 | A potential therapeutic approach for Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by synaptic failure, amyloid protein deposition, and memory loss. Despite significant efforts in recent decades to develop efficient therapies, the search for treatments remains a challenge and, therefore, the development of new approaches and therapeutic strategies is necessary. Physical activity exercise has been investigated as a non-pharmacological therapy for AD since preclinical studies demonstrate neuroprotective effects related to physical exercise. A current challenge is to identify molecules that are responsible for the beneficial effects of exercise on the central nervous system. Our group showed that FNDC5/irisin, a hormone released by the muscle during physical exercise, mediates the protective actions of exercise in cognition in AD models. Here, we tested an approach to increase irisin using adeno-associated virus vector (AAV). Our results show that increasing FNDC5/irisin through AAV attenuates cognitive impairment in a transgenic mouse model of AD. These findings reinforce the role of FNDC/irisin attenuating memory impairment in AD.

MTU08-02 | Immunomodulatory sphingosine-1-phosphates as plasma biomarkers of Alzheimer's disease and vascular cognitive impairment

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There has been ongoing research impetus to uncover novel blood-based diagnostic and prognostic biomarkers for Alzheimer's disease (AD), vascular dementia (VaD), and related cerebrovascular disease (CEVD)-associated conditions within the spectrum of vascular cognitive impairment (VCI). Sphingosine-1-phosphates (S1Ps) are signaling lipids which act on the S1PR family of cognate G-protein-coupled receptors and have been shown to modulate neuroinflammation, a process known to be involved in both neurodegenerative and cerebrovascular diseases. However, the status of peripheral S1P in AD and VCI is at present unclear. We obtained baseline bloods from

individuals recruited into an ongoing longitudinal cohort study who had normal cognition (N = 80); cognitive impairment, no dementia (N = 160); AD (N = 113); or VaD (N = 31), along with neuroimaging assessments of cerebrovascular diseases. Plasma samples were processed for the measurements of major S1P species: d16:1, d17:1, d18:0, and d18:1, along with pro-inflammatory cytokines interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF). Furthermore, in vitro effects of S1Ps on cytokine expression were also studied in an astrocytoma cell line and in rodent primary astrocytes. Of the S1Ps species measured, only d16:1 S1P was significantly reduced in the plasma of VaD, but not AD, patients, while the d18:1 to d16:1 ratios were increased in all cognitive subgroups (CIND, AD, and VaD). Furthermore, d18:1 to d16:1 ratios correlated with levels of IL-6, IL-8, and TNF. In both primary astrocytes and an astroglial cell line, treatment with d16:1 or d18:1 S1P resulted in the upregulation of mRNA transcripts of pro-inflammatory cytokines, with d18:1 showing a stronger effect than d16:1. Interestingly, co-treatment assays showed that the addition of d16:1 reduced the extent of d18:1-mediated gene expression, indicating that d16:1 may function to "fine-tune" the proinflammatory effects of d18:1.

MTU08-03 | Predictive markers of multiple sclerosis progression measurable in vivo using the MRSI in the basal ganglia

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Multiple sclerosis (MS) is proposed to be a primary degenerative disease linked with local inflammation and the release of antigenic cell components such as myelin oligodendrocyte glycoprotein, myelin basic protein, and proteolipid protein, leading to the multifunctional failures within the central nervous system. Despite the widely accepted neurodegenerative hypothesis of MS, the mechanisms of neurodegeneration are not yet sufficiently understood.

This study evaluated metabolic alterations in the basal ganglia region, that is, putamen and caudate nucleus that may be enrolled in the MS progression, physical disability, or cognitive impairment due to the ability to release neurotransmitters and peptide neuromodulators. Thirteen adult MS patients (35 ± 9 years of age, 7 men/ 6 women, 2 ± 1 Expanded Disability Status Scale) and thirteen age- and gender-matched healthy controls were recruited for this study. Based on the

neurotransmitter-edited proton magnetic resonance spectroscopy imaging (MRSI) at 3T MR scanner (Siemens Magnetom PrismaFit) were evaluated in vivo metabolites like tNAA (N-acetyl-aspartate/aspartyl-glutamate), mIns (myoinositol), tCr and tCho (choline & creatine-containing compounds), GABA (gamma-aminobutyric acid) and Glx (glutamate/glutamine).

In MS patients, it was apparent that significantly decreasing GABA with increasing Glx in the putamen; however, in the caudate nucleus was more pronounced the elevated tCho and mIns. Furthermore, metabolic alterations in the putamen of MS patients strongly correlated with neurological disability and conducted high predictive power in multivariate analysis. This study was supported by VEGA 1/0092/22 and APVV-19-0222, co-funded by EU sources.

MTU09-01 | Transient synaptic density unbalancing in the motor cortex after peripheral nerve injury: involvement of microglial

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Peripheral nerve injury can lead to a remodeling of body representation areas in different parts of the brain such as in the primary motor cortex (M1). This plasticity is a consequence of several cellular events such as the emergence and elimination of synapses in these areas. Beside neurons, microglial cells are the class of cells intimately involved in synapse plasticity, especially in the synaptic pruning. In this work, we investigate the transient changes in synaptic density in M1 after different types of peripheral nerve injury and how microglial cells behave in each scenario. Male C57/B6 mice were divided in control group (no injury), crush group (sciatic crush) and transection group (sciatic transection). For investigation of the role of microglial cells, animals from the same experimental groups received daily dose of minocycline or PBS. Both types of sciatic lesion led to a significant decrease of synaptophysin and PSD95 compared to control animals 4 days after lesion (DAL), which was stabilized 7 DAL and sustained until 14 DAL. The synaptic density alterations correlated with changes in microglial cell density and its morphology, with significantly more ramified microglial cells at injured animals M1 on day 1 and 4 DAL. Although the decreased synaptic density overlapped with increased microglial cells number, there was no increased number of lysosomes in these cells on day 4 after lesion. Surprisingly, the daily administration of minocycline increased microglial cell number and PSD95 density 14 DAL. Taken together, we described transient changes in synaptic density in M1 related to peripheral injury and the participation of microglia in this plasticity process although not directly related to synaptic engulfment.

**MTU09-02 | Microglia depletion and repopulation alleviates vascular damage and neuronal loss in the diabetic retina**

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Diabetic retinopathy (DR), is the leading cause of blindness amongst working age adults. Microglia, the resident phagocytes of the retina, are believed to influence the development of DR, but their exact contributions to vascular integrity and neuronal loss are unknown. Previous studies have shown that diabetic retinas with aberrantly activated microglia in the absence of CX3CR1 or CX3CL1, show (1) robust microglial activation and elevated release of pro-inflammatory mediators-IL-1 β and TNF- α and decreased production of anti-inflammatory cytokines IL-10 and IL-13, (2) cellular clustering around blood vessels and (3) robust fibrinogen deposition and vascular damage. Therefore we hypothesize that acute depletion and *repopulation* of CX3CR1^{CreER}:R26^{idTR} microglia during the course of disease when microglia receive the early-stage environmental cues of retinal inflammation due to hyperglycemia will induce neuroprotective cues to alleviate neuronal and axonal loss and prevent fibrinogen deposition into the diabetic retina. Utilizing a genetic model to express diphtheria toxin receptor (DTR) under the tamoxifen (TAM) inducible CX3CR1-promoter, microglia become susceptible to the effects of diphtheria toxin (DTx). Microglia were depleted in diabetic and control mice for two weeks from six to eight weeks of diabetes. *Following a two week microglia repopulation*, vascular and neuronal damage were assessed at ten weeks of diabetes. Our findings revealed that transient depletion of microglia induced neuroprotective cues to prevent NeuN⁺RBPMs⁺ neuronal loss and induced thicker TUJ1⁺ axons. Transient depletion of microglia also decreased fibrinogen extravasation into the diabetic retina. Repopulating microglia displayed a resting phenotype mirroring those of non-diabetic TAM controls in contrast to diabetic mice displaying microglia with amoeboid morphology and decreased the percentage of Ly6C⁺ infiltrating monocytes into brain and spinal cord tissues. Microglia depletion and repopulation during the course of disease when microglia receive early-stage activation cues, is neuroprotective by inducing the proliferation of a homeostatic microglia cell population that prevents neuronal loss and supports vascular repair.

MTU09-03 | The role of astrocyte Gq signalling in neuroinflammation and cognition

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Astrocytes sense and modify neuronal activity, contribute to synaptic plasticity and cognitive functions, and are essential players in neuroinflammation. Acetylcholine (ACh) regulates cognitive processes and modulates immune responses, potentially via astrocytic signalling. Indeed, previous experiments have demonstrated that activation of hM3Dq designer receptor exclusively activated by designer drugs (DREADDs) in astrocytes facilitated synaptic plasticity and improved contextual memory in mice. We generated mice expressing hM3Dq specifically in astrocytes to further investigate the role of astrocytic muscarinic signalling in brain function. Treatment of cultured primary astrocytes with CNO increased intracellular calcium only in DREADD expressing astrocytes, demonstrating that CNO activates hM3Dq and initiates Gq signalling. To investigate whether activation of hM3Dq specifically in astrocytes modulates network organization, we completed a brain-wide functional connectome analysis using resting-state fMRI. Intravenous injection of CNO resulted in changes in functional connectivity in mice expressing DREADDs in astrocytes, but not in control mice, suggesting that Gq signalling in astrocytes modulates brain networks. Furthermore, activation of hM3Dq via intraperitoneal injection of CNO in mice, resulted in increased expression of proinflammatory cytokines, TNF α , IL1- β , and IL-6, in the cortex. Despite this increase, CNO did not affect motor activity, anxiety-like behavior, or working memory determined with alternations in the Y maze. In contrast, local activation of astrocyte DREADDs in the hippocampus facilitated location discrimination performance in mice expressing DREADDs in astrocytes, but not in controls. Our results suggest that Gq signalling in astrocytes can have a profound influence in network activity, activation of cytokine expression, and modulation of specific types of cognition.

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MTU09-05 | Responses of oligodendrocytes to different FGF-family members: Uncoupling structure–function relationship within FGF subfamilies

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Fibroblast growth factors (FGFs), a family of 22 members is divided into 7 sub-families based on their structural similarities. Most FGFs regulate cellular functions via the activation of four FGF-receptors. In addition, numerous heparan sulfate proteoglycans (HSPGs) serve as co-receptors. At least 18 different FGFs are expressed in a temporally and spatially regulated manner in the brain, including regions in which oligodendrocyte (OL) progenitors originate, migrate or differentiate. FGF-2 (basic FGF) is thought to be a prototype FGF and much of the information on the effects of FGF-signaling on OL-lineage cells has evolved from the study of FGF-2. To examine the effects of other FGFs on OL-lineage cells and determine the role of HSPGs in this regulation, we selected FGFs from 5 subfamilies, namely, FGF1 (FGF1,2), FGF4 (FGF4,5,6), FGF8 (FGF8,17,18) and FGF9 (FGF9,16,20), all of which are (a) expressed in embryonic, postnatal &/or adult brain and (b) signal via the activation of FGF-Receptors. Specifically, using a well-defined culture system of isolated cells from neonatal rat brain, we investigated the effects of different FGFs on OL progenitor proliferation and terminal differentiation as well as on cell cycle re-entry of OLs, down-regulation of their myelin proteins, process growth and MAPK activation. Several conclusions were drawn from this study. (a) While all of these responses were elicited strongly by FGF-2, other FGFs showed only a subset of them; (b) some, but not all responses were restored in the presence of exogenously added heparin (a pan-FGF co-receptor), emphasizing the selectivity and importance of HSPGs as FGF co-receptors for the OL lineage cells; (c) FGFs within structurally similar subfamilies did not always show similarities in their effects on OL-lineage cells, suggesting that the sequence similarities among FGF-subfamily members is an imperfect predictor of their function in the OL-lineage cells. NIH Grant R37NS038878 to RB.

MTU09-06 | Astrocyte senescence is associated with lamin-B1 loss, nuclear deformations, and synaptogenic capacity impairments

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Brain aging is associated with an accumulation of senescent cells, synapse loss, and cellular dysfunctions. Astrocytes undergo senescence in vitro and in neurodegenerative diseases, although astrocytic senescence-associated biomarkers are not well known. Further, there is a lack of information about senescent glial cells during normal brain aging and the functional impact of these cells on age-related cognitive decline. Here, we investigated the phenotype and function of senescent astrocytes in physiological aging. We used hippocampal tissue from young (2–3 months) and aged (18–24 months) C57BL/6 mice and post-mortem human hippocampal tissue from middle-aged and elderly donors. We also established an in vitro model to study astrocyte senescence, in which cultures were maintained for 30–35 days in vitro (DIV) (senescent astrocytes) or 7–10 DIV (control astrocytes). We observed a significant loss of lamin-B1, a major nuclear lamina component, as a hallmark of senescent astrocytes in vitro and in the hippocampus of aged mice and old humans. The lamin-B1 reduction was associated with nuclear deformations, such as nuclear invagination and loss of nuclear circularity. Senescent cultured astrocytes also showed a reactive phenotype based on LCN2 (lipocalin 2), C3, and Serping3n immunostaining. The conditioned medium from these cells exhibited reduced capacity to support neurite outgrowth and synaptogenesis in neuronal cultures. Our findings indicate morphological and functional changes in astrocytes with age, suggesting their involvement in age-related cellular dysfunctions and cognitive decline.

The protocols of this study were approved by the Research Ethics Committees of the UFRJ, UMCU, BABSG. Support: CNPq, CAPES, FAPERJ, Ministério da Saúde, ZonMW Memorabel.

MTU09-07 | Ginger-garlic supplementation attenuates hippocampal astrocytic response following lead (Pb) exposure in adult female Wistar rats

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The mechanisms by which lead affects astrocytes are unknown. Probably the astroglial changes induced by lead intoxication produce microenvironmental modifications that may disturb the neuronal function. This study showed the effect of garlic-ginger



supplementation in attenuating astrocytic response in thirty (30) lead-induced wistar rats. The animals were grouped as follows: normal control group (rat feed and water *ad libitum*), group administered low-dose Pb acetate (25 mg/kg), untreated high-dose Pb group (100mg/kg), treated group of mixed ginger/garlic juice (200mg/kg), treated group of low dose Pb plus ginger and garlic extract (25 mg/Kg lead acetate + 200mg/kg ginger/garlic juice) and treated group of high dose Pb plus ginger and garlic extract (100mg/Kg lead acetate + 200mg/kg ginger/garlic juice). The groups of intoxicated animals and the controls were sacrificed by perfusion-fixation after days of exposure. Staining of GFAP-positive cells demonstrated an astroglial transformation from the quiescent to the reactive state, characterized by an increase in GFAP. In the control rats, no change in GFAP immune staining was observed. Thus the intensity of the astroglial response showed an increment in GFAP immune reactivity. Quantification of these changes was made by computerized image analysis, confirming that the sectional areas of the astroglia in lead-exposed animals were larger than those in controls. These results were also consistent with ultrastructural alterations. Ginger and garlic supplementation was able to ameliorate these observations.

MTU09-08 | Sphingolipid-dependent membrane organization and signaling orchestrating myelin repair

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Recombinant human IgM22 (rHlgM22) binds to myelin and oligodendrocytes (OLs) and promotes remyelination in mouse models of multiple sclerosis. However, target antigen and signaling mechanisms through which rHlgM22 exerts its function are still unclear.

In vitro analysis revealed that rHlgM22 binds to sulfatide, phosphatidylinositol and phosphatidylserine. Moreover, the composition of the lipid microenvironment of its antigen can modulate the affinity of the antibody, suggesting reorganization of lipid membrane might be relevant in its biological activity.

In rat mixed glial cells (MGC), rHlgM22 induces an increase in PDGF α R levels and a dose-dependent proliferative response in all cells in the culture, with the most significant response associated with astrocytes. Moreover, rHlgM22 increases production and release of sphingosine 1-phosphate (S1P) in MGC while total levels of ceramide remain unchanged. Furthermore, release of S1P is strongly reduced by a selective inhibitor of PDGF α R. Remarkably, rHlgM22 treatment does not induce changes in the production and/or release of S1P in astrocytes, but it increases its release in BV2 cells, suggesting that rHlgM22 indirectly influences the proliferation of astrocytes in MGCs, by affecting ceramide/S1P balance.

Analysis of the effect of rHlgM22 on glycosphingolipid metabolism in MGC and astrocytes revealed no significant effects on the lipid pattern, while in OPCs and OLs the levels of gangliosides GM3 and GD3, known for their ability to interact with and modulate the activity of growth factor receptors, are increased.

Considering all this, we propose rHlgM22 protective effects might be mediated by alterations of lipid-dependent membrane organization and/or signalling in different glial cells and that a complex cross talk between these cells is underlying the repair effect elicited by this antibody.

MTU10-01 | The antidepressant-like action of ethanolic extract of garlic is mediated by its antioxidant function

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Depressive disorders are among most common psychiatric diseases and second most common form of psychiatric illness globally. Commonly available chemical drugs used for treatment of nervous system disorders exert undesirable effects. Therefore, there is a growing need towards exploring novel antidepressants of herbal origin. This article presents the antidepressant potential and neuroprotective property of ethanolic extract of garlic using the reserpine-induced depressed rats. Rats were divided into five different groups: (1) control, (2) reserpine, (3) fluoxetine, (4) reserpine with garlic extract and (5) reserpine with fluoxetine. The reserpine-induced depressed animal model was validated by forced swimming test (FST) and open field test (OFT). This depressed experimental animal model is also utilised to assess the activity of garlic extract against depression. The results indicated significant rise in immobility duration of depressed rats in both of aforesaid tests (FST and OFT) thereby confirming the development of depression in rats. The depressed rats displayed sharp decrease in the levels of GSH and significant rise in extent of lipid peroxidation. The reserpine treatment resulted in significant loss in activities of CAT, GST and SOD. The serotonin content, activities of AChE and LDH and levels of TNF- α and IL-6 (inflammatory markers) were sharply perturbed in the reserpine treated rat brain. The garlic extract helped improve the altered levels of these parameters. GC-MS analysis of garlic extract yielded 35 compounds, which might be associated to antioxidative/antidepressant function and brain protection. Using computational analysis, these compounds were screened by docking on to serotonin transporter / leucine receptor. This resulted in identification of top ten compounds with low binding energy. These compounds were subjected to physicochemical and bioactivity studies for their drug like activity. Dihydrocitrinellol, was found to be most active as selective serotonin reuptake inhibitor (SSRI), which can act as a potential antidepressant.

MTU10-02 | Amelioration of cognitive deficit by propranolol in a scopolamine-induced Alzheimer's disease-like condition in a rat model

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MTU10-03 | Methyl jasmonate inhibits MPP+-induced neuroinflammation and oxidation in BV2 microglia cells Via NF-kB(p65)/Nrf2 pathway

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Parkinson's disease (PD) is the most common neurodegenerative movement disorder characterized by neuroinflammation, oxidation and apoptosis. Compounds and mechanisms that regulate these hallmarks are great targets for PD therapy. Methyl Jasmonate (MJ) has been conferred with anti-inflammatory, anti-oxidative, and anti-apoptotic properties, but its role in BV-2 models of PD is unknown. This study investigated the neuroprotective role of methyl jasmonate on BV-2 microglia cells stimulated with 1-methyl-4-phenylpyridinium (MPP+) neurotoxin. The objectives of the study were to: (i) investigate the neuroprotective effects of MJ on (MPP+)-induced neurotoxicity in BV-2 microglia cells; and (ii) determine the mechanisms by which MJ exerts these neuroprotective effects. BV-2 microglia cells were pre-treated with MJ for 30 minutes and stimulated with MPP+ for 24 hours. The treatment groups are: (A) control (dimethyl sulfoxide); (B) MPP+ (100µM); (C) MJ (5µM) plus MPP+ (100µM); (D) MJ (10µM) plus MPP+ (100µM); (E) MJ (20µM) plus MPP+ (100µM); and (F) MJ (40µM) plus MPP+ (100µM). Cell viability, pro-inflammatory cytokines, and enzymes levels were assayed. Levels of iNOS, cyclooxygenase-2, DNA binding and Nrf2 were also determined. One-way analysis of variance and post hoc Tukey's test were used for statistical analysis at $p < 0.05$. The findings of the study showed that MJ significantly ($p < 0.05$): (i) inhibited MPP+-induced neurotoxicity and promoted the viability of BV2 microglia cells; (ii) reduced MPP+-induced production of cytokines; (iii) suppressed MPP+-induced expression of pro-inflammatory enzymes when compared with MPP+ only group; (iv) inhibited MPP+-induced neuroinflammation via NF-kB inactivation and suppressed MPP+-induced nuclear accumulation of NF-kB when

compared with MPP+ only group; (v) suppressed MPP+-induced expression of Nrf2 when compared with MPP+ only group. This study concluded that methyl jasmonate protected BV-2 microglia cells against (MPP+)-induced neuroinflammation and oxidation via NF-kB(p65)/Nrf2 pathway.

MTU10-04 | In silico study of Date Palm phytochemicals against Cdk5/p25 Activation in the treatment of Alzheimer's disease

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Alzheimer's disease is on the rise and has caused alarm around the world. Alzheimer's disease will most certainly continue for decades due to the incapacity of current medicines to slow down degradation. It is critical to develop medications that can slow down the progression of degeneration. The date palm includes non-proteinogenic amino acids and associated chemicals whose significance, along with its human health advantages, remains to be explored. They have been linked to lowering or preventing the risk of a variety of chronic diseases due to their high polyphenol content. The results of virtual screening of cyclin-dependent kinase-5 as a target for novel inhibitors from Date palm phytochemicals utilizing the molecular docking method are presented here. To determine the best match, Date compounds retrieved from the Pubchem database were docked with cyclin-dependent kinase-5 protein (PDB ID- 1UNG) using PyRx Virtual screening tool to locate compounds with high binding affinity. PreADMET and Molinspiration webserver were further used to screen the top-ranked compounds expected to interact with 1UNG by predicting their toxicity, drug-likeness predictions, and biological activity. The top hit compounds were then screened using PreADMET and Molinspiration webserver to predict their toxicity, drug-likeness, and biological activities based on parameters such as blood-brain barrier, human intestinal absorption (HIA), Caco-2 cell permeability, hERG gene inhibition, toxicity, Pgp substrate/inhibitor identification, Cyp450 metabolism, and carcinogenicity. Verbanol, cyclododecane and Isoamyl benzoate were discovered to be intriguing candidates that can be further investigated in the treatment of Alzheimer's disease.

MTU10-05 | MTBR-243 in the early diagnoses of AD and the role of microglia/carbenoxolone as localized therapeutic interventions in AD rats

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Background: During Alzheimer's disease (AD) progression cascade, insoluble tau protein spreads throughout the brain and other regions including cerebrospinal fluid (CSF) and spinal cord. Microtubule-binding region MTBR-243 is an insoluble tau protein suggested to be found in the CSF and spinal cord during the early AD stage. Glial cells, such as microglia have been proved to be involved in brain functionality by maintaining an M1-M2 balanced homeostatic brain environment. It is suggested that the administration of microglia may play a vital role in alleviating AD hallmarks depositions and related cognitive dysfunctions. Carboxoxolone (Cbx) is a known gap junctional blocker that cannot penetrate the blood-brain barriers (BBB). Its localized administration could be a favorable intervention following AD acting as a potent antioxidant and anti-inflammatory. Aim: To reveal the degree of success in using MTBR tau in the early diagnosis of AD. To find out whether the localized administration of healthy isolated microglia and Cbx can be used for plaques engulfing and regeneration of damaged brain tissue in AD.

Methods: Male albino rats were divided into four groups: control, Lipopolysaccharide (LPS)-induced AD rats, microglia-treated AD rats, and Cbx-treated AD rats. Behavior tests, brain tissue, and spinal cord isolation along with the collection of different biological fluids and the estimation of immunological-inflammatory related were conducted among all groups.

Results: MTBR-243 tau was identified in the CSF and spinal cord of AD-induced rats. The localized administration of microglia and Cbx revealed promising AD therapeutic interventions with more relevant effects following microglia administration. Conclusion: The concept of using fluid biomarkers in reflecting the degree of brain damage may be successfully established by examining the ability of CSF MTBR tau protein in predicting the early AD stage. These finding can be considered potential as it opens up the opportunity for new AD therapeutic alleviating protocols.

MTU10-06 | Neuroprotective consequence of embelin in experimental model of multiple sclerosis: behavioral and biochemical aspects

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Multiple sclerosis (MS) is an autoimmune progressive neurodegenerative disease characterized by behavioral and biochemical alterations following demyelination of the central nervous system. Embelin, a para-benzoquinone has been shown the neuroprotective and anti-inflammatory potential in several experimental model of neurodegeneration. Herein, embelin (1.25, 2.5 and 5 mg/i. p) is assessed for its neuroprotective potential in ethidium bromide (EB)-induced demyelination in Wistar rats. EB (0.1%/10 μ l/ICP) from day 1st to 7th to induce MS-like symptoms. Behavioral activities were assessed through locomotor activity, Morris-water maze test, rotarod test, and narrow beam walking

test on day 1st, 7th, 14th and 21st. and biochemical alteration were observed in terms of Oxidative and nitrosative markers. Embelin treatment from day 8th to 21st improves behavioral impairments and biochemical alterations compared with EB treated rats. Therefore, embelin appears to improve MS-related motor neuron dysfunctions.

MTU10-07 | Attenuation of amyloid β -induced cognitive decline by averting mitochondrial injury in Alzheimer's disease

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Alzheimer's disease (AD) has been well associated with the progression of synaptic failure, neuronal cell death, and mitochondrial dysfunctions. The proposed study is aiming at repurposing a selected drug Atorvastatin (ATOR) based on the docking score and in vitro results. ATOR is an FDA-approved and cost-effective drug and works by reducing the amount of cholesterol made by the liver. We have investigated the neuroprotective potency of ATOR against Amyloid beta ($A\beta$) dysfunction. In vitro $A\beta$ model was established and also confirmed by FTIR, HPLC, and immunoblotting. After in vitro, $A\beta$ model validation with N2a cell line, cell cytotoxicity, and cell viability assay was done through MTT, LDH, and Live/ dead staining against all the selected conc. of ATOR (0.1- 10 μ M) at 24 and 48 hrs. Our data also showed that ATOR significantly reduced the accumulation of intracellular ROS in a dose-dependent manner against DCFH2-DA (2',7'-dichlorofluorescein diacetate) probes when compared to the $A\beta$ group. SOD and LPO activity were significantly reduced in the $A\beta$ group when compared to the control group and also showed significantly increased levels with ATOR groups with different conc. in a time-dependent manner (24 and 48h). ATOR prevented the $A\beta$ -induced high expression of apoptotic factors Bax, cleaved caspase 3 but the expression of Bcl 2 and Caspase 3 were restored, which confirms the anti-apoptotic property after 24 and 48h against $A\beta$.

MTU10-08 | Sustained release triple drug loaded colloidosomes for management of Parkinson's disease

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Parkinson's disease (PD) is well-known as a progressive and degenerative disease of the nervous system. The degeneration of dopaminergic neurons in the substantia nigra, and a reduction in the amount of the neurotransmitter dopamine available in the striatum relate symptoms of this disease. It is hypothesized that a drug delivery system that provides controlled and sustained release of PD drugs would afford better management of PD. Hollow microcapsules composed of PMMA (polymethyl methacrylate) and

poly (caprolactone) (PCL) are prepared through a modified double-emulsion technique. They are loaded with three PD drugs, that is, levodopa (LD), carbidopa (CD), and entacapone (ENT), at a ratio of 4:1:8.

Microcapsules were prepared through a double emulsion (W1/O/W2) solvent evaporation method with modifications to produce hollow microspheres. Microcapsules were then spray coated along with ENT. The microcapsules were analyzed for size distribution and zeta potential using Zetasizer. Shape and surface morphology were studied using SEM. Transmission electron microscope (TEM) was used as a visualizing aid for particle morphology. The average particle size and polydispersity index were determined by optical microscopy using a calibrated occludometer, drug entrapment, CLSM, Buoyancy tests and in vitro drug release was studied.

LD and CD are localized in both the hollow cavity and PMMA/PCL shell, while ENT is localized in the PMMA/PCL shell. Release kinetics of hydrophobic ENT is observed to be relatively slow as compared to the other hydrophilic drugs. It is further hypothesized that encapsulating ENT into PCL as a surface coating onto these microcapsules can aid in accelerating its release. Now, these spray-coated hollow microcapsules exhibit similar release kinetics, according to Higuchi's rate, for all three drugs.

The results suggest that multiple drug encapsulation of LD, CD, and ENT in gastric floating microcapsules could be further developed for in vivo evaluation for the management of PD.

MTU10-09 | Carrier-mediated delivery system bearing dopamine for effective management of parkinsonism

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

the bilayer. In vivo study reveals that the animals receiving uncoupled liposomes showed partial reduction and animals that received coupled liposomes showed almost complete reduction in catatonia. Fluorescence study clearly indicates the uptake of 6-CF in blood vessels and accumulated in brain. This could be due to enhanced uptake of Lysine coupled liposomes through amino acid transporters present at BBB surface

MTU10-10 | Intranasal delivery of insulin for the restoration of memory signaling in Alzheimer disease

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Alzheimer's disease (AD), a form of dementia, is progressive, degenerative brain disease characterized by marked atrophy of cerebral cortex and loss of cortical and sub-cortical neurons. Weakening of insulin receptor signaling is involved in ageing-related brain degeneration such as AD. Objective of this study is to develop delivery-system to overcome BBB by employing novel, non-invasive approach via nasal route. The olfactory neural pathway provides both intra-neuronal and extraneuronal pathway into brain. In present study delivery of antibody appended Insulin encapsulated carrier, PEGylated nanoparticle coated with chitosan to facilitate nasal absorption for efficient transfer to brain.

PEGylated-PLGA nanoparticles were prepared by modified Double Emulsification method and coated with chitosan by freeze drying. Characterization was done by FTIR, NMR and in vitro for shape, size, and drug-entrapment. In vivo study comprised biodistribution in various organs and fluorescence microscopy, estimation of anti-A β antibody, PET-imaging of brain, hemolytic toxicity studies, histopathology of nasal mucosa and brain with periodic blood glucose level monitoring.

Nanoparticles were spherical in shape and smooth. Degree of hemolysis showed PEGylated nanoparticles (PEG-NPs) and chitosan coated nanoparticles (cPEG-NPs) were less toxic. Blood glucose monitoring indicates reduction in blood glucose level in cPEG-NPs. Biodistribution assessment suggests nanoparticles showed maximum availability at olfactory bulb entrance. Chitosan coating increased CSF availability of drug even at initial period of administration. Uptake study shows intense fluorescence in brain revealing higher uptake of nanoparticles. These studies highlight possible biological significance of cPEG-NPs for delivery to brain.

Results from various studies suggest nanoparticles are effective delivery system for targeted delivery of insulin in brain for an extended period. Chitosan coating elicits associated benefits in addition to prolonging uptake via intranasal route. This project may provide sound platform towards employment of this modified nanoparticulate carrier for brain delivery of proteins and peptides towards intranasal delivery of insulin for restoration of memory signaling in Alzheimer patients.



MTU10-11 | The molecular tweezer CLR01 reduces motor impairments and neuropathology in spinocerebellar ataxia type 3 in vivo models

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During the process of protein aggregation, folding intermediates and misfolded states of proteins likely accumulate, and the intermolecular contacts between non-native states result in the formation of various aggregate species, including oligomers, amorphous aggregates, and amyloid fibrils. Amyloid fibrils are often thermodynamically more stable than the native state, favoring their formation, ultimately leading to cell dysfunction and death. Therefore, inhibiting the formation and toxicity of these aggregated species is a very exciting therapeutic route to follow. Molecular tweezers are inhibitors of abnormal protein self-assembly and toxicity. Among these, CLR01 has been found to inhibit the formation of toxic oligomers and aggregates of multiple disease-associated proteins by binding to positively charged lysine residues, temporarily reversing their charge from positive to negative, and disrupting hydrophobic interactions. Here, we aimed at testing the impact of CLR01 in in vivo models of spinocerebellar ataxia type 3, a neurodegenerative disease caused by expansion of a polyglutamine tract in the ataxin-3 protein (ATXN3), that makes it prone to aggregate and toxic for neurons involved in movement regulation. Pre-symptomatic chronic treatment of *C. elegans* expressing the human mutant ATXN3 protein with CLR01 ameliorated their neuronal dysfunction. Importantly, when CLR01 was administered to the animals post-symptomatically, it was still able to suppress mutant ATXN3-mediated motor behavior defects, mimicking the most frequent clinical situation of symptom-driven diagnosis and treatment initiation in SCA3 patients. CLR01 chronic and early symptomatic treatment also delayed disease installation and significantly improved motor behavior and rescued motor neuron pathology of a SCA3 transgenic mouse model. Intriguingly, these important beneficial effects observed in the two SCA3 animal models were segregated from its effect on mutant ATXN3 end-stage aggregates, which were unaffected by CLR01 treatment. These results suggested the amyloid inhibitor

CLR01 as a potentially effective therapy for SCA3, acting mostly on early-stage toxic protein species.

MTU10-12 | Levels of activating transcription factor 6 alpha (ATF6α) in Alzheimer's disease

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disease worldwide. More than 40 million people currently suffer from AD, and such prevalence tends to increase considerably in the coming decades due to increased human longevity. Nonetheless, despite intense research efforts, no therapy has yet proven effective in attenuating or reversing the progression of AD. The unfolded protein response (UPR) is an adaptive signaling mechanism that aims to maintain cell viability under protein folding stress. The accumulation of misfolded proteins is associated with several neurodegenerative diseases, such as AD, leading to synaptic loss and neuronal death. UPR activation depends on stress sensors such as ATF6α (activating transcription factor 6 α). Recently, ATF6α factor gained importance as a therapeutical target in some disorders and pharmacological activation of this pathway has already been shown to be protective in heart disease. Nonetheless, the association between the ATF6α pathway and AD is not fully understood yet. Here we investigated whether dysfunctional ATF6α associates with AD. Initial results using *postmortem* tissue from the cerebral cortex suggested that ATF6α protein levels are reduced in AD patients. Using an online database (Aging, dementia and TBI study from the Allen Institute), we found reduced ATF6α mRNA expression, in relation to the Braak scale of tau pathology in the parietal neocortex and hippocampus. Conversely, we found increased ATF6α protein levels in the hippocampus of APP/PS1 transgenic mouse model of amyloid pathology. Together, our initial results suggest that ATF6α may be differentially altered in AD. Further investigation of the ATF6α pathway in AD may offer a novel therapeutic perspective for cognitive decline.

MTU10-13 | Brain cell type-specific insulin resistance exacerbates Alzheimer's disease-like phenotypes in 5xFAD mice

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Brain insulin signaling controls peripheral energy metabolism and plays a key role in the regulation of mood and cognition. Dysregulation of this signaling has been implicated in brain disorders including Alzheimer's disease (AD). Epidemiological studies have suggested that insulin resistance is a major risk factor for AD, little is known, however, of exactly how insulin resistance in the brain contributes to the comorbidity of T2D and AD. Here, we aim to understand the roles of insulin signaling in AD progression, with a particular focus on astrocyte and microglia, two major disease-associated brain cell types that are heavily implicated in AD pathology. To this end, we established several new mouse models, by crossing 5xFAD mice with mice that have selective insulin receptor (IR) deficiency in neurons, astrocytes, or microglia. We hypothesize that insulin resistance in selective brain cell types drives comorbidity of T2D and AD. We performed behavioral and biochemical characterizations at key disease stages that allow for the understanding of the dynamic role of insulin signaling in AD progression. Our results show that 5xFAD mice with astrocytic IR knockout exhibited cognitive impairment and abnormal contextual fear conditioning at 6-month age. This was associated with an elevated expression of AD risk genes and key blood-brain barrier genes in the brain. By mapping A β deposits in the whole brain using a tissue CLARITY approach, we generated a brain atlas that allows for identifying brain areas that show the highest level of vulnerability to A β toxicity. To understand the mechanisms that contribute to these AD-like phenotypes, we infected primary astrocytes with adenovirus to knockout IR in vitro. This resulted in the loss of astrocytic insulin signaling impaired A β uptake and reduced ATP production and glycolytic capacity. In conclusion, we find that insulin signaling in the brain, especially in astrocytes, contributes to AD pathology, highlighting the importance of a deeper understanding of insulin signaling in different brain cell types in brain disorders. This may also lead to the development of new cell-specific therapeutics for patients with T2D and AD.

MTU10-14 | The study of depression and cognitive comorbidities in a mouse model of spinocerebellar ataxia type 3

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Spinocerebellar ataxia type 3 (SCA3) is a neurodegenerative disease characterized by progressive motor deficits that impair normal daily activities and lead to an early death. Clinical and functional studies have linked cerebellar dysfunction not only to motor coordination

impairments but also to cognitive and mood-related disturbances—the so-called cerebellar cognitive affective syndrome (CCAS). However, the prevalence and severity of CCAS in SCA3 is poorly characterized, as these comorbidities are often underdiagnosed. Although SCA3 patients report depressive and anxiety symptoms that are often attributed to the negative prospects of their disease, these symptoms can, alternatively, be inherent to the disease state, as part of the CCAS—a question that remains to be elucidated being difficult to tackle in humans. Therefore, and knowing that rodents have no awareness of their disease condition, we aimed to study: (i) the presence of CCAS-like manifestations in a SCA3 mouse model and (ii) the impact of a chronic unpredictable stress (CUS) protocol on the motor function of SCA3 mice. Longitudinal behavioral evaluations were performed in the CMVMD135 transgenic mouse, in which we have previously detected alterations in the expression of the glucocorticoid receptor in the brain and in corticosterone levels in the blood. Different tests were applied to assess mood, anxiety, and cognition throughout disease progression. Overall, SCA3 mice did not exhibit anhedonia, anxiety, lack of coping behavior or cognitive deficits. Also, no major impact on disease severity or progression was observed following a 6-week CUS protocol. We conclude that a CCAS-like profile is not present in this mouse model, and that it does not show an enhanced sensitivity to chronic stress. Ongoing analysis are being conducted to further explore the HPA-axis-mediated stress response and to measure neurotransmitter levels, at late stages of the disease, in basal conditions and in response to stress.

MTU10-15 | Unveiling the astrocytic contribution for synaptic dysfunction in ALS

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Amyotrophic Lateral sclerosis (ALS) is a neurodegenerative disease, affecting mainly motor function, as well as, in some cases, cognitive function. The relevance of astrocytes in excitotoxicity and neurodegeneration, in ALS, has been highly recognized. Thus, we aimed to study the astrocyte contribution for synaptic activity and plasticity in the hippocampus and motor cortex of SOD1G93A (mSOD1) and wild-type (wt) mice.

Pre-symptomatic (4–6w) and symptomatic (14–18w) mSOD1 mice, as well as age-matched wt mice were used. Astrocytic metabolism was selectively reduced using fluorocitrate (FC), and synaptic plasticity and transmission was assessed by eliciting long-term potentiation (LTP) protocols and recording input/output curves, respectively,



while recording field excitatory postsynaptic potentials in the CA1 area of hippocampal slices and layer II of primary motor cortex slices. In the presence of FC (200 μ M), hippocampal synaptic responses were significantly lower in pre-symptomatic mSOD1 mice, when compared with wt mice. In the symptomatic phase, mSOD1 mice exhibited higher post-tetanic potentiation and LTP magnitudes, when compared with wt mice. However, astrocytic inhibition impaired significantly LTP, as well as synaptic responses, in both wt and mSOD1 mice. Regarding the motor cortex, pre-symptomatic mSOD1 mice showed an impairment in LTP magnitude and basal synaptic transmission. Interestingly, presence of FC (100 μ M) led to an impairment of LTP only in wt mice, to similar levels that of mSOD1 mice, in both stages of disease.

Altogether, we further explored alterations in synaptic plasticity and transmission, as well as the role of astrocytes, in two affected regions of the mSOD1 mice model. These findings suggest that, in the hippocampus, astrocytes are essential for the maintenance of LTP in healthy and ALS conditions. More importantly, in the motor cortex, mSOD1 mice present early alterations in synaptic function and plasticity, and astrocytes seem to be impaired even before the onset of symptoms.

MTU10-16 | Geldanamycin and spironolactone enhance the degradation of C9orf72 ALS/FTD dipeptide repeat proteins

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Intronic GGGGCC (G4C2) hexanucleotide repeat expansion within the human C9orf72 gene represents the most common cause of familial forms of amyotrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD) (C9ALS/FTD). Repeat-associated non-AUG (RAN) translation of repeat-containing C9orf72 RNA results in the production of neurotoxic dipeptide-repeat (DPR) proteins. DPR proteins misfold and aggregate into cytoplasm or nuclei of motor neuron. Here they can alter the proteotoxic response machinery. The protein quality control (PQC) system maintains protein homeostasis by re-folding (by chaperone) or

by degradation (by autophagy or proteasome) of misfolded proteins to counteract proteotoxicity. We developed a high-throughput drug screen for the identification of positive and negative modulators of DPR levels. We identify forskolin, a cAMP-elevating compounds, as positive modulators of DPR protein levels. Interestingly, PKA inhibition (by H89) or knockdown reduced translation efficiency of DPRs, while the PKA inhibitor H89 reduced endogenous DPR protein levels in C9ALS/FTD patient-derived iPSC motor neurons. In motor neuron-like cells (NSC34), we evaluated the role of the selected compound in the regulation of the two main degradative pathways of PQC. Using RT-qPCR, WB and IF analysis, we observed that none of the compounds were able to modulate TFEB, SQSTM1/p62, and LC3 expression and localization. Nevertheless, the reduction of DPR levels observed in cells treated with geldanamycin (an HSP90 inhibitor) and with spironolactone (an aldosterone antagonist) is counteracted by autophagy and proteasome inhibitor suggesting that these compounds promote DPR proteins degradation via the proteasome and autophagy pathways, respectively. Together, our results suggest degradative systems as drugable pathways modulating DPR protein levels in C9ALS/FTD.

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MTU10-17 | Intranasal administration of nootropics reverses motor symptoms and loss of midbrain dopamine neurons in PINK1 knockout rats

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Background: Parkinson's disease (PD) is a, chronic neurodegenerative disease characterized by the progressive loss of *substantia nigra* (SN) neurons that leads to the onset of motor and non-motor symptoms. Current standard of care consists of replenishing the loss of dopamine through oral administration of Levodopa. However, this treatment is not disease-modifying as PD patients eventually develop resistance to standard-of-care with disease progression. A decrease in protein kinase A (PKA) signaling and a reduction in brain-derived neurotrophic factor (BDNF) contributes to neuropathology in murine models of Parkinson's disease. We have published that intraperitoneal administration of forskolin, a labdane dipertene that enhances cyclic AMP-dependent PKA activity, reverses motor symptoms, increases brain energy production, reverses the loss of hindlimb strength and neurodegeneration of midbrain dopamine neurons PTEN-induced kinase-1 (PINK1) knockout rats, a *bone fide* genetic model of PD. In this study, we developed an intranasal formulation to deliver several nootropic agents including foorskolin with high bioavailability in the brain to activate neuroprotective PKA and neurotrophic signaling pathways.

Objective: We surmised that intranasal administration nootropic agents that can pharmacologically PKA signaling and neurotrophic

support enhance can reverse motor symptoms and loss of midbrain neurons in symptomatic PINK1-KO rats.

Methods/Results: By using a beam balance and a grip strength analyzer, we show that intranasal administration of formulation containing forskolin (10 μ M) and Noopept (10 nM), termed CNS/CT-001, reverses motor symptoms and loss of hindlimb strength, a therapeutic benefit associated with increased PKA activity and level of neurotrophic factors (NGF and BDNF) in the cortex. By using immunohistochemical assays, treatment of PINK1-KO rats with CNS/CT-001 completely reversed the loss of SN neurons, and reduced the amount of α -synuclein—a proxy of Lewy bodies—in symptomatic PINK1-KO rats.

Conclusions: Overall, our preclinical evidence data show that intranasal administration of CNS/CT-001 is a promising disease-modifying therapeutic alternative for treating PD.

MTU10-18 | Diagnosing learning and memory dysfunction in a neurodegenerative disease: The case of Alzheimer's disease

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Early diagnosis improves prognosis and treatment outcome. Failure to diagnose learning and memory deficit in patients with Alzheimer's disease (AD) is the most common mistake medical doctors or caregivers do. It makes the disease reach its irreversible stage when eventually, the clinical symptoms become clear. Here, our objective was to evaluate the diagnostic correctness of methods for diagnosing learning and memory deficit in patients with AD. A systematic literature search was conducted in Pubmed, MEDLINE, EBSCOHost, PsycINFO, EMBASE, Scopus and Google Scholar (inception from January 1, 2010) and relevant data were submitted to a meta-analysis. We found that, the pooled prevalence of learning and memory deficit and diagnostic correctness estimated is usually evaluated using random effects models. Further, when possible, diagnostic correctness was estimated among the best-reported cutoffs from each case study and among specific cutoffs where neuroinflammation and non-motor symptoms were prevalent. The pooled prevalence of learning and memory deficits in AD was lower always questioning the diagnostic tools used (the Montgomery-Asberg learning and memory rating scale, the unified AD rating scale, the Beck learning and memory Inventory I, the 15-item Geriatric learning and memory Scale). Therefore, numerous valid methods for diagnosing learning and memory dysfunction in patients with AD are still needed although each medical practitioner may choose the one that suits their clinical practice and the affordability of the patient.

MTU10-19 | Hippocampal histone trimethylation and neurogenesis are associated with resilience in a rat model with depressive-like behavior

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We know that major depressive disorder (MDD) is multifactorial, influenced by genetic, epigenetic, and environmental factors, in which stress plays a prominent role. However, the exact mechanisms by which epigenetics or other neuronal endophenotypes might be associated with the disorder are still to be unraveled. Thus, we investigated this further using a translational model of MDD. Male Wistar rats underwent Chronic Unpredictable Mild Stress (CUMS). The sucrose consumption test was performed to classify the animals regarding behavior induction into "resilient-like" and "depressive-like" phenotype groups. We established that 11 animals (52.38%) developed the depressive-like phenotype, while 8 animals (47.62%) did not change their sucrose consumption in comparison to the control group. Next, animals were euthanized to remove the blood and the hippocampus to evaluate the pattern of trimethylation of lysines, neurogenesis through DCx immunoreactivity, and aquaporin-4 (AQP-4) immunoreactivity. We found that H3K9me3 is hypermethylated in the hippocampus of rats with the resilient-like and depressive-like phenotypes ($p < 0.0001$ and $p = 0.0002$). H3K4me3 and H3K36me3 are hypermethylated in rats with a resilient-like phenotype compared to the control group ($p = 0.048$, 0.003) and compared to rats with the depressed-like phenotype ($p = 0.031$, $p = 0.036$). Rats with depressive-like phenotype have a lower DCx immunoreactivity compared to control animals ($p = 0.014$), although no differences comparing the control group with rats with resilient-like phenotype were observed, showing that rats with depressive-like phenotype, but not resilient-like, have impaired neurogenesis in the hippocampal CA3 subregion. Moreover, an increase in AQP-4 hippocampal immunoreactivity was observed comparing rats with a resilient-like phenotype and the control group ($p = 0.038$). We show that AQP-4 might play a key role in depression etiology and that its influence is possibly related to the methylation of histones and neurogenesis in the hippocampus.

**MTU10-20 | ATF4 promotes PUMA/Bax-dependent neuronal apoptosis via the mTOR pathway in Parkinson's disease models**Matthew Demmings¹, Erica Kane², Kate Hurley¹, Sean Cregan¹¹Neuroscience, Robarts Research Institute, Schulich School of Medicine and Dentistry, London, Canada; ²Anatomy and Cell Biology, Robarts Research Institute, Schulich School of Medicine and Dentistry, London, Canada

Parkinson's disease (PD) is highlighted by the progressive loss of dopaminergic neurons in the substantia nigra. Mechanisms underlying this neuronal loss remain largely unknown. Previously we have demonstrated that activating transcription factor-4 (ATF4), a key mediator of the Integrated Stress Response (ISR), is upregulated in dopaminergic neurons following exposure to MPP+, 6-OHDA, or humanized alpha-synuclein pre-formed fibrils. Specifically, we determined that ATF4 induction promotes neuronal death through transcriptional activation of known death genes including pro-apoptotic BH3-only protein PUMA. Despite ATF4 being required for PUMA activation, chromatin immunoprecipitation experiments have revealed that ATF4 does not interact directly with the PUMA promoter. In the current study, we aim to characterize the indirect mechanism by which ATF4 activation signals PUMA-dependent neuron loss. Using primary mouse neurons, we demonstrate that ectopic ATF4 expression results in activation of Trib3, SESN2, and DDIT4, which have previously been implicated to have inhibitory activity on mTOR. Ectopic expression of ATF4 in neurons also results in downregulation of mTOR signaling and increases in neuronal apoptosis. Importantly, our current investigations show that ATF4-deficient neurons are resistant to mTOR inhibition induced by exposure to a-syn PFFs or PD toxins. In addition, our mechanistic studies show that pharmacological inhibition of mTOR results in significant increases in PUMA activation, which promotes dopaminergic neuron loss via an intrinsic mitochondrial apoptotic pathway. In line with these findings, we provide novel evidence that Bax-deletion protects dopaminergic neurons from alpha-synuclein toxicity. These results provide a novel signalling mechanism by which ATF4 indirectly activates PUMA through suppression of mTOR signalling and provides further evidence for targeting the ISR for the development of therapeutics to prevent neuronal apoptosis in PD.

MTU10-21 | Studying the role of autophagy in krabbe diseaseNarayan Dhimal¹, Nadav Weinstock¹, Edward Hurley³, Karrar Aljanahi⁴, Daesung Shin², Lawrence Wrabetz^{3,1}, M.Laura Feltri^{1,3}¹University at Buffalo, Biochemistry, Buffalo, United States; ²University at Buffalo, Biotechnical and Clinical Laboratory Sciences, Buffalo, United States; ³Institute of Myelin and Glia Exploration, Neurology, Buffalo, United States; ⁴University at Buffalo, Biological Sciences, Buffalo, United States

Krabbe disease (KD) is an inherited autosomal recessive, debilitating, lysosomal storage disorder (LSD) caused by mutations in galactosyl-cerebrosidase (Galc) gene. Galc deficiency affects both the peripheral nervous system (PNS) and the central nervous system (CNS) and if untreated leads to rapid demyelination, neurodegeneration, and death. Although therapies that delay the disease exist, there is currently no available cure for KD. Schwann cells-specific deletion of Galc (Galc-SC-cKO) in mice leads to a PNS pathological phenotype of KD, which includes accumulation of undigested substrates accompanied by demyelination, neurodegeneration, and a neuropathy very similar to the one observed in KD patients (Weinstock et al. 2020). Using this validated Galc-SC-cKO model, this study aims to investigate autophagy in the PNS to understand its contribution to the pathogenesis of KD. Preliminary data indicate that macroautophagy (or autophagy) may be defective in Schwann cells (SC) when GALC is absent. Western blot results suggest that autophagy might be increased at the beginning of the disease, but it halts and decreases during disease progression. Autophagosome accumulation in SCs is observed under electron microscopy (EM) during disease progression. Furthermore, using a pH sensitive fluorescent autophagy reporter model, we see a significant increase in the number of SCs with autophagosome in Galc-SC-cKO in vivo. Thus, we plan to manipulate autophagy in Galc-SC-cKO mice in vivo, and this work is currently in progress. Understanding specific mechanistic changes in degradative pathways could help pinpoint the cause and timing of substrate accumulation in KD and other LSDs. Such studies may also be relevant to other more common diseases caused by failure in lysosomes, autophagy, and proteostasis.

MTU10-22 | iPSC-derived in vitro striatal circuits to understand GBA-associated Parkinson's diseaseQuyen Do^{1,2}, Bryan Ng¹, Nora Bengoa-Vergniory^{1,2}, Richard Wade-Martins^{1,2}¹University of Oxford, Physiology, Anatomy and Genetics, Oxford, United Kingdom; ²Oxford Parkinson's Disease Center, Physiology, Anatomy and Genetics, Oxford, United Kingdom

Objectives: Dysfunction of medium spiny neurons (MSNs) are implicated in motor impairments in Parkinson's disease (PD). However, most research on MSN pathology in PD has relied on animal models of toxin-induced PD which fail to recapitulate the human nature, genetics and temporal aspects of PD pathogenesis. By developing an induced pluripotent stem cells (iPSCs)-derived cortico-striato-nigral mini-circuit, we recapitulate the complex connectivity received by MSNs and examine temporal changes of human striatal electrophysiology due to DaNs harbouring PD-relevant mutations.

Methods: Healthy control iPSC-derived cortical neurons CNs and MSNs, together with dopaminergic neurons (DaNs) derived from either healthy controls or patients carrying GBA N370S mutation were co-cultured in custom microfluidic devices. We combined



molecular and electrophysiological techniques to investigate electrophysiological properties of MSNs.

Results: We have established an all iPSC-derived cortico-striato-nigral mini-circuit mimicking the dual glutamatergic and dopaminergic pre-synaptic inputs onto MSNs. Coculturing of MSNs with CNs did not affect MSNs intrinsic properties but improved MSNs synaptic activities which was consistent with CN-MSN increased spine density. Meanwhile, DA reduced intrinsic excitability of aged CN-MSNs, potentially via an increase in voltage-gated potassium conductance. CN-MSNs cocultured with GBA N370S DaNs displayed significantly increased intrinsic excitability and reduced sodium and potassium conductance in early MSNs.

Conclusions: Our results highlight the utility of modelling neurons in a highly physiological manner recapitulating their endogenous connectivity, particularly the importance of dual glutamatergic and dopaminergic pre-synaptic inputs in improving electrophysiological maturation of human MSNs in vitro. They also suggest early electrophysiological dysfunctions in human MSNs induced by surviving GBA N370S DaNs, providing insights into early pathology of MSNs in PD and relevant potential therapeutic target.

MTU10-23 | ALS astrocytes impair neuronal firing in ALS and healthy iPSC-derived motor neurons

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease leading to progressive paralysis. One of the earliest clinical observations ALS patients is hyperexcitability of motor neurons in the cortex and spinal cord prior to hypoexcitability and deterioration of motor neuron function. The mechanisms that underlie the alterations in electrical signalling of ALS motor neurons throughout disease progression are not yet fully understood. Our previous work has demonstrated that ALS-causing mutations, CCNF^{S621G} or C9ORF72^{exp}, increase neuronal excitability in induced pluripotent stem cell (iPSC)-derived motor neurons. However, as astrocytes are known to be impaired in ALS, the aim of this study was to further investigate how co-culture of ALS astrocytes with motor neurons affects motor neuron excitability and function. Whole-cell patch clamping was used to assess electrophysiological properties of co-cultured motor neurons. The addition of CCNF^{S621G} or C9ORF72^{exp} astrocytes caused the loss of neuronal firing in ALS and control motor neurons. Moreover, Na⁺ and K⁺ currents, which govern

neuronal excitability, were reduced by up to 55% and 30%, respectively, in both ALS and control motor neurons co-cultured with ALS astrocytes. This suggests that ALS astrocytes may be involved in the transition from motor neuron hyperexcitability to hypoexcitability. The findings highlight that the cellular crosstalk between motor neurons and astrocytes play a significant role in altering intrinsic neuronal excitability, which could impact ALS progression.

MTU10-24 | A fantastic beast or a fantastic drug? Doxy (doxycycline) attenuates L-DOPA-induced dyskinesia in hemiparkinsonian mice

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Pharmacologic manipulation of neuroinflammation appears to be a promising strategy to alleviate L-DOPA-induced dyskinesia (LID). Recently, our group demonstrated that anti-inflammatory drugs alleviate LID in hemiparkinsonian mice. Here we addressed the hypothesis that LID could be reversed by sub-antibiotic doses of the antibiotic doxycycline (Doxy), that is, doses that maintain its anti-inflammatory properties without acting on bacterial structure. To this aim, we used 6-OHDA-lesioned C57BL/6 mice with a partial nigrostriatal lesion and treated them with L-DOPA (L-DOPA 25 mg/kg + benserazide 10 mg/kg i.p. for 21 days). These animals developed severe axial, limb and orofacial abnormal involuntary movements (AIMs). Sub-sequentially, the animals were exposed to Doxy (20 or 40 mg/kg s.c.) 30 minutes prior to L-DOPA for 5 days. Sub-chronic treatment with Doxy (20 and 40 mg/kg s.c.) in dyskinetic mice presented a significant effect on reducing the already established LID without affecting the locomotor activity improved by L-DOPA. Doxy at 40 mg/kg had a more robust effect over AIMs compared to Doxy 20 mg/kg, as it reduced AIMs by 65%, 20% more than Doxy 20 mg/kg. However, since Doxy 20 mg/kg (the lower dose, closest to sub-antibiotic activity) caused a significant decrease of LID, we chose this dose for molecular analysis. After LID attenuation, we performed immunohistochemistry for the markers Fos-B and cyclooxygenase-2 (COX-2), and ELISA for cytokines TNF- α , IL-1 β and IL-6 and for COX-2 metabolite PGE2 in the lesioned dorsal striatum of hemiparkinsonian mice. LID decrease was accompanied by the reduction of COX-2 expression and of cytokines TNF- α and IL-1 β and COX-2 metabolite PGE2 in this area. Overall, we conclude that sub-antibiotic doses of Doxy are responsible for anti-inflammatory actions that are mandatory for LID attenuation and might be an interesting and alternative way to use Doxy as treatment for LID in Parkinson's disease.

**MTU10-25 | On cholinergic regulation in Alzheimer's disease: transcriptomic analysis at the single-cell level**Serafima Dubnov^{1,2}, Hermona Soreq^{1,2}¹The Hebrew University of Jerusalem, The Edmond & Lily Safra Center for Brain Sciences, Jerusalem, Israel; ²The Hebrew University of Jerusalem, The Alexander Silberman Institute of Life Sciences, Jerusalem, Israel

Studies show that neurodegeneration associated with Alzheimer's Disease (AD) starts in the basal forebrain, affecting cholinergic neurons before other cells. However, it is widely unknown why cholinergic neurons in this brain region are particularly vulnerable to the pathology. Furthermore, concrete molecular pathways by which the impairment in cholinergic signaling causes cognitive decline are yet to be discovered. Although it is known that various cell types take part in cholinergic signaling, in-depth understanding of cholinergic identities and concrete roles of each cell type await further deepening. Single-cell RNA-seq (scRNA-seq) allows to study gene expression as a function of a cell type, providing an opportunity to classify cell types based on their transcriptome and to track major biological mechanisms involved in cholinergic signaling.

We have analyzed a single nucleus RNA-seq dataset from human prefrontal cortices derived from AD patients and age-matched controls, focusing both on the 'classical' cholinergic genes like choline acetyl transferase (CHAT) and acetylcholinesterase (ACHE) and on additional molecular agents linked to cholinergic signaling. We have identified several subpopulations of cholinergic disease-associated cells, consisting of both neurons and glia, which are mostly characterized by genes involved in circadian rhythm regulation. Additionally, we found cell type-specific differential expression of muscarinic receptor subunits in AD patients.

To challenge the concept of cholinergic regulation of inflammation in the CNS, we used correlation-based computational analysis mediated by microglia, as a part of the cholinergic anti-inflammatory pathway. Notably, microglia from donors with elevated representation of ChAT featured significant downregulation of known proinflammatory factors, such as APOC1, CD81, SPP1, MAF and LAPTM5. Furthermore, the same genes were found to be significantly upregulated in patients with AD-associated cognitive decline. Thus, our results demonstrate that dysfunction of the cholinergic anti-inflammatory pathway in AD patients might lead to elevated immune response in microglia, which correlates with cognitive impairment.

MTU10-26 | Studies on the role of B to Z DNA conformational transition in neuronal cell death: relevance to neurodegenerative disordersLakshmi Sowmya Emani¹, Jayanth KS Rao², Muralidhar L Hegde³, Jagannatha Rao Kosagisharaf¹¹Koneru Lakshmaiah Deemed to be University, Department of Biotechnology, Guntur, India; ²University college of Dublin, Graduate Medical student in Medicine, Dublin, Ireland; ³Methodist Research Institute, Department of Neurosurgery, Houston, USA

DNA is a dynamic and crucial molecule whose conformation kinetics plays a major role in biological function. Reports from our lab and elsewhere indicated the presence of non-B-DNA forms of conformations in neurodegenerative diseases like fragile X-syndrome, Huntington's chorea, Alzheimer's and others. Recently, our laboratory discovered the presence of Z-DNA in the hippocampal region of severely affected Alzheimer's disease (AD) brain samples and modified B-conformation in Parkinson disease. The alternate purine-pyrimidine bases are the potential sequences adopting Z-DNA, and these are present in the promoter regions of AD-specific genes like amyloid precursor protein (APP), Presenilin and ApoE. We hypothesized that Z-DNA might be involved in the expression of these pathologically important genes and the production. In the present paper, we have developed a theoretical model on the possible mechanisms/hypothetical proposition of Z-DNA transition and its implications in AD. We developed a model where we try to understand that Z-DNA is formed in the promoter region of the APP, and Presenilin genes and this conformation may absorb the negative supercoils at that region. The decrease in the supercoil density may alter the native supercoiling domain and positively regulate gene expression of like APP and Presenilin. We further try to understand that Z-DNA may be involved in the down-regulation of genes involved in A β clearance defense mechanisms in AD. Also we developed novel hypothesis on B-Z transition role in producing excess unnecessary RNA pool in causing cell death and the possible role of Strip1 in cell survivability. The proposed model tries to understand the behavioral pathology like emotions, eating behavior memory loss, and coordination failure in neurodegenerative disorders.

MTU10-27 | Structure–function studies of Alzheimer's disease

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According to the National Institute of Aging (NIA) AD is an irreversible, progressive brain disorder that slowly destroys memory, thinking skills, and, eventually, the ability to conduct the simplest tasks. People with the late-onset show symptoms in their mid-sixties. Early-onset AD occurs between 30 to 60 years of age and is exceedingly rare. AD is the most common cause of dementia among older adults. Dementia is a loss of thinking, remembering, and reasoning skills. It is not a normal part of aging. Increasing life expectancy and AD go hand in hand, medicines can slow down but cannot stop it. Therefore, early diagnosis is mandatory. To monitor the disease progression closely and enhance the accuracy of diagnosis, it is essential to identify extremely sensitive and robust biomarkers of AD. Literature indicates that the neural tracts and nuclei of the brain stem and midbrain connect the ear to the auditory cortex and play an essential part in the translation of sound waves into auditory perception. Thus, it is possible that tracking aural pathway function may allow us to identify individuals who are at risk of developing dementias such as Alzheimer's disease in the future. This may also be helpful in the early diagnosis of AD and may delay or stop its progression. Neuropsychological testing MMSE, MRI, and MRS examinations were done. Typical bitemporal atrophy with consecutive dilatation of temporal horn, hippocampal atrophy, and marked enlargement of the Sylvian was present in patients with MMSE values of 10–20 points. Whereas MRS results demonstrate inferior colliculi show degeneration earlier than the noticeable landmark structural changes in the brains of patients with AD. Furthermore, we observed not only biochemical but also electrical and functional deviations prior to these structural changes for the diagnosis of cognitive decline.

MTU10-28 | Strain-typing of scrapie and bovine spongiform encephalopathy using ovine PrP (ARQ/ARQ) overexpressing transgenic mice

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The pathological prion protein (PrP^{Sc}) causes transmissible spongiform encephalopathies (TSE), which can harm a wide range of animals. Furthermore, varying amounts of strains are found in classical scrapie isolates. Although isolate characterization, traditionally executed in the wild type mouse models, has progressed to using transgenic mice, the procedure is not standardized yet. In this study, two transgenic mouse models (TgshpIX and TgshpXI) overexpressing the sheep ARQ/ARQ PrP were injected intracerebrally with confirmed ovine scrapie isolates from different host genotypes, bovine

spongiform encephalopathy (BSE) as well as ovine BSE isolates, and incubation period, lesion profile, and PrP^{Sc}-profile were evaluated. All isolates infected both mouse models with a nearly 100% attack rate, although with genotype dependent differences, with incubation times nearly twice as long after infection with VRQ strains. Incubation times after BSE infection were longer in Tgshp XI mice than in Tgshp IX mice. There were no evident variations in the histopathological lesion profiles between the isolates in both models, but the immunohistochemistry-based PrP^{Sc} profiles clearly allowed the discrimination of BSE isolates from scrapie. For this the overall PrP^{Sc} distribution pattern in certain brain areas as well as the cellular staining reaction were characterized. Taken together, Tgshp IX and XI mice were successfully used to characterize TSE isolates.

MTU10-29 | *Nigella sativa* oil modulated hippocampal endophenotypes in dizocilpine-induced schizophrenia in BALB/c mice

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Schizophrenia is a neuropsychiatric disorder characterised by behavioral symptoms. *Nigella sativa* oil (NSO) is a medicinal plant notable for its dietary, neuroprotective and anti-inflammatory properties. However, there is paucity of information on its neuroprotection in schizophrenia. This study was designed to investigate the modulatory effects of NSO on the hippocampus of dizocilpine-induced schizophrenia in mice.

Sixty 14-week-old male BALB/c mice (23–25g) were divided into five groups ($n = 12$); control (normal saline, 1 mL/kg), NSO (1 mL/kg), dizocilpine-control (0.5 mg/kg) all for 7 days, while NSO (1 mL/kg for 7 days) + dizocilpine (0.5 mg/kg, for another 7 days) for preventive measure, and dizocilpine (0.5 mg/kg for 7 days) + NSO (1 mL/kg for another 7 days) for reversal. Dizocilpine and NSO were administered intraperitoneally and orally, respectively. Open field box was used for stereotypic popping; while anxiety and recognition memory were measured using the elevated plus maze and novel object recognition tests, respectively. Animals were euthanised after behavioral studies, and harvested brains were weighed. Hippocampal glutamate was determined spectrophotometrically. Neuronal arrangement, sizes and densities were determined in perfused brain tissues using H&E stain. Dendritic arborisations were assessed using Golgi stain. mGluR-2 and GFAP were evaluated immunohistochemically. Data were analysed using descriptive statistics and ANOVA at $\alpha 0.05$. Stereotypic popping was observed only in untreated dizocilpine-control animals. NSO increased open arm entry and exploration index, novel object recognition index, relative brain weight and hippocampal glutamate levels in the treated groups. It also modulated hippocampal neuronal density and significantly inhibited neuronal de-arborisation, increased mGluR-2 expression and reduced GFAP expression in the treated groups.



Nigella sativa oil mitigated schizophrenic symptoms induced by dizocilpine in mice via modulation of hippocampal glutamate, metabotropic glutamate receptor-II upregulation, astrogliosis inhibition and neuroprotective mechanisms.

MTU10-30 | Microglial large extracellular vesicles propagate early synaptic dysfunction in Alzheimer's disease by moving at the axon surface

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Synaptic dysfunction occurs early in Alzheimer's disease (AD), involving progressively larger areas of the brain over time. However, how it starts and propagates is unknown. We show that amyloid-beta ($A\beta$) released by microglia in association with large extracellular vesicles ($A\beta$ -EVs) alters dendritic spine morphology in vitro and impairs synaptic plasticity both in vitro and in vivo in the entorhinal cortex-dentate gyrus circuitry. 1h after $A\beta$ -EV injection into the mouse entorhinal cortex (EC), long-term potentiation (LTP) is impaired in the EC, while 24 h later it is impaired also in the dentate gyrus (DG), revealing a spreading of LTP deficit between the two synaptically connected regions. Similar results are obtained by injecting EVs carrying $A\beta$ naturally secreted by CHO7PA2 cells, while neither $A\beta_{42}$ alone nor inflammatory EVs devoid of $A\beta$ are able to propagate LTP impairment. Using optical tweezers coupled to time-lapse imaging to study $A\beta$ -EV-neuron interaction, we show that $A\beta$ -EVs move anterogradely at the axon surface and that their motion can be blocked by annexin-V coating. Importantly, when $A\beta$ -EV motility is inhibited, no propagation of LTP deficit occurs along the EC-DG circuit, implicating large EV motion at the neuron surface in the spreading of LTP impairment. The influence of mesenchymal stem cell (MSC) indirect co-culture with microglia primed

with $A\beta$ on cell phenotype, EVs and functions is currently being explored.

Our data indicate the involvement of large microglial EVs in the rise and propagation of early synaptic dysfunction in AD, and suggests a new mechanism controlling the diffusion of large EVs and their pathogenic signals in brain parenchyma, paving the way for novel therapeutic strategies to delay the disease.

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MTU10-31 | Role of metabolism in pathological aggregation of TDP-43 and its down-stream toxicity

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Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar dementia (FTLD) are two fatal neurodegenerative disorders with considerable molecular overlap. Cytoplasmic aggregation of transactive response DNA binding protein of 43 kDa (TDP-43) in neurons is a consistent feature of a majority of ALS and FTLD cases. Conditions associated with a conventionally risky metabolic profile, such as type 2 diabetes mellitus, high body mass index, and dyslipidaemia have been associated with delayed onset, slower disease progression, and longer survival in both ALS and FTLD. This study aimed to investigate how TDP-43 nuclear loss of functions, which is considered an early event in the pathological aggregation of TDP-43, dysregulate cellular metabolic cascades. TDP43 nuclear loss of function in NSC-34 mouse motor neuron-like cells revealed an alteration in neuronal energy metabolism, particularly mitochondrial oxidative phosphorylation and the activity of key enzymes involved in lipid and glucose metabolism. Functional validation of these findings showed that TDP43 loss-of-function enhances energy substrate uptake but perturbs intracellular metabolism, culminating in aberrant energy production and reduced survival of neurons. Furthermore, glucose manipulation of our TDP-43 pathology model exacerbates impaired neuronal health at low doses but improves neuronal viability at moderately high doses. Cumulatively, our study has underscored the putative interplay between TDP-43 loss-of-function and neuronal metabolism. Our ongoing investigations involve elucidating the subcellular mechanisms driving the purported interplay and modelling of TDP-43 pathology in brain organoids reprogrammed from induced pluripotent stem cells (iPSCs) derived from patients' fibroblasts.



MTU10-32 | Approaching promising markers for Parkinson's disease using serum cytokines and DAMPs along to clinical parameters

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Currently, the major factors to diagnose Parkinson's disease (PD) are the appearance of typical symptoms, such as movement alleviation, tremor or stiffness, and an important improvement when initiating treatment with L-DOPA. PD patients also present depression, REM sleep disorder, hyposmia and constipation. The cause of neurodegeneration in PD is not completely understood. Peripheral inflammation plays an important role in disease initiation and progression, exacerbating the neuroinflammation and synergistically driving neurodegeneration. Cytokine and chemokine respond to danger associated molecular patterns (DAMPs) during stressor and inflammatory process. The inflammatory status and course of PD is highly variable and there are no established prognostic biomarkers. We evaluate serum cytokines, chemokines, and some DAMPs that are RAGE agonists, in parallel with clinical parameters of PD, including motor symptoms and NMS. Blood samples were collected from PD patients and healthy controls at the Hospital de Clínicas de Porto Alegre (HCPA), Brazil. Serum parameters were measured by Multiplex-based assay and ELISA. Bioinformatic analysis was performed to identify predictors of disease and clustering patients by serum profile. Serum DAMPs and RAGE ligands were lower in PD when compared to HC, and decay over time. At the same time, PD patients present an anti-inflammatory profile of cytokines that changed towards pro-inflammatory profile after a year, even without progression of disease staging. We also observed two distinct clusters of PD patients according to serum profiles, wherein DAMPs/RAGE ligands varied inversely to cytokines. Together, the 12 serum markers

analyzed by machine learning model (HMGB1, HSP70, S100B, 4-HNE, CML, TNF- α , IL-1 β , IL-2, IL-4, IL-6, IFN- γ , IL-10) were able to predict PD in HY scale 2. Financial support: CNPq, FAPERGS, CAPES and Propesq/UFRGS

MTU10-33 | Role of pharmacological antagonism of A2AR in the acquisition of morphine withdrawal-induced CPA

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Opioid like morphine are substances with a great ability to induce addiction. The purinergic system, through the A2A adenosine receptor (A2AR), is involved in addiction induced by different drugs of abuse. It has been previously demonstrated that A2A gene deletion in mice modifies some of the behavioral effects of morphine. To discriminate whether the A2AR participates in the acquisition or in the expression of morphine-induced behaviors and, thus, to design effective pharmacological treatments targeted to A2AR to prevent behaviors responsible for the maintenance and relapse opiate addiction, we have used the condition place aversion (CPA) paradigm and administered an A2AR antagonist to morphine-dependent and control mice prior the conditioning sessions with naloxone. To induce opiates dependence mice were subcutaneously implanted with morphine (MOR) or placebo (PLA) pellet. An A2AR antagonist (sch58261; 1 mg/kg) or its vehicle was administered intraperitoneally 15 min before the conditioning sessions with naloxone (NX; 0.1 mg/kg, s.c.). CPA has been used to study the aversive emotional aspects of naloxone-precipitated morphine. As expected, opiate dependent animals spent significantly less time in the morphine withdrawal-paired chamber during the CPA test comparing to PLA mice. Nonetheless, when sch58261 was injected before naloxone conditioning to morphine-dependent mice they spent more time in compartment associated with withdrawal than MOR vehicle mice (&& $p < 0.01$ vs MOR vehicle). Percentage of entries in NX-paired chamber was significantly reduced in vehicle MOR animals and no decrease was observed in A2AR blockade MOR mice. Our results reveal that the blockade of A2AR in MOR dependent mice prevented the aversion to the withdrawal paired-compartment indicates that this receptor is essential for the acquisition of morphine withdrawal-induced behaviors, pointing out that A2AR antagonists might be effective pharmacological tools to treat addiction to opiates.

**MTU10-34 | Pre-clinical study of a 5HT_{1A} specific agonist in the CMVMJD135 mouse model of Machado–Joseph disease**

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Machado–Joseph Disease (MJD), an autosomal dominant neurodegenerative disease caused by an expansion of a CAG repeat tract in the ataxin-3 gene (ATXN3) is mainly characterized by a late onset of motor dysfunction. Currently, no disease-modifying therapy is available. Recent findings showed that NLX-112, a highly selective and fully efficacious 5-HT_{1A} receptor agonist, improved motor dysfunction and reduced mutant ATXN3 aggregation in a *Caenorhabditis elegans* MJD model. This study aims to analyze the therapeutic impact of NLX-112 in the CMVMJD135 MJD mice motor function. After drug doses' selection, based on plasma/brain exposure levels and animal welfare, two pre-clinical trials were performed: WT and MJD animals were treated with NLX-112 pre-symptomatically via drinking water for 35 weeks, using tandospirone as reference, and post-symptomatically via intraperitoneal injections for 16 weeks. Animals' welfare, body weight and temperature were regularly assessed, along with motor function using the beam walking (BWT) and motor swimming (MST) tests. These experiments revealed that doses up to 8 mg/kg for NLX-112 and 80 mg/kg for TD were safe and well-tolerated. Pre-symptomatically, NLX-112 significantly improved motor coordination and balance of MJD animals in the BWT, at advanced stages of the disease. Contrarily, TD exhibited no therapeutic effect on motor function. Post-symptomatically, NLX-112-treated animals' coordination was improved in the MST early on the disease, while minor alterations were observed in other motor parameters. Overall, NLX-112 showed a beneficial effect on MJD mice motor function, reinforcing the potential role of serotonergic signaling modulation as a promising therapeutic target for MJD.

MTU10-38 | Ouabain prevents NMDA elicited neuronal calcium overload in brain cortical slices: role of membrane cholesterol

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In neurons of primary culture 1 nM ouabain does not inhibit Na/K-ATPase but promotes intracellular regulatory effects. At concentrations

of 1 nM and below, ouabain induces neuroprotection in excitotoxic stress caused by agonists of glutamate receptors because calcium entry through glutamate-activated NMDA receptor channels is compensated by extrusion via Na/Ca-exchanger co-localized with the receptors in lipid membrane microdomains. Here in cortical neurons in P1-3 rat brain slices, we studied the 1 nM ouabain effect on 100 μ M NMDA (+30 μ M glycine as co-agonist) elicited calcium responses. For this purpose by the use of confocal microscopy, we measured the fluorescence of cells loaded with calcium-sensitive dye Fluo-8 AM. In experiments NMDA was applied to slices for 150 s, then ouabain was added to NMDA for the next 150 s. Ouabain caused a significant decrease of NMDA elicited calcium responses. Therefore 1 nM ouabain can rescue adult cortical neurons from NMDA receptor-induced calcium overload. A similar protocol was used on slices pretreated with methyl- β -cyclodextrin. It is supposed that this procedure causes plasma membrane cholesterol extraction, lipid microdomains dissipation, and decoupling of ion transport via Na/K-ATPase, NMDA receptor, and Na/Ca-exchanger. Cholesterol extraction prevented ouabain effects. Most probably ouabain action depends on local functional interaction between NMDA receptors providing the calcium entry, Na/Ca-exchanger extruding the excessive calcium, and Na/K-ATPase, which interaction with ouabain enhances the Na/Ca-exchanger function. Cholesterol extraction with methyl- β -cyclodextrin probably breaks this functional coupling and prevents ouabain effects on calcium responses. The study is supported by the RSF grant #21-15-00403.

MTU 10-39 | A new insight on Al-maltolate-treated aged rabbit as Alzheimer's animal model: a new approach in drug discovery

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Lack of an adequate animal model for Alzheimer's disease (AD) has limited an understanding of the pathogenesis of the disease and the development of therapeutic agents targeting key pathophysiological processes. There are undoubtedly few satisfactory animal models for exploring therapies targeting at amyloid beta (A β) secretion, deposition, aggregation, and probably the inflammatory response. However, an understanding of the complex events – tau, A β , oxidative stress, redox active iron, etc. – involved in the neuronal cell loss is still unclear due to the lack of a suitable animal model system. The use of neurotoxic agent's particularly aluminum–organic complexes, especially Al-maltolate, expands the scope of AD research by providing new animal models exhibiting neurodegenerative processes relevant to AD neuropathology. Examination of different species of aged animals including the rapidly advancing transgenic mouse models revealed very limited AD-like pathology. Most other animal models have single event expression such as extracellular A β deposition, intra-neuronal-neurofilamentous aggregation of proteins akin to neurofibrillary tangles, oxidative stress or apoptosis. To date, there are no paradigms of any animal in which all the features of AD were evident. However, the intravenous injection of

Al-maltolate into aged New Zealand white rabbits results in conditions which mimics a number of neuropathological, biochemical and behavioral changes observed in AD. Such neurodegenerative effects include the formation of intraneuronal neurofilamentous aggregates that are tau positive, immunopositivity of A β , presence of redox active iron, oxidative stress and apoptosis adds credence to the value of this animal model system. The use of this animal model should not be confused with the ongoing controversy regarding the possible role of Al in the neuropathogenesis, a debate which by no means has been concluded. Above all this animal model involving neuropathology induced by Al-maltolate provides a new information in understanding the mechanism of neurodegeneration.

MTU11-01 | Agonist activation of sigma-1R upregulates Kv1.2 trafficking at the plasma membrane through anterograde and recycling pathways

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Brain functions are maintained by a strict regulation of neuronal excitability, in which voltage-gated ion channels, and especially potassium channels (Kv), play a key role. Thus, controlling expression and proper targeting of Kvs to the plasma membrane (PM) is essential for the regulation of this process. Our previous work has identified Kv1.2 and sigma-1R (S1R) protein as critical players in cocaine-induced neuronal hypoactivity in nucleus accumbens neurons, a pivotal brain region involved in the reward circuits. While this neuroadaptation has been shown to contribute to addiction-relevant behaviors, its cellular underpinnings remain elusive. S1R protein is a ligand-activated chaperone residing at the ER that is involved in the maturation and trafficking of several Kv channels to the PM, including Kv1.2. Determining the molecular mechanisms and trafficking routes by which S1R is able to regulate this channel expression at the PM is critical to elucidating the neurobiological basis of cocaine-induced neuronal hypoactivity.

Using a transient expression system and ELISA assays, we confirmed that S1R exposure to agonist (e.g., by cocaine) increases Kv1.2 expression at the PM. Using co-immunoprecipitation (CoIP) and GST-Pull down analyses, we validate S1R interaction with Kv1.2 while also exposing its predominant binding to Kv1.2 N-terminal domain, more precisely its T1 domain. Next, we reveal that inhibition of both ER-to-Golgi trafficking pathways and endosomal recycling pathways blocked S1R modulation of Kv1.2 cell surface expression. Taken together, these data show that S1R activation by cocaine modulates anterograde and recycling pathways, which enhances Kv1.2 PM expression. Interestingly, a recent study demonstrated that cocaine

activation of S1R stimulates extracellular vesicles secretion through an interaction with ARF6 (a small GTPase). Knowing the involvement of small Rab GTPases in the trafficking pathways mentioned above, we decide to evaluate a specific Rab proteins' association with S1R and test their impact on Kv1.2 PM expression.

MTU11-02 | Novel uptake and degradation pathway of proteins by lysosomes required for neuromuscular homeostasis

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Protein is one of fundamental cellular components to construct biological substances. Both synthesis and degradation of protein are essential for maintaining homeostasis. Accumulating evidence shows the importance of lysosomal degradation of intracellular proteins: Dysfunctions in multiple pathways to deliver cytosolic substrates into lysosomes are related to various diseases, including neurodegenerative diseases and myopathies. Here, we show that cytosolic proteins are directly imported into lysosomes by a mechanism distinct from any known pathways and degraded. We found that a lysosomal membrane protein, SIDT2, which was previously reported as a putative nucleic acid transporter, is involved in the translocation of substrate proteins in this system. Gain- and loss-of-function analyses revealed that SIDT2 contributes conspicuously to the lysosomal degradation of a wide range of cytosolic proteins in cells at the constitutive level. Furthermore, we identified a dominant-negative type of mutation in SIDT2 which causes familial rimmed vacuolar neuromyopathy in humans. Typical features of rimmed vacuolar neuromyopathy, including atrophy and accumulation of cytoplasmic inclusions in skeletal muscles, were recapitulated Sidt2 knockout mice. These results reveal a

previously unknown pathway of protein degradation in cells, and highlight the importance of noncanonical types of lysosomal degradation pathway in human physiology and pathophysiology.

MTU11-03 | Sigma-1R is involved in Kv1.2 channel stability

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Voltage-gated ion channels (VGICs) are multi-spanning transmembrane proteins that play crucial role in electrical signaling of cells. Amongst VGICs, potassium channels (Kvs) represent the most complex class from both functional and structural standpoints. The diversity of Kv channels plays an essential role in fine-tuning neuronal excitability, thereof neurons' capacity to generate and propagate information within brain circuits. Therefore, understanding the cellular and molecular mechanisms modulating Kv channels activity and expression is of the utmost importance. Kv channels are fully functionals when bound to auxiliary subunits. These subunits can modulate the channels' plasma membrane (PM) targeting, compartmentalization, intracellular trafficking, conductance, and gating (open or closed state). Interestingly, studies have identified Sigma-1R (S1R) as an atypical auxiliary subunit for several Kv α -subunits. As an endoplasmic reticulum (ER) ligand-operated chaperone, S1R has been shown to have activity-dependent and -independent effects on many VGICs. However, the molecular and cellular mechanisms involved in S1R regulation of Kv channels is still poorly understood.

In this study, we observe that expression of Kv1.2 with increasing amount of S1R reduces the channel expression level while using transient expression experiments in HEK 293T cells. Based on these observations, we next evaluate the channel stability by using cycloheximide chase assay. As expected, we found that Kv1.2 turnover is increased when co-expressed with S1R. Importantly, using cell-based ELISA assay, we also notice that Kv1.2 cell surface expression is decreased when expressed with S1R. Given that S1R is a well-known chaperone involved in ER-associated degradation (ERAD), unfold protein response (UPR) and autophagy, we will further investigate proteasomal and endo-lysosomal pathways involvement in these observations. This ability to regulate Kv basal expression level and PM expression suggests that S1R may act as a regulator of the homeostasis of neuronal excitability.

MTU12-01 | Jigsaw-shaped self-assembling peptide (JigSAP) hydrogels efficiently incorporate and release VEGF and promote brain regeneration

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During injured tissue regeneration, the extracellular matrix (ECM) plays a key role in controlling and coordinating various cellular events by binding and releasing secreted proteins in addition to promoting cell adhesion. Herein, we developed a novel cell-adhesive fiber-forming peptide that mimics the jigsaw-shaped hydrophobic surface in the dovetail-packing motif of glycophorin A (GYPA) as an artificial ECM for regenerative therapy. The jigsaw-shaped self-assembling peptide (JigSAP) formed several-micrometer-long supramolecular nanofibers through a helix-to-strand transition to afford a hydrogel under physiological conditions and three-dimensionally homogeneously dispersed in the hydrogel. JigSAP was three-dimensionally homogeneously dispersed in the hydrogel. The molecular- and macro-scale supramolecular properties of the jigsaw-shaped self-assembling peptide JigSAP hydrogel allowed efficient incorporation and sustained release of vascular endothelial growth factor (VEGF), and showed cell transplantation-free regenerative therapeutic effects on a subacute-chronic phase mouse stroke model. This research highlights a therapeutic strategy for injured tissue regeneration using the jigsaw-shaped self-assembling peptide supramolecular hydrogel.

MTU12-02 | A new strategy to improve central neuronal regeneration after glial scar formation

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The glial scar translates the direct impact of astrocytes upon injury to the CNS, being the lesion type and location a key differential factor in CNS response. Astrocytes, the main constituent of glial scar, were shown to be mostly inhibitors of lesion spread. The simultaneous injection of immature astrocytes and chondroitin sulphate proteoglycans (CSPGs) degrading enzyme succeeded in crossing the lesion while guiding axonal processes. Thus, our work aimed to characterize the glial scar following traumatic injury to the hippocampus (HSI), a model in which we aim to study both the physical side of regeneration and functional side (memory behaviour, synaptic aptitude).

Our results show that HSI mice have memory deficits and LTP impairment. In the Morris Water Maze, injured mice performed significantly worse than sham controls, failing to learn the task. In HSI mice with the 560µm injury have shown a significant decrease on both synaptic transmission and long-term potentiation (LTP), evidence for a synaptic aptitude decrease in injured mice. Moreover, immunohistochemistry analysis demonstrated different glial scar profiles according to injury size and location. Particularly, GFAP (astrocyte marker), CS56 (CSPG marker) and NG2+ cells marker were more expressed in HSI mice. GFAP overexpression was not exclusive to injury, where it had the strongest signal as expected, but was present throughout the brain as well. With these results in mind, we expect our future treatment of the lesion with immature astrocytes together with Lv-ChABC will rescue memory and LTP impairments, through the formation of astrocytic bridges that are important for axonal regrowth.

Our data suggest that hippocampal glial scar formation impacts on synaptic plasticity and cognition, and its modulation can act as a neuroprotective strategy against specific brain injuries. Thus, the implications of this project are far-reaching. Considering that any major degenerative disease or injury to the CNS has glial scar-like formation, our project can possibly change the scope of how we look at therapeutical interventions for CNS injury. Furthermore, this project presents, for the first time, an HSI model with memory and synaptic deficits.

MTU12-03 | BDNF, adenosine A2AR and cannabinoid receptors: a triad of regulators of postnatal oligodendrogenesis

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Oligodendrocytes (OLs) are the myelin-forming cells in the central nervous system of vertebrates. The role of modulators such as brain-derived neurotrophic factor (BDNF), cannabinoid receptors type 1 and 2 (CBRs) and adenosine A2A receptors (A2ARs) on adult oligodendrogenesis from subventricular zone neural stem cells (SVZ-NSCs) remains unknown. Hence, we aimed at studying how these modulators and the putative crosstalk between them can influence OL differentiation from postnatal SVZ-NSCs. Results obtained using SVZ-NSCs cultures pharmacologically treated with adenosine A2ARs modulators, CBR ligands or BDNF, show that treatment with BDNF tends to increase oligodendrocyte precursor cell (OPC) formation (NG2/PDGFRα-positive cells) after 4 days in vitro (DIV) ($n = 3$; CTRL set to 100%, BDNF 203.8 ± 27.59 ; $p = 0.0548$), whilst significantly increasing the number of OPCs at DIV7 ($n = 7-8$; CTRL

set to 100%, BDNF 210.2 ± 21.87 ; $p < 0.0001$) without affecting OL maturation (MBP-positive cells). Importantly, BDNF effects on OPC formation at DIV7 were partially abrogated by the A2AR antagonist ($n = 4-8$; CTRL set to 100%, BDNF+ZM 174.0 ± 8.951 ; $p < 0.01$), while the antagonist by itself had no effect when comparing with control (ZM 117.6 ± 15.47 ; $p > 0.05$). No changes were observed after treatment with the A2AR agonist at these timepoints in both OPC formation and OL maturation. This work outlined the role of BDNF in promoting the formation of OPCs from SVZ-NSCs. Now we are addressing if the effect of BDNF is dependent of A2ARs, and if a simultaneous activation of CBRs is required to potentiate adult oligodendrogenesis, which will ultimately contribute to the development of alternative therapeutic targets for OL formation and remyelination.

Also, we are currently exploring how physical exercise-induced neurotrophic factors (BDNF, insulin growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF)) can potentiate adult oligodendrogenesis in vivo using the cuprizone model of demyelination.

MTU13-03 | A remyelination-promoting antibody modulates sphingolipid metabolism in microglial cells

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Strategies aimed at enhancing re-myelination efficiency represent a promising option for the cure of multiple sclerosis (MS).

Recombinant human IgM22 (rHlgM22) effectively promotes re-myelination in all mouse models of MS. Its effects, being still under characterization, seems to be mediated by the reorganization of membrane lipid microenvironment or relevant membrane receptors, thus affecting their downstream signalling. Indeed, cells belonging to the oligodendrocyte lineage (likely the main cellular target of rHlgM22), showed an altered sphingolipid (SL) metabolism upon rHlgM22 treatment. On the other hand, re-myelination may occur only after myelin debris clearance by microglia. Indeed, rHlgM22 increase phagocytic activity in microglial cells (Zorina, Y. et al., Sci Rep, (2018).

We hypothesize that rHlgM22 might act by modulating SL metabolism in microglia cells.

Since fewer information was present in literature about SL composition in microglia, we started to characterize the sphingolipid pattern of the microglial cell line BV-2. Using mass spectrometry analysis, we found a different ganglioside composition between adherent and floating cells, two co-existing subpopulations likely representing different phenotypes of microglia.

Subsequently we evaluated that a single dose 24 hrs treatment with rHlgM22 had different effects on sphingolipid synthesis in BV-2 cells. In detail, rHlgM22, respect to Human IgM used as the negative control, induced a marked decrease of ceramide, sphingomyelin, neutral glycosphingolipids, and gangliosides GM1, GM2, GD1a,



GD1b synthesis in adherent cells whereas it induced their increase in floating cells.

These results suggest that the effects of rHlgM22 in BV-2 cells could be mediated by an alteration in the plasma membrane sphingolipid composition of these cells. In fact, gangliosides are well known to interact with and modulate the activity of several membrane receptors (including PDGFR, likely implicated in the effect of rHlgM22). On the other hand, changes in ceramide levels are known to induce the formation of transient signalling platforms. 7ytfs3

MTU14-01 | Caveats to use of the NeuN antibody in formalin-fixed human infant brain tissue

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Neuronal nuclei (NeuN), a neuron-specific nuclear protein, is reported to be stably expressed in most postmitotic neurons of the vertebrate central nervous system. Some studies have interpreted a reduction in NeuN immunostaining to indicate a loss of cell viability, while others suggest that this could result from changes in antigenicity of the target epitope. Antigenicity can be greatly affected by methods of handling and storing tissue, such as the post-mortem interval or fixation method and its duration. Infant brain tissue is commonly only available for retrospective research purposes, where these parameters are not able to be controlled by the researcher. Pre-treatments to enhance immunoreactivity of target epitopes can be applied; however, this is not always to a level that is sufficient for cell quantification. Preliminary studies in our laboratory found low immunostaining for the NeuN antibody on formalin-fixed and paraffin-embedded (FFPE) human brain tissue. Therefore, we attempted to enhance the staining by altering various steps in our immunohistochemistry protocol. In parallel, we stained for NeuN in FFPE piglet brain tissue, where methodological parameters were tightly controlled, including those for tissue fixation and storage. Results showed that in FFPE human brain tissue, modified pre-treatments could not enhance NeuN immunostaining to a degree that was suitable for cell counting. By comparison, high levels of immunostaining were seen consistently in the piglet tissue. We conclude that processes used for tissue fixation and storage are responsible for reduced immunostaining and emphasize that a cautionary approach should be undertaken when interpreting outcomes of NeuN staining in archival human brain tissue.

MTU14-02 | Measuring amyloid- β dynamics in response to selective excitation and inhibition of glutamatergic and GABAergic signaling

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Amyloid- β (A β), the primary component of AD plaques, is known to be released into the ISF and increases during periods of increased synaptic activity. It is currently unclear which types of neurons are producing A β in vivo. The Cirrito Lab has developed a microimmuno-electrode (MIE), which is an antibody-based implantable electrode that can be used in awake and behaving mice to detect changes in protein levels (depending on the antibody) every minute for several hours. The MIE can detect A β 40 and A β 42 with at least 8000-fold selectivity over mouse A β in vivo. To parse out the neuronal source of amyloid generation under the endogenous amyloid precursor protein promoter, the APP-NLF/NLF knock-in mouse model was used. The model produces human A β with two mutations known to accelerate pathology in familial AD. Manipulating the activity of cells expressing transporters for either GABAergic or glutamatergic signaling was achieved by crossing the APP-NLF/NLF model with either a Vgat-Cre or Vglut2-Cre mouse; this provided the ability to specifically target inhibitory and excitatory signaling, respectively. To modulate signaling in vivo, Cre-dependent DREADD (Designer Receptors Exclusively Activated by Designer Drugs) G-protein receptors were expressed selectively in the hippocampus using a viral (AAV) vector. These receptors could activate (hM3Dq) or inhibit (hM4Di) the cell type selected by the Cre driver upon systemic administration of the inert compound clozapine-N-oxide. Our preliminary results demonstrate that A β 40 generation is potentiated by inhibition of VGAT expressing cells in the hippocampus. Activation of VGAT and VGlut2 neurons did not result in increased A β 40 release. A future direction will be to measure EEG activity under similar conditions to verify how network neuronal activity is modulated.

@RDHendrix on Twitter.

MTU15-01 | Modulation of BDNF By gabapentin, indomethacin and their low-dose combination in inflammatory adjuvant model of rat

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Background: Chronic inflammatory pain is a throbbing sensation that leads to physical and psychological disability. Therapies like NSAIDs, opioid analgesics, muscle relaxants, anxiolytics, and corticosteroids are

available with good potential in reducing chronic pain. However, these therapies failed to respond in advanced stages either due to pathway selectivity or loss of sensitivity. We hypothesize that the N-methyl-D-aspartic acid (NMDA) receptor coordinates with overexpressed brain-derived neurotrophic factor (BDNF). The over-expressed BDNF also activates nociceptors at peripheral nerve endings for an extended period to stimulate and sustain the inflammatory stimuli. Pain can be controlled by reducing the over-expressed BDNF.

Objective: We investigated the modulation of BDNF by gabapentin, indomethacin, and their low-dose combination on adjuvant-induced inflammatory arthritis (in vivo model of chronic pain).

Methodology: Mycobacterium tuberculosis H37Ra strain was used as an adjuvant which was injected into the tail base of the rats. Gabapentin (5 mg/kg), indomethacin (5 mg/kg) and gabapentin + indomethacin (1.5 mg/kg + 2.5 mg/kg) combination were treatment groups. Paw oedema and nociception were measured by plethysmometer and plantar apparatus, respectively. Nitric oxide, peroxide, and superoxide dismutase levels were measured to analyse the free radicals and antioxidants in different treatment groups.

Results: Low-dose combination shows a significant reduction of nitric oxide and peroxide concentrations. Immunohistochemistry and PCR data analysis demonstrated that the low dose combination has better potential in lowering the BDNF expression as compared to their monotherapy counterparts.

Conclusion: The present study shows that a low-dose combination has an additive impact on lowering chronic inflammatory pain. We anticipated that our study should be the starting point for more in-depth molecular studies to understand chronic inflammatory pain management.

MTU15-02 | Behavioral, neurodevelopmental and gut microbiome changes following gestational exposure to high housing temperature in rats

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Adverse effects of changing climate have been associated with increase average global temperature resulting in environmental changes. We set out to investigate effects of environmental stress due to increased heat exposure on developmental milestones, behavior, gut microbiota and neuroarchitecture in rat pups.

Pregnant rats were kept in standard temperature (ST) ($26 \pm 2^\circ\text{C}$; control) or high temperature (HT) ($40 \pm 2^\circ\text{C}$) housing. After parturition, a cohort of the HT group and their pups were moved to the ST (gestational-only exposed pup [GE]) while the other subset remained in the HT housing (gestational and postnatal exposed pups [GE+PE]). At different time points, we examined neurodevelopmental milestones and behavior in the pups. Changes in gut microbiota, neuroarchitecture, cytokine levels (TNF- α , IL-4, IL-10), SOD, MDA, expression of MBP, NeuN and GFAP were also determined.

We observed impaired positioning of paws, prolonged righting reflex, delayed ear opening and significant decreased body weight gain in HT pups when compared with control. We noted a significant difference in *Firmicutes* count between GE and GE+PE pups at P15. Furthermore, TNF- α , IL-4, IL-10 and MDA levels were increased in GE and GE+PE pups. There was also a reduction in MBP expression in HT pups.

Our results revealed a delay in neurodevelopmental milestones in pups exposed to high temperature during gestation and post natal life. Pups whose dam were exposed to HT during gestation also showed some set back but improved over the course of testing.

MTU15-03 | Association between cardiorespiratory fitness and metabolic syndrome

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Previous studies have shown that cardiorespiratory fitness can attenuate the risk of developing metabolic syndrome. This study aimed to evaluate the association between cardiorespiratory fitness and metabolic syndrome in Korean adults. A cross-sectional study was conducted using data obtained from the database of the Seoul National University Hospital Health Promotion Center from 1995 to 2008. A total of 43,369 participants aged 20 or older were obtained and underwent health screenings with fitness testing. The level of cardiorespiratory fitness was defined as low ($\text{VO}_{2\text{max}} \leq 21 \text{ mL/kg/min}$), moderate ($21 \text{ mL/kg/min} < \text{VO}_{2\text{max}} \leq 28 \text{ mL/kg/min}$), or high ($\text{VO}_{2\text{max}} > 28 \text{ mL/kg/min}$). Multivariate logistic analysis was conducted after adjusting the age, sex, smoking status, alcohol intake, physical activity, and family history of cardiovascular diseases in model 1 and for variable in model 1 plus fat ratio in model 2. In addition, a stratified analysis was conducted in both sexes and various age groups. Approximately 17.37% of the participants presented with metabolic syndrome, of which 20.94% were male and 12.55% were female. Among metabolic abnormalities, the prevalence of high blood pressure (47.77%) was the highest. In the multivariate logistic regression analysis, the adjusted prevalent odds ratios (adjusted pORs) of the participants with moderate and high cardiorespiratory fitness were 0.59 (95% CI: 0.55–0.63) and 0.47 (95% CI: 0.44–0.50) in model 1, 0.72 (95% CI: 0.67–0.77) and 0.70 (95% CI: 0.65–0.76) in model 2, respectively. A similar inverse associations were observed after stratification in both sexes and all age groups. A significant inverse association was observed between cardiorespiratory fitness and metabolic syndrome in Korean adults. The participants with high level of fitness were less likely to have metabolic syndrome. The results were clear and significant in both sexes and various age groups.

**MTU15-04 | Characterization of nuclear lamina alterations in astrocyte senescence**

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Cellular senescence is a hallmark of aging and age-related neurodegenerative diseases. In the central nervous system (CNS), senescent astrocytes exhibit a specific phenotype, including transcriptomic and morphological alterations. Lamin-B1, a component of nuclear lamina, has been shown to be reduced in senescent cells from several tissues. However, little is known about whether lamin-B1 levels and integrity of nuclear lamina change in senescent astrocytes in physiological aging models. Here, we investigated nuclear lamina alterations in an in vitro model of astrocyte senescence, based on a long-term astrocyte culture (30-35 DIV) compared to control cultures (7-10 DIV). We showed that senescent astrocytes present higher levels of senescence hallmarks compared to the control group, such as increased β -galactosidase activity, production of reactive oxygen species, and elevated immunolabeling for p16^{INK4a}. Furthermore, immunofluorescence and western-blotting assays revealed reduced lamin-B1 levels. We also detected significant morphological alteration in nuclear lamina such represented by increased invaginations, which led to disrupted nuclear circularity in senescent astrocytes in vitro. In contrast, we observed an upregulation of lamin-B2 levels, another lamin subtype which is homologue to lamin-B1, in in vitro senescent astrocytes, through immunofluorescence, western-blotting and qRT-PCR assays. Moreover, immunohistochemical assays demonstrated increased intensity of lamin-B2 in hippocampal dentate gyrus granular cell layer of elderly C57Bl6 male mice (≥ 18 months old) compared to their controls (young C57Bl6 male mice, 3 months old). Our data suggest that astrocytic senescent phenotype is accompanied by loss of lamin-B1, gain of lamin-B2 and altered nuclear morphology in aging. Support: CNPq, CAPES, FAPERJ, Ministério da Saúde.

MTU15-05 | GPR27-mediated increase in cytosolic L-lactate in 3T3 embryonic cells and astrocytes

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G-protein coupled receptors (GPCRs) consist of over 800 members; one third of these represent targets for approved drugs. Here we investigated GPR27 an orphan GPCR belonging to the family of super conserved receptor genes expressed in the brain (SREB), with unknown function. Cytosolic levels of L-lactate ([lactate]_i), the end-product of aerobic glycolysis were measured by the fluorescence

resonance energy transfer (FRET)-based nanosensor Laconic in single 3T3 embryonic cells and astrocytes upon application of 8353, a surrogate agonist of GPR27. CRISPR-Cas9 was used to generate 3T3 cells with knocked-out (KO) GPR27. To rescue the responses in GPR27KO3T3 cells we used the transfection of a GPR27-carrying plasmid into the GPR27KO3T3 cells. In 3T3 cells the application of 8353, a surrogate agonist, known to activate GPR27, resulted in an increase in [lactate]_i, the end-product of aerobic glycolysis. A similar increase was recorded in astrocytes, an abundant type of neuroglial cells providing homeostatic support to neuronal networks in the central nervous system, which express glycogen and enzymes of aerobic glycolysis. The 8353-induced increase in [lactate]_i, was reduced in GPR27KO3T3 cells in comparison to the wild type controls. However, the transfection of a GPR27-carrying plasmid into the GPR27KO3T3 cells rescued the 8353-induced increase in [lactate]_i. These results indicate that stimulation of GPR27 enhances aerobic glycolysis and L-lactate production in 3T3 cells and astrocytes. Moreover, in the absence of GPR27, resting [lactate]_i was reduced in 3T3 cells in comparison to controls, further supporting the view that GPR27 regulates L-lactate homeostasis in the absence of any stimulation.

MTU15-06 | Chronic vanadium neurotoxicity in mice: Oxidative stress and neuroinflammatory consequences

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Vanadium, an environmental pollutant, induces oxidative damage in the brain following acute exposure. However, prolonged exposure may produce severed destruction of the neurological system. This study was designed to ascertain neurodegenerative consequences of chronic vanadium administration and withdrawal from initial exposure. A total of 85, four weeks male BALB/c mice were used and randomly divided into three major groups of vanadium treated (sodium metavanadate 3 mg/kg, intraperitoneally (i.p), 0-18 months), matched controls and animals that were exposed to vanadium for 3 months and thereafter withdrawn from exposure. Mice at post-exposure were sacrificed at different time points. Metal profiling was done using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) and brain homogenates were subjected to antioxidant assays. Also, Brain sections were stained with haematoxylin and eosin, and immuno-histochemically probed to demonstrate microglia (IBA-1), astrocytes (GFAP), and neurons (Neu-N with DAPI) for nuclear morphology and cytological study. Vanadium exposure significantly increased the levels of oxidative stress markers with a concomitant decrease in activities of intrinsic antioxidant enzymes. Metal profiling revealed increasing vanadium absorption



with regional variabilities following chronic exposure, but the brains had diminishing residues of the metal after withdrawal. There was progressive disruption of layering pattern, degeneration, and necrosis in the prefrontal cortex, hippocampal pyramidal cells, and Purkinje cells of the cerebellum in vanadium-exposed brains. With prolonged exposure (15–18 months), the evident neuropathology was microgliosis, while progressive astrogliosis, was evident only till 12 months. All the observed oxidative effects and cellular changes were ameliorated but not completely restored after vanadium withdrawal. In conclusion, chronic vanadium administration in mice caused severe brain damage through oxidative stress and neuroinflammation. The metal load and neuropathological effects remained evident following vanadium withdrawal.

MTU15-07 | Arsenic induces differential neurotoxicity via an interdependent ER α , Wnt and EGFR pathway in male and female rats

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Arsenic contamination of groundwater has been reported worldwide, affecting around 150 million people. We previously reported that environmentally relevant concentrations of arsenic, a potent neurotoxicant, induced hippocampal neuronal damage and cognitive deficits in rats and effects were more in male and estrogen (E2)-deficient female rats compared to the normal and E2-replete ones. Our study indicated that arsenic reduced hippocampal neuronal estrogen receptor- α (ER α), and co-treatment (hippocampal insertion) with ER α agonist, PPT, offers protection against arsenic-induced neurotoxicity. ER α links with Wnt pathway of neuroprotection, and for current study we explored whether the latter played any role in discrepant effects of arsenic. We treated male and female rats with arsenic from postnatal day (PND)-60 to PND120. We performed Ovariectomy (OVX) in female rats and treated OVX rats with E2 for generating E2-deficient and E2-replete sets, respectively. Examining the effect on neuronal Wnt/ β -catenin pathway, revealed a biphasic effect of arsenic, marked by a reduced Wnt levels in the hippocampus until PND-90, followed by an increase until PND-120. Similarly, the levels of β -catenin, a key signaling molecule of canonical Wnt pathway, showed an increase until PND-90 followed by a decrease (PND-120) upon arsenic exposure. Arsenic-induced alteration in hippocampal Wnt/ β -catenin activation was significantly more in male and OVX females compared to normal and E2-replete rats and which then restored on treatment with PPT. Since growth factor signaling undergoes significant cross-talk with Wnt pathway, we screened for growth factor receptors upon arsenic treatment. We identified a reduction in hippocampal epidermal growth factor receptor (EGFR) activation, which when restored, using its ligands, resulted

in recovery of Wnt/ β -catenin pathway. We finally related the above findings with neurobehavior, and detected that modulator of Wnt/ β -catenin and EGFR, as well as ER α , reduced the arsenic-induced cognitive dysfunction, detected through Y-Maze tests of Learning-memory. Overall, our study suggests that adult males and E2-deficient may be more susceptible than normal (sufficient E2) females to arsenic-induced neurotoxicity, where a dysregulated hippocampal ER α , biphasic Wnt/ β -catenin and EGFR signaling plays a key role.

MTU15-08 | A novel method for isolation of spontaneously released small extracellular vesicles from mouse and human brain

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Extracellular vesicles (EVs) were considered for a long time cell waste disposers. However, evidence has demonstrated their relevance in cell-cell communication implicating them in essential physiological and pathological processes. Nonetheless, the importance of EVs for central nervous system homeostasis is still poorly understood, with recent studies suggesting a role for EVs in nervous system development and neuronal and synaptic homeostasis. Importantly, both clinical and experimental evidence highlight a pathological role for EVs in gliomas and neurodegenerative diseases, among other pathological conditions. Furthermore, EVs are considered potential disease biomarker carriers with great diagnostic and prognostic relevance in the new era of Precision Medicine, as EVs cargo reflects the status of the cell of origin and they cross the blood-brain barrier, being detected in biofluids (e.g., blood, CSF). Thus, the collection and characterization of physiologically relevant EVs are of the utmost importance. Currently, brain EV isolation methods rely on tissue dissociation, which might contaminate EV fractions with intracellular vesicles and other contaminants. Based on multiscale analytical platforms such as cryo-EM, label-free proteomics, advanced flow cytometry, and ExoView analyses, we hereby present an efficient purification method that captures a more physiologically relevant, sEVs-enriched population spontaneously released by mouse and human brain tissue. This spontaneous release



method may contribute to the characterization and biomarker profile of physiologically relevant brain-derived exosomes in brain function and pathology.

MTU15-10 | Cellular, synaptic, and behavioral effects of the anti-epileptic drug cenobamate (YKP3089)

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Antiepileptic medication is the mainstay of treatment for seizures, and adjunctive therapy is widely used to achieve adequate seizure control. Adjunctive treatment of the newly developed antiepileptic drug cenobamate (YKP3089) improved seizure control in patients with uncontrolled focal seizures. However, the behavioral and synaptic actions of cenobamate are unclear. Here, we show that cenobamate affects long-term memory and hippocampal synaptic

plasticity. A single administration of cenobamate attenuated novel object recognition and object location memory in mice. Cenobamate enhanced inhibitory postsynaptic potentials by prolonging inhibitory postsynaptic current (IPSC) decay without affecting presynaptic GABA release or the peak amplitude of IPSCs. In addition, cenobamate suppressed hippocampal excitatory synaptic transmission by reducing the excitability of Schaffer collaterals and interfered with the induction of long-term potentiation. A reduction in neuronal excitability induced by cenobamate was associated with an elevation of action potential (AP) threshold, and which progressively increased in later APs during repetitive firing, indicating the activity-dependent modulation of neuronal sodium currents. Cenobamate suppressed neuronal excitability under the condition that GABAergic neurotransmission is excitatory, and administration of cenobamate rapidly enhanced the phosphorylation of eukaryotic elongation factor 2 in the hippocampus of adult and neonatal mice. Collectively, these results suggested the mechanism underlying the antiepileptic action of cenobamate in which modulation of the sodium currents exerted a stronger influence on neuronal excitability than potentiation of the GABAergic postsynaptic response.

ABSTRACT

Poster Sessions Wednesday/Thursday

WTH01-01 | A possible pathogenic PSEN2 Gly56Ser mutation in a Korean patient with early-onset alzheimer's disease

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Early-onset Alzheimer's disease (EOAD) is characterized by the presence of neurological symptoms in patients with Alzheimer's disease (AD) before 65 years of age. Mutations in pathological genes, including amyloid protein precursor (APP), presenilin-1 (PSEN1), and presenilin-2 (PSEN2), were associated with EOAD. Seventy-six mutations in PSEN2 have been found around the world, which could affect the activity of γ -secretase in amyloid beta processing. Here, a heterozygous PSEN2 point mutation from G to A nucleotide change at position 166 (codon 56; c.166G>A, Gly56Ser) was identified in a 64-year-old Korean female with AD with progressive cognitive memory impairment for the 4 years prior to the hospital visit. Hippocampal atrophy was observed from magnetic resonance imaging-based neuroimaging analyses. Temporal and parietal cortex hypometabolism were identified using fluorodeoxyglucose positron emission tomography. This mutation was at the N-terminal portion of the presenilin 2 protein on the cytosolic side. Therefore, the serine substitution may have promoted AD pathogenesis by perturbing the mutation region through altered phosphorylation of presenilin. In silico analysis revealed that the mutation altered protein bulkiness with increased hydrophilicity and reduced flexibility of the mutated region of the protein. Structural changes were likely caused by intramolecular interactions between serine and other residues, which may have affected APP processing. The functional study will clarify the pathogenicity of the mutation in the future.

WTH 02-01 | Respiratory Neuron Identification using Cell-type specific expression by channelrhodopsin-2 in vivo

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Breathing is a behavior that requires complex patterns of neuronal activity, driving respiratory muscles contraction. The region in the ventrolateral medulla, which is kernel of the respiratory rhythm generation is the pre-Bötzinger Complex (preBötC). The glutamatergic neurons in the preBötC are crucial for respiratory rhythm generation and inhibitory interaction between other brainstem regions (i.e. Bötzinger complex) and the preBötC causes the breathing phases. Here, we analyzed the function of inhibitory neurons within the respiratory network by in vivo electrophysiology and optogenetic techniques. To express Channelrhodopsin-2 in glycinergic neurons we crossbreed Tg (Slc6a5icre) 121Veul with 129S-Gt (ROSA) 26 Sortm32 (CAG-COP4*H134R/EYFP)Hze/J mice. Adult mice were anesthetized by ketamine, Medetomidine and Lidocaine and the breathing was recorded by the piezo sensor located underneath the animal. For simultaneous juxtacellular recording and optogenetic activation we used a special glass pipette holder (Optopatcher). The Optopatcher was connected to a 450-460nm high-power LED. The tip of the capillary coated with black nail polish, which restricts optogenetic activation to the recorded neuron. By navigating the recording electrode with the robot stereotaxic instrument, we searched for respiratory rhythmic neurons. Cycle triggered averaging revealed that glycinergic neurons were among the population of inspiratory preBötC-neurons.

To conclude, respiratory neurons mapping in terms of their neurotransmitter-type can be done by our method. Additionally, according to our data, juxtacellular recordings and identification of respiratory neurons optogenetically using the single automated stereotaxic arm is feasible by the optopatcher pipette holder.

**WTH 02-02 - Bolaji Oyenike Oyetayo | Glutamate-dependent increase in DNA Methylation in Cultured Bergmann Glia Cells**

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Glutamate (Glu) is the major excitatory neurotransmitter in the vertebrate Central Nervous System (CNS). Glu plays an important role in CNS development and function. The release of Glu is followed by an immediate uptake activity by glial Glu transporters (GLAST and Glt-1). A tight regulation of the uptake activity is needed to avoid an over-stimulation of extra-synaptic glutamate receptors, that has been linked to excitotoxic insults. Currently, an increasing number of the so-called neurodegenerative diseases are associated with glutamatergic system dysregulation.

Glutamate transporters are regulated at different levels: transcription of its gene(s), translation of its mRNA(s) or, insertion of the protein to the plasma membrane, transporter phosphorylation, phosphorylation of accessory proteins. Cerebellar Bergmann glia express the GLAST subtype of Glu transporters and its activity has proven to be fundamental for the function of the Parallel fiber-Purkinje cell synapses that these cells wrap completely. An exquisite biochemical coupling between Bergmann glia and both granule and Purkinje cells is needed for Glu recycling as well as for energy supply in a bona fide tripartite synapse. Dynamic DNA methylation is a critical regulatory epigenetic mechanism in the CNS that recently has been documented to play a major role in neuronal plasticity, and that is modified in a number of diseases.

WTH 02-03 | BDNF upregulates the synaptic expression of NMDA receptors in hippocampal neurons by a mechanism dependent on local translation

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The effects of brain-derived neurotrophic factor (BDNF) in long-term synaptic potentiation (LTP) are partly mediated by local protein synthesis. However, BDNF-induced alterations in the synaptic proteome coupled to synaptic strengthening are poorly understood. In this work we investigated the effects of BDNF on the synaptic accumulation of GluN2A- and GluN2B-containing NMDA receptors (NMDAR) in cultured hippocampal neurons, using immunocytochemistry and live staining of the surface receptors with antibodies against extracellular epitopes. Activity of synaptic NMDA receptors was assessed by measuring the receptor mediated mEPSC.

Incubation of hippocampal neurons with BDNF increased the amplitude of NMDA receptor mediated mEPSC, and this effect was correlated with the synaptic accumulation of GluN2A- and GluN2B-containing NMDA receptors. However, the effects on the two groups of subunits followed a distinct kinetics. Using the FUNCAT-PLA method we found that BDNF induces the local synthesis of the tyrosine kinase Pyk2 at the synapse, which was found to mediate the effects of the neurotrophin on the surface expression of GluN2B-containing NMDA receptors. The effects of BDNF on the surface expression of GluN2A were only partly mediated by Pyk2. In addition, the upregulation in synaptic GluN2A and GluN2B-containing NMDA receptors was correlated with an increase in their stability in this compartment, as determined by single particle quantum dot imaging. Finally, the activation of Pyk2 downstream of TrkB receptors was blocked by PKC inhibition, as determined by western blot with an antibody against the phosphorylated form of Pyk2. The results show a key role for Pyk2 synthesis at the synapse as a mediator of the effects of BDNF on the synaptic distribution of NMDA receptors, which may have an impact on LTP. (Supported by FCT, Portugal)

WTH 02-04 | Desensitization of Mu-opioid receptors in the spinal cord dorsal horn is reduced by endogenous TRPV1 agonist

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Activation of opioid receptors exerts inhibitory control of nociceptive transmission throughout the afferent pain pathway inducing analgesia. This is also strongly evident at central terminals of primary afferent neurons in the spinal cord dorsal horn (SCDH). At these presynaptic endings of the first nociceptive synapse, opioid receptors co-express with the transient receptor potential vanilloid type 1 (TRPV1) channel, known for its role in the development of thermal and mechanical hyperalgesia during pathological states. Our study focused on the interaction of TRPV1 and μ -opioid receptor (MOR) in agonist-induced desensitization of MOR. We used whole-cell patch-clamp recordings of miniature excitatory postsynaptic currents (mEPSC) from SCDH neurons in rat spinal cord slices and behavioral measurement of the mechanical paw withdrawal threshold and thermal plantar test. Application of MOR agonist DAMGO (1 μ M, 3 min) robustly depressed mEPSC frequency to $62.3 \pm 3.6\%$. To induce MOR desensitization, we incubated slices with DAMGO for 2h. Acute application of DAMGO in slices after the incubation failed to depress the mEPSC frequency. In experiments where endogenous TRPV1 agonist N-oleoyldopamine (OLDA) was added with DAMGO during the incubation, MOR desensitization was prevented and short DAMGO application evoked a decrease of mEPSC frequency to $66.94 \pm 9.6\%$. In slices from animals after chronic constriction injury (CCI) model of neuropathic pain, DAMGO application induced reduced inhibition compared to the controls. Furthermore,



the effect of TRPV1 activation on CC-chemokine ligand 2 (CCL2) induced modulation of MOR function was also studied. Our data suggest that activation of TRPV1-mediated pathways by endogenous agonist in the SCDH may interact with MOR function, reduce MOR desensitization and promote the efficacy of opioid-induced analgesia. Further study of the underlying mechanisms could contribute to improved opioid-mediated analgesia in patients. Supported by GACR 20-19136S.

WTH 02-05 | MIF inhibitor (ISO-1) reduces pain hypersensitivity in a model of peripheral neuropathy

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Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine, and its genetic deletion prevents the development of hypersensitivity induced by peripheral nerve injury or inflammation. MIF exerts its biological functions mainly through binding to the putative membrane receptor, CD74. MIF tautomerase inhibitor (S,R)-3(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1) binds to the MIF active site and blocks the MIF-CD74 binding. Our aim was to examine whether systemic treatment with ISO-1 alleviates the hypersensitivity induced by peripheral neuropathy and modulates synaptic transmission at the spinal cord level. Peripheral neuropathy was induced by chronic constriction injury (CCI) of the sciatic nerve. Electronic Von Frey test to assess mechanical sensitivity and patch-clamp recordings of excitatory and inhibitory postsynaptic currents (EPSCs, IPSCs) from superficial dorsal horn neurons in mouse spinal cord slices were used. Inhibitory currents were evoked by optical stimulation of channelrhodopsin containing inhibitory neurons. Mechanical allodynia induced by CCI was prevented with the ISO-1 treatment. CCI also significantly increased the spontaneous sEPSCs frequency and decreased the amplitude of the light-evoked IPSCs in excitatory neurons. The systemic ISO-1 treatment largely diminished these pathological effects on nociceptive synaptic transmission induced by the CCI. Our results suggest that ISO-1 treatment reduces the development of hypersensitivity after nerve injury. One of the underlying mechanisms of the ISO-1 effect could be balancing the CCI-induced changes of excitatory and inhibitory synaptic transmission in the spinal cord dorsal horn. Previous work from our group has demonstrated that Bergmann glia Glu exposure results in a differential DNA methylation. A decrease in DNA methylation alters GLAST expression and uptake activity. Using the well-established model of chick cerebellar Bergmann glia cells (BGC), in this contribution we explored the signaling cascades involved in Glu-mediated changes in methyl cytosine content. A time

and dose-dependent effect in dynamic DNA methylation was found. The involvement of both Glu receptor and transporters was evident.

WTH 02-06 | Progesterone and allopregnanolone attenuates the effects of cocaine on dopamine release in the NAc and hypermobility in rats

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Currently, there is no effective drug to treat cocaine use disorders, which affect millions of people worldwide. Neurosteroids, such as progesterone and allopregnanolone are potential therapeutic candidates since microdialysis and voltammetry studies have shown that they can decrease dopamine release in the nucleus accumbens of rodents. Dopamine regulates fundamental aspects in the disbalance of chronic exposure to cocaine in the central nervous system such as, motivation, learning and plasticity. So, the attenuation of the rapid actions of cocaine administration in the dopaminergic system could be a promising pharmacological strategy. We tested whether the systemic administration of 30 mg/kg of progesterone or 10 mg/kg of allopregnanolone could block the effects of 20 mg/kg cocaine on electrically evoked dopamine release in the nucleus accumbens in adult male and female Wistar rats measured by fast-scan cyclic voltammetry. Also, we tested if these neurosteroids could reduce the hypermobility and stereotypy produced by cocaine. Our results demonstrate that progesterone decrease basal accumbal dopamine release with a stronger effect in females compared with males. Furthermore, we found that previous administration of progesterone and allopregnanolone attenuates the high and medium mobility increased by cocaine administration. Our results suggest that positive GABA-A receptors must attenuates the disbalance on dopaminergic dysregulation produced by cocaine.

WTH03-01 | Hippocampal-dependent spatial memory impairment is associated with neuroinflammation after mild traumatic brain injury in mice

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Traumatic brain injury (TBI) is a major public health concern. Cognitive deficits are well known sequelae of TBI. However, the pathophysiology of TBI-associated cognitive deficits remains to be further explored. Emerging evidence supports the hypothesis that TBI-associated cognitive decline might be inflammatory based. Herein, we aim to investigate the role of inflammatory response in cognitive impairment secondary to mild TBI (mTBI). The study was conducted according to the Ethics Committee on Animal Experimentation of UFMG (250/2017). C57BL/6 mice were submitted to a weight-drop or a sham injury. The open field was employed to evaluate locomotor activity at 6, 24, 72h, and 30days after mTBI. Barnes-Maze test was performed to assess hippocampal-dependent spatial memory at 72h and 30days after mild TBI. In parallel with cognitive analysis, levels of inflammatory mediators (IL-1 β , IL-6, IL-10, IL12p70, IFN, TNF, MCP-1, KC, CX3CL1) and neurotrophic factors (BDNF, GDNF, NGF) were analyzed at 6, 24, 72h, and 30days after mTBI in the ipsilateral and contralateral hippocampus and prefrontal cortex, by ELISA and CBA approaches. We used the principal components analysis (PCA) and the generalized additive models (GAM) for global comprehension of inflammatory mediators and neurotrophic factors. TBI-mice presented a locomotor impairment at 6- and 24-hours post-injury, compared to sham ($p < 0.05$). Also, we found a hippocampal-dependent spatial memory impairment at 72h and 30days after mTBI, compared to sham ($p < 0.05$). The PCA and GAM analysis revealed that two PCs represent 59.1 % of the variance in data and PC1 (cytokines and chemokines) and PC2 (neurotrophic factors) has a different pattern of expression. Our results suggest that neuroinflammatory response may underlie mTBI-associated cognitive deficits.

WTH03-02 | Social isolation impact on the development of neuropsychiatric disorders and neuroinflammation—an aging study in a mice model

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Social isolation is classified as a chronic mild stressor, which can lead to the development of neuropsychiatric disorders, including anxiety and depression. Chronic stress can induce an activation of neuroinflammatory mediators, being this molecular response also linked with depressive disorders. NLRP3 inflammasome activation integrates the stress-associated signals and the consequent release of cytokine IL-1 β is associated with a higher risk of depression. Currently, there is a knowledge gap regarding glial cells and neuroinflammatory alterations induced by social isolation.

Aim: To explore the effects of isolation regarding neuroinflammation in a rodent model.

Methods: Young and Aged C57BL7/J male mice were studied and divided into two groups: Group housed (GH) and socially isolated (SI) for 3 weeks. Depression and anxiety were evaluated through Force Swim Test (FST) and Open Field (OF), respectively. Morris Water Maze (MWM) was performed to assess spatial memory. Molecular approaches were performed to evaluate glial cell and neuroinflammatory markers in hippocampus region.

Results: Aged-SI mice shown a depressive and anxiety-like behavior during FST and OFT, respectively. Moreover, social isolation induced spatial memory impairment in Aged mice. Microglia activation analyses demonstrated an increase of Iba-1 expression in Aged-SI group and a decrease of anti-inflammatory microglia marker (Ym1) in Young-SI. Also, socially isolated groups shown a higher NLRP3 protein expression. Pro-inflammatory cytokines levels were higher in Aged groups.

Conclusions: Depressive- and anxiety-like behavior and cognitive impairment was observed as well as a neuroinflammatory response due to social isolation in Aged mice.

WTH03-03 | Anti-neuroinflammation of black seed oil against D-galactose- and lead-induced Alzheimer-like disease model in rats

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Alzheimer's disease (AD) has been reported as the most prevalent neurodegenerative disease affecting elderly people and



worsening the global economic burden related to aging society. However, *Nigella sativa* (NS) seeds and its derivative, have been used as a spice, food preservative, and as well as a protective and curative medication for neurodegenerative disorders like Alzheimer's due to its anti-inflammatory, anti-oxidative and neuro-protective effects. Hence, this propose study is aimed to evaluate anti-neuroinflammatory effects of black seed oil (BSO) against D-galactose- and Lead-induced Alzheimer-like disease model in rats. Fifty (30) rats will be randomly divided in to five groups ($n = 6$) and administered with lead and D-galactose, and after 30 min, followed by treatment with BSO for 6 weeks. Neurobehavioral studies will be carried out using Eight-arm radial water maze test (8-ARWMT) and object recognition paradigms to assess learning and memory. The levels of amyloid beta ($A\beta_{42}$), Tumor Necrosis Factor Alpha (TNF- α), Interleukin 1 Beta (IL-1 β), Interleukin-10, nuclear factor-kappa B (NF- κ B), nuclear factor E2-related factor 2 (Nrf2) and glutathione (GSH) using the respective Enzyme-linked Immunosorbent Assay (ELISA) kits. Expression of $A\beta$, growth-associated binding protein 43 (GAP-32), Synaptophysin (SYP) and neuron-specific nuclear protein (NeuN), using immunohistochemical methods. The results obtained from: cognitive functions; levels of $A\beta_{42}$, TNF- α , IL-1 β , Interleukin-10, NF- κ B, Nrf2 and GSH; and expression of $A\beta$, GAP-32, SYP and NeuN, could suggest that BSO therapy might recover memory loss through new neural circuits in Alzheimer-like model rats, possibly by regulating amyloid beta production and inhibiting neuroinflammation.

WTH03-04 | Extracellular DJ-1 plays a key role in the induction of sterile inflammation after ischemic stroke

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Objective: Post-ischemic inflammation plays a pivotal role in the progression of ischemic stroke pathologies. However, the detailed molecular mechanisms underlying the activation of infiltrating immune cells which trigger sterile post-ischemic inflammation have not been sufficiently clarified. We tried to identify the previously unknown damage-associated molecular patterns (DAMPs), inflammatogenic self-molecules derived from damaged tissue.

Methods: Among the candidate proteins which were detected from brain lysate by mass spectrometry, recombinant proteins were generated and added to the culture of bone marrow-derived macrophages (BMMs) to examine the expression of inflammatory cytokines. To determine the important peptide sequence for DAMP activity, deletion mutant peptides were generated. We finally examined the extracellular release of candidate DAMPs by using a mouse model of transient middle cerebral artery occlusion (MCAO). Neutralizing

antibodies or KO mice were used for compromising DAMP activity in ischemic stroke.

Results: We successfully identified DJ-1 (Also known as Park7) as a novel DAMP in brain lysate. Recombinant DJ-1 protein activated BMMs only through TLR2 and TLR4. The expression of inflammatory cytokines was induced in a DJ-1 dose-dependent manner in vitro. DJ-1 had a unique peptide sequence (aG-aH helix), which was not related to its antioxidant activity, to trigger the production of inflammatory cytokines.

In the ischemic brain, we observed the induction of DJ-1 expression within only ischemic neuronal cells 6–12h after stroke onset. Twenty-four hours after stroke onset, DJ-1 was passively released into extracellular space from necrotic brain cells and directly contacted with the surface of infiltrating myeloid cells. DJ-1 deficiency significantly reduced the expression of inflammatory cytokines after the stroke. Administration of DJ-1-neutralizing antibody suppressed the expression of inflammatory cytokines and reduced the infarct volume and improved neurological deficits.

Conclusion: DJ-1 was released into extracellular space in the ischemic brain and functioned as the previously unknown DAMP that directly activated infiltrating myeloid cells and induced sterile inflammation. Thus, extracellular DJ-1 would be a prominent therapeutic target for ischemic stroke.

WTH03-05 | Dietary inclusion of *bryophyllum pinnatum* modulates markers of inflammation and neurodegeneration in ulcerative colitis rats brain

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Ulcerative colitis (UC) is a chronic inflammatory disease that damages the colonic mucosa, and further potentiates other complications. Neurodegeneration is suspected to be one of the complications of UC after the identification of vagus nerve as an integral circuit in the gut-brain axis. *Bryophyllum pinnatum* (BP) is an edible polyphenol rich medicinal plant which are responsible for various pharmacological effects such as treating inflammation and Alzheimers disease as reported in traditional medicine. Dietary approach has been suggested a possible alternative natural treatment strategy for the management of UC and neurodegeneration (its attendant complications). In this study, the protective effect of dietary supplemented BP were investigated on C-Reactive protein (CRP) and inflammatory cytokines (TNF α , NF κ B, IL-6, and IL-10) contents as well as acetylcholinesterase (AChE), Butyrylcholinesterase (BChE), adenosine deaminase (ADA), and myeloperoxidase (MPO) activities in the brain of acetic acid-induced UC rats. Thirty-five (35) male Wistar rats were used and divided into five groups ($n = 7$). Group 1-normal control rats; Group 2-UC rats; Group 3- UC rats + sulfasalazine; Group



4-UC rats + 10% BP; Group 5-UC rats + 15% BP. After 21 days of experiment comprising of acclimatization, dietary manipulation, pre-treatment with BP supplemented diet, the animals were sacrificed and the rats' brain and blood were collected for subsequent analysis.

The results demonstrated that the elevated CRP, TNF- α , NF- κ B, and IL-6 contents and AChE, BChE, ADA, and MPO activities in the UCs rats were significantly reduced when compared with the control rats. This study demonstrated the neuroprotective property of BP as typified by the modulation of inflammatory markers, as well as cholinergic and non-cholinergic enzymes linked with neurodegeneration in UC state.

WTH03-06 | Therapeutic inhibition of HMGB1 suppress seizures and ameliorates memory impairment in adult zebrafish

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The mechanism of seizure generation in epilepsy is not well understood, however epilepsy affects 70 million population throughout the globe. In recent years, HMGB1 has been implicated in the mechanism of seizure generation mainly via interacting with its principal receptors TLR4 and RAGE. HMGB1 targeting strategies has gained much attention against neuroinflammation mediated pathologies, however, anti-HMGB1 monoclonal antibody (mAb) and Glycyrrhizin are the mostly used one to inhibit HMGB1 function and release. In the second hit PTZ-induced chronic seizure model in adult zebrafish, pre-treatment with anti-HMGB1 mAb (1, 2.5, and 5 mg/kg, i.p.) and Glycyrrhizin (25, 50, and 100 mg/kg, i.p.) suppressed seizures and related memory impairment as evident by the decrease in the seizure scores and increase in the inflection ratio at T maze test respectively. Moreover, anti-HMGB1 mAb and Glycyrrhizin pre-treatment modulates the concentration of neurotransmitters implicated in epilepsy (GABA, Glutamate). Upregulation in the mRNA expression level of neuroinflammatory genes (HMGB1, TLR4, NF- κ B, and TNF- α) in the second hit PTZ group were downregulated upon pre-treatment with anti-HMGB1 mAb Glycyrrhizin reflecting its anti-neuroinflammatory potential. Our findings indicate anti-HMGB1mAb, and Glycyrrhizin pre-treatment attenuates second hit PTZ induced seizures, ameliorates related cognitive impairment, and downregulates the seizure induced upregulation of inflammatory markers which possibly protect the zebrafish from the incidence of further seizures through mainly via modulation of neuroinflammatory pathway.

WTH03-07 | Receptor for advanced glycation end products mediates LPS-induced chronic neuroinflammation in the substantia nigra

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The receptor for advanced glycation end products (RAGE) is a protein of the immunoglobulin superfamily capable of regulating inflammation through interaction with its ligands. Considering the role of this receptor in the initiation and establishment of neuroinflammation, and the lack of investigations addressing its function in the maintenance of this condition, the present study describes the effects of RAGE inhibition in the brain through an intranasal treatment with its antagonist FPS-ZM1, in an animal model of chronic neuroinflammation induced by an acute intraperitoneal injection of lipopolysaccharide (LPS). Seventy days after LPS administration (2 mg/kg, i.p.), the rats used in the study received intranasally, over 14 days, 1.2 mg of FPS-ZM1. The animals were perfused and euthanized on day 90 after the intraperitoneal injection of the compound, having their brains extracted for immunofluorescence analysis. Our results indicate that RAGE encephalic blockade attenuates—in the substantia nigra—the LPS-induced chronic neuroinflammation, marked by reduced levels of gliosis markers, RAGE's ligands, and phosphorylated nuclear factor kappa-B. Additionally, the treatment also reverses the high levels of S100 calcium-binding protein B in the cerebrospinal fluid and the cognitive-behavioral deficits promoted by the model. In summary, this work demonstrates the prominent role of RAGE in the maintenance of a chronic neuroinflammatory state, pointing to possible future RAGE-based therapeutic approaches to treat diseases characterized by this condition.

WTH03-08 | Effects on parvalbumin neurons and perineuronal nets in Alzheimer's disease mouse models are not mediated by C5a-C5aR1 signaling

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Perineuronal nets (PNNs), an extracellular matrix structure, primarily surround parvalbumin inhibitory (PV) neurons. Together, PVs and PNNs are critical for proper long-term memory. Loss of PNNs and PV neurons has been demonstrated in both Alzheimer's disease (AD)



and animal models of AD and shown to be microglial dependent in mice. The complement (C') system, a critical component of the innate immune system, is activated in the context of AD pathology, and genetic ablation or pharmacologic inhibition of the complement receptor, C5aR1, has been demonstrated to provide protection from gliosis in animal models. The objective of this study was to determine if PV expressing interneurons and PV neurons surrounded by perineuronal nets (PV+ PNNs) are reduced in the Arctic (Arc) and Tg2576 mouse models of AD and if these reductions are rescued by inhibition of C5a-C5aR1 signaling. Briefly, 30 μ m coronal brain sections were obtained from 10-month Arc and Arc C5aR1 knock-out mice and 15-month Tg2576 mice previously treated with the C5aR1 inhibitor, PMX205, for 12 weeks. Using immunohistochemistry, the tissue was stained for PV and PNNs and quantified using Imaris. While no reductions in PV interneurons were found between any of the genotypes, significant reduction in the percentage of PV+ PNNs in both the cortex and hippocampus in Arc versus WT animals was observed. This reduction was not rescued by genetic deletion of C5aR1. No significant loss of PV interneurons nor the percentage of PNN+ PV neurons was observed in Tg2576 relative to WT regardless of PMX205 treatment. These results indicate that C5a-C5aR1 signaling does not contribute to PNN loss in the Arc mice model. Our results also indicate there are differences among the mouse models in damage to PNNs and PV interneurons, providing opportunities to dissect pathways that lead to PNN loss.

WTH03-09 | Analysis of motor and anxiety-like behavior and autoantibody binding after passive transfer of IgG from SPS patients into mice

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Stiff Person Syndrome (SPS) is a rare autoimmune disease of the central nervous system (CNS). The more severe form is called progressive encephalomyelitis with rigidity and myoclonus (PERM). About 20 % of SPS patients and 50 % of PERM patients have autoantibodies (aAbs) against the glycine receptor (GlyR). The GlyR is a synaptic target protein, which plays an important role in inhibitory signal transmission. aAb binding blocks the GlyR, which impairs its ion channel function, leading to decreased inhibitory signaling. For many patients, this results in a phenotype presenting with stiffness and high muscle tone. Additionally, some patients show strong startle responses and task-specific phobias.

So far, the pathomechanism of glycine receptor aAb associated SPS has mainly been studied *in vitro*, while *in vivo* models have only rarely been used.

Using sera from patients with GlyR aAbs, we detected specific GlyR aAb binding in cell-based and tissue-based assays. In murine

brain and spinal cord sections, specific binding was located in various areas of the CNS, mainly in the spinal cord and brainstem. First analysis of aAb binding in tissue-based assays suggests an association between the aAbs' binding patterns and the patients' specific symptoms.

To further elucidate the underlying pathomechanism of the patients' symptoms, passive transfer experiments addressing possible effects of GlyR aAb injections on motor and anxiety-like behavior are ongoing. Mice are injected with purified IgG from GlyR aAb positive patients on six consecutive days. Changes in motor and anxiety-like behavior are investigated using Rotarod, Catwalk, Open Field, and Elevated Plus Maze before and after injections.

Analysis of post-mortem immunohistochemical stainings are expected to reveal binding patterns similar to the ones previously detected and will unravel the connection between the location of aAb binding and the animals' symptoms evoked by GlyR aAb passive transfer.

WTH03-10 | An in-vitro oxygen-glucose-deprivation model to investigate the challenges of implant-based drug delivery for human glia cells

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Diseases of the brain have high mortality rates and their treatment is oftentimes difficult. Local administration of drugs from brain implants could improve bioavailability and decrease side effects of therapeutics, because they allow higher on-site concentrations. However, implantation of a material into the brain itself causes an injury, loss of perfusion, thus leading to hypoxia and inflammation in the surrounding tissue. Subsequent glial foreign body reaction may severely impair brain integrity and drug release.

We have established an *in-vitro* oxygen-glucose-deprivation (OGD) model for human astrocytes and microglia cells to investigate the impacts of hypoxia and inflammation on glia cells. Furthermore, we used resveratrol, a natural phenol produced by plants, to protect the glia cells against the effects of OGD.

In our studies, we showed an increased production of the pro-inflammatory cytokines IL6 and IL1 β in microglia cells and we found higher levels of Galectin-3, a marker for activated astrocytes in the OGD-treated samples. However, production turned back to baseline when resveratrol was added. Also, resveratrol protected astrocytes from changing to an apoptotic state under OGD conditions and production of hypoxia inducible factor 1 α was reduced.

For both types of glia cells, we found increased levels of reactive oxygen species (ROS) in the OGD-treated samples. ROS are correlated to adverse pro-inflammatory processes in tissue and, again, this effect was diminished upon addition of resveratrol.



In conclusion, we established a model to deepen the understanding of processes following implantation into the brain. Furthermore, with resveratrol we identified a compound that can milden the harmful effects of implantation and thereby facilitate a local, more effective and better-tolerated treatment for patients with brain diseases.

WTH03-11 | Hypothalamic inflammation induced by high-fat high sugar diet is a gender-specific, reversible process

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Hypothalamic inflammation was previously shown in diet-induced obese and diabetic models, namely with reactive gliosis that promotes hypothalamic insulin resistance. It remains unknown whether long-term hypothalamic inflammation induced by obesogenic diets is an irreversible process. In this study, we aimed at evaluating sex-specific hypothalamic neuroinflammation, gliosis and their reversibility in mice fed a fat- and sugar-rich diet.

Mice were exposed to 60%-fat diet and 20% (w/v) sucrose in drinking water (HFHSD) for 3 days, 24 weeks, or 16 weeks followed by 8 weeks of diet normalization (reverse diet group, RD). Control mice received a composition-matched 10%-fat diet for 3 days or 24 weeks. Expression of pro-inflammatory (IL-1 β , IL-6, IL-18, IFN- γ , TGF- β , and TNF- α), anti-inflammatory (IL-10 and IL-13) hypothalamic cytokines and inflammatory cell markers (IBA1, CD68, GFAP and EMR1) was evaluated by RT-qPCR. Hypothalamic microglia (IBA-1+/CD11b+ cells) and astrocytes (GFAP+ cells) were analyzed by immunofluorescence confocal microscopy.

Female mice presented an increase in pro-inflammatory IL-6 while male mice displayed a decrease in anti-inflammatory IL-10 after 3 days on HFHSD. Both genders evidenced lower IBA-1 and GFAP mRNA levels than controls (CD).

Female mice fed HFHSD for 24 weeks presented a generalized increase in pro-inflammatory cytokines mRNA levels, while RD females showed similar levels to CD. On the other hand, male mice fed HFHSD showed a decrease in pro-inflammatory cytokines mRNA levels that were reverted to control levels after diet normalization. Both sexes show a decrease in anti-inflammatory cytokine mRNA levels but only female mice showed reversibility to control levels upon diet normalization. Both sexes presented HFHSD-induced microgliosis and astrogliosis that was not irreversible by diet normalization.

We conclude that HFHSD-fed mice display reversible inflammatory profiles that are sex-specific, despite irreversible gliosis in the hypothalamus.

WTH03-13 | Restoring heart failure-induced long-term memory impairment by targeting the cystic fibrosis transmembrane regulator

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Over 64 million people worldwide live with heart failure (HF). Despite improved acute phase management, long-term consequences that include HF-associated cognitive impairment increasingly impact patients' well-being. The shortage of effective therapies for HF-associated cognitive decline can largely be explained by the lack of mechanistic insight. We previously identified impaired cystic fibrosis transmembrane regulator (CFTR) expression as critical contributor to cerebral perfusion deficits associated with structural and functional alterations in HF brains.

In a murine model of HF, reduced hippocampal neuronal dendrite length and spine density coincide with an apparent reduction of neuronal CFTR expression. Co-occurrence of hippocampal microglia activation and elevated interleukin (IL)-1 β and IL-18 transcripts led us to investigate the link between inflammation and neuron-specific CFTR expression. Conditioned media from lipopolysaccharide-stimulated microglia (LCM) profoundly reduces neuronal cell CFTR protein and PSD-95 mRNA expression, as measured by western blot and qPCR. Acutely inhibiting CFTR channel activity (10 μ M CFTR(inh)-172) mediates a significant downregulation of PSD-95 also in the absence of LCM. CFTR corrector treatment (13 μ M Lumacaftor) increases CFTR expression on the cell surface of CFTR⁺ neuronal cells, and corrects both, the LCM-mediated overall CFTR protein reduction and PSD-95 mRNA down-regulation.

Collectively, these results suggest that cytokines released from activated microglia can mediate CFTR downregulation in neurons and that the loss of CFTR in neurons is detrimental to their health. *In vivo*, pharmacological CFTR correction in HF mice normalizes hippocampal neuron CFTR expression associating with improved memory function and alleviation of HF-induced reduction in hippocampal neuron dendrite length and dendritic spine density.

In conclusion, these results suggest that adequate neuronal CFTR expression is critical for maintaining neuronal integrity and that pharmacological correction of CFTR protects neuronal structure and preserves memory function during HF.



WTH03-14 | Embryonic LPS-administration induces developmental neurotoxicity via HDAC inhibitor-like neuronal alteration

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In recent years, patients with autism spectrum disorders (ASD) or mental disorders have been on the rise, and the cerebellum is one of the pathological focal points in early ASD. We have investigated cerebellar degeneration using ASD-model rats treated with sodium valproate (VPA) and have confirmed malformation of Purkinje cells and hyperplasia between the V and VI lobules of the vermis. The effects of VPA would depend on the inhibition of HDACs. In this report, we report developmental neurotoxicity of Lipopolysaccharide (LPS); LPS, material in the outer membrane of Gram-negative bacteria, is a pro-inflammatory factor via binding to Toll-like receptor 4 and may cause mental disorders. Inflammation and neurodegenerative diseases have been linked, as studies have shown that LPS levels are elevated in the brain of Alzheimer's disease. LPS-administration is one of the regular models of neuronal cell death or disease. We administered 100 µg/kg of LPS to pregnant rats on day 16 of gestation and observed the cerebellum of pups at 7 and 14 days after birth (P7, P14, respectively). We report that LPS-administration to embryonic animals showed a similar symptom of VPA-models in the early phase and progressed neuronal cell death gradually. Rats treated with PBS were used as a vehicle. In LPS-exposed rats at P7, we observed earlier and more increased NeuN⁺ developed cells than vehicles, similar to VPA-model pups. During P14, a decrease in Purkinje cells and excessive folding of cerebellar V and VI lobules were observed in LPS-exposed rats. Alteration in internal immunity due to infection or antibiotics is associated with mental disorders. We suggest that LPS would induce neurodevelopmental alteration due to immune malformation and eventually neuronal cell death.

WTH03-15 | Illustrating synergistic mechanism of herbal compound-formula in targeting neuroinflammation

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Chronic neuroinflammation is an important therapeutic target for neuronal degeneration and death. However, therapeutic agents that are effective in reducing neuroinflammation are lacking. The multi-component and multi-target approach in herbal formulations may provide a practical strategy to help address the complex pathological mechanisms in neuroinflammation.

This study aimed to develop a synergistic herbal compound-formula from six promising phytochemicals including luteolin,

andrographolide, tetrandrine, 6-shogaol, curcumin, and baicalein in reducing neuroinflammation.

Network pharmacology was conducted to identify the key gene targets of each compound acting in relation to neuroinflammation. Our results suggested that one of the top overlapping gene targets for the six bioactive compounds against neuroinflammation was NOS3. The molecular docking analysis showed that all of the six compounds had a strong binding affinity to inducible nitric oxide synthase (iNOS) which partially validated the predicted target of NOS3. Our experimental results showed that the combination of andrographolide and 6-shogaol exhibited the most prominent NO inhibitory effect in lipopolysaccharide (LPS)-activated microglia N11 cells with IC₅₀ value of 6.10 µM. This combination continued to show a promising effect in LPS activated tri-culture system (microglia N11, endothelial bEnd3 cells, and neuron N2A cells) that reduced NO production and enhanced neuronal survival.

The illustration from the network pharmacology on potential targets of the compounds will advance the understanding of their potential synergistic action in inhibiting neuroinflammation. Furthermore, it will guide the subsequent experimental study on their mechanical action in the gene and protein levels.

WTH 03-16 | Social isolation-induced depression promotes ethanol intake through microglia-derived neuroinflammation

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Social isolation is very common in modern society and it causes depressive disorders. People who are depressed are highly vulnerable to alcohol use, and alcohol abuse is a well-known obstacle to treating depressive disorders. Using a mouse model involving isolation stress (IS) and/or ethanol intake, we investigated the reciprocal influence between IS-derived depressive and ethanol-seeking behaviors along with the underlying mechanisms. IS increased ethanol cravings, strongly exacerbated depression-like behaviors. Ethanol intake activated the mesolimbic dopaminergic system via dopamine/tyrosine hydroxylase double-positive signals in the ventral tegmental area and c-Fos activity in the nucleus accumbens. IS-induced ethanol intake also downregulated serotonergic activity, as evidenced by microglial hyperactivation in raphe nuclei, that was notably attenuated by a microglial inhibitor (minocycline). Our study showed that activation of microglia is a key mediator in the vicious cycle between depression and alcohol consumption. We also suggest that dopaminergic reward may be involved in this pathogenesis.

WTH04-01 | Reduced chronic restraint stress in mice overexpressing hyperactive proteasomes in the forebrain

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While chronic restraint stress (CRS) results in depression-like behaviors possibly through oxidative stress in the brain, its molecular etiology and the development of therapeutic strategies remain elusive. Since oxidized proteins can be targeted by the ubiquitin-proteasome system, we investigated whether increased proteasome activity might affect the stress response in mice. Transgenic mice, expressing the N-terminally deleted version of $\alpha 3$ subunit ($\alpha 3\Delta N$) of the proteasome, which has been shown to generate open-gated mutant proteasomes, in the forebrain were viable and fertile, but showed higher proteasome activity. After being challenged with CRS for 14 d, the mutant mice with hyperactive proteasomes showed significantly less immobility time in the forced swimming test compared with their wild-type littermates, suggesting that the $\alpha 3\Delta N$ transgenic mice are resistant to CRS. The accumulation of ER stress markers, such as polyubiquitin conjugates and phospho-IRE1 α , was also significantly delayed in the hippocampus of the mutants. Notably, $\alpha 3\Delta N$ mice exhibited little deficits in other behavioral tasks, suggesting that stress resilience is likely due to the degradation of misfolded proteins by the open-gated proteasomes. These data strongly indicate that not only is the proteasome a critical modulator of stress response in vivo but also a possible therapeutic target for reducing chronic stress.

WTH04-02 | SARS-CoV-2 affects the dopamine metabolism in human iPSCs-derived dopaminergic neuronsGiulia Lunghi¹, Emma Veronica Carsana¹, Silvia Breviario¹, Gioia Cappelletti², Claudio Fenizia², Massimo Aureli¹¹University of Milano, Department of Medical Biotechnologies and Translational Medicine, Milano, Italy; ²University of Milano, Department of Biomedical and Clinical Sciences "L. Sacco", Milano, Italy

Increasing evidence related to the onset of neurological symptoms is emerging from a high proportion of patients affected by COVID-19 pathology, suggesting the possible neuroinvasiveness of SARS-CoV-2. Recent studies show that an increasing number of patients, even with mild COVID-19, experiences symptoms even weeks or months after the infection. These symptoms comprise a wide range of neurological conditions such as memory and cognitive dysfunction, post exertional malaise, brain fog, neurological sensations, headaches, insomnia, balance and speech issues, anxiety, and depression.

These premises suggest that SARS-CoV-2 infection is not restricted to the respiratory system but reaches also the central nervous system and the COVID-19-related symptomatology let to hypothesize that dopaminergic neurons might be particularly affected. However,

no scientific evidence has been produced so far, and the neuroinvasive mechanism of SARS-CoV-2 remains unknown.

To investigate this aspect, human iPSCs were differentiated into dopaminergic neurons and infected with three different SARS-CoV-2 variants (EU, Delta, and Omicron). 96h after infection with EU and Delta variants, but not with Omicron, neurons showed a reduced intracellular content and extracellular release of dopamine. Moreover, neurons infected with the EU SARS-CoV-2 variant were characterized by a reduced protein levels of Tyrosine hydroxylase and dopamine transporter (DAT) together with a reduced mRNA expression of DOPA-decarboxylase, DAT, and VMAT2 transporter. In addition, the infected neurons displayed the onset of neurodegeneration, demonstrated by the reduction in MAP2 and TAU content. Finally, we found an intense activation of antiviral intracellular innate response and an increase in neuronal stress markers.

Taken together these preliminary observations let us to speculate that neurons are affected by SARS-CoV-2 infection, with particular consequences on the dopamine production and metabolism, explaining some of the neurological symptoms developed upon SARS-CoV-2 infection.

WTH04-03 | Altered secretion of astrocyte-derived extracellular vesicles contribute to metabolic and redox imbalance in Huntington's diseaseGonzalo Mayorga-Weber^{1,2}, Felipe A. Beltrán^{1,2}, Leandro Torres^{1,2}, Paulina Troncoso-Escudero^{1,2}, Juan Villalobos-González^{2,1}, Marcelo Lara⁴, Adriana Covarrubias-Pinto^{1,2}, Sharin Valdivia^{1,2}, Isidora Vicencio^{1,2}, Eduardo Papic^{1,2}, Alejandro Rojas^{2,3}, Luis Lamberti⁴, Felipe Court⁵, Abraham Rosas-Arellano⁶, Federico Bätz⁷, Patricio Rojas⁸, Maite A. Castro^{1,2,9}¹UACH, Instituto de Bioquímica y Microbiología, Valdivia, Chile;²UACH, Center for Interdisciplinary Studies on Nervous System (CISNe), Valdivia, Chile; ³UACH, Instituto de Medicina, Valdivia, Chile;⁴Universidade de Sao Paulo, Faculdade de Medicina de Ribeirao, Sao Paulo, Brazil; ⁵Universidad Mayor, Center for Integrative Biology, Santiago, Chile; ⁶UNAM, Instituto de Neurobiología, Querétaro, Mexico; ⁷Universidad de Los Andes, Centro de investigación biomédica, Santiago, Chile; ⁸USACH, Laboratorio de Neurociencias, Santiago, Chile; ⁹Janelia Research Campus HHMI, Janelia Research Campus HHMI, Ashburn, USA

Huntington's disease (HD) is a neurodegenerative disorder caused by a glutamine expansion at the first exon of the huntingtin gene. Huntingtin protein (Htt) is ubiquitously expressed and it is localized in several organelles, including endosomes. HD has been associated with a failure in energy metabolism and oxidative damage. Ascorbic acid is a powerful antioxidant highly concentrated in the brain where it acts as a messenger, modulating neuronal metabolism. During synaptic activity, ascorbic acid is released from glial intracellular reservoirs and it is taken up by neurons. Using an electrophysiological approach in YAC128 HD slices, we observe a



decreased ascorbic acid flux from astrocytes to neurons, which is responsible for alterations in neuronal metabolic substrate preferences. Ascorbic acid efflux and recycling was decreased in cultured astrocytes from YAC128 HD mice. Our findings were confirmed in experiments using GFAP-HD160Q, a HD mice model expressing mutant N-terminal Huntingtin mainly in astrocytes. We demonstrated that ascorbic acid is released from astrocytes through extracellular vesicles (EV). Decreased number of particles and exosomal markers were observed in EV fractions obtained from cultured YAC128 HD astrocytes, as well as, from Huntingtin KO cells. Using electronic microscopy, we observed a decreased number of multivesicular bodies (MVBs) in the striatum of YAC128 HD mice. This support the idea that MVBs biogenesis is altered in presence of mutant Htt. Therefore, we conclude that a decrease in EV-mediated ascorbic release from astrocytes would be responsible for the early metabolic failure in HD.

WTH04-04 | TET3 modulates mitochondrial genes and mitochondrial pathophysiology after stroke

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The CNS-enriched epigenetic modification known as 5-hydroxymethylcytosine (5hmC) has been shown to play an important role in neuroprotection in the diseased brain. We previously showed that the ten-eleven translocase 3 (TET3), a hydroxylase enzyme involved in producing 5hmC, reduces brain damage and promotes recovery after stroke. As mitochondria play a major role in the development of stroke pathophysiology, we wanted to determine whether TET3-mediated 5hmC modulates genes related to mitochondrial function in the post-stroke brain. We subjected adult C57BL6/J mice to transient focal ischemia by middle cerebral artery occlusion following TET3 knockdown or TET3 overexpression. Hydroxymethylation DNA immunoprecipitation sequencing revealed hundreds of TET3-dependent differential hydroxymethylation regions on genomic loci associated with mitochondrial function in the peri-infarct cortex. Gene ontological analysis identified several mitochondrial processes related to the 5hmC-associated genes including mitochondrial membrane potential, mitochondrial bioenergetics, mitochondrial calcium homeostasis, mitochondrial structural components, and mitochondrial-mediated apoptosis. Real-time PCR analysis showed that increased gene promoter levels of 5hmC were associated with increased expression of mitochondrial genes. Furthermore, TET3 maintained mitochondrial integrity and inhibited mitochondrial-mediated apoptosis following stroke. Collectively this evidence indicates that TET3 may protect the brain after stroke by modulating mitochondrial gene expression and mitochondrial function.

WTH04-05 | Synergistic effect of fibrinogen and A β peptides on neuroinflammation and synaptotoxicity in Alzheimer's disease research models

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Alzheimer's disease (AD) is characterized by the presence of hyperphosphorylated tau and different isoforms of β -amyloid peptides (A β). Mutations in presenilins and the amyloid precursor protein lead to the production of longer A β peptides, which are believed to be more pathogenic. Furthermore, there is strong evidence of cerebrovascular deficits in AD. We and others have shown that fibrinogen extravasates from the vessels into the brain parenchyma. A β interacts with fibrinogen, leading to fibrinolysis-resistant clots in the brain and around blood vessels. Although this interaction is well recognized, whether vascular damage contributes to synaptic dysfunction and how it synergizes with amyloid pathology to cause neuroinflammation and cognitive decline remain poorly understood. Here we show how different lengths of A β interact with fibrinogen to synergistically induce neuroinflammation and synaptic loss.

We examined the morphology of fibrin clots with A β peptides and their binding affinities. Organotypic hippocampal cultures (OHC) from mice were exposed to different length A β oligomers, fibrinogen, or their complexes. Cell death and synaptotoxicity were analyzed. Furthermore, A β oligomers, fibrinogen, or their complexes were intracerebroventricular (ICV) injected into mice. AD markers, neuroinflammation, and synaptotoxicity responses were also observed.

A β oligomers incubated with fibrinogen showed morphological differences. While A β 38-46 had similar binding affinities for fibrinogen, A β 40 showed significantly less affinity. OHC treated with low dose A β oligomers or fibrinogen alone showed no alteration in synaptophysin and PSD-95 synaptic markers. However, when treated with complexes, levels of these synaptic markers decreased significantly. ICV injection showed that low dose A β oligomers with fibrinogen induced greater neuroinflammatory activation in the hippocampus. CD68+ cells were also identified specifically in the CA1 region. Injection of the complexes led to a decrease in synaptophysin but no change in PSD-95. Interestingly, although we did not observe A β plaque formation in these mice, a significative increase in p-tau181 was observed.

WTH04-06 | Early cellular and molecular alterations in a novel mouse model expressing human ApoE4

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The E4 allele of apolipoprotein E (ApoE) is a key genetic risk factor for late-onset Alzheimer's disease (AD), increasing the risk of



developing the disease by up to three-fold. However, the mechanisms underlying how ApoE4 contributes to AD pathogenesis are poorly understood. Here, we utilized a targeted-replacement mouse model expressing different isoforms of human ApoE to evaluate the effects of the ApoE4 allele on a wide range of cellular, molecular, and biochemical processes in the brain. We demonstrate that ApoE4 mice begin to show early changes in the expression of several genes, leading to alterations in downstream pathways related to neural cell maintenance, insulin signaling, amyloid processing, and cognition. In addition, we reveal a strong involvement of intermediate filaments such as GFAP in the regulation of several aspects of intracellular trafficking in astrocytes, a process that is disrupted in ApoE4-expressing astrocytes. These alterations may result in the early accumulation of pathological proteins such as beta-amyloid, which may build up within cells due to impaired vesicular trafficking and clearance, leading to the accelerated degeneration of neurons and astrocytes as is observed in ApoE4-positive individuals. We also reveal previously unknown interactions between intermediate filaments and Rab proteins—small GTPases that play a key role in the vesicular trafficking machinery. We demonstrate that these interactions regulate the transport of cargo between different compartments of the endolysosomal pathway, and that they are disrupted in ApoE4 astrocytes. Taken together, these findings reveal new proteins and pathways that could mediate ApoE4-related AD risk, and may help identify more tractable therapeutic targets for treating ApoE4-mediated AD.

WTH04-07 | Mechanisms for brain region and sex-specific alterations in *circHomer1* expression in adult mouse brain following PAE

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Circular RNAs are a novel category of non-coding RNAs derived from the back-splicing and covalent joining of exons or introns. Recent studies have suggested that circRNAs are preferentially generated from synaptic plasticity-related genes and are particularly enriched in the brain. Although some circRNAs have been found to sequester microRNAs and others to associate with RNA-binding proteins (RBPs), the mechanism of action of the majority of circRNAs remains poorly understood. Moreover, little is known about the potential involvement of circRNAs in Fetal Alcohol Spectrum Disorders (FASD). Using circRNA-specific quantification, we have found that *circHomer1*, an activity-dependent circRNA derived from Homer protein homolog 1 (*Homer1*), is significantly downregulated in the male hippocampus and frontal cortex of mice subjected to prenatal alcohol exposure (PAE). Our data suggest that the CREB-regulated RNA-binding protein Eukaryotic initiation factor 4A-III (Eif4a3) binds at the *circHomer1* back spliced junction and can promote its biogenesis. Interestingly, levels of Eif4a3 are significantly reduced in the

hippocampus of male PAE mice, whereas expression of an imprinted and sexually-dimorphic long non-coding RNA, capable of inhibiting Eif4a3 function, is significantly upregulated in the frontal cortex of male PAE mice. Furthermore, *in vivo* shRNA-mediated knockdown of *circHomer1* in mouse frontal cortex suggests that is capable of modulating synaptic gene expression, neuronal function, and coordinated cortical activity, as well as behavioral flexibility. Taken together our work introduces novel molecular networks with potential importance for FASD.

WTH04-08 | Rapid MSO-sensitive extracellular accumulation of taurine in hippocampus of young rats during initial pilocarpine-induced seizures

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We recently showed that pretreatment with a non-convulsive dose of glutamine synthetase (GS) inhibitor, methionine sulfoximine (MSO) delays the onset and mitigates the intensity of initial seizures in juvenile rat Li-Pilo model of temporal lobe epilepsy, by both a canonical (GS inhibition) and non-canonical mechanism (Pawlik M. *et al.*, Brain Res. 2021, PMID: 33422530; Pawlik M. *et al.*, IJMS 2021, PMID: 34681786). Here we examined EEG profiles and extracellular (hippocampal microdialysate) levels of amino acid neurotransmitter Glu and GABA, their precursor Gln, and osmosensitive neuromodulator Tau, measured by HPLC, in Li-Pilo –treated rats, subjected or not to MSO pretreatment. A rapid, gradual accumulation of Tau was noted upon initiation of seizures (the initial 60–75 min post Pilo), and the effect was significantly less pronounced in animals pretreated (for 2.5 h) with MSO. Relative EEG power including that of high frequency oscillations recorded during the initial seizure, were significantly reduced in MSO-pretreated rats ($p < 0.0001$) and a change of power over time strongly correlated with levels of Tau, both in MSO-treated and untreated rats ($R^2 = 0.9433$, $p < 0.0001$ and $R^2 = 0.8167$, $p < 0.0001$, respectively). MSO reduced Gln, but did not affect the GABA and Glu contents in the microdialysates. Considering Tau is a marker of cellular osmotic stress, these results indicate that MSO delays and alleviates the initial pilocarpine-induced seizures by relieving edema of the brain. Given high abundance of Gln and its contribution to the pool of brain osmolytes, attenuation of edema could be related to the MSO-evoked decrease of Gln content.

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WTH04-09 | The role of third intracellular loop in the trace amine associated Receptor 1 mechanism. Toward new opportunities for drug design

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The rising population and the increase in life expectancy makes the nervous system disorders one of the top 10 causes of death in the world. One of the neuropsychiatric disease hypotheses, have been related to the dysregulation of neurotransmitters which are controlled by enzymes, transporters and receptors. Thus, the recent discovery of trace amine-associated receptors (TAARs), particularly the TAAR1 receptor and its expression in areas known to be monoaminergic, provides an opportunity to explore new areas of research related to the treatment of neurodegenerative and neuropsychiatric disorders. Here, we carried out *in silico* studies in order to obtain new structural insights of the of the hTAAR1 receptor interacting with its endogenous agonist *p*-Tyramine (*p*-TA) and in absence of *p*-TA. Thus, the comparative study evaluating different conformational states of hTAAR1 shows a crucial role of the third intracellular loop (IL3) in the activation process. Our results lead us to propose a concatenated mechanism dependent on the presence of the *p*-TA and IL3. These results provide a new possible target for the treatment of neuropsychiatric disorders, being able to control the activation or inactivation of receptors based on their conformational mechanisms.

WTH04-10 | New mechanism involved in rat kainate-induced status epilepticus

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Alterations in brain-derived neurotrophic factor (BDNF) and its full-length tropomyosin-related kinase B (TrkB-FL) receptor have been suggested to contribute to epilepsy. Under excitotoxic conditions, TrkB-FL receptor is cleaved, forming an intracellular fragment (TrkB-ICD). In an *in vitro* model of *status epilepticus* (SE) a putative TrkB-FL cleavage was also suggested. Therefore, we studied whether the TrkB-FL cleavage occurs in an *in vivo* model of SE and chronic epilepsy and if it is related with seizure severity, neurogenesis and memory. A rat model of epilepsy was induced with kainate (KA, 10mg.kg, intraperitoneal) and two groups were obtained: SE group and chronic

group. Seizure severity was classified using the modified Racine's Scale.

In the SE group, animals with the highest seizure score showed a significant decrease of TrkB-FL protein levels and increased levels in TrkB-ICD/TrkB-FL ratio. Moreover, a positive significant effect of TrkB-ICD levels on the number of seizures was observed, suggesting that animals with more seizures have higher levels of TrkB-ICD. Nevertheless, no differences were found in Ki67⁺ proliferative cells comparing with saline-treated animals.

Regarding the chronic group, although no evidence of TrkB-FL cleavage was observed, a positive significant effect of proliferative Ki67⁺ cells in the number of spontaneous seizures per hour was found. Also, a preliminary novel object location test revealed a loss of preference for the displaced object in the epileptic animals, indicating memory impairment. Taken together, our results suggest that TrkB-FL is cleaved in an *in vivo* KA-induced epilepsy model, a mechanism that appears to occur during SE, affecting later neurogenesis and memory.

WTH04-11 | Identification of a novel lysosomal release pathway regulated by Parkinson's disease kinase LRRK2

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Leucine-rich repeat kinase 2 (LRRK2) is a protein kinase associated with Parkinson's disease (PD) and phosphorylates a subset of Rab GTPases including Rab10. We have previously shown that lysosomal overload stress induces the extracellular release of lysosomal contents that is regulated by Rab10 phosphorylation by LRRK2, but more detailed mechanism of release is largely unknown. Here we investigated the LRRK2-mediated release upon lysosomal overload stress by focusing on three known endo-lysosomal pathways, that is, calcium-dependent lysosomal exocytosis, secretory autophagy, and exosome-mediated secretion. We first confirmed that LRRK2 was not required for the so-called lysosomal exocytosis induced by a Ca²⁺ ionosphere ionomycin. We found that the autophagy pathway proteins ATG5 and ATG16L1, but not autophagy upstream components, regulate LRRK2-mediated release upon lysosomal overload stress. We also found that the LRRK2-mediated release involves the secretion of exosomes that unexpectedly contain lysosomal luminal proteins. These data collectively support the existence of a novel type of lysosomal release under lysosomal stress that is mediated by LRRK2. Understanding the physiological and pathological roles of this pathway will help elucidate LRRK2-associated mechanisms that lead to PD.



WTH04-12 | Genome-wide CRISPR screens to identify modulators of TDP-43 aggregation in ALS

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The formation of inclusions comprised of TAR DNA-binding protein-43 (TDP-43) throughout the brain and spinal cord in ALS is strongly correlated with motor neuron degeneration. However, the processes that initiate the aggregation of TDP-43 and mediate its toxicity remain unknown. The objective of this study is to identify key genes and biological processes that modulate TDP-43 inclusion formation and toxicity using a pooled genome-wide CRISPR knockout screen. Analysis of the genome-wide CRISPR knockout screen data revealed seven distinct groups of "TDP-43 modifier genes" that have functionally distinct roles in the cell, including inhibiting or enhancing TDP-43 aggregation and protecting or sensitizing to TDP-43 toxicity. Gene knockouts that sensitize or protect cells against mutant TDP-43 toxicity were enriched with genes associated with endoplasmic reticulum to cytosol transport and autonomic nervous system development, respectively. Gene knockouts that enhance or inhibit mutant TDP-43 aggregation were enriched in genes associated with neuron projection organization and response to interferon-beta, respectively. Using this combination of state-of-the-art analysis algorithms for genome-wide CRISPR knockout data (e.g., MAGeCK) and top gene ontology pathways (WebGestalt), we short-listed 323 "TDP-43 modifiers". These gene targets are the subject of further investigation for prioritization of genes that decrease TDP-43 aggregation and enhance neuronal survival. Using arrayed CRISPR knockout tools combined with multi-parametric high-content imaging, we have developed sophisticated analytical pipelines to cluster targets that cause statistically similar phenotypic effects. This has allowed us to rapidly identify and triage our highest confidence targets. This research will lead to a greater understanding of the pathogenesis of ALS and identify avenues for better therapeutic interventions aimed at protecting neurons against TDP-43 pathology.

WTH04-13 | Alterations in miRNA and proteome profile identify miR-29b: A potent regulator of arsenic-induced neurodegeneration

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Neurodegeneration involves a highly dynamic and interactive system of multiple layers of molecular markers. Epidemiological

studies have revealed the possible neurodegenerative potential of different environmental toxins. Among heavy metals, arsenic exposure represents a major public health threat for populations worldwide, which causes a wide range of neurological complications by involving pathogenic mechanisms that are extremely complex and likely multifactorial. The present study aimed to identify potent regulatory modules between miRNAs and proteins that might uncover pathophysiological mechanisms underlying arsenic-induced neuronal death. Integrated Open array-based miRNA profiling and unlabeled LC-MS/MS global protein profiling were carried out in differentiated human neuroblastoma SH-SY5Y cells with/without arsenic exposure. Furthermore, cellular bioenergetics assays were performed to explore the role of identified miRNA in arsenic-induced mitochondrial dysfunction by employing Seahorse XFe analyzer. Arsenic exposure to SH-SY5Y cells dramatically increased the expression of mmu-miR-153, hsa-miR-29b, hsa-miR-221, hsa-miR-199a, and hsa-miR-503, while hsa-miR-126, hsa-miR-138, and hsa-miR-664 have shown maximum downregulation. The proteomics analysis revealed that proteins involved in oxidative stress, mitochondrial function, oxidative phosphorylation, glycolytic process, mitophagy, protein folding, autophagy, and apoptosis were found to be significantly deregulated in the proteome profile of SH-SY5Y cells receiving the toxic insult of arsenic. Furthermore, YY1 regulated gene PGC-1 α as well as genes involved in mitochondrial fusion and fission were also found to be deregulated upon arsenic exposure. Integrative analysis discovered novel miRNA-protein regulatory modules with miR-29b as a potential regulator of key proteins involved in cellular and molecular mechanisms of mitochondrial functions in neuronal cells. Gain- and loss-of-function studies confirmed the involvement of miR-29b in the regulation of major mitochondrial pathways underlying arsenic-induced neuronal damage. Furthermore, miR-29b induction leads to reduction in OCR and other bioenergetics parameters in neuronal cells. These findings identified miR-29b as a novel target that could develop as a tool to study neurodegenerative events associated with arsenic exposure.

WTH04-14 | Reference gene stability within the rat brain in the lithium pilocarpine epilepsy model: Timing and region specificity

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Reverse transcription followed by quantitative polymerase chain reaction (qRT-PCR) is a powerful and commonly used tool for gene expression analysis. It requires the right choice of stably



expressed reference genes for accurate normalization. In this work, we aimed to select the optimal reference genes for qRT-PCR normalization within different rat brain areas at different stages of the Li-pilocarpine model of acquired epilepsy. We have tested the expression stability of nine housekeeping genes: *Actb*, *Gapdh*, *B2m*, *Rpl13a*, *Sdha*, *Ppia*, *Hprt1*, *Pgk1*, and *Ywhaz*. We have developed set of original multiplex qPCR assays allowing to analyze expression of aforementioned genes in 3 reactions. Designed assays demonstrate optimal efficiency and repeatability. Based on geometric averaging of ranks obtained by four common algorithms (geNorm, NormFinder, BestKeeper, Comparative Delta-Ct), we found that the stability of tested reference genes varied significantly between different brain regions after pilocarpine induced status epilepticus and depends on timing (3 days, 7 days in latent phase of the model, or 2 months, i.e., chronic phase). *Pgk1* and *Ywhaz* were the most stable, while *B2m* demonstrate the lowest stability in the analyzed brain areas. *Gapdh* expression was one of the most stable in the hippocampus, but it has low stability in the medial prefrontal and temporal cortical areas, and amygdala. High reference gene stability were detected in the medial prefrontal cortex, amygdala, and dorsal hippocampus, whereas in the ventral hippocampus and temporal cortex 4–5 of 9 analyzed genes were unstably expressed and inappropriate for expression normalization. Within the analyzed brain regions, the stabilities of tested reference gene expression were lower 3 days (early latent phase of the model), and especially 2 months after pilocarpine induced seizures compared to 7 day post-seizure rats. Thus, the reference genes for RT-qPCR data normalization in the rat Li-pilocarpine should be differentially selected based on specific brain area and time after the induction of epileptogenesis.

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WTH04-15 | Towards understanding the role of CRMs as key regulators of autophagy in the brain: An in-silico molecular docking approach

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Autophagy is a conserved catabolic process that helps in the removal and recycling of aggregated toxic proteins and damaged cell organelles. The AMPK (AMP-activated protein kinase) and mTOR (mammalian or mechanistic target of rapamycin) are two important regulators of the autophagy process. AMPK is a nutrient/fuel sensing protein that gets activated in nutrient deficiency. The nutrient deficiency triggers the process of autophagy by activating the AMPK pathway. The AMPK inhibits the mTORC1 which is a cell proliferation pathway that otherwise inhibits autophagy. A class of drugs called Caloric Restriction Mimetics (CRMs) has been seen in the induction of autophagy, but the process is still unclear. In this study, we have

utilized in silico molecular docking approaches to understand the binding mechanisms of the CRMs like spermidine, chlorogenic acid (CGA), fisetin, and 2-deoxy-D-glucose (2DG) to the autophagy regulators, AMPK and mTOR. The molecular docking results indicated the binding affinity of the selected ligands towards AMPK in increasing order as: CGA > Fisetin > 2DG > Spermidine. Similarly, the binding affinity of the CRMs to mTOR indicates that CGA showed the strongest binding affinity followed by Fisetin > 2DG > Spermidine. The study will provide insights into the structural features of the binding mechanism. Furthermore, we are validating the findings of the in-silico study in in-vitro conditions.

WTH04-16 | Type 1 diabetes mediated increased capillary stalling and impaired behavioral performance in mice

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Introduction

Recent work from our lab has shown that the brain capillaries routinely get clogged by cells and debris even under healthy conditions. The present study was undertaken to determine how experimentally induced Type 1 diabetes affects this phenomenon, and whether obstructions contribute to cognitive decline.

Methods

C57BL/6 male and female mice were injected with streptozotocin to induce type 1 diabetes. These mice were implanted with cranial windows and cortical volumes were repeatedly imaged from 3–9 weeks after induction of diabetes. To model susceptibilities to short or long lived obstructions, we injected (i.v.) 5 µm diameter fluorescent microspheres in diabetic and control mice at 30 min and 3 days before euthanasia. The density of microsphere obstructed capillaries was quantified across 15 different brain regions. To determine the impact of diabetes on cognitive and sensorimotor activity, mice were subjected to a battery of behavioral tests.

Results

2-photon imaging indicated that diabetic mice have higher rates of capillary stalling in somatosensory cortex that became more pronounced with duration of diabetes. The majority of stalls in diabetic mice were associated with Rhod6G labeled cells, suggesting that leukocytes play a key role. Increased stalling also led to greater pruning of cortical capillaries. Consistent with these observations, our fluorescent microsphere obstruction assay yielded significantly higher levels of short and long lived capillary obstructions in both male and female diabetic mice, including those treated with insulin. Behaviorally, diabetic mice were not different from controls in tests of ambulatory activity (open field), nor did they show any differences in visual function. However, diabetic mice were significantly impaired in learning/memory tests such as the novel object recognition, water maze learning or reversal learning.

Conclusions



These studies suggests that diabetes is associated with greater risk for capillary obstructions in the brain as well as learning/memory deficits. Our future aims will provide a mechanistic understanding of how diabetes elevates one's susceptibility to capillary obstructions and cognitive decline, and whether manipulating certain immune signaling pathways can alleviate these impairments.

WTH04-17 | Rapid demise of cholinergic-targeted brain transfer RNA fragments in cognitively declined women with Alzheimer's disease

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Cholinergic neurons die first in Alzheimer's disease (AD) brains, and women AD patients deteriorate faster than men, but the underlying mechanisms are unclear. Here, we report that microRNAs and transfer RNA fragments carrying complementary sequence motifs to at least five cholinergic genes each (CholinomiRs, CholinotRFs) may be dually involved in these AD traits. Small RNA-sequencing of numerous brain region tissues (89 females, 70 males) revealed massively changed small RNA levels, especially in the cholinergic nucleus accumbens. Importantly, these changes associated both with the AD pathophysiological hallmarks, including beta amyloid plaques and neurofibrillary tangles, and with patients' cognitive impairments. However, while men tissues primarily presented changes in miR levels, women patient brains exhibited greater changes in CholinotRFs carrying complementary sequences to key cholinergic transcripts. Specifically, tRFs targeting the phosphatidylcholine biosynthesis regulator PCYT1A and the acetylcholinesterase (AChE)-targeting miR-132 were all declined, accompanying larger AChE mRNA increases in the brains and the cerebrospinal fluid (CSF) of women than men. Moreover, women showed greater decrease of mitochondrially-originated tRFs with complementary motifs to AD-risk genes, including the circadian regulator NEBL. That women AD-associated changes in tRFs and miRs correlate with altered levels of their targeted cholinergic transcripts points at a novel mechanism underlying the sex-specific cholinergic decline in AD.

WTH04-18 | The unique biology of RNA-granules in vulnerability and treatment of Stress and Alzheimer's Disease brain pathologies

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AD is an age-related-neurodegenerative disorder, with many identified risk factors, for example, age and sex, and more recently clinical studies suggest that stressful life experiences and daily stress are etiological risk factors for AD. Given that the modern lifestyle increasingly exposes individuals to high-stress loads, it is clear that understanding the mechanistic links between chronic stress and AD pathogenesis may facilitate AD treatment helping a large population worldwide. Unfortunately, very little is known about how stress and its molecular signaling contribute to AD precipitation and whether it may contribute to therapy. So far, we know that cellular stress dysregulates the homeostasis of RNA binding proteins (RBPs) and mRNAs leading to the formation of stress granules (SGs); granules that become persistent under conditions of cellular stress, which are found in long-lasting, age-related neurodegenerative disorders such as AD. Using in vitro and in vivo models of Tauopathy, we have shown for the first time that SG persistency appears to act as a nucleating factor of aggregation for disease-related proteins such as Tau, and chronic stress/GC deregulate RBPs homeostasis leading to their accumulation and intraneuronal translocation into SGs; in addition, in vitro blockade of SG formation attenuated GC-driven Tau aggregation and downstream neurotoxicity suggesting the precipitating role of SG on Tau Tau-related neurodegeneration. Altogether, our findings suggest causality between Tau aggregation, SG formation, and stress-induced neurodegeneration leading to associated synaptic toxicity.

We are clarifying and dissecting the precise SG-related signaling cascades and the SG contribution to both parameters of Tau-related neurodegeneration induced by chronic stress. We want to understand the emerging role of unique RNA granules biology in the precipitating impact of chronic stress on AD disease pathology and also study their potential contribution to AD treatment.



WTH04-19 | Prenatal hypoxia affects nicotine consumption and withdrawal in adult rats via impairment of the glutamate system in the brain

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The role of damaging factors in the prenatal period as a basis for drug addiction in offspring is of great interest. In this study, we aim at deciphering the effects and possible mechanisms of prenatal severe hypoxia (PSH) on predisposition to nicotine addiction in adult rats. In PSH rats, we found an increasing tendency to nicotine consumption in the two-bottle choice test. After two weeks of chronic treatment with nicotine via osmotic minipump (9 mg/kg per day), we assessed the symptoms of withdrawal in the conditioned place aversion test after mecamylamine (an antagonist of nicotinic acetylcholine receptors, nAChR) treatment. We showed that the mecamylamine-precipitated withdrawal aversion was stronger in the PSH group than in the Control group. This suggests that PSH acts as a predisposing factor for developing nicotine addiction in adulthood. PSH rats also demonstrated an increased level of phosphorylated DARPP-32 protein (known as the relay for dopamine and glutamate signaling) at 34 threonine (pThr34DARPP-32) in relation to its total amount in the nucleus accumbens of the striatum (NAc). Meanwhile, no changes in both the content of dopamine in the mesolimbic pathway and the first type of dopamine receptors (DAR1) in NAc were found. The increased rate of DARPP-32 phosphorylation in adult PSH rats might result from excessive glutamatergic stimulation of the dopaminergic (DA) neurons of the ventral tegmental area (VTA) caused by activation of presynaptic nAChR by nicotine. This hypothesis is supported by the observed increase in VGluT2 positive terminals to Nurr1 positive neuronal bodies in VTA in PSH animals. Thus, the altered glutamate signaling phenotype might play a significant role in the development of PSH-related nicotine addiction.

WTH04-20 | Dynamics of microtubules and MAPs under the physiological condition that cause high phosphorylation state of tau protein

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Tau is a microtubule-associated protein (MAP) that has the abilities to bind to microtubules (MTs) and to stabilize them. In the affected neurons with tau-inclusion bodies in the brains of dementia,

tauopathy, tau protein is hyperphosphorylated and lost its functions. It is considered that the cellular conditions in neurons that alter the phosphorylation state of tau are involved not only in its physiological functions but also in the pathogenesis of tauopathies. However, the molecular mechanisms underlying tauopathies *in vivo* remain unknown. Previously, we established a procedure to quantify stable MTs, labile MTs, soluble-tubulin dimers, as well as MT-binding activity of MAPs in mouse tissues. Using this method, here we analyzed the behavior of MTs and MAPs in the brains of two models that promote tau phosphorylation.

Firstly, we analyzed the postnatal mice brains, in which tau is highly phosphorylated in the first week of life, but is nearly completely dephosphorylated by P14. According to its dephosphorylation along with development, we found the increase of the association of tau with MTs besides the stabilization of MTs. Secondly, we analyzed anesthesia-induced hypothermia model that has the transient hyperphosphorylation of tau. Compared with normothermia, both labile-MTs-bound and stable-MTs-bound tau significantly decreased in hypothermia model. Furthermore, MT-unbound tau in hypothermia mice lost the ability to bind MTs, that in normothermia mice still had. Interestingly, despite the dissociation of tau by transient phosphorylation, the amount of stable MTs was increased. Then, we focused on the dynamics of other MAPs in hypothermia model and found an increase of stable-MTs-bound MAP1B. Additionally, MAP6 and MAP7 rigidly bound to stable MTs in any conditions.

These findings suggest that physiological conditions that cause tau phosphorylation affects the functions of tau and dynamics of MTs in mice brain, and the robustness of stable-MTs is coordinately maintained by MAPs.

WTH04-21 | Quantum dot conjugates in cellular models derived from patients: Towards molecular characterization and personalized medicine

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease (NDs) characterized by motor neuron (MN) death that yields in progressive paralysis. Currently, drug development is hampered due to the heterogeneity of the disease and the lack of knowledge of the mechanism triggering selective MN death.^[1] In this way, ALS and other NDs will greatly benefit from molecular profiling studies as it allows to unravel complex molecular pathways that underlie physiological and pathological processes.

Quantum dots (QDs) are luminescent nanoparticles with a high potential to become promising tools to detect molecular mechanisms at the subcellular level enabling multiplexing applications.^{[2][3]} Using this technology, different ALS targets and processes such as intracellular transport which is known to be hampered in ALS, will be



analyzed at the single-cell level in human cell models derived from immortalized lymphocytes from ALS patients. This characterization at the single-cell level not only will allow to better understand the pathology but also a proper characterization of patients attending to their molecular fingerprint.

In addition, the scientific aim of this project is to explore molecular changes in key protein targets and processes such as intracellular transport and exosome production, upon pharmacological treatment to help select therapeutic candidates with a molecular pathology modulation. Our group holds already promising therapeutic candidates from the treatment of ALS, such as IGS2.7, a CK-1 inhibitor that has reached pre-clinical phases. The molecular modulation of IGS2.7, has been studied using these tools, alone and in combination with Riluzol, the only drug approved by the EMA for ALS treatment, showing promising results.

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WTH04-22 | Under similar synaptic patterns, microglia unveils sex-differences in the valproic acid model of autism spectrum disorder

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairments in social communication and repetitive-stereotyped behaviors. It is reported a male bias in prevalence and different type and grade of symptoms between sexes. Both microgliosis and synaptic alterations have been found in patients and animal models of ASD. The valproic acid (VPA) model is a well-validated experimental model of ASD. The aim of this work was to study synaptic and microglia features in male and female VPA rats in brain areas related to ASD core symptoms: the prefrontal cortex (PFC) and the hippocampus. Synaptophysin and Iba1 immunostaining were performed to evaluate synapses and microglia on tissue slices of juvenile control and VPA rats of both sexes. The PFC of both male and female VPA rats showed an increase in synaptophysin suggesting a higher number of synapses and a microgliosis characterized by a higher proportion of Iba1 (+) unramified cells. However, in the hippocampus, even when reduced synaptophysin was a common trait in both sexes, microgliosis

was only evident in female VPA rats. To evaluate this distinctive microglia reactivity between sexes we performed microglia primary cultures from control and VPA neonates. Using Iba1 immunolabeling, we studied microglia morphology under basal conditions and after exposure to either a pro-inflammatory (lipopolysaccharide) or a phagocytic (synaptosomes) stimulus. Cortical microglia from both male and female VPA animals showed morphological changes under basal conditions and a preserved response to a pro-inflammatory stimulus compared with their sex-matched controls, but only cortical microglia from males showed resistance to a phagocytic stimulus. Hippocampal microglia from both sexes were capable of responding to pro-inflammatory and phagocytic stimuli, even when only those from females showed altered morphology under basal conditions. In conclusion, microglia from VPA rats show sex-dependent features which may contribute to sex-differences in ASD.

WTH04-23 | Role of miR-802 in brain insulin signaling and its impact on Down syndrome

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Down syndrome (DS) individuals are characterized by a variety of pathological phenotypes that manifest with wide variability in the different tissues. At the level of the central nervous system, the accelerated aging phenotype is associated with the risk to develop Alzheimer-like dementia. A central aspect of neurodegeneration is the close association between metabolic disorders and cognitive decline. Multiple studies have suggested a link between metabolic disorder and microRNAs (miR), small non-coding RNAs acting as post-transcriptional regulators of a plethora of genes. Among triplicated miRNAs on chromosome 21, we focus on miR-802 because recent studies demonstrated its association with development on insulin resistance in obesity and diabetes. Considering these findings and based on the "gene dosage hypothesis" of DS, the goal of the study is to decipher how miR-802 may contribute to aberrant insulin signaling (IS) and, in parallel to the risk to develop dementia early in life in DS.

Methods. The miR-802 expression, protein levels and activation state of main components of the IS were evaluated (i) in the brain of autaptic cases from DS, DSAD and age-matched controls and (ii) in the brain of euploid and Ts65Dn mice (a model of DS). Further, using bioinformatic tools we identified miR-802 predicted target genes that are involved in the IS (*PTEN* and *GSK-3β*).

Results and Conclusions. The IS alterations worsen in the transition from DS to DS/AD and similar findings were collected in Ts65Dn mice where IS dysregulation persists with aging where neurodegeneration becomes significant. Intriguingly, these latter changes were driven by the over-expression of miR-802, which negatively regulates *PTEN* and *GSK-3β* mRNA in the brain. In this picture, the identification of specific targets modulated by miR-802 and involved in IS pathway,



will provide molecular basis to develop novel therapeutic strategies to prevent/delay the onset of brain insulin resistance in DS.

WTH04-24 | HIF1 induces pentose phosphate pathway dysfunction and neuronal death in rat hippocampus after severe hypoxia

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Introduction: The pentose phosphate pathway (PPP) of glucose metabolism in the brain serves as a primary source of NADPH which in turn plays a crucial role in multiple cellular processes, including maintenance of redox homeostasis and antioxidant defense.

Methods and Results. In our model of protective mild hypobaric hypoxia in rats (3MHH), an inverse correlation between hypoxia-inducible factor-1 (HIF1) activity and mRNA levels of glucose-6-phosphate dehydrogenase (G6PD), the key enzyme of PPP, was observed. In the present study, it was demonstrated that severe hypobaric hypoxia (SH) induced short-term upregulation of HIF1 α -subunit (HIF1 α) in the hippocampal CA1 subfield and decreased the activity of G6PD. The levels of NADPH were also reduced, promoting oxidative stress, triggering apoptosis, and neuronal loss. Injection of a HIF1 inhibitor (HIF1i), topotecan hydrochloride (5 mg/kg, i.p.), before SH prevented the upregulation of HIF1 α and normalized G6PD activity. In addition, HIF1i injection caused an increase in NADPH levels, normalization of total glutathione levels and of the cellular redox status as well as suppression of free-radical and apoptotic processes. The universality of the mechanism of HIF1-dependent negative regulation of G6PD expression was confirmed in *in vitro* experiments on human HEK cell culture transfected with luciferase under the HIF-dependent promoter.

Conclusion. These results demonstrate a new molecular mechanism of post-hypoxic cerebral pathology development which involves HIF1-dependent PPP depletion and support a recently suggested injurious role of HIF1 activation in the acute phase of cerebral hypoxia/ischemia. Application of PPP stimulators in early post-hypoxic/ischemic period might represent a promising neuroprotective strategy.

WTH04-25 | Mitragynine induced cognitive impairments via histones regulation at the epigenetic level

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Background: Mitragynine, an indole alkaloid from Kratom (*Mitragyna speciosa*) was previously reported to cause addiction and spatial memory deficit, however, the mechanism through which it impairs memory has not yet been established.

Aims: In this work, we investigated the impact of 14 days of mitragynine exposure on the modulation of histone expression associated with cognitive functions in the rats hippocampus.

Methods: Sprague Dawley's (SD) rats were treated (intraperitoneally) daily with mitragynine (1, 5, 10, and 30mg/kg) for 14 days. Effects of the treatments on addiction and cognitive behavior were determined using global scoring and passive avoidance tasks respectively. Animals were then euthanized using pentobarbital and the hippocampus were removed, snapped frozen in liquid nitrogen, and immediately stored at -80°C for further study. Histone expression such as H3K9, H4K12, and HDAC-2 gene were determined using western blot.

Results/outcome: Mitragynine (5, 10, and 30mg/kg) significantly ($p < 0.05$) impaired step-through latency as compared to control. We found a significant decrease ($p < 0.05$) in both H3K9 and H4K12 protein expressions in all mitragynine treated groups as compared to control. A significant HDAC-2 ($p < 0.05$) overexpression was detected in 5, 10 and 30mg/kg mitragynine treated rats as compared to control.

Conclusion/interpretation: Our data indicate that changes in histone (H3K9, H4K12, & HDAC-2) expressions may be responsible for mitragynine induced cognitive impairment following repeated exposure, which provides an insight on the potential therapeutic target in managing cognitive impairments as seen in various diseases such as Alzheimer's Disease.

WTH05-03 | Biotinylation by neurofascin antibody recognition maps the extracellular axon initial segment proteome

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The nodes of Ranvier and axon initial segment (AIS) are important for the generation and propagation of action potentials, and regulation of neuronal polarity. It is well established that AnkyrinG (AnkG) and β 4-spectrin play key roles in the formation and maintenance of the nodes and AIS. Interestingly, nodes and AIS share a common molecular organization. Recently, some new node-specific and AIS-specific or -enriched proteins have been discovered (e.g., Trim46, NuMA1, TRAAK1), but the molecular mechanisms that stabilize the AIS and control neuronal polarity remain obscure. Recently, we identified candidate AIS-associated proteins using proximity biotinylation and



proteomics. In the current study, to further investigate these candidates, we have used an AAV-based CRISPR-mediated homology independent knock-in method for high-throughput modification of endogenous proteins. We found that Cntn1 a GPI-anchored cell adhesion molecule, is enriched at the AIS. CRISPR knock-out of Cntn1 in cultured neurons reduces the accumulation of the extracellular matrix protein, Tenascin-R, at the AIS. We further focused on the assembly of axo-axonic synapses at the AIS. Cntn1 knock-out animals showed weak and abnormal axo-axonic contact at Purkinje neuron AIS. These results suggest that Cntn1 plays important roles at the AIS for interacting with extracellular matrix proteins and forming axo-axonic synapses.

WTH05-05 | The Unfolded Protein Response (UPR) in the piglet brainstem following exposure to intermittent-hypercapnic-hypoxia (IHH)

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The UPR is known to be affected by hypoxia and cellular stress. Yet the effects of IHH on UPR marker expression at the cellular level within the brain has not yet been studied. This study aimed to determine whether the immunohistochemical expression of key UPR markers p-PERK and ATF6 are affected within the young developing piglet cerebellum, brainstem (pons & medulla), and hypothalamus after IHH exposure. IHH exposure was either acute (for 1 day; $n = 7$), or repeated (for 4 days; $n = 7$) and compared to piglets exposed to their respective control group (air; 1 day $n = 5$, 4 days $n = 4$). In the cerebellum, a significant increase was seen in ATF6 ($p < 0.01$) and p-PERK ($p < 0.05$) after 1 day IHH. In the pons, only p-PERK was increased after 1d-IHH ($p < 0.05$) and this was evident in both the facial nucleus and locus coeruleus. In the medulla, changes were only seen after 4-days IHH and included increased ATF6 and p-perk in various nuclei ($p < 0.05$). No changes were evident for either marker in orexin neurons of the hypothalamus. These results indicate for the first time that IHH affects UPR expression and that the two arms of the UPR we studied were affected differently and at different time-points of exposure, based on the brain region analyzed. The regions/nuclei particularly affected relate to proprioception and arousal, which links to the physiological findings in these piglets of depressed arousal.

WTH05-06 | Environmental toxicant bisphenol alternatives (BPS & BPF) mediated effect(s) on neurogenesis in the rat hippocampus

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Bisphenol S (BPS) and Bisphenol F (BPF) are environmental toxicants, which are used in the production of plastic products. BPS and BPF are the analogs of Bisphenol A (BPA). These are endocrine disruptors and mimic the structure of the estrogen hormone. Previously, our lab found that BPA exerts an adverse effect on neurogenesis, mitochondrial dynamics, biogenesis, and cognitive deficits. Herein, we studied the impact of bisphenol alternatives (BPS and BPF) on neurogenesis in rat brain hippocampus. In this study, we assessed the neurotoxicity of bisphenol alternatives on neurogenesis in the rat brain hippocampus. To explore the effects of Bisphenol S and F on learning and memory function, we performed the behavioral experiment and found a significant decrease in learning and memory behavior. We have investigated the gene expression and protein levels of parental factors of cellular proliferation, stem cell renewal, and differentiation (Wnt/ β -catenin) after exposure of BPS and BPF in the developing rat brain hippocampus. BPS and BPF reduce the expression of neurogenic and the Wnt pathway genes and proteins. Bisphenol alternatives reduce the Gsk3 α levels and increase the levels of phosphorylated form of β -catenin proteins. We also performed Transmission Electron Microscopy (TEM) and found that BPS and BPF damage cellular architecture. Overall, our results conclude that BPS and BPF impaired neurogenesis via alteration Wnt/ β -catenin signaling pathway

WTH05-07 | Visualization of newly formed oligodendrocytes in mice

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Oligodendrocytes (OLs) show dynamic morphological changes during differentiation exemplified by elongating processes. Along with the morphological diversity of OLs, a recent single-cell analysis indicated that OLs exhibit spatiotemporally regulated heterogeneous gene expression patterns. Although detailed morphological and genetic characteristics of OLs have been elucidated separately, few have reported what correspondences exist between the two. The single-cell analysis conducted by He et al has revealed that *LncOL1* is a long non-coding RNA specifically expressed in newly



formed OLs. To examine the spatiotemporal expression pattern of *LncOL1*, we performed in situ hybridization and validated that *LncOL1* transcripts are widely distributed throughout the brain, not only in the spinal cord on which He et al focused in the 2017 paper. To identify and label *LncOL1*-expressing newly formed OLs in mouse brains, we used a bigenic approach comprising a tetracycline-controlled gene induction system. We generated tTA-knockin mice, in which a tTA-polyA cassette is inserted into immediate downstream of the *LncOL1* transcription initiation site. We crossed the line with a tetO-YFP line, in which a tetO gene cassette is knocked into the β -actin locus. In obtained tTA::tetO double transgenic mice, a subset of myelinating OLs was positive for YFP, indicating that tTA protein was functionally synthesized despite the usage of the non-coding RNA frame. YFP-positive cells were also positive for PLP but negative for a pre-myelinating OL marker, *Gpr17*, confirming that they myelinated. Sparse labeling of these newly formed OLs enabled us to describe brain region-dependent OL shape diversities. This new tTA mouse line can be used for multiple experimental purposes. By crossing with other tetO lines, we can exclusively induce a variety of functional proteins such as calcium indicators in newly formed OLs. Here we provided a refined genetic tool for dissecting how these OLs contribute to developmental myelination.

WTH05-08 | Neurodevelopment and pharmacological studies: in vitro and in vivo models for neuroactive drug testing

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According to last epidemiology studies, brain-related illnesses will take first place in the global burden of diseases by the 2020s, for example, depression but also other psychotic disorders and neuro-oncology. Many neurological diseases begin during embryogenic and fetal development, some of these can be caused or be precipitated by drug use during pregnancy, for this reason, we need platforms for testing pharmaceuticals in the developing brain—the only way to do that is by employing adequate *in vitro* and *in vivo* models. Perinatal drug exposure testing and pregnant women are a typically excluded cohort in pre/clinical drug efficacy and safety assessment, in addition to the current international lack of knowledge about the possible long-term effect of pharmaceuticals on human neurodevelopment. The aim of the study was to investigate whether antidepressants and antiepileptic drugs commonly used by pregnant women show to have neuro-pathological effects and to shed the light and give insights into the potential signaling pathways and mechanisms involved in pathogenesis. We recapitulate neurodevelopmental events and test the effects of neuroactive drugs in clinically relevant concentrations, with help of neuronally-differentiated PC12, SH-Sy5y and Ntera-2 cells, as well as their use in 3D cultures, but also chicken embryos at a late stage to model situation *in vivo*, and human-induced pluripotent stem cells

and -derived patterned brain organoids. We've used multiple classical (e.g., histology, morphometry, etc.) and modern (promoter assay, confocal and live-cell imaging, MEA, etc.) methods to show that exposure to neuroactive drugs during neurodevelopment provoked differential maturational changes and may reflect some of the underlying molecular deviations observed in human ASD pathology. The project outcomes might eventually help to impact national/international trials of pharmaceuticals and predict the drug of choice to avoid irreparable brain changes in the fetus.

WTH06-01 | The expression of pyruvate carboxylase in human astrocytes cell line

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The biosynthetic process in human brain depends on the withdrawal of several intermediates from the Krebs cycle (KC). Anaplerotic reactions can compensate for KC intermediates' loss and prevent a collapse of the mitochondrial metabolism. To investigate the expression and significance of pyruvate carboxylase (PC) for astrocytes cells, we evaluated the effect of the PC inhibition on survival of cultured human astrocytes cells and the expression of PC in cultured astrocytes cells as well as among human brain cortex samples by immunoprobable methods. The application of PC inhibitor negatively affects the survival of all types of tested cultured astrocytes cells. We correlate the cytotoxic effect of PC inhibitor with measurement of PC activity *in vitro*. The supplementation of culture media with PC inhibitor together with metabolites from KC, reverted the lethality of PC inhibitor. We looked at metabolomics changes, for example: cholesterol and ketone bodies synthesis, levels of metabolites from KC, lactate/glucose ratio, NADH/NAD ratio, after incubation the cultured astrocytes cells with CDP inhibitor. Immunocytochemical examination of cultured cells confirmed the expression and mitochondrial localization of PC. In addition, we confirmed the presence of PC in human brain astrocytes and neurons by immunoprobable method. Our results indicate that PC is expressed in human brain astrocytes and brain astrocytes cells, and the anaplerotic activity of PC plays a significant and in synthesis of cholesterol, in preventing lactic acidosis role and in sustaining their viability. This work was supported by the Slovak Research and Development Agency under Contract No. APVV-18-0088 and VEGA 1/0255/20.

**WTH06-02 | ATP depletion in cultured primary astrocytes**

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Adenosine triphosphate (ATP) is the central energy currency of all cells. In order to maintain a high cellular ATP level, the cellular consumption of ATP has to be compensated by continuous ATP synthesis. Cultured primary rat astrocytes contain a specific cellular ATP content of 32.4 ± 3.7 nmol/mg, which corresponds to a cytosolic ATP concentration of around 8 mM. During an incubation in the absence of glucose, this high ATP level was maintained for at least 6 h, whereafter the cellular ATP levels declined to around 30% of the initial value within the following 18 h. This observed depletion of cellular ATP content during glucose-deprivation was fully prevented by incubation of astrocytes in the presence of glucose or mannose, while substitution of glucose by mitochondrial substrates such as lactate, pyruvate, ketone bodies or acetate did not prevent cellular ATP depletion. Furthermore, ATP levels in glucose-starved astrocytes were depleted already within 30 min by the presence of the respiratory chain inhibitor antimycin A (10 μ M) or the mitochondrial uncoupler Bam15 (1 μ M) to 1% (antimycin A) and 20% (Bam15) of the initial value. In contrast, ATP levels in glucose-fed (5 mM) astrocytes were not affected by application of Bam15, while in the presence of antimycin A the cells maintained around 60% of the initial cellular ATP content. These data demonstrate that mitochondrial ATP production is essential to maintain a high cellular ATP content in astrocytes. In addition, antimycin A-inhibited mitochondrial respiration accelerated glycolytic flux in glucose-fed astrocytes, which appears to be insufficient to fully maintain the initial high cellular ATP levels.

WTH06-04 | Brain metabolic network trajectories during aging

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Aging changes many physiological parameters and constitutes the main risk factor for the development of several diseases, among them, neurodegenerative diseases. Considering the increase in life expectancy, it is necessary to understand how energy metabolism is affected in different brain regions during aging and which mechanisms underlie it. This study aimed at evaluating brain glucose

metabolism ([¹⁸F]FDG-PET), glial markers (GFAP and MAOB), and behavioral parameters in adult, middle-aged, and aged rats Wistar rats. Results were analyzed by one-way ANOVA, with statistical significance set at $p < 0.05$. Glucose metabolism showed no differences between young and aged rats. The middle-aged rats, however, presented hypometabolism in all regions (except cerebellum) compared to young rats, with maximum site in the parietal cortex ($p < 0.0001$). Compared to the aged rats, the middle-aged group presented decreased [¹⁸F]FDG uptake in the frontal cortex and striatum. Interestingly, we identified that the brain metabolic network was increasingly hyposynchronous with increased age. Aging also increases GFAP in the cortical region and MAOB in the hippocampus. No behavioral changes were detected in the working memory and attention. Conclusion: Our results show that the brain glucose metabolism and glial markers undergo changes without causing loss of memory in physiological aging. The brain metabolic network is increasingly hyposynchronous during aging, which may be related to an initial metabolic decline (middle-aged group) and subsequent astrocytic reactivity (aged group), considering that astrocytes largely contribute to the signal of [¹⁸F]FDG-PET, as we previously showed. We hypothesize that astroglial reactivity and loss-of-function in the elderly are key events that explain the higher susceptibility of the aged brain to develop neurodegenerative diseases. Other experiments are being conducted to better understand the changes the brain undergoes throughout aging.

WTH06-05 | Protective effect of *Rhus Verniciflua* extract on mitochondrial fitness status during the progression of Parkinson's disease

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It is well known that mitochondrial abnormality causes a variety of neurological diseases, such as Parkinson's disease (PD). However, their etiology is still not fully understood. PD's effects on the CNS are persistent and usually, by the time a patient exhibits classic motor symptoms, widespread loss of brain functions has already occurred. Therefore, evaluation of actual physiological state of mitochondria would enable early detection of PD and close monitoring of disease progression. Our work aimed to 1) establish a clinically applicable algorithm using an ex vivo model to assess actual physiological status of mitochondria and 2) examine the changes after application of plant-isolated mitochondria-like nanoparticles Lu120819in. Mitochondrial fitness was determined in PBMC cells isolated from



the peripheral blood of healthy and PD patients through molecular, physiological, and biochemical analysis. The application of 62 µg/ml or 186 µg/ml Lu120819 had a dose-dependent stimulating effect on complex I activity as well as overall respiration in PD patients with a disease duration of more than 6 years on Levodopa with a high degree of individuality in patients with the same disease characteristics was confirmed, highlighting the importance of personalization. While there were no significant changes in respiration at the concentrations studied in healthy subjects and newly diagnosed patients. Interestingly, we have found that diet (especially high protein) in healthy individuals influenced the enzyme activities of mitochondria in the naïve state as well as after the application of Lu120819. In conclusion, the mitochondrial fitness algorithm could serve as a dynamically responding marker reflecting the current status of mitochondria in the initialization/progression of the disease and the subsequent therapeutical response of PD patients.

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WTH06-06 | Intranasal insulin elicits different intracellular signaling responses in the hippocampus and frontal cortex of the mouse

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While brain glucose uptake is insulin-insensitive, insulin is necessary for normal function of the brain, particularly during periods of high metabolic demand, such as learning. Indeed, loss of insulin sensitivity is proposed to underlie cognitive dysfunction in type 2 diabetes or Alzheimer's disease. Intranasal insulin administration (IN) is a useful tool for testing brain insulin sensitivity or for therapy testing in neurodegeneration models. However, few pharmacokinetic studies have been performed to determine the optimal IN insulin dose for different target brain areas. The present study aimed at determining the effective insulin dose capable of triggering the activation of the insulin signaling pathway (ISP) in the mouse frontal cortex and hippocampus. Intranasal insulin (0.5–2 IU) was administered to 3 months-old C57BL6 mice. After 30 min, the frontal cortex and the hippocampus were collected for evaluating protein levels and phosphorylation of IR, IRS1, Akt, and GSK3β. Insulin levels were determined by ELISA in these brain areas and also in plasma samples collected at the same time. Plasma glucose was assessed by glucose oxidase assay. Intranasal insulin triggered a robust activation of IR and IRS1 in the hippocampus (increased phospho-IR^{Tyr1146/Tyr1150/Tyr1151}/IRβ and phospho-IRS1^{Tyr612/Ser636}/IRS1 ratios). In frontal cortex, activation was observed in phospho-IRS1^{Ser636}/IRS1, phospho-GSK3β^{Ser9}/GSK3β and phospho-Akt^{Ser473}/Akt ratios. Insulin levels increased with increased intranasal dose administered to similar levels

in the cortex and hippocampus, as did plasma insulin. Nevertheless, raising of plasma insulin was not sufficient to reduce plasma glucose. Altogether, these results suggest that the hippocampus and cortex are likely respond to intranasal insulin at different rates. Future studies should determine such kinetics.

WTH06-07 | β-lapachone-mediated WST1 reduction as indicator for the cytosolic redox metabolism of cultured primary astrocytes

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Electron cyclor-mediated extracellular reduction of the water-soluble tetrazolium salt 1 (WST1) is frequently used as tool for the determination of cell viability. We have optimized this method to investigate the cellular redox metabolism of cultured primary astrocytes by using the reduction of the electron cyclor β-lapachone by cytosolic NAD(P)H:quinone oxidoreductase 1 (NQO1). Cultured astrocytes that had been exposed to β-lapachone in concentrations of up to 3 µM remained viable and showed a linear extracellular accumulation of WST1 formazan for the first 60 min, while higher concentrations of β-lapachone caused oxidative stress and impaired cell metabolism. β-lapachone-mediated WST1 reduction was inhibited by the NQO1 inhibitors ES936 and dicoumarol in a concentration-dependent manner, with half-maximal effects observed at around 0.3 µM. β-lapachone-mediated WST1 reduction depended strongly on glucose availability, while mitochondrial substrates such as lactate, pyruvate or ketone bodies allowed only residual β-lapachone-mediated WST1 reduction. Accordingly, applying the mitochondrial respiratory chain inhibitors antimycin A and rotenone hardly affected astrocytic WST1 reduction. Both NADH and NADPH are known to supply electrons for reactions catalyzed by cytosolic NQO1. Around 60% of the glucose-dependent β-lapachone-mediated WST1 reduction was prevented by the presence of the glucose-6-phosphate dehydrogenase inhibitor G6PDi-1. In contrast, the glyceraldehyde phosphate dehydrogenase inhibitor iodoacetate had only little inhibitory potential in this process, suggesting that pentose phosphate pathway-generated NADPH, and not glycolysis-derived NADH, is the preferred electron source for cytosolic NQO1-catalyzed reductions. These findings demonstrate that extracellular WST1 reduction in the presence of the redox cyclor β-lapachone is a suitable test system to investigate the metabolic pathways involved in cytosolic NADPH regeneration in cultured astrocytes.

**WTH07-01 | Brain volume and proteome profiles changes in the aging rat brain and impacts on cognitive and locomotor functions**

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Aging is a progressive physiological change in an organism that led to decline of various biological and molecular functions. Rising incidence of neurodegenerative diseases in aging populations is creating a sense of urgency for understanding of structural and functional changes in the aging brain. The aging brain process is also not well understood at a molecular level. Therefore, the current study was designed to examine proteome profiles in the specific brain regions, as well as brain volume measurements, behavioral evaluations, and biochemical analyses in middle- to late-aged rats. In this study, Sprague Dawley rats aged 14–27 months old were used. Magnetic resonance imaging was performed to measure the brain volumes. Behavioral studies were conducted using open field and Morris water maze tests. Blood was taken to assess malondialdehyde levels in plasma and erythrocyte antioxidant enzymes activity. Proteome profiling in the brain was performed through liquid chromatography tandem mass spectrometry (LC-MS/MS). Increased volume of lateral ventricle, and decreased medial prefrontal cortex, hippocampus, and striatum volumes were observed in late-aged rats. The late-aged rats also demonstrated impairment in exploratory behavior and memory, higher malondialdehyde levels, and lower glutathione peroxidase activity. Proteomics analysis by LC-MS/MS identified a total of 1074, 871, and 241 proteins, of which 97, 25, and 5 were expressed differentially with age in the hippocampus, medial prefrontal cortex, and striatum, respectively. Aging altered levels of several proteins potentially related to energy metabolism, glutathione metabolism, and calcium signaling pathways. In conclusion, age-related increases in oxidative stress, cognitive and locomotor functions decline, changes in brain volume, and alterations of proteins expression level in the brain were observed, with the most marked impairments observed in later age.

WTH07-02 | Influence of strength training on behavioral parameters in an animal model of alcoholism

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Abusive use and dependence on alcohol are considered a serious public health problem. Treatment for alcoholism is still very inefficient, considering the high prevalence of relapses and low adherence to treatment. Despite physical exercise being a safe and effective form of and therapeutic resource for various health problems, little is known about the benefits of physical exercise with the benefits of alcohol use. In this study, behavioral changes were investigated as result of acute intervention with strength training in alcohol-dependent rats. Male Wistar rats ($n = 96$) were divided into six groups: Trained Control (CT), Abstinent Sedentary (WS), Abstinent Trained (WT), Alcohol Sedentary (AS), and Alcohol Trained (AT). The induction of alcohol dependence occurred through the 28 days voluntary consumption protocol. Then, the animals were strength trained, which consists of 2-week of vertical climbing. Voluntary alcohol consumption, spontaneous locomotor activity and anxiety-like behaviors were evaluated. The 2-week strength training did not change the consumption behavior or preference for alcohol of AT, these animals showed anxiety-like behavior after the period of intervention with physical exercise. Physical strength training prevented anxious-like behavior in WT animals, possibly related to the anti-inflammatory effects of exercise. We suggest an intervention with strength training during alcohol withdrawal as the anti-inflammatory and antioxidant effects of exercise can help prevent brain damage and anxiety disorders.

WTH07-03 | Safety profile of localized non-invasive brain stimulation using uHD-PES: Histological analysis

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Transcranial electrical stimulation has been widely used for noninvasive neuromodulation of the central nervous system to induce a psychological and behavioral response. Despite its success in enhancing learning and memory and its applications in mitigating different neurological disorders, high resolution targeted non-invasive electrical stimulation of the brain remains elusive. We have recently demonstrated a focused minimally invasive ultra-high-density pulse electrical stimulation (uHD-PES) method to evoke neural activity in rodent models. Here, we show that this is a safe modality for non-invasive neural stimulation. Ultra-high-density flexible surface



electrode arrays are placed on the mice skull ($n = 3$) to apply pulsed electric currents with higher resolution to achieve localized brain stimulation. The evoked activity was confirmed using c-fos immunoreactivity, which is a marker of neural activation. Further safety profile of the stimulation approach was characterized with immunofluorescence analysis of different cellular markers (e.g., NeuN, GFAP, Iba1) and apoptotic markers (Caspase-3). Our results show an elevated c-fos expression following stimulation in the motor cortex of the stimulated hemisphere in comparison to the non-stimulated hemisphere. Evoked activity was localized to the motor cortex of the stimulated hemisphere within 200–400 μm of the simulated stimulation focus. Interestingly, no significant changes were observed in neuronal density between the stimulated and non-stimulated hemispheres. Similarly, no neuronal damage was observed between two different hemispheres as indicated by lack of apoptosis, that is, caspase-3 expression. Also, uHD-PES did not cause any gliosis as no significant difference in intensity of glial and astrocytes markers were observed. This indicates lack of reactive microglial or astrocytes in response to the uHD-PES method. In conclusion, uHD-PES is confirmed to be an effective approach for localized noninvasive brain stimulation without any significant tissue and cellular damage.

WTH07-04 | Memory consolidation related computational markers utilizing in vivo electrophysiology

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Learning and memory are the integral part of the regular brain functioning. Previous literature has established several memory related molecular markers as a target for memory formation based on neurobehavioral tasks on rodents, however, there is paucity of literature which could decipher the memory formation markers in real time. *In vivo* rodent electrophysiology is a robust tool to explore the dynamics of memory formation in real time. The present study aims to identify and validate the memory formation in rodent brain utilizing *in vivo* electrophysiology data and machine learning tools. Wistar rats were surgically implanted with 16-channel head stage electrodes at the CA1 hippocampal region, connected through adaptor cable to the signal acquisition system. Rats were subjected to 3 days Novel Object Recognition Task. The neural spike signals and local field potentials were recorded during the behavioral task. The bio-signals acquired were then subjected to machine learning algorithms using MATLAB for identification of computational markers of memory formation. The identified computational markers were then validated through behavioral data analysis and established memory related molecular markers estimation. The results showed that neural signals are altered during the neurobehavioral task and neural signal features are characteristic of the memory formed during the training

task. Taken together, our results establish that memory formation is not necessarily translated into behavioral data, but computational markers provide robust and novel insight on the dynamics of memory consolidation mechanism independent of behavioral analysis.

WTH07-05 | Astrocytic mGluR3 and related metabotropic receptors alter spatial memory in a sex-specific manner

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Astrocytes regulate cognition and behavior, but the exact mechanisms are not clear. Metabotropic glutamate receptor 3 (mGluR3) is highly enriched in astrocytes. As a central astrocytic sensor of glutamate, mGluR3 likely regulates cognition and other neural processes. mGluR3 alterations are implicated in diverse neurological disorders that cause cognitive dysfunction, including neurodegenerative and neuropsychiatric diseases. However, the roles of astrocytic mGluR3 in cognitive function are not known. Here, we investigated the effects of astrocytic mGluR3 and related receptors on learning and memory using complementary transgenic and chemogenetic approaches. First, we utilized CRISPR-Cas9 to inducibly knock down astrocytic mGluR3 in the hippocampal formation of adult mice. Remarkably, astrocyte-targeted mGluR3 knockdown caused prominent sex-specific effects on spatial memory, despite similar rates of learning and similar reductions in mGluR3 across groups. Notably, mGluR3 knockdown impaired memory in females, but improved memory in males, suggesting that astrocytic mGluR3 regulates memory in a sexually dimorphic manner. We next increased astrocytic mGluR3 expression in a cell-selective and brain region-specific manner using Cre-dependent viral vectors that induced modest increases in astrocytic mGluR3 expression. These increases improved memory in females, but not males. Furthermore, we determined if these sex-specific effects are recapitulated with *in vivo* receptor stimulation, and whether these effects are unique to mGluR3 or also pertain to other Gi/o-coupled receptors. Thus, we used *in vivo* chemogenetics to selectively stimulate Gi/o- or Gs-coupled receptor signaling in hippocampal astrocytes. Analogous to the sex-specific effects of modulating astrocytic mGluR3, activating astrocytic Gi/o-coupled signaling improved memory in females, but impaired memory in males. Conversely, activating astrocytic Gs-coupled signaling impaired memory in females but not males. Thus, our findings demonstrate that astrocytic mGluR3 and other metabotropic receptors regulate neural function in a sex-specific manner and may promote sex differences in neurodegenerative and neuropsychiatric diseases that involve alterations in astrocytic signaling.

**WTH07-06 | Unraveling caffeine influence on adult neurogenesis and behavior**

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Neurogenesis occurs in the adult mammalian brain, primarily in the subventricular zone and dentate gyrus of the hippocampus, allowing the generation of new neurons that form synapses and integrate pre-existing circuits. Caffeine is a potent non-selective adenosine receptor blocker and the most widely used psychostimulant. However, its impact on the regulation of events related to adult brain plasticity has been largely overlooked. Therefore, the main objective of this work is to dissect the effect of caffeine on the differentiation, maturation and integration of cells born during the neonatal or at adult stage.

For this purpose, *in vitro* and *in vivo* approaches are being pursued. Using the neurosphere assay, our results indicate a dose-dependent role of caffeine in the regulation of postnatal neurogenesis *in vitro*, namely a tendency to promote proliferation at an intermediate concentration (125 μ M) whilst decreasing it at higher concentration (1 mM) in both SVZ and DG cells ($n = 3-8$). Moreover, caffeine at 1 mM induced neuronal differentiation in the SVZ-derived neurospheres while showing a tendency to increase the number of mature neurons at 125 μ M in DG-derived neurospheres ($n = 3-6$). Surprisingly, caffeine administration (0.3 g/L in drinking water) in adult male mice showed a tendency to impair spatial learning and memory but did not induce anxiety-like behavior ($n = 5-7$). Currently, we are analyzing caffeine behavioral influence in female mice. Further behavioral (namely recognition memory, sociability, and social discrimination behaviors), cellular (specifically to evaluate neurogenesis in both neurogenic niches), molecular (adenosine receptors levels and signaling as well as neurogenic markers), and electrophysiological features analysis are required to fully ascertain caffeine effects on adult neurogenesis and behavior.

Taken together, this project will unveil the mechanisms underlying caffeine effects in postnatal and adult neurogenesis giving novel insights about its effects in brain physiology, which will have a relevant impact in public health.

WTH07-07 | Anxiolytic effects of aqueous methanolic extract of *Allium sativum* and enriched environment in an open field test in Wistar rats

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Murine models of anxiety have been used to study anxiety-related behaviors in mammals. The open-field test explores the natural

aversion of rodents to exposed fields and is based on anxiogenic agents such as an open area with surrounding walls to prevent animal escape and the anxiety level is expressed by the number of entries and the length of time spent in the aversive area. Enrichment of the environment has long been proposed as a treatment or strategy for increasing well-being in rodents. In this study, we looked at the individual as well the combined effects of garlic extract and enriched environment on anxiety behaviors in an open-field test.

Forty-two male albino mice were used for this study. They were divided into seven groups of six mice each. Group 1 was given distilled water; Groups 2 and 3 received 200 mg/kg and 400 mg/kg of aqueous-methanolic extract of *Allium sativum* respectively; while Groups 4 and 5 were in addition to receiving 200 mg/kg and 400 mg/kg of the extract, housed in an enriched environment cage; Group 6 was only housed in an enriched environment cage and Group 7 receives 20 mg/kg of imipramine. The experiment lasted for three weeks after which the mice were subjected to an open-field test to assess anxiety-related behaviors. MDA, GPx and TNF- α levels were assayed in homogenate from brain tissues harvested. Aqueous-methanolic extract of *Allium sativum* and enriched environment ameliorated anxiety behaviors in mice through the reduction of grooming, rearing, defecation, stretch attend and immobility time while the number of center crossing, center square duration and line crossing increases. There was a reduction in MDA and TNF- α levels but an increased GPx level.

Anxiety-related behavior is ameliorated by an enriched environment and *Allium sativum* and therefore could be used in the management of anxiety.

WTH07-08 | Supplementation with medium-chain triglycerides (MCT) improves memory and alters glutamatergic gene expression in the rat brain

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Medium-chain triglyceride (MCT) supplementation improves cognition in young and aged healthy subjects, and in neurodegenerative conditions. Although the benefits of MCT are linked to ketogenesis, which provides alternative fuel for the brain, the exact mechanisms remain poorly understood. Changes in number and subunit composition of NMDA- and AMPA-ionotropic glutamatergic receptors (iGR) underly long-term potentiation, one of the key mechanisms in learning. We studied the effects of MCT feeding on learning and mRNA expression of the iGR subunit and EAAT2 (glutamate transporter) genes in medial prefrontal cortex (mPFC), dorsal (DH) and ventral hippocampus (VH), of adult male Wistar rats. The animals



were tested in Y-maze (YM), Open Field (OF) tests. After 2 weeks of daily o/g treatment with 2 ml/kg MCT (water as control), the animals were tested in YM, OF, and Morris water maze (MWM) tests. Gene expression was assessed with RT-PCR. After the last MCT treatment, animals were sacrificed, blood was collected for biochemical tests. β -hydroxybutyrate level was elevated, while pyruvate and cholesterol levels decreased after MCT treatment compared to control. In YM, the rate of spontaneous alternation decreased in control but not MCT-fed animals, indicating that MCT improved working memory. In OF, MCT animals demonstrated higher extinguishing in exploratory activity, indicating better memory of the surroundings. In MWM, MCT animals spent more time in the target sector, indicating improved spatial memory. GluN2a- and GluN2b- NMDA-iGR subunit and GluA1- and GluA2 AMPA-iGR subunit expression was upregulated in mPFC of MCT animals, suggesting enhanced memory consolidation. MCT feeding increased EAAT2 expression in DH and decreased it in VH. Therefore, memory-enhancing effects of the intermittent ketosis established by MCT feeding are at least partially mediated via the effect on glutamatergic transmission. Funded by the Russian Science Foundation, grant 19-75-10076.

WTH07-09 | Effect of taurine on GABA efflux during alcohol withdrawal and re-exposure in the nucleus accumbens of rats

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Taurine modulates GABAergic and glutamatergic systems, both related to progression from occasional alcohol intake to dependence. We investigated the effect of taurine treatment on GABA levels in the nucleus accumbens (NAcc) during alcohol withdrawal and re-exposure of rats. Adult male Wistar rats were allowed to choose from two bottles containing alcohol (20%) and vehicle solution (AL group) or two bottles containing vehicle (CT group), 24h/day, for 4 weeks (CEUA-UFRGS#36606). On day 22nd, half of AL rats had their alcohol bottle substituted for vehicle (WH group). CT, AL, and WH groups were subdivided to receive 100 mg/kg taurine or saline i.p. (CTS; CTT; ALS; ALT; WHS; WHT; $n = 6$ /group), 1x/day, 6 days. On day 20th, a guide cannula was inserted into NAcc (stereotaxic surgery), and 7 days after, microdialysis was performed along 5h, with samples collected every 30 min. Baseline GABA levels were determined until 5th sample (UFLC-MS). Immediately after, rats received taurine/saline, and its acute effect was observed over the next 150 min (GABA% from baseline). Later, rats from WH groups were re-exposed to alcohol for 24h. Taurine

decreased baseline GABA of ALT group. In WHT group, taurine prevented the lower baseline GABA found in WHS group. Acute taurine increased GABA efflux 30 min after injection in CTT group, and after 60, and 90 min in ALT group. In WHT group, acute taurine constantly reduced GABA efflux from 30 min. Taurine doubled the alcohol intake of the ALT group from the 3rd day and decreased by 64% the alcohol re-exposure intake of WHT group. Taurine produces alcohol anti-addictive effect dependent on the abstinence condition, and this may be related to the restoration of GABA levels in NAcc impaired by alcohol withdrawal.

WTH07-10 | Cognitive abilities and hippocampal structure in aged brevicin and neurocan double knock-out mice

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The attenuation of learning and memory during aging is associated with an increase in neural extracellular matrix (ECM) components. Accumulation of brevicin, neurocan and link protein HAPLN1 at hippocampal synapses might attenuate plasticity and diffusability of receptors and neuromodulators, thus contributing to declining learning abilities. Cognitive functions can be preserved during aging through exercise. Enriched environment with voluntary running affects the composition of the ECM and activity of matrix-degrading enzymes. Beneficial effects of exercise during aging could thus be potentially mediated via ECM composition.

We explored the effects of voluntary running on learning abilities in aged brevicin and neurocan double knock-out (DKO) mice to elucidate the significance of those ECM components for cognitive decline during aging.

Wildtype and DKO mice were kept under standard or exercise conditions throughout their life. At 19–20 months, we tested spatial learning in the water-cross maze and fear learning via trace fear conditioning (TFC). After behavioral testing, brains were collected for protein quantification and immunohistochemical staining.

Both genotypes showed age-related increase in glial fibrillary acidic protein, even after lifelong exercise. Immunoblotting data also confirmed high expression of hippocampal HAPLN1. Normal spatial learning and spatial flexibility were observed in all groups. Lifelong exercise positively affected spatial accuracy during maze training in both genotypes. Brevican and neurocan DKO mice were deficient in conditioned stimulus learning, but there were no group differences



in context fear learning. This deficiency could be due to a dysplasia in the CA1 region of the DKO mice, especially since the CA1 is pivotal for temporal association learning in TFC. Thus, brevicin and neurocan are essential for conditioned stimulus learning in TFC in aged mice, but those ECM components seem not to mediate beneficial effects from lifelong exercise.

WTH07-11 | A cartographic and morphometric analysis of neuronal spines in m6A reader-deficient mice

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The morphological features of dendritic spines are highly dynamic and correlated to synapse maturation, plasticity, learning and memory. Recently, a new mode of gene regulation, epitranscriptomics, has emerged as a versatile and powerful post-transcriptional regulatory pathway to regulate spines and synapses; the prevalent mRNA modification N6-methyladenosine (m6A) is a critical component in multiple learning and memory paradigms such as motor learning, spatial memory, fear consolidation and extinction, etc. Previously, we have cataloged thousands of m6A-modified RNAs in the synaptic compartments and demonstrated that the loss of the cytoplasmic “reader” proteins YTHDF1 and YTHDF3 cause reduced synaptic transmission. However, the role of YTHDF proteins in neurons over the regulation of spine morphology in vivo is unknown. In the current study, we have performed high-resolution morphometric analysis on a large number of dendritic spines over wide brain regions by combining modified Dil-labeling with high-speed, high-resolution, position-registered imaging followed by computer-aided spine detection and quantification. We applied this technique to analyze dendritic spines in YTHDF1 or YTHDF3-conditional knockout mice (CaMKIIa-Cre: to specifically inactivate YTHDF1 or YTHDF3 in mature forebrain excitatory neurons) and quantified parameters such as spine volume, diameters of the spine head and neck, as well as the length of spine neck, etc. We found massively altered spine morphometry and density at the loss of YTHDFs in different brain regions. Intriguingly, morphometric measurements revealed diverse responses to the loss of YTHDFs in different neurons, and even within the neurons in difference subcellular compartments. Golgi staining supported the analysis with consistent albeit less accurate measurements. Further detailed anatomical analysis will be applied in the future studies to understand the role of m6A signaling in regulating spine dynamics.

WTH07-13 | Role of hippocampal AMPA-R trafficking with STEP signaling in cadmium induced cognitive deficits in rats Amelioration by quercetin

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The study is aimed to identify targets in cadmium induced cognitive deficits with focus on to assess the integrity of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA-R) receptors and STRiatal Enriched protein tyrosine Phosphatase (STEP) signaling in rat hippocampus. Further, protective potential of quercetin, a natural bioflavonoid, was also assessed.

Rats were randomly divided into four treatment groups and exposed to cadmium (5mg/kg, body weight p.o. for 28days) or quercetin (25mg/kg body weight p.o. for 28days) alone or simultaneously. A group of rats treated with vehicle that served as control was also included. Rats were sacrificed 24h after the last dose of treatment and hippocampus was dissected out and processed for transcriptional, translational, and ultrastructural studies.

A decrease in mRNA expression and protein levels of AMPA-R subunits (GluA1, GluA2, and GluA3) was observed in cadmium treated rats as compared to controls. Also, there was a decrease in the levels of proteins associated with AMPA-R downstream signaling: GRIP1, Synaptophysin, FyN/pFyN, PyK2/pPyK2, and PTPa/pPTPa in hippocampus of cadmium treated rats. These changes were associated with increase in the levels of STEP61/pSTEP61 that resist synaptic plasticity in hippocampus. Damage in myelin sheath, synaptic membrane and synaptic vesicles in hippocampus of cadmium treated rats was also evident in ultrastructural studies as compared to controls. Simultaneous treatment with quercetin was found to protect cadmium induced changes in rat hippocampus.

The decrease in the expression of AMPA-R subunits and its signaling proteins associated with increased expression of STEP in hippocampus may be attributed to impaired learning and memory in cadmium treated rats. It is interesting that quercetin has the ability to modulate these receptors/signaling targets and ameliorate cadmium induced synaptic and memory loss.

WTH07-14 | Brain mapping of c-Fos in binge-like drinking mice reveals that alcohol memories are stored in networks in remote brain regions

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Alcohol consumption is a social problem linked to many harmful consequences both for the individual drinker and the society as a whole. Even episodic alcohol consumption may shift toward severe alcohol-related problems. While some drinkers will not develop a dependence, others will do. Alcohol experience activates different population of neurons that undergo persistent changes to become an engram, that is, groups of neurons holding a specific memory. Neurons create functional networks changing over time, since brain regions work together differently in different contexts. These networks are subsequently reactivated by alcohol-related cues. The aim of this study was to perform functional brain mapping in binge-drinking animals. For this we used global brain c-Fos profiling. c-Fos protein is greatly increased after exposure to novelty. It serves as an activity marker that mediates the long-term potentiation of excitatory synapses. The present study investigated c-Fos expression after long-term alcohol withdrawal in a model of binge-like drinking in socially-housed female mice. We developed a dedicated image computational workflow to identify c-Fos-positive cells in three-dimensional images obtained after optical brain clearing and imaging in the light-sheet microscope. We report a map of the engram complexes for alcohol reexposure by characterizing activated neuronal ensembles in multiple, distinct brain regions. We provide c-Fos expression profile data for 169 brain structures and indicate that after binge-like drinking alcohol reexposure produces disconnection of functional networks of the brain toward the formation of fine circuitries that are involved in the development of addiction and processing of emotional information.

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WTH07-15 | Intermittent ketosis induced by MCT supplement: Dose-dependent differential effects on cognitive and metabolic parameters in rats

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Medium-chain triglycerides (MCT) demonstrated a range of neuroprotective effects, mostly attributed to their ketogenic properties.

However, the exact mechanisms of the MCT effects on the brain and the scope of targetable deficits and cognitive functions are largely unknown. Long-term metabolic consequences of MCT supplementation in cognition-enhancing doses have not been rigorously investigated. We administered MCT oil (1, 3, or 6g/kg/day) or water (control) orogastrically to 2.5-m.o. male Wistar rats for 28 days, without limiting access to standard chow, and assessed working memory in Y-maze, behavior in the Open Field, spatial learning in Morris water maze (MWM), and weight of internal organs. In a separate experiment, we evaluated the effects of MCT (3g/kg) or lard (3g/kg, control) supplementation for 28 days on blood biochemical parameters. In Y-maze, MCT-1 and MCT-3 treatment improved working memory. In MWM, MCT-6 treatment improved spatial memory. The MCT-6 treatment increased liver weight and the brown/white adipose tissue ratio. Chronic MCT treatment (3g/kg) did not affect the blood levels of glucose, lactate, pyruvate, acetoacetate, β -hydroxybutyrate, total and HDL cholesterol, triglycerides, malondialdehyde, and aspartate transaminase and alanine transaminase activities. Chronic MCT supplementation of regular chow demonstrated a non-uniform dose-dependent effect on the measured cognitive parameters. For spatial memory, the effect disappeared at the highest dose, which was also associated with changes in organ weight and adipose tissue metabolism. Further detailed studies are needed to better understand the neuroprotective effects of MCT. Supported by Russian Science Foundation (RSF, project no. 19-75-10076).

WTH07-16 | Length impairments of the axon initial segment in rodent models of attention-deficit hyperactivity disorder

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The axon initial segment (AIS) is a structural neuronal compartment of the proximal axon that plays key roles in sodium channel clustering, action potential initiation, and signal propagation for neuronal outputs. Mutations in constitutive genes of the AIS, such as ANK3, have been identified in patients with neurodevelopmental disorders. Nevertheless, morphological changes in the AIS in neurodevelopmental disorders have not been characterized. In this study, we investigated the length of the AIS in animal models of attention-deficit hyperactivity disorder (ADHD). We observed abnormalities in AIS length in the rodent models. In ADHD model mice and rats, we observed shorter AIS length in layer 2/3 (L2/3) neurons of the medial



prefrontal cortex (mPFC) and primary somatosensory barrel field (S1BF). Further, we observed shorter AIS length in S1BF L5 neurons of ADHD model mice. These results demonstrate that AIS length is altered in specific brain regions in ADHD rodent models, and AIS abnormalities may be conserved across species. Our findings provide novel insight into the potential contribution of the AIS to the pathophysiology and pathogenesis of neurodevelopmental disorders.

WTH08-01 | No association exists between non-alcoholic fatty liver disease and dementia risk: A meta-analysis of real-world evidence

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Introduction: Dementia remains a global public health challenge and imposes a significant humanistic and epidemiologic burden on patients and healthcare payers. Epidemiological studies presented inconsistent findings on the association between non-alcoholic fatty liver disease (NAFLD) and dementia risk. Therefore, this study aimed to meta-analyze the association between NAFLD and the risk of dementia.

Methods: A systematic search of MEDLINE and Embase was conducted to identify articles assessing the association between NAFLD and dementia. The quality of individual studies was assessed using the Newcastle-Ottawa scale (NOS). Literature screening, quality assessment, and data extraction were performed independently by two authors. The risk of dementia in NAFLD patients was computed as the primary outcome of interest. Subgroup analysis based on sex was also performed. Certainty of findings was assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) criteria. RevMan v 5.5 was used to perform the meta-analysis.

Results: This meta-analysis was based on a total of four real-world studies with a total of 2,059,253 participants. The mean age of the NAFLD patients was 62.2 ± 16.71 years and the mean follow-up period was 10.25 years. Included studies were found to be of high quality. No significant association exists between NAFLD and dementia risk with a pooled relative risk of 1.03 (95% CI: 0.91–1.16); $p = 0.62$. Subgroup analysis based on sex also revealed non-significant association of NAFLD with the dementia risk in the female with a pooled relative risk of 1.09 (95% CI: 0.32–3.70), $p = 0.88$. GRADE criteria revealed low certainty of evidence.

Conclusion: NAFLD was not found to be associated with the risk of dementia. Future large well-designed epidemiological studies are needed to confirm the findings.

WTH08-02 | Differential expression of brain endothelial cell markers in the human brain: Implications in the pathogenesis of cerebral malaria

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Cerebral microvascular endothelial cells (CMVECs) line the vascular system of the brain and are the chief cells in the formation and function of the blood brain barrier (BBB). These cells are heterogeneous along the cerebral vasculature and any dysfunctional state in these cells can result in a local loss of function of the BBB in any region of the brain. There is currently no report on the distribution and variation of the CMVECs in different brain regions in humans. This study investigated microcirculation in the adult human brain by the characterization of the expression pattern of brain endothelial cell markers in different brain regions. Five different brain regions consisting of the visual cortex, the hippocampus, the precentral gyrus, the postcentral gyrus, and the rhinal cortex obtained from three normal adult human brain specimens were studied and analyzed for the expression of the endothelial cell markers: Cluster of differentiation 31 (CD31) and von-Willebrand-Factor (vWF) through immunohistochemistry. We observed differences in the expression pattern of CD31 and vWF between the gray matter and the white matter in the brain regions. Furthermore, there were also regional variations in the pattern of expression of the endothelial cell biomarkers. Thus, this suggests differences in the nature of vascularization in various regions of the human brain. These observations also suggest the existence of variation in structure and function of different brain regions, which could reflect in the pathophysiological outcomes in a diseased state.

WTH08-03 | A systematic review of the psychological stress on cancer recurrence risk

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Cancer recurrence is an important clinical issue in cancer treatment. It is well known that psychological stress contributes to the incidence and progression of cancer; however, its effect on cancer recurrence remains unclear. We conducted a systematic review to examine the current evidence published by May 2021 from the three databases: Medline (PubMed), Embase and Cochrane Library. Of the total 35 relevant articles, six studies (10 data points) were finally selected. This study included 26,329 patients (26,219 breast cancer patients except hepatocellular carcinoma patients in 1 study), four cohort studies (8 data points) and two RCTs (2 data points). Among the eight data points in cohort studies, four psychological stress-related factors (2 'anxiety', 1 'depression', and 1 'hostility') were moderately related to increase the risk for cancer recurrence, while 'loss of partner' resulted in opposite



results. The 'emotional' and 'mental' health factors showed conflicting results, and the meta-analysis of RCTs proved the positive effects of psychotherapies in reducing the cancer recurrence risk among breast cancer patients (HR = 0.52; 95% CI 0.33–0.84). In spite of some limitations, this study exhibits comprehensive information about the effect of psychological stress on cancer recurrence and provides reference data to clinicians for future studies.

WTH09-01 | Ontogeny and early-life stress: microglial and behavioral sex-specific responses of young mice to infant maternal separation

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Early-life stress is etiologically linked to many neuropsychiatric disorders, and several emerge during adolescence, another sensitive developmental window. One common feature of both periods is neuronal circuit-remodeling in response to environmental inputs. Microglia is highly responsive and involved in developmental processes, and its malfunctioning is seen in neurodevelopmental disorders.

This study aimed to evaluate the effects of maternal separation (MS – early-life stress protocol) on behavior and microglial morphology of prepubescent male and female GFP-expressing microglia transgenic mice (heterozygote CX3CR1:GFP). Pups were subjected to 3h of MS (P2–P14), and tested for anxiety- and depressive-like behaviors at P30; other subgroups were euthanized at P15 or P30 and hippocampal tissue was collected. Microglial numbers and morphology were analyzed by confocal microscopy. Data were analyzed using Student's *t*-test or Two-Way ANOVA and Tukey test.

Our results showed a decrease in microglial density within the hippocampus from P15 to P30 in males, in concordance with the literature; however, females do not follow the same pattern and, compared to males, females have fewer microglial cells already at P15. On the other hand, female microglial cells had longer dendrites than males at P30. Not only microglial developmental dynamics were different in males and females, but they also responded differently to MS: males had smaller cells, whereas females had a decrease in general cell complexity. Interestingly, we also see anxiety- and depressive-like behavioral phenotypes in males subjected to MS, whereas females were less responsive to the behavioral tests and exhibited only an increase in exploration and motor behavior. Thus, our study shows a sex-specific ontogeny pathway in microglial morphology, as well as sex-specific responses to MS, with a decrease in cell complexity only in females; although we are not able to provide a causal link, we also identify a sex-specific behavioral effect after MS, with a stronger effect in males. Further investigation of the mechanisms behind sex- and stress-related differences are needed to understand how microglial physiology interferes with developmental pathways in health and brain disorders.

WTH09-02 | Adenosine -CB1 receptors crosstalk in astrocytes: implications in medial prefrontal cortex synaptic function

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The mPFC (medial prefrontal cortex) is involved in cognitive functions such as working memory and emotional responses. This brain region features not only neurons but also glial cells such as astrocytes that have an impact on mPFC activity. Astrocytic CB1R activation elicits Ca²⁺ transients, which lead to the modulation of synaptic transmission. Astrocytes also express adenosine A₁ and A_{2A} receptors (A₁R, A_{2A}R), whose interactions with CB1R in mPFC astrocytes are not known. Thus, this work aims to fill this gap by studying the crosstalk between astrocytic CB1R and adenosine receptors and its role in mPFC activity.

Primary astrocytic cultures were prepared from Sprague Dawley pups where a physical interaction was observed between CB1R with A1R as well as with A2AR by Proximity Ligation assay (PLA) and Bioluminescence Resonance Energy Transfer (BRET). By performing Ca²⁺ imaging in astrocyte cultures, it was observed that CB1R activation led to Ca²⁺ transients which were significantly increased by the previous activation of A₁R and diminished while activating A_{2A}R. Field excitatory postsynaptic potentials were recorded in mPFC from an IP3R2 knockout mouse (which obliterates the GPCR-dependent astrocytic Ca²⁺ responses), and the magnitude of long term potentiation (LTP) was evaluated on both IP3R2-WT and IP3R2-KO mice. CB1R activation led to a significant increase of LTP magnitude in IP3R2WT while it had a significant decrease in IP3R2KO mice. Our data, so far, indicates that in IP3R2-WT the blockade of A1R abolishes the CB1R effect in mPFC LTP, however further experiments are needed.

Thus, it was shown that CB1R activation has an opposite effect on mPFC synaptic plasticity depending on the presence of astrocytic Ca²⁺ signaling. Regarding CB1R and adenosine receptors, both receptors crosstalk with CB1R and A1R increases CB1R-mediated Ca²⁺ signaling while A2AR decreases it, demonstrating a direct functional crosstalk between cannabinoid and adenosine receptors in astrocytes.

**WTH09-03 | Fractalkine isoforms differentially regulate microglia activation and vascular damage in the diabetic retina**

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Diabetic retinopathy (DR) is a microvascular complication and leading cause of blindness worldwide. Hallmarks of DR include loss of neurons, microglia activation, and vasculature damage. Inflammation caused by microglia exacerbates the retinal integrity, resulting in glial cell dysfunction. The microglia-neuronal crosstalk mediated by CX3CR1/FKN signaling provides a neuroprotective environment in several neurological diseases. However, CX3CR1 polymorphic variants are present in about 30% of the population decreases FKN binding affinity to CX3CR1, and their role in microglia function in the retina during diabetes is unknown. Moreover, FKN exists as a membrane-bound (mFKN) adhesion molecule. When cleaved by a disintegrin metalloproteinase (ADAM) protein, the soluble (sFKN) form exerts chemoattractant properties. Studies show that diabetic CX3CR1-KO and FKN-KO mice display worse retinal pathology, and intra-vitreous administration of recombinant FKN to diabetic FKN-KO mice rescued vascular pathology. The mechanisms by which mFKN and sFKN regulate retinal function are still unknown. We hypothesize that during diabetes, the over-expression of sFKN using recombinant adeno-associated viruses (rAAVs) will prevent vascular and neuronal damage, and improve visual function. To test this hypothesis, rAAVs expressing mFKN or sFKN were delivered to FKN-KO retinas. We validated the expression of FKN levels in retinal protein extracts in mice that received rAAVs expressing mFKN or sFKN. Immunofluorescence and high-resolution confocal imaging show that sFKN minimizes microglial activation, reduces fibrinogen deposition, and rescues neuronal loss, compared to mice administered rAAV-mFKN. Diabetic mice with rAAV-sFKN had improved visual function by implementing a two-choice visual discrimination task through learning-based behavior. In conclusion, our data suggest that rAAVs expressing mFKN or sFKN are efficient means to deliver FKN isoforms to the retina. Delivery of rAAV-sFKN, compared to rAAV-mFKN, ameliorates activation of microglia, vascular damage and prevents neuronal loss. rAAV-sFKN correlates with the success rate of the mice finding the reward based on their ability to distinguish between spatial gradients. FKN appears to be a neuroprotective agent in the diabetic retina, potentially serving as an alternative pathway to implement translational and therapeutic approaches to minimize retinal pathology and improve visual function.

WTH09-05 | The role of L-MPZ in the aging of the peripheral nervous system

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Large-myelin protein zero (L-MPZ) is an isoform of myelin protein zero (MPZ, P0) produced by translational readthrough of the MPZ stop codon. L-MPZ mice produced by genome-editing technique for the synthesis of only L-MPZ but not P0 show motor disturbance and abnormal morphologies both in myelin and axon after ten weeks of age. However, the physiological role of L-MPZ in the peripheral nervous system remains unknown. To clarify the functional significance of L-MPZ *in vivo*, we generated another genome-edited mouse line, L-MPZ KO mouse that produces only P0 but not L-MPZ. L-MPZ KO mice were born normally and indistinguishable from wild type during development and no significant abnormality was found in motor function and myelin morphology in 8-week-old mice as previously reported. In this study, we examined the influence of L-MPZ deficiency in pathological condition and in aging. Demyelination ratio by intraneural injection of lysophosphatidylcholine did not show difference between L-MPZ KO and wild type mice at 8–10 weeks of age, indicating no myelin fragility in these younger mice. In 6- and 12-month-old mice, although no apparent abnormality was found in behavioral tests, the electrophysiological study showed significant decrease of the amplitude of the compound muscle action potential in L-MPZ KO mice. Taken together with our previous results, loss of L-MPZ does not show large influence in young animals, but L-MPZ is indispensable for the normal axonal function in aged peripheral nervous system. Thus, regulation of L-MPZ level by translational readthrough is important for the peripheral nervous system.



WTH09-06 | The transcriptional regulator DREAM modulates glial cell proliferation dependent on the regional inflammatory microenvironment

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The transcriptional regulator downstream regulatory element antagonistic modulator (DREAM), also known as potassium channel interacting protein 3 (Kcnp3) was shown to modulate the cell cycle, pain perception, acute lung inflammation and developmental gliogenesis. We here investigate the role of DREAM in CNS-resident cell proliferation and neuroinflammation, which was previously unaddressed. DREAM^{-/-} mice showed increased proliferation of microglial cells in the hippocampus and thalamus and astrocytes in the thalamus, while the proliferation of oligodendrocyte progenitor cells (OPCs) as well as adult hippocampal neurogenesis were not altered. In mice overexpressing human tumor necrosis factor (Tnf) in the periphery, a model of regional neuroinflammatory response in the thalamus, but not the hippocampus, loss of DREAM did not affect neuroinflammatory gene expression or absolute microglial numbers. Interestingly, DREAM^{-/-}/TNF double transgenic mice showed boosted astrocyte and OPC proliferation, whereas the number of proliferating microglia was increased only in the thalamus. Collectively, these data indicate that DREAM modulates glial cell proliferation dependent on the inflammatory milieu, but does not affect neuroinflammation or adult hippocampal neurogenesis.

WTH09-07 | Exposure to insecticide mixture of cypermethrin and dichlorvos increased the volume of astrocyte and glucose in substantia nigra

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Objective: The widespread resistance of malaria vector reported by world health organization in 2017 has increased the intensity of the use of more than one insecticide, thus this research was aimed at assessing the impact of insecticide mixture of cypermethrin and dichlorvos on the volume of astrocyte and glucose in substantia nigra.

Methods: The research exploited, thirty-two adult Wistar rats, divided into 4 groups; group A (control) inhaled fresh air, groups B, C, and D were exposed to a mixture of 5mg/m³ (4.4 ppm) of dichlorvos and 10

mg/m³ (8.7 ppm) of cypermethrin insecticide for 2h/day, 3h/day, and 4h/day respectively. The animals were exposed for 5 days a week in 4 weeks. Twenty-four hours after the last exposure following the neurobehavioral test, the animals were weighed, then anaesthetized under chloroform vapor and the animal skull dissected by occipitofrontal incision. The brain tissues were harvested from the cranial vault, some of the tissues were prepared for biochemical analysis through the process of homogenization while the remaining tissues were fixed in 10 % Neutral Buffered Formalin for 48h and grossed to isolate the brain tissue of interest for immunohistochemical investigations.

Results: The neurobehavioral test presents statistical non-significant decrease in the motor function of the exposed groups when compared to the control group ($p < 0.05$). The astrocytes and glucose level of all the exposed groups were significantly higher than the control ($p < 0.05$). There was an impairment of glycolytic capacity and an increased absorption of circulatory glucose by the proliferated astrocyte which resulted to increased availability of glucose to sustain the motor function of substantia nigra in one month toxic exposure.

Conclusion: The results presented in this study indicates that in cases of short term xenobiotic toxicity, astrocyte and glucose are simultaneously increased to maintain the motor activity of substantia nigra.

WTH09-08 | Microglia derived from stem cells exhibit a more homeostatic and adult-like phenotype in brain organoids than in a monoculture

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Microglia (the innate immune cells of the brain) help maintain homeostasis, regulate synaptic development, and facilitate the maturation of other cell types in the central nervous system. Historical approaches to the study of functions of microglia have significant disadvantages. For example, microglia in monoculture do not resemble their counterpart *in situ* and most, if not all, chemical or genetic approaches to eliminate microglia *in vivo* have substantial off-target consequences. Our objective is to establish a platform for the more effective study of human microglial function. To this end, we focused on brain organoids, a three-dimensional culture of different cell types. At maturity, these organoids emulate functions of the human brain and exhibit relevant cytoarchitectural layering, yet microglia are absent from cultures generated by current brain organoid protocols. We developed a reproducible method to selectively embed microglia into organoid cultures. We demonstrate that iPSC-derived microglia grown in monoculture display a protein signature representative of immune activation, whereas these same cells exhibit a mature, non-activated signature when in brain organoids. Furthermore, levels of synaptic markers are associated with



the levels of transmembrane protein 119 (TMEM119, a marker of mature microglia), suggesting that, when incorporated into brain organoids, microglia exhibit their normal synapse-regulating functions. Taken together, iPSC-derived microglia embedded in brain organoids exhibit physiology and functions similar to primary microglia *in situ* and our organoid model allows researchers to study functions and responses of microglia in an environment that is easily manipulatable and, importantly, more relevant to the human context.

WTH10-01 | Safety profile of localized non-invasive brain stimulation using uHD-PES: histological analysis

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Transcranial electrical stimulation has been widely used for noninvasive neuromodulation of the central nervous system to induce a psychological and behavioral response. Despite its success in enhancing learning and memory and its applications in mitigating different neurological disorders, high resolution targeted non-invasive electrical stimulation of the brain remains elusive. We have recently demonstrated a focused minimally invasive ultra-high-density pulse electrical stimulation (uHD-PES) method to evoke neural activity in rodent models. Here, we show that this is a safe modality for non-invasive neural stimulation. Ultra-high-density flexible surface electrode arrays are placed on the mice skull ($n=3$) to apply pulsed electric currents with higher resolution to achieve localized brain stimulation. The evoked activity was confirmed using *c-fos* immunoreactivity, which is a marker of neural activation. Further safety profile of the stimulation approach was characterized with immunofluorescence analysis of different cellular markers (e.g., NeuN, GFAP, Iba1) and apoptotic markers (Caspase-3). Our results show an elevated *c-fos* expression following stimulation in the motor cortex of the stimulated hemisphere in comparison to the non-stimulated hemisphere. Evoked activity was localized to the motor cortex of the stimulated hemisphere within 200-400 μ m of the simulated stimulation focus. Interestingly, no significant changes were observed in neuronal density between the stimulated and non-stimulated hemispheres. Similarly, no neuronal damage was observed between two different hemispheres as indicated by lack of apoptosis i.e., caspase-3 expression. Also, uHD-PES did not cause any gliosis as no significant difference in intensity of glial and astrocytes markers were observed. This indicates lack of reactive microglial or astrocytes in response to the uHD-PES method. In conclusion, uHD-PES is confirmed to be an effective approach for localized noninvasive brain stimulation without any significant tissue and cellular damage.

WTH10-02 | An Early Call to Prevent Valproic Acid Toxicity in Antiepileptic Therapy

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Background: Valproic acid (VPA) is one of the most prescribed drugs for epilepsy. Extended use of VPA not only induces hepatotoxicity but also impairs the cognitive functions.

Objectives: Metformin has been reported to prevent epileptogenesis and enhance memory. To counter the VPA-induced adverse events, it is hypothesized that combination of sub-therapeutic dose of VPA with metformin may attenuate the toxicity stemming from the therapeutic dose of VPA.

Methods: Pentylentetrazole (PTZ)-induced kindling model of epilepsy in mice was used to assess the combined effects of sub-therapeutic dose of VPA (100 mg/kg) and metformin (200 mg/kg). The memory performance was analyzed by passive avoidance test, while alkaline comet assay was used to determine genotoxicity. Histopathological examination and serum biochemical analysis was performed to determine hepatotoxicity.

Results: Combination dose of VPA with metformin reduced seizure scores. VPA (300 mg/kg) administered as a single agent did not enhance memory impairment caused by PTZ, however, combination of sub-therapeutic dose of VPA with metformin enhanced memory function. Furthermore, in alkaline comet assay, combination therapy demonstrated reduced genotoxicity compared to the VPA 300 mg/kg. Histopathological examination of liver and analysis of serum hepatic enzymes revealed that combination therapy (VPA + metformin) reversed the toxicity as seen in case of PTZ or VPA (300 mg/kg) treated animals with no other treatment given.

Conclusion: Based on the study data, it is concluded that the combination of sub-therapeutic dose of VPA with metformin might be used for epileptic seizures. This will prevent the hepatotoxicity and enhanced memory functions as compared to the VPA given as a single agent therapy.

WTH10-03 | Pioglitazone mediated transitory upregulation of neuronal uncoupling protein-4 and -5 in adult nonhuman primate's brain

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While mitochondrial dysfunction and oxidative stress are known key pathogenic events that underlie Parkinson's disease (PD), no current treatment halts the disease progression. However, there



is hope that existing small molecules may possess properties that counteract the consequences of neurochemical dysfunction in PD. Neuronal uncoupling protein-4 and -5 (UCP-4/-5) are mitochondrial proteins which help to minimize ROS generation via a "mild uncoupling phenomenon". Particularly fascinating is their higher expression in dopamine-rich regions of the brain that are vulnerable in PD. UCP-4/-5 have been documented to protect neurons due to their antioxidant and anti-apoptotic activities. Moreover, in vitro studies have shown that UCP-4/-5 overexpression results in better preservation of mitochondrial membrane potential, cellular ATP levels, and lower oxidative stress induced by the parkinsonian toxin, MPP⁺. In our study, we found that there is no sex-based bias in expression of UCP-4/5 in striatum and substantia nigra of infant African green monkeys whereas we discovered that adult male monkeys exhibit significantly greater expression of both isoforms than females in substantia nigra but not in striatum. These striking findings on age- and sex-dependent UCP-4/-5 expression in nigra stimulated our interest in activating UCP-4/-5 in vivo as a strategy to halt PD progression, particularly as PD is 2-2.5 times more prevalent in males than females. Preclinical and clinical studies indicate that the PPAR γ activator, pioglitazone, is neuroprotective in PD, however, its precise mechanism has not been elucidated. We hypothesized that pioglitazone's action involves induction of UCP-4/-5 and tested this in male African green monkeys that were treated with oral pioglitazone (5 mg/kg/day) or vehicle for 1 or 3 weeks. Pioglitazone significantly increased UCP-4/5 expression in substantia nigra and striatum at 1 week, but after 3-weeks of treatment the effect on UCP-4/5 had waned. An explanation for the short-term effect might be lie in homeostatic mechanisms that are activated in the non-diseased brain. These studies establish the therapeutic potential of pharmacologically stimulating UCP-4/5. We anticipate that these outcomes will contribute to the development of neuroprotective treatments for PD.

WTH10-04 | Behavioral and molecular aspects of AlCl₃ induced neurotoxicity in zebrafish

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Aluminium is linked to neurotoxicity in the brain, causing neurodegeneration and impairing memory and cognition. AlCl₃ has been shown to increase reactive oxygen species and inflammatory indicators, both of which contribute to neuron degeneration. AlCl₃ treated zebrafish show behavioral, biochemical, and neurochemical alterations in the brain. In our protocol, zebrafish were administered AlCl₃ at three different doses (50 μ g/L, 100 μ g/L, and 200 μ g/L) for 4 days. On days 1st and 4th, zebrafish were tested for anxiety using a novel diving test. On days 3rd and 4th, zebrafish were tested for memory, using T maze, and novel object recognition with the help of ANY-maze software. Zebrafish were sacrificed on the 4th day, and

whole brains were used to conduct biochemical, and neurotransmitter analysis. Our study demonstrated that AlCl₃ exposure markedly reduced the overall distance traveled and the number of entries in the top zone while increasing the time spent in the bottom zone in the novel diving test. In the T maze test, AlCl₃-treated zebrafish had considerably longer transfer latency and time spent in the favorable zone, as well as a higher number of entries into the unfavorable zone. After AlCl₃ treatment, zebrafish show significantly decreased exploration duration with a novel object in a novel object recognition test. Furthermore, AlCl₃-treated zebrafish show a lower level of GSH and SOD and a higher level of MDA, indicating strong oxidative stress. The neurochemical level was also disrupted, as evidenced by considerably reduced GABA, DA, NE, and 5-HT levels in contrast to an increase in glutamate in the brains of zebrafish treated with AlCl₃. These data suggest that AlCl₃ has a major impact on zebrafish behaviors, biochemicals, and neurotransmitters which leads to neurodegeneration.

WTH10-05 | Repurposing of Nrf2 activator attenuates mitochondrial-mediated apoptosis in experimental tMCAO model of ischemic stroke

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Mitochondrial dysfunction is one of the characteristics of ischemic stroke. As a result, mitochondria are a key therapeutic target in the treatment of stroke. Patients with multiple sclerosis and psoriasis have utilized dimethyl fumarate (DMF) as an antioxidant. We predicted that DMF could protect against mitochondrial dysfunction. tMCAO surgery was performed in male Wistar rats. DMF (25mg/kg & 50mg/kg) was administered orally twice a day, for 7 days prior to tMCAO surgery. After an experimental period of 24 h, neurobehavioral tests were done, and then animals were sacrificed for infarct volume estimation, evaluation of mitochondrial parameters, and morphological alterations. DMF treatment showed significant improvements in neurobehavioral output and infarct damage (TTC staining). DMF diminished the mitochondria ROS and the membrane potential loss. Pre-treatment of DMF significantly elevated levels of electron transport chain enzyme activity and ATP content. DMF treatment significantly reduced the Bax/Bcl2 ratio, mitochondrial Bax, and cytoplasmic Cyt c expression. Additionally, DMF caused a significant reduction in the number of reactive astrocytes and morphological alterations. This study demonstrated the efficacy of DMF treatment in ischemia/reperfusion injury by modulating mitochondrial-mediated apoptosis and astrogliosis in the penumbral region. This suggests the potential of DMF in attenuating disease progression by targeting mitochondria.

**WTH10-06 | Functional association and validation of genes of Parkinson's disease: A potential susceptibility Biomarker**Vinay Yadav*CSIR-Indian Institute of Toxicology Research, Systems Toxicology and Health Risk Assessment Group, Lucknow, India*

Neurological disorders often result from alterations in brain metabolism. The genetic polymorphism of the drug-metabolizing enzymes is responsible for the altered metabolism of endogenous substances like neuro-hormones, drugs, and other toxic compounds. Even the small amounts produced in the brain are of significance as they could act at the pharmacodynamics level directly on receptors or other active sites leading to altered brain function and brain disorders. Parkinson's disease (PD) is a result of a decrease in the secretion of dopamine in the Substantia nigra region of the brain. Through in-silico studies, we have identified putative biomarkers of Parkinson's disease. Gene expression analysis shows a significant association with multiple human tissues and multiple genes together with NUCKS1. Furthermore, the post-mortem brain samples showed diverse expressions of the NUCKS1 gene in PD patients compared to healthy samples. Besides, the metabolite analysis shows a significant association with serotonin a known neurotransmitter, and other 15 metabolites. In addition, NUCKS1 also showed co-expression with ZNF43 and PLIN1 genes involved in cell cycle regulation indicating their association with Parkinson's disease. We now propose to clinically validate the NUCKS1 and other gene RPS4Y1, SNCA, NAE1, VDAC1, DDX3Y, SRMM2, and UBE2G1 biomarkers in the peripheral blood of Parkinson's disease patients. The validation of these genes as a potential disease susceptibility biomarker for PD will help in the early diagnosis of such neurological disorders and better therapeutic management of the patients.

WTH10-07 | Neuroprotective action of eugenol on oxidative stress-dependent apoptotic death of DA neurons in a Parkinson rat modelAbhishek Kumar, Bechan Sharma*University of Allahabad, Department of Biochemistry, Prayagraj, India*

Parkinson's disease (PD) being one of the most common neuro-degenerative disease is primarily caused by the degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta of the midbrain. Mitochondrial dysfunction and oxidative stress characterize major factors involved in the activation of complex processes corresponding to apoptosis-mediated neuronal senescence of dopaminergic neurons (DA) in Parkinson's disease (PD). The studies have proven to determine treatment for symptomatic relief but till date, no cure has been identified for PD. The aim of this study was to evaluate the neuroprotective efficacy of eugenol in MPTP-induced mice model of PD. The results indicate that eugenol treatment significantly improved the motor coordination of the

MPTP-intoxicated rats. Eugenol also alleviated the fall in activity of mitochondrial complexes I, IV, and V in accordance with ameliorating the level of superoxide dismutase and mitochondrial glutathione in the midbrain of MPTP-induced rats. Eugenol inhibited the activation of proapoptotic proteins including caspase-3 and Bax, while upshifting the expression of antiapoptotic protein like Bcl-2 consequently preventing the MPTP-mediated apoptotic progression. After eugenol supplementation, the activity of pAkt1 was promoted, which has further inhibited the apoptosis of DA neurons. The treatment with eugenol after MPTP intoxication showed reduced expression of TNF- α and IL-6; the proinflammatory cytokines. Our findings signify that eugenol may possess pharmacological properties and contribute to neuroprotection against MPTP induced toxicity in a PD rat model associated with phosphorylation of GSK3 β via activating Akt/ERK signaling in the mitochondrial intrinsic apoptotic pathway. Thus, eugenol treatment may arise as a potential therapeutic candidate for mitochondrial-mediated apoptotic senescence of DA neurons in PD.

WTH10-08 | Doing more with less: Maximizing data yield of small-scale in vitro neurotoxicology investigations using morphological profilingNils-Anders Labba¹, Lars Nilsson¹, Ragnhild E. H. Paulsen², Ragnhild Eskeland³*¹University of Oslo, Department of Pharmacology, Institute of Clinical Medicine, Oslo, Norway; ²University of Oslo, Section for Pharmacology and Pharmaceutical Biosciences, Department of Pharmacy, The Faculty of Mathematics and Natural Sciences, Oslo, Norway; ³University of Oslo, Department of Biochemistry, Institute of Basic Medical Sciences, Oslo, Norway*

Most *in vitro* neurotoxicology is hypothesis-driven in that it seeks to address a specific question. In cases where the hypothesis turns out to have been wrong, extensive methodological troubleshooting is often followed by abandonment of the line of inquiry in question, leading to wasted time and resources. Hypothesis-generating research, for example, omics methodology, is often prohibitively time-, personnel-, and resource-intensive for small-scale investigations characteristic of sparsely-funded academic toxicity studies. *In vitro* neurotoxicology employing cellular material derived from hiPSCs/hESCs also suffers from extensive differentiation-associated variability, in addition to large time- and resource costs. Here we showcase a cost-effective, imaging-centered, microplate-format method based on executing as many intercompatible procedures as possible on the same sample material, yielding a large volume and type-variety of data from a single experiment. Live-cell imaging of neuroglial mixed culture with live staining is combined with multiple medium-sampling steps for ELISAs followed by a non-destructive cell viability assay prior to PFA-mediated fixation of cells, whereupon cells are stained with labile fluorescent dyes, imaged, purged for dyes, then subjected to multiple rounds of immunocytochemistry. Images are analyzed and cross-compared using machine learning approaches



to yield cell-type-specific morphological profiles, migration velocities, clustering tendencies, and structural and topological data on the neurite network. This hybrid biased/unbiased approach maximizes data-generation on a per-experiment basis while enabling small-scale hypothesis-driven inquiries to tap into the hypothesis-generating benefits of the emergent omics-like field of morphological profiling.

WTH10-09 | A convincing pathophysiological mechanism of ME/CFS

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Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) has become a medical concern awaiting solution in modern society. Nobody found indisputable evidence for pathological mechanisms, although possible hypotheses were suggested to understand the molecular biology of ME/CFS. Based on the clinical evidence, we hypothesized that specific neuronal hyperactivity is leading cause of ME/CFS and its major symptoms. Our hypothesis was experimentally investigated using novel mice model subjected to repeated chemical injection for 28 days. Chemical induced notable fatigue-like behaviors and ME/CFS-related comorbidities. As our expectation, intra and extracellular neuronal over-activity was showed by immunofluorescent analysis and microdialysis in raphe nuclei region. Furthermore, our findings were confirmed using receptor agonist challenge and rAAV-mediated CRISPR knockdown system, showing considerable fatigue-like behavior and low glucocorticoid response. We provide a first evidence for particular neuronal hyperactivation depending on desensitized receptor as a persuasive pathophysiological caused of ME/CFS.

WTH10-10 | Brain FNDC5/Irisin in depressed patients and its modulation in mouse models of major depression

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Regular physical exercise has been associated with reduced incidence and severity of major depressive disorders (MDD), a severe

group of mood disorders that has been a major cause of disability in adults. We previously reported that the exercise-induced myokine FNDC5/irisin is neuroprotective in Alzheimer's disease and mediates the beneficial actions of exercise in the brain. Irisin originates from the precursor protein fibronectin type III domain-containing protein 5 (FNDC5), which is expressed in the skeletal muscle and notably in brain regions linked to memory and mood, such as the hippocampus and the frontal cortex. Nonetheless, region-specific FNDC5/irisin expression in animal models of depressive-like behavior and in humans with MDD is warranted. Here we demonstrate that *fnDC5* expression is selectively increased in the mouse frontal cortex by chronic fluoxetine treatment, but not other antidepressants. Surprisingly, chemical induction of depressive-like behavior by corticosterone or lipopolysaccharide (LPS) increased *fnDC5* expression selectively in the hippocampus. On the other hand, a chronic social isolation protocol did not alter cortical or hippocampal *fnDC5* expression. Finally, we assessed *fnDC5* gene expression in postmortem tissue from subjects diagnosed with MDD with or without psychotic features (MDD-P). We found a marked reduction of *fnDC5* gene expression in the medial prefrontal cortex of depressed subjects as compared to controls. Moreover, we used a lentiviral vector to *knockdown fnDC5* (ShFNDC5) in mouse brains and observed no changes in depressive-like behavior or monoamine turnover. By using mouse models and human samples, we demonstrate that the *fnDC5* gene expression is responsive to depressive contexts. Our results indicate that different depression-linked stimuli trigger opposing responses and may stimulate future studies aimed to determine the region-specific relevance of FNDC5 to depressive behaviors.

WTH10-11 | Oxidative stress in Q175 mouse model of Huntington's disease

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Huntington's disease (HD) is a neurodegenerative disorder that initially affects the striatum and later the cortex resulting in motor, cognitive and psychiatric dysfunction. Oxidative stress, mitochondrial dysfunction, and neurotoxicity are proposed as pathogenic mechanisms. Oxidative stress is generated by high levels of reactive oxygen species (ROS) which can be reduced by antioxidant molecules such as glutathione (GSH) and the activity of the mitochondrial enzyme superoxide dismutase 2 (SOD2). UCP4 is an uncoupling protein involved in the reduction of mitochondrial ROS levels which dissipates proton gradient. We evaluated motor performance, ROS and GSH levels, as well as SOD2 and UCP4 expression in striatum and cortex of Q175 knock-in HD mice and WT mice at the ages of 4 months (4m) and 8 months (8m). We observed reduced motor performance in Q175 mice in the open field test at 4m and 8m ($p < 0.05$).



In Q175 mice's striatum, we found no difference in ROS levels, while GSH levels were reduced at 8m ($p < 0.05$), SOD2 expression was reduced at 4m and increased at 8m ($p < 0.05$), and UCP4 expression was reduced at both 4 and 8m ($p < 0.05$). In Q175 mice's cortex, we observed reduced ROS levels at 8m ($p < 0.01$), no difference in GSH levels, an increase in SOD2 expression at 8m ($p < 0.05$), and an increase in UCP4 expression at 4m and a reduction at 8m ($p < 0.05$). Thus, while ROS levels are mostly maintained, evidence of oxidative stress is shown in the striatum of HD mice where UCP4 and SOD2 expression and GSH levels are modified. Cortex, which presents a later onset of the disease, started to exhibit some of these alterations at 8m. Mechanisms involved in oxidative stress in HD are largely unexplored and could represent targets for treating HD.

WTH10-14 | A novel tropomyosin-related kinase-B (TrkB) receptor activator: HIFN (2-fluoro-N-(2-(5-hydroxy-1H-indol-3-yl)ethyl)nicotinamide

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Purpose: Our lab has previously reported a synthetic molecule, HIOC, could activate TrkB receptor and prevent vision loss from ocular trauma or phototoxic injury. In the present study we synthesized and tested novel HIOC analogs that could effectively activate TrkB receptor with greater potency.

Methods: Using HIOC as a lead compound, over 20 different analogs were synthesized. In preliminary screening for TrkB activation, these compounds (10nM) were tested in NIH-3T3-TrkB cells and primary cortical neurons using western blot. Promising TrkB activators were further tested for protection against blast overpressure injury (20psi) in C57BL/6J mice. Animals received analog (40mg/kg) or vehicle, 30min after blast and treatment was continued for another 6 days. Visual function (contrast sensitivity and visual acuity) and retinal function tests [scotopic ERG and pattern ERG (pERG)] were used to assess vision. A TrkB antagonist, ANA-12 (0.5mg/kg), was administered to animals 2.5h prior to treatment with TrkB activator to test specificity.

Results: A fluorine substituted pyridine analog (HIFN) showed better TrkB activation than the parent compound HIOC. HIFN prevented the deficit in visual acuity ($p \leq 0.05$) and contrast sensitivity after ocular blast ($p \leq 0.05$). HIFN proved better than HIOC in preserving contrast sensitivity in animals at 7-week post blast ($p \leq 0.05$). Treatment also prevented the decline in the pERG P1 amplitude ($p \leq 0.05$) and N2 amplitude ($p = 0.06$). ANA-12 (TrkB inhibitor) blocked TrkB activation by HIFN and prevented the protective effects in blast animals. Dose response studies using different HIFN concentrations (0–100mg/kg) were carried out. Dose dependent rescue of visual function deficit was seen reaching significant levels at dose of 30mg/kg for contrast sensitivity ($p \leq 0.0001$) and

10mg/kg ($p \leq 0.05$) for visual acuity. At a dose of 30mg/kg, HIFN also prevented ($p \leq 0.05$) the decline in retinal ganglion cell function, assessed by pERG.

Conclusion: HIFN activates TrkB receptor and protects against trauma-induced vision loss.

WTH10-15 | Ethinyl estradiol and levonorgestrel improves spatial memory and induces neurodegeneration in the limbic system of Wistar rats

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Oral contraceptives have gained global publicity. Inconsistent reports on the neurological impact of oral contraceptives necessitated the study on the effect of oral contraceptives on the limbic system. Twenty four adult female wistar rats weighing 180–220g were divided into four groups designated A, B, C, and D. Group A was the control and did not receive any treatment, Groups B, C, and D were treated with combined oral contraceptives containing 0.002mg/kg levonorgestrel and 0.0043mg/kg estradiol for 21, 42 and 63days respectively via orogastric tube. The animals were exposed to Morris' water maze after 24h of the last administration for each group. Blood was collected for serum estimation of serotonin, malondialdehyde, alpha feto protein and cortisol levels. All brains were perfused fixed weighed and post fixed in 10% buffered formalin. The hypothalamus, hippocampus and amygdala were dissected and processed for histological studies using Hematoxylin and eosin method, cresyl fast violet method for Nissl substance and immunolabeled for Glial fibrillary acidic protein and neuron specific enolase. There was significant decrease in brain weight in Group B compared with control. There was no significant ($p < 0.05$) change in the serum levels of serotonin, malondialdehyde, cortisol alpha feto protein the test groups compared with control. Histological studies revealed pyknotic nuclei and reduced number of neurons with Nissl substances in the treated groups. Immunohistochemical sections revealed reactive astrocytes and reduced enolase activity in the treated groups indicating neurodegeneration. Ethinyl estradiol and levonorgestrel induced neurodegenerative changes in the hippocampus, amygdala and hypothalamus in rats and may influence their functions

WTH10-16 | Anthracene and benz[a]anthracene induced neurodegeneration, altered behavior and increased SKN-1 expression in *Caenorhabditis elegans*

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Anthracene (ANT) and benz[a]anthracene (BEN) are polycyclic aromatic hydrocarbons (PAHs) ubiquitous in the environment. These PAHs have been identified in ambient air, surface and drinking water, smoked foods, exhaust emissions, smokes of cigarettes and edible aquatic organisms. In this study, we investigated the neurotoxicity of ANT and BEN via their effects on cholinergic, dopaminergic and GABAergic neurons, locomotion and behavior and genes linked to antioxidant defense system in *Caenorhabditis elegans*. Wild-type worms and worms expressing green fluorescent protein (GFP) in either cholinergic, dopaminergic or GABAergic neurons were treated with ANT and BEN for 48h. ANT and BEN (50–1000 μ M) reduced the survival of the worms after 48h with a median lethal dose (LD₅₀) of 251.3 and 201.5 μ M. Morphological changes were observed after ANT and BEN exposure. Fluorescent micrographs revealed that ANT and BEN induced degeneration of cholinergic, dopaminergic and GABAergic neurons with increasing concentrations. ANT and BEN also reduced locomotor behavior (basal slowing response and locomotion speed) of the worms. Real-time polymerase chain reaction data showed a significant increase in *skn-1* (a homolog of Nrf2), *gst-4*, and *cat-1* expression after exposure to ANT and BEN. Our findings revealed that ANT and BEN induced upregulation of genes related to antioxidant defense system, which suggests redox imbalance and overproduction of reactive oxygen species in the worms and may contribute to degeneration of cholinergic, dopaminergic and GABAergic neurons and altered locomotor behavior.

WTH10-17 | Effect of curcumin and gallic acid on behavioral and neurochemical indices in diesel exhaust particle-induced neurodegeneration

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This study seeks to determine the effects of gallic acid and curcumin on behavioral and toxicological indices, redox status, inflammatory and neurotransmitter activities in diesel exhaust particle (DEP)-induced neurodegeneration in *Drosophila melanogaster*. *D. melanogaster* were fed with basal diet containing dietary inclusions of DEP (0.3 μ g/g) and treated with dietary supplementation of gallic acid and curcumin (0.1 mg/g and 1.0 mg/g) respectively for 14 days. Behavioral indices (survival, aversive phototaxis, and negative

geotaxis) to assess toxicology, locomotion and memory retention were conducted. Non-enzymatic (lipid peroxidation and reactive oxygen species) and enzymatic (catalase, superoxide dismutase, glutathione-S-transferase) antioxidant indices and monoaminergic neurotransmitters (dopamine and L-dopa) were measured as well as the activities of acetylcholinesterase (AChE), monoamine oxidase (MAO) and myeloperoxidase enzymes (MPE) were evaluated in the head regions of *D. melanogaster*. The results indicated that both curcumin and gallic acid caused a notable dose-dependent increase in the cognitive and locomotor activity that was initially deranged in DEP-exposed flies. Furthermore, the polyphenolic compounds exerted a reduction in the high levels of thiobarbituric acid reactive substances and reactive oxygen species observed in the DEP-exposed flies. Gallic acid and curcumin also reversed the observed reduction in catalase, superoxide dismutase and glutathione-S-transferase enzyme activities in the DEP-exposed flies. Lastly, the increased enzymatic activities of AChE, MAO, and MPE in DEP-exposed flies were ameliorated by gallic acid and curcumin while the concentrations of monoaminergic neurotransmitters were also restored. The immediate restoration of altered behavioral and biochemical conditions including the anti-inflammatory action exerted in DEP-exposed flies by these natural polyphenols, therefore, validates the promising use of nutraceuticals as a therapeutic candidate for restoring neuronal health linked with DEP-induced oxidative stress.

WTH10-18 | Morin ameliorates rotenone-induced Parkinson's Disease in mice through anti-oxidation and anti-neuroinflammation

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Background: Parkinson's disease (PD) is the second most common neurodegenerative disorder and a range of nonmotor symptoms precede the motor phase. Various studies showed that the neuropathological process leading to PD starts from the gut before spreading to the CNS affirming the role of environmental toxicants such as rotenone. Morin (3, 5, 7, 2', 4'-pentahydroxyflavone) is a potential neuroprotective and anti-oxidant product that could be beneficial in PD.

Objectives: This study was designed to investigate the neuroprotective effect of morin on rotenone-induced PD in mice.

Methods: Male albino mice (18–23g) were randomly divided into groups ($n = 10$) as follows: Group 1: Normal saline (10ml/kg, *p.o.*); Group 2: rotenone (1mg/kg, *p.o.*, 0.5% in CMC); Group 3–5 morin (5, 20 or 80mg/kg, *i.p.*)+rotenone (1mg/kg, *p.o.*) respectively, Group 6: morin (20mg/kg only, *i.p.*) and treated for 28 consecutive days. Animals were treated orally with freshly prepared rotenone an hour after pretreatment with morin. Behavioral tests were carried out weekly, 1h after rotenone administration and results obtained were recorded. Mice were sacrificed on the 28th day and different



brain sections were collected for measurement of oxidative stress parameters.

Results: Pretreatment with morin reversed rotenone-induced behavioral deficits (motor incoordination, movement deficit and depression). Also, rotenone significantly increased lipid peroxidation (MDA), acetylcholinesterase (AChE) with a concomitant decrease in glutathione (GSH) and superoxide dismutase (SOD) activities in discrete regions of the brain which were attenuated by the pretreatment of mice with morin.

Conclusion: This study showed that morin had neuroprotective effect in the rotenone model of PD through enhancement of antioxidant defense mechanism.

WTH10-19 | Influence of curcumin on cholinesterase activity and gene expression in brain of scopolamine-induced rats treated with donepezil

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Combination therapies involving cholinesterase inhibitors with other therapeutic agents have been found to have better effects in the management of dementia related diseases but there has been a dearth of information on their synergistic effects with dietary polyphenols. Hence, this study sought to assess the influence of curcumin (the major polyphenol in turmeric) on some therapeutic properties of donepezil (an acetylcholinesterase inhibitor) on cognitive function in scopolamine-induced model of amnesia in rats. Pretreatment with curcumin (50 mg/kg) and/or donepezil (2.5 mg/kg; p.o.) was done for seven successive days. Dementia was thereafter induced with scopolamine (1 mg/kg; i.p.) administration. Thereafter, the changes in spatial memory were determined. This was followed by determination of activities of cholinesterases, adenosine deaminase, nitric oxide level and oxidative stress markers. Furthermore, RT-qPCR for expressions of cholinesterases, and glial fibrillary acidic protein (GFAP) genes were carried out. Histological examination of the brain tissues for neuronal damage and GFAP protein immunoreactivity were also carried out. Results showed that rats from the Scopolamine-induced groups showed impaired learning and memory, with concomitant increased activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), adenosine deaminase (ADA), and lipid peroxidation as well as decrease in levels of nitric oxide (NO), Glutathione-S-transferase (GST) activity, and total thiol production when compared with control. There was also an upregulation in the

AChE, BChE, and GFAP genes, as well as increased immunoreactivity of GFAP protein in the brain tissues. However, it was observed that the combination of curcumin and donepezil ameliorated these impairments when compared to the scopolamine-induced group, with a notable ameliorative effect in the scopolamine-induced group administered curcumin plus donepezil. Thus, this finding provides more evidence to support that combination of curcumin with donepezil offers significant therapeutic intervention in rat model of memory impairment

WTH10-20 | Hippocampal inflammation disrupts REM sleep in a neurodegeneration model induced by systemic LPS

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Intraperitoneal injection of lipopolysaccharide (LPS) can induce an acute inflammatory response, leading to sustained brain inflammation in Wistar rats. This neuroinflammatory state leads to progressive neurodegeneration in the dopaminergic axis, cognitive impairment, and hippocampal degeneration accompanied by hippocampal vascularization and metabolism changes. We investigated whether an acute systemic inflammatory stimulus would induce hippocampal neuroinflammation and neurodegeneration and alter sleep patterns. Fifty-day-old male Wistar rats were treated with LPS (2 mg/kg i.p.) or saline, and 3 or 5 months post-injection, they were either euthanized or subjected to video-EEG recordings during sleep. Elevated expression of proinflammatory cytokines tumoral factor- α (TNF α) and interleukin beta (IL1 β) and the receptor for advanced glycation end products (RAGE), summed by raised levels of microglial marker ionized calcium-binding adaptor molecule 1 (Iba1), RAGE protein, and nuclear factor kappa B (NF- κ B) activity, indicate an environment of hippocampal inflammation three months post-injection. In 5 months, neuroinflammatory parameters reached control levels. Treated animals displayed neurodegeneration and synaptic alterations cues such as diminished neuronal nuclei protein (NeuN), neurofilament light (NEFL), and Synaptophysin. Although there were no significant differences between wakefulness (WK), rapid-eye-movement (REM), and non-REM (NREM) sleep epochs between groups, REM sleep episode duration was reduced 3 months after treatment. Moreover, Theta power diminished significantly during REM sleep 3 months after LPS exposure. However, Theta and Delta peaks at REM and NREM sleep remained unaltered. These results indicate



LPS's ability to induce chronic hippocampal inflammation, leading to alterations in REM sleep quality and neurodegeneration.

WTH10-21 | Melatonin attenuates the bidirectional gut-brain axis mediated inflammation caused by PM_{2.5} and averts neurodegeneration

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The microbiota and brain communicate through a bidirectional neurohumoral system called the gut-brain axis. Recent reports revealed the role of air pollution-derived ambient particulate matter and its profound effect in causing disturbance to gut microbiome homeostasis and neurodegeneration. The smaller size particles such as PM_{2.5} exposed to lung epithelial cells (Beas2b) at a dose of 60 µg/ml showed a significant increase in reactive oxygen species (ROS), extracellular cytokine level (IL-1β and TNF-α), and inflammatory proteins. The PM_{2.5} exposed media was collected from Beas2b cells and further treated to human microglial cells (HMC-3) and human colon cells (CCD-841). Melatonin treatment at a dose of 100 µM significantly alleviates the ROS levels, mitochondrial membrane potential damage, mitochondrial fission, iba-1, and CD-68 expression. To correlate the *in-vitro* and *in-vivo* models, C57BL/6 mice were exposed to PM_{2.5} at a dose of 60 µg/ml for 90 days. Neurobehavioral analysis revealed a decline in spatial memory, learning, and cognitive function after chronic exposure to PM_{2.5}. Astrocyte activation and neurodegeneration was observed in the olfactory bulb and hippocampal regions of the mice. A profound decline in the levels of neurotransmitters such as ACh, GABA, Glutamate, and NE responsible for neuronal survival and memory formation was observed in the mice brain. Further, V3/V4 sequencing of the microbiome revealed changes in the chao1 and Shannon coefficient in the alpha diversity. Histology and immunofluorescence of the colon revealed neutrophil infiltration and an increase in MPO levels. Melatonin treatment to mice at a dose of 50 mg/kg restored the oxidative stress and inflammation associated with neurodegeneration by PM_{2.5}. In the current investigation, we explored and identified novel mechanisms involved in neurodegeneration and the effect of melatonin in halting the progression of oxidative stress and inflammation induced by PM_{2.5}.

WTH10-22 | Integrated microRNA and protein profiling reveals microRNAs-mediated regulation of apoptosis and autophagy flux in PD

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Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects the aged population. Accumulating evidence reveals that the expression of miRNAs mediates various processes involved in PD pathogenesis via controlling the expression of genes involved in apoptosis and autophagy. Despite an abundance of potential and budding options, the precise mechanism driving the loss of dopamine neurons in PD remains enigmatic. The integration of multiple omics datasets provides a useful tool for biologists who are investigating biological networks and seeking to identify direct regulators of proteins involved. The current study employed integrated miRNA and unlabeled LC-MS/MS global protein profiling of the rotenone-induced *in-vitro* cellular PD model. Our miRNA profiling results have identified a significant increase in the expression of hsa-miR-29b, hsa-miR-490, mmu-miR-499, hsa-miR-543, and hsa-miR-494, while maximum downregulation was observed in hsa-miR-331-5p, hsa-miR-182, and bta-miR-139. The proteomics analysis revealed that proteins involved in the apoptosis and autophagy pathways, including SIRT-1, PARP-1, BCL2, ATG4B, NAVI, and others, were found to be deregulated. Furthermore, the transcriptional changes in genes regulating rotenone-induced apoptosis and autophagy were in concurrence with translational changes for the same genes following the rotenone exposure. Gain and loss of function studies were conducted to explore the role of miR-29b and miR-490 in intrinsic apoptotic pathways and autophagy impairment in neuronal cells. Mimics and inhibitors studies revealed miR-29b as a direct regulator of SIRT-1 mediated caspase-dependent neuronal death via regulating the expression of SIRT-1, PARP-1, CYT-C, BCL-2, CAS-3, and CAS-9; whereas miR-490 regulates the expression of CAS-3, P62, ATG-4B, BCL-2, NAV1, and LC-III leading to induction of apoptosis and impairment in autophagy. Collectively, we have revealed that miR-29b and miR-490 as a direct modulators of the apoptosis and autophagic flux in cellular model of PD. Thus, miR-29b and miR-490 could develop as exciting novel targets for potential therapeutic interventions in PD.

**WTH10-23 | Learning from the (ir)reproducibility of an amyloid- β -induced model of Alzheimer's disease**

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In recent years, Alzheimer's disease (AD) models achieved by intracerebral injections of amyloid- β (A β) have been increasingly reported. Although these models attempt to mimic the initial stages of AD, prior to plaque deposition, they may present significant challenges. Focusing on classical mechanisms of neural plasticity related to the hippocampus, we have characterized a rat model obtained by a single intracerebroventricular (icv) injection of soluble amyloid- β 42 (A β 42). We found that while cell proliferation and neuronal differentiation in the dentate gyrus was preserved 3 weeks post-A β 42 injection, dendritic morphology of immature granule neurons was abnormally enhanced. Animal behavior analyzed 2 weeks after A β 42 infusion unexpectedly did not reveal changes in cognitive performance (Y-Maze spontaneous or forced alternation, and Morris water maze) nor in locomotor (open field) and anxious-related activity (elevated plus maze). Moreover, brain-derived neurotrophic factor related-signaling was unaltered at 3 and 14 days after A β 42 injection, as evaluated by the levels of hippocampal tropomyosin receptor kinase B isoforms. Similarly, in these same time points, astrocytic and microglial markers of neuroinflammation in the hippocampus were unchanged. In the present work, we point out a low success rate for attaining a model that resembles the behavior and neural plasticity impairments shown in AD. Together, our data highlight a high variability and lack of reproducibility associated with these A β infusion-based models, which may lead to publication bias. Further optimization as well as concurrent use of other AD models such as transgenic mouse models is encouraged, aiming at tackling the gap between human and preclinical AD models.

WTH10-24 | Proteomic landscape of Alzheimer's disease

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Mass spectrometry-based proteomics empowers deep profiling of proteome and protein posttranslational modifications (PTMs) in Alzheimer's disease (AD). Here we present the advances and limitations in historic and recent AD proteomic research. Complementary

to genetic mapping, proteomic studies not only validate canonical amyloid and tau pathways, but also uncover novel components in broad protein networks, such as RNA splicing, development, immunity, membrane transport, lipid metabolism, synaptic function, and mitochondrial activity. Meta-analysis of seven deep datasets reveals 2698 differentially expressed (DE) proteins in the landscape of AD brain proteome ($n = 12,017$ proteins/genes), covering 35 reported AD genes and risk loci. The DE proteins contain cellular markers enriched in neurons, microglia, astrocytes, oligodendrocytes, and epithelial cells, supporting the involvement of diverse cell types in AD pathology. We discuss the hypothesized protective or detrimental roles of selected DE proteins, emphasizing top proteins in "amyloidome" (all biomolecules in amyloid plaques) and disease progression. Based on the identified aggregated proteome in AD, we introduce a novel disease model of dysfunctional U1 snRNP-mediated RNA splicing. Thus, proteomics-driven systems biology presents a new frontier to link genotype, proteotype, and phenotype, accelerating the development of improved AD models and treatment strategies.

WTH10-25 | Gene therapy approach for SPG4-based hereditary spastic paraplegia

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Mutations in the *SPAST* gene (called *SPG4*) are the most common cause of Hereditary Spastic Paraplegia (HSP). To date, haploinsufficiency has been the prevalent explanation for HSP-*SPG4*. A case for haploinsufficiency has been made based on the genetics and because spastin dosage is tightly regulated to ensure the vitality of the axon, but other evidence suggests a gain-of-function pathology of the mutant spastin protein. *SPAST* has two start codons producing a longer and a shorter isoforms called M1 and M87 (M85 in rodents), respectively. M87 is widely distributed, while M1 is only detectably present in the adult cortex and spinal cord. Studies using a variety of experimental systems have consistently shown that mutant M1 protein is far more toxic than its M87 counterpart bearing the same mutation. Mutant M1 protein is more stable than mutant M87, and this might cause M1 to accumulate in the corticospinal tracts when mutated. Thus, we have posited that mutant forms of spastin with toxic properties may accumulate in the corticospinal tracts and cause degeneration. Recently, by comparing anatomical and behavioral changes using a mouse model that only has gain-of-function toxicity of mutant spastin, a mouse model that only has loss-of-function (due to knockout of one spastin allele) and a new mouse model resulting from the crossbreeding of the two, we conclude that gain-of-function toxicity is the cause of corticospinal degeneration, but that loss-of-function is an exacerbating factor. Here, we propose an AAV



gene therapy-based approach, which allows us to turn off expression of the mutant spastin and replace it with wildtype spastin under the control of its endogenous promoter. Early intervention with such a therapy would theoretically succeed regardless of mechanism involved and could be extended to other forms of HSP as well.

WTH10-26 | Inhibiting SUMOylation promotes aggregophagy, mitophagy and ferritinophagy and SENP3 deSUMOylase is increased in Parkinson's disease

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There is growing evidence for a link between the Small Ubiquitin-like Modifier (SUMO) pathway and Parkinson's disease (PD)^{1,2,3}. In previous studies, inhibiting SUMOylation in a potassium depolarization SH-SY5Y human neuroblastoma cell model of PD was found to induce autophagy-dependent clearance of alpha-synuclein aggregates⁴. In the current study, immunofluorescence and Western analysis revealed that inhibiting SUMOylation in the same cell culture model resulted in clearance of accumulated ferritin, the major cellular iron storage protein, due to ferritinophagy and increased mitophagic activity. SUMO pathway components were investigated in cell lines derived from biopsies of olfactory epithelium from PD patients ($n = 8$) and age-matched controls ($n = 8$). Western analysis and immunofluorescence showed changes in certain SUMO-1 conjugates in PD compared to normal controls. Western analysis also revealed that total levels of the deSUMOylase, SENP3, which has been linked to the induction of mitophagy upon iron chelation⁵, were significantly increased in PD compared to normal controls. SENP3 expression was also increased in the SH-SY5Y model and also in PD compared to normal human brain tissue homogenates from substantia nigra ($n = 17$) and striatum ($n = 21$). Expression of other SUMO pathway enzymes (Ubc9 and SAE2) was not significantly different. The SUMO pathway may be altered in PD, possibly linked to changes in the autophagy-lysosome system and could provide targets for disease modulation.

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WTH10-27 | 6BIO is a potent dual inhibitor of Gsk3 beta and Cdk5 to protect by Alzheimer disease in experimental models

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Aim: The main objective of study was to determine the role of 6-bromo indirubin-3-oxime (6BIO) a dual inhibitor of GSK3B and cdk5 in Insilco, *in vitro* and *in vivo* models of Alzheimer's disease.

Method: Firstly, GSK3B protein crystal structure was selected (PDB:1UV5), then performed docking with library of indirubin compounds. Next we performed molecular dynamic simulation and ADMET profile of selected compounds. In invitro study, SH-SY5Y cell line were treated with A β -42 (20 μ m) for the induction of AD model for 24, 48, and 72h. Then treated with 6BIO in 5, 10, 15 for 24h. MTT assay, ROC estimation and NeuN expression were analyzed. In In-vivo study, A β -42 was administrated ICV to induce AD in male Wister rat. 6BIO was given daily in three different doses (5.95 μ g/kg, 11.9 μ g/kg, 23.8 μ g/kg *i.p* dose) for 7 days after 14 days of A β -42 administration. At the end various neuro-behavioral parameters (MWM, EPM) were assessed and correlated with biochemical and molecular markers (oxidative stress, Elisa, RT-PCR and morphological alteration by H&E staining).

Result: Insilco study shown that among all compound 6BIO were shown favourable pharmacokinetics, negative Ames test, cross BBB, good docking score: -10.451 and Dynamics score (RMSD:1.8Å, RMSF:1Å. In in-vitro, 6BIO was shown to have neuroprotective effect and enhance neurite growth access by decreasing pGSK3 β , pTau, dkk1, p β -catenin expression. Significant deterioration of memory, GSH, SOD, catalase levels while rise in A β , GSK3B(P216), pTau, p β -catenin level, quantitative RT-PCR expression dkk1 were observed in A β treated rats as compared to the control rats. Treatment with 6BIO at the dose (23.8 μ g/kg) markedly improved memory along with modulation of oxidative stress parameters, A β , GSK3B(P216), pTau, p β -catenin level, quantitative RT-PCR dkk1. Marked improvement in morphological changes were observed in 6BIO (23.8 μ g/kg) as compared to A β treated group.

Conclusion: 6BIO may proves to be a useful therapeutic target of Alzheimer disease. Protective effect of 6BIO is via its dual inhibitory effect on GSK3B and CDK5 activity.

WTH10-28 | Curcumin modulates Nrf2-Keap1-p62 mediated autophagy in rotenone intoxicated Parkinsonian mouse model

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Autophagy mediates self-digestion of abnormally aggregated proteins and organelles present in cytoplasm. This mechanism may prove to be neuroprotective against Parkinson's disease (PD) by clearing misfolded alpha-synuclein (α -syn) aggregates from



dopaminergic neurons. p62, an adaptor protein acts as a selective substrate for autophagy and regulates the formation as well as the degradation of protein aggregates. p62 sequesters Keap1 freeing Nrf2 and consequently activating the transcription of its target genes. In the present study, we aimed to investigate the role of Curcumin in activation of autophagy via Nrf2-Keap1 pathway. The mice were subcutaneously intoxicated with rotenone (2.5 mg/kg bw) and co-treated with oral administration of curcumin (80 mg/kg bw) for 35 days. The results revealed the down-regulation of Nrf2 in the rotenone-intoxicated mice along with decrease in tyrosine hydroxylase (TH) level. However, the level of both Nrf2 and TH were significantly restored on the treatment with curcumin. Autophagy was assessed by evaluating the level of autophagic markers, p62 and LC3. Both p62 and LC3 decreased in the rotenone mouse model of PD increasing the aggregation of misfolded protein α -syn. Whereas, curcumin administration showed the significant increase in expression of autophagic markers along with down-regulation of α -syn expression. Consequently, the findings reveal the neuroprotective role of curcumin in rotenone-intoxicated mice by activating p62-dependent Keap1-Nrf2 autophagy pathway.

WTH10-29 | Neuroprotective and computational investigations of metal-hydroxyquinoline complexes against neurodegenerative diseases

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Recently, several bioactive metal complexes and quinoline derivatives containing antioxidant, anticancer, and antimicrobial properties have been reported. However, the neuroprotective effects of novel transition metal complexes with quinoline used in this study have not been documented. Thus, the protective effects of metal-hydroxyquinoline hybrids against H₂O₂-induced neuronal damage were investigated in this work. The human neurons were pretreated with various concentrations of hydroxyquinoline-based metal complex for 3 h followed by H₂O₂ exposure for 24 h. The result revealed that metal-hydroxyquinoline hybrids presented no neurotoxicity at the concentrations of 0.1 and 1 μ M. Biological assays of rhodamine, DCFDA, and annexin V staining were further evaluated. Pretreatment with synthetic compounds significantly increased mitochondrial membrane potential, being responsible for such effects as decreasing cellular ROS production and apoptosis in

H₂O₂-intoxicated neuronal cells. Additionally, binding interaction of the synthetic compounds to SIRT1 protein is elucidated and characterized by a molecular docking. Moreover, the computed prediction of Lipinski's rule indicated that metal-hydroxyquinoline hybrids hit all the requirements, as well as adequate blood-brain barrier permeability and oral bioavailability. These findings highlighted the interesting neuroprotective properties of hydroxyquinoline-based metal complexes which were able to prevent neurotoxicity, balance oxidative stability, restore mitochondrial membrane potential changes, and mitigate apoptosis via SIRT1 signaling pathway. Overall, these synthesized agents might be promising candidates which play potential roles for the drug development of neurodegenerative diseases.

WTH10-30 | Delineating the effect of deferoxamine on iron homeostasis in cypermethrin-induced Parkinsonism

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Iron plays a key role in the pathogenesis of Parkinson's disease (PD). Substantial evidence reports the accumulation of iron in substantia nigra (SN) of PD patients, and dysregulation of metal transporter proteins in the SN of both neurotoxin-induced PD models and PD patients. Increased availability of free iron pool has the ability to generate free radicals via Fenton reaction, promote α -synuclein aggregation, and aggravate the process of neurodegeneration. Thus, the chelation of iron can be a promising strategy for improving PD. Deferoxamine mesylate (DFO), an FDA approved iron chelator is widely reported to reduce iron-induced oxidative stress. Cypermethrin, a pyrethroid pesticide is a well-established PD model, known to cause mitochondrial dysfunction, autophagy impairment and oxidative stress. However, the role of iron accumulation and the effect of DFO in the cypermethrin-induced PD model is still elusive. The current study was designed to delineate the role of iron and its transporter protein in the aforementioned model. Male Wistar rats were treated with cypermethrin and/or DFO using standard procedure. Rats were sacrificed and the level of iron was measured employing atomic absorption spectroscopy. Nitrite content and lipid peroxidation (LPO) were also measured to assess the nitrogen and oxygen-derived free radicals. Besides, the expression of divalent metal transporter-1 (DMT-1), ferroportin, and hepcidin was checked employing western blotting. Cypermethrin increased the level of iron, nitrite, and LPO in rat nigrostriatal tissue. While the expression of DMT-1 and hepcidin was augmented, the expression of ferroportin was reduced in the nigrostriatal tissue of cypermethrin-treated rats. Treatment with DFO reversed the cypermethrin-induced



changes towards normalcy. These results demonstrate that DFO mitigated iron homeostasis by chelating the labile pool of iron thus protecting the cells from oxidative stress.

WTH10-31 | Activation of DJ-1/Nrf2 signaling pathway ameliorates cypermethrin-induced oxidative damage in SH-SY5Y cells

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Oxidative stress-induced damage to proteins such as modification by 4-hydroxy-2-nonenal (4-HNE) and 3-nitro tyrosine, have been implicated in the pathophysiology of Parkinson's disease (PD). Silencing of protein deglycase-1 (DJ-1), a PD associated redox-sensitive protein, was recently found to exacerbate the α -synuclein expression and 4-HNE modification of proteins in cypermethrin-induced Parkinsonism. However, the precise molecular mechanisms have remained to be elucidated. Nuclear factor erythroid-2-related factor 2 (Nrf2) is a redox-sensitive transcription factor that regulates the expression of several antioxidant enzymes, thereby protecting cells from oxidative stress. The present study aimed to investigate whether activation of the Nrf2 pathway is responsible for the DJ-1-mediated neuroprotection against cypermethrin-induced neurotoxicity. DJ-1 was silenced with small interfering RNA in the presence or absence of cypermethrin treatment in the human neuroblastoma SH-SY5Y cells. The expression of peroxiredoxin 3 (Prx3), thioredoxin 2 (Trx2) and Nrf2 proteins were measured employing western blot analysis. Furthermore, neuroblastoma cells were treated with sulforaphane, an Nrf2 activator, and the expression of these redox-sensitive proteins along with α -synuclein expression, mitochondrial membrane potential (mMP), reactive oxygen species (ROS) content and 4-HNE modification of proteins were also examined using standard procedures. The results obtained thus demonstrate that DJ-1 knockdown increased the sensitivity of cells to cypermethrin treatment and caused a pronounced reduction in the expression of Prx3 and Trx2 proteins and inhibition of nuclear translocation of Nrf2 in cypermethrin-treated cells. However, sulforaphane-mediated Nrf2 activation restored the level of these redox-sensitive proteins and mMP, which in turn reduced α -synuclein expression, ROS production and 4-HNE modification of proteins thereby providing protection against cypermethrin-induced neurotoxicity. These findings indicate that activation of the Nrf2 pathway is a critical mechanism by which DJ-1 upregulates mitochondrial antioxidative enzymes and attenuates cypermethrin-induced α -synuclein expression and oxidative damage.

WTH10-32 | Neuroprotective role of chlorogenic acid in arsenite-induced oxidative damage via modulation of redox homeostasis and inflammation

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Objective: Arsenic is a well-known environmental toxicant of global concern. It is usually found in underground water as a result of rock/soil erosion or industrial processes. Chlorogenic acid (CGA) is a dietary phenolic acid widely found in coffee beans. Intake of arsenic-contaminated ground water causes cellular damage to various organs of the body. The present study was undertaken to explore the possible neuroprotective efficacy of CGA against sodium arsenite (SA) induced neurotoxicity in the albino wistar rat model.

Experimental Design: The rats were randomly divided into five groups ($n = 5$): Group 1: control (normal saline), Group 2: CGA control (300mg/kg), Group 3: sodium arsenite (10mg/kg), Group 4: CGA (150mg/kg) + arsenite, and Group 5: CGA (300mg/kg) + arsenite. All animals were treated with CGA or SA orally for 28 days. Different behavioral, biochemical, and histological biomarkers were assayed to evaluate the neuroprotective and neuromodulatory effect of CGA.

Results: Experimental findings revealed that the arsenite exposure in the rat brain significantly elevated the malondialdehyde, nitric oxide, protein carbonyl and proinflammatory cytokines (TNF- α , IL-6, IL-1 β) levels. A decrease in antioxidant enzyme (SOD, CAT, GST), reduced glutathione (GSH), AChE, total protein and antiinflammatory cytokines (IL-10) level were observed in SA treated rat brain tissue. Arsenite treated rats also showed the decrease in rotorod timing, hanging test and increase in narrow beam timing compared to normal rats. The behavioral and biochemical findings were further validated by histopathological analysis of rat brain. CGA fed rats showed significant reversal in all these altered behavioral, biochemical and histological markers induced by sodium arsenite.

Conclusion: Our experimental findings suggest that CGA has potential to protect rat brain tissue against SA-induced neurotoxicity through its antioxidant and anti-inflammatory activity.



WTH10-33 | Impact of small molecules on Tau-Microtubules interaction and tau aggregation in primary peripheral neurons and neuronal models

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Tau protein, a member of the Microtubule Associated Proteins family, is crucial for neuronal cytoskeleton being and motor coordination within the cell. Tau is found in abundance within neuronal axons of both of the central and peripheral nervous systems of vertebrates. In fact, tau is involved in the regulation of microtubules (MTs) polymerization, it is estimated that almost 80% of tau has close interaction with neuronal MTs. Different tau conformational changes can lead to pathological tau aggregations causing abnormal aggregations known as neurofibrillary tangles. This results in neurodegenerative diseases development at the end, for example, Alzheimer's disease. In order to identify, characterize and try to cure such issue, we conducted a comprehended study relating tau, MTs assembly and neuronal dysfunction using known and novel small molecules. These molecules were examined through *in vitro* experiments on two model neuronal cells: PC12 cells and mice dorsal root ganglia neurons primary culture. Different tau protein isoforms (fetal and adult tau) were transfected into the neurons with a green fluorescent protein tag. Tau-MTs interaction and tubulin polymerization were imaged in real time using live cell imaging techniques with the aid of Laser scanning microscopy. Moreover, we did comprehended studies on the small molecules used. We assessed their neuroprotection and cytotoxicity potential on a neuroblastoma cell line. In addition, we measured the liability of those compounds to cross the blood brain barrier through conducting an *in vitro* blood brain barrier penetration assay. This study allowed us to create a comprehended clear picture of neuronal cell behavior and structural modifications in response to different treatments/conditions.

WTH10-34 | Molecular mechanisms of BDNF-mediated mitochondrial remodeling and function in dendrites

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Introduction: Brain-Derived Neurotrophic Factor (BDNF) is a neurotrophin that promotes dendrite outgrowth by binding to the tropomyosin receptor kinase B (TrkB).¹ PTEN-induced Kinase 1 (PINK1) is

a serine/threonine kinase that activates downstream Protein Kinase A (PKA) in the cytosol to modulate the synthesis and posttranslational cleavage of BDNF.² Previous studies have shown BDNF can confer neuroprotection in part by elevating oxidative phosphorylation.³ The molecular mechanisms by which the mature form of BDNF modulates mitochondrial function remains to be elucidated. Here, we investigated the molecular players that regulated BDNF-elicited energy production, mitochondrial movement and morphology in dendrites. We hypothesized that BDNF modulates mitochondrial function, morphology, and content in dendrites to maintain healthy neurons and reverse dendrite/ mitochondrial pathology in a PINK1-dependent manner.

Methods: Wild-type & PINK1-deficient primary cortical neurons cultured in chambered cover glasses were transfected with mitochondrial-targeted RFP (Mito-RFP), treated with exogenous human BDNF, and imaged for mitochondrial movement, content and morphology by employing confocal & standard epifluorescence microscopy. To monitor bioenergetics, primary cortical neurons were treated with BDNF, and mitochondrial respiration and glycolysis were measured with an XF[®]24 Extracellular Flux Analyzer.

Results: Treating neurons with exogenous human BDNF increased anterograde movement of mitochondria within dendrites leading to increased mitochondrial content, mitochondrial fusional PKA-mediated phosphorylation of Drp1, and significantly increased oxidative phosphorylation and glycolysis. Additionally, exposure of primary cortical neurons to exogenous human BDNF protected dendrites from degeneration by rotenone, a complex I inhibitor.

Discussion: Here, we show new molecular mechanisms of BDNF-mediated neuroprotection of dendrites against oxidative stress by enhancing oxidative phosphorylation, enhancing mitochondrial motility and content in dendrites, and promoting mitochondrial fusion. Based on our collective data, we posit that pharmacological and molecular biology approaches that enhance PINK1-PKA-BDNF signaling can lead to neuroprotective strategies for reversing neurodegeneration in brain-related diseases affected by low PKA and BDNF signaling.

WTH10-35 | Rod bipolar cell degeneration in chronically hypoglycemic mouse retina

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Mice rendered chronically hypoglycemic by a null mutation of the glucagon receptor gene (*Gcgr*) experience an age-related loss in retinal and visual sensitivity along with a loss of synaptic contacts in the outer retina. Acuity and contrast sensitivity, as measured by the optomotor response, begin to decline at 9–13 months. Similarly, retinal function, as measured by the electroretinogram (ERG), decreases more than 100-fold in the same period. Because the ERG b-wave is the first signal to decrease in amplitude, we investigated the response of rod bipolar cells in 11–13 month-old *Gcgr*^{-/-} mice using



the whole-cell patch-clamp technique. We included L-AP4 (4 μ M) (an agonist at the mGluR6 receptor) in the external solution, to mimic a dark-adapted condition. To simulate light responses we applied CPPG (600 μ M) using a puffer pipette positioned near the dendrites of rod bipolar cells (RBCs). CPPG antagonizes L-AP4 at the mGluR6 receptors in bipolar cell dendrites, resembling the decrease in glutamate release that follows photoreceptor light activation. We found that the responses of RBCs in *Gcgr*^{-/-} mice are smaller than in control *Gcgr*^{+/-} mice. In voltage-clamp mode and a holding potential of -62 mV, 2-s applications of CPPG evoked maximal inward currents of 6.8 ± 4.7 pA and 18 ± 14 pA in *Gcgr*^{-/-} and *Gcgr*^{+/-} mice, respectively (*Gcgr*^{-/-}, *n* = 35; *Gcgr*^{+/-}, *n* = 38). In current-clamp mode the voltage responses peaked at 18 ± 14 mV and 31 ± 18 mV in *Gcgr*^{-/-} and *Gcgr*^{+/-} mice, respectively. Dendrites of Lucifer yellow-filled RBCs appeared to be shorter in *Gcgr*^{-/-} mice than in control animals. These results suggest that chronic hypoglycemia causes postsynaptic alterations in the photoreceptor to RBC synapse in agreement with observed losses in visual and retinal sensitivity.

WTH10-36 | Cellular analysis of Autism Spectrum Disorder using co-culture system of hiPSC-derived neurons and macrophages from patients

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Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by deficits in social communication and interaction, and presence of restricted or repetitive behavior and interests. Although its etiology and pathogenesis have not been elucidated, a growing body of evidence suggests that immune dysfunction and inflammation, both in the peripheral and in the central nervous system, are associated with the neurodevelopmental deficits observed in ASD. In the periphery, pro-inflammatory cytokines were found to be elevated in plasma, serum and peripheral blood mononuclear cells of patients with ASD, and these cytokine expression levels were associated with severity of behavioral impairments and with symptoms in ASD children. In the previous study, our group reported that TNF- α expression in differentiated M1 macrophages and TNF- α expression ratio in differentiated M1/M2 macrophages were markedly higher in patients with ASD than in TD individuals (Yamauchi et al, Autism Research, 2021), however, it remained unclear how the highly expressed TNF- α affect the brain or neuronal cells. In rodent models, it was shown that IL-1 α and TNF- α , expressed in microglia after repeated social defeat stress, lead to shortening and reduced branching of dendrites of neurons, and lead to social avoidance behavior.

In this study, to elucidate the effect of macrophages to human neuronal cells, we used the co-culture system of control human iPS cell-derived neurons and differentiated macrophages obtained from peripheral blood mononuclear cells. Our results show that the macrophages actually cause neuronal morphological changes via secretion of pro-inflammatory cytokines, and that the macrophages derived from ASD patients have worse impact than those from controls. These results may support immune dysfunction hypothesis of ASD, giving new insight to its pathology.

WTH10-37 | The role of the GluN2D subunit in mediating cognition in schizophrenia

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Introduction: Cognitive dysfunction including deficits in working memory (WM) are a core feature of schizophrenia and predictive of functional outcomes. However, there are currently no treatments for cognitive symptoms. Hypofunction of the glutamate NMDA receptor has been suggested to underlie many symptoms in schizophrenia including cognitive dysfunction. In healthy humans and animal models, NMDA receptor antagonists (receptor blockers) reproduce behavioral and molecular schizophrenia-like phenotypes. In the GluNR2D knockout mouse, the psychosis-inducing effects of the NMDA receptor antagonist, phencyclidine (PCP), is reduced. However, the role of GluNR2D in mediating NMDAR antagonist-induced cognitive dysfunction, particularly within the working memory domain, is currently unknown.

In the current study, we aimed to identify the role of the GluNR2D subunit in mediating NMDAR antagonist-induced WM disturbances, which are analogous to WM disturbances found in schizophrenia.

Methods: The Trial Unique Non-Matching to Location (TUNL) task in the touchscreen system was used to assess WM in wildtype (WT) and GluNR2D-knockout (KO) male and female mice (*n* = 44). Mice were then challenged with four different NMDA receptor antagonists, MK-801, PCP, S-ketamine, and R-ketamine during working memory performance on the TUNL task.

Results: There were no differences in baseline WM between GluNR2D-KO mice and WT. However, PCP impaired WM in WT but not in GluNR2D-KO mice (*p* = 0.0035), suggesting that PCP might act through GluNR2D to produce its cognition-impairing effects. In contrast, MK-801 impaired WM in both sexes and genotypes while R-ketamine and S-ketamine did not impair overall accuracy in the TUNL task irrespective of genotype or sex.

Discussion: This research uncovers a novel role of the GluNR2D subunit in mediating PCP-induced WM deficits. Differential effects of the various NMDAR-antagonists, sheds further light on the pharmacological action of the different drugs and may lead to the



identification of a molecular pathway that could be integral to working memory deficits in schizophrenia.

WTH10-38 | Disease- and sex-specific RNA surveillance in cholinergic and dopaminergic circuits of schizophrenia and bipolar disorder brains

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Schizophrenia (SCZ) and bipolar disorder (BD) are complex psychiatric diseases affecting ca. 3% of the world's population. While SCZ and BD show similar prevalence between sexes, both disease progression and treatment efficiency and options seem to be strongly sex dependent. To address this matter, we mined an RNA-sequencing dataset (CommonMind, NIH) seeking disease- and sex-specific changes in SCZ and BD dorsolateral prefrontal cortices and investigating their links to the changes in cholinergic and dopaminergic pathways. We found modulation of the tetracycline (TTP)-dependent post-transcriptional regulation, which contributes to decay of mRNAs with 3'-UTR AU-rich elements (ARE), including the brain-specific NOVA1 regulator of alternative splicing and nonsense mediated mRNA decay (NMD). Correspondingly, middle temporal gyrus Netherlands Brain Bank brain tissues from BD patients showed female-specific TTP upregulation and modified levels of the post-transcriptional circular RNA regulator, circSLC8A1. CircSLC8A1 'sponges' brain-specific miR-128 and incapacitates degradation of miR-128 target transcripts involved in neurodevelopment and NMD, including ARPP21, BDNF, RELN, and UPF1. Indeed, downregulation of circSLC8A1 corresponded to decline of these miR-128 targets, although miR-128 levels remained unchanged. Also, the ARE database predicts direct TTP targeting of cholinergic and dopaminergic transcripts. Supporting this notion, either cholinergic or dopaminergic differentiation of neuroblastoma cell lines of human male and female origin modified TTP and/or NOVA1 levels, caused neurotransmitter- and sex-dependent changes in circSLC8A1 and miR-128 targets, and revealed alternative regulation of BDNF in female-originated neurons. Finally, blood samples showed TTP upregulation in leukocytes of women with BD which might reflect changes in pro-inflammatory cytokines. Taken together, our findings confirm functional sex-specific changes in SCZ/BD post-transcriptional regulators that might alter disease progression, indicating that targeting specific pathways might offer novel sex-specific therapeutic venues for mental diseases.

WTH10-39 | Behavioral and molecular changes resulted from different environmental lead exposures: a comparative study

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Lead is a heavy metal that widespread use has resulted in environmental contamination and significant health problems, particularly if the exposure occurs during developmental stages. It is a cumulative toxicant that affects multiple body systems. Chronic lead exposure has been defined as a cause of behavioural changes, inflammation, hypertension, and autonomic dysfunction. However, different environmental lead exposure paradigms can occur and the different effects of these have not been described in a broad comparative study. In the current study, the effects of developmental (DevPb), intermittent (IntPb), and permanent (PerPb) exposure to Pb upon behavioural, memory impairment, molecular changes within the brain and physiological parameters have been examined in rats of both sexes exposed to lead acetate (0.2% w/v) drinking solution. DevPb pups were exposed to lead until 12 weeks of age (n=13), IntPb pups ingested lead until 12 weeks of age, tap water until 20 weeks and leaded water for the second time from 20 to 28 weeks of age (n=14) and the PerPb animals drunk leaded water until 28 weeks of age (n=14). A control group (CTL), matching in age and sex was used. All Pb-exposed groups lead to hypertension, partly explained by a baroreflex impairment. Long-term memory impairment associated with reactive astrogliosis and microgliosis in the dentate gyrus of the hippocampus, indicating the presence of neuroinflammation were also observed. However, these alterations seem to reverse after lead abstinence for a certain period (DevPb) and are enhanced when a second exposure occurs (IntPb), along with a synaptic loss. The results suggest that the duration of Pb exposure is more relevant than the time of exposure, since the PerPb group, in general, showed greater impairment than the other groups, even though a double hit on the system leads to stronger effects. In summary, our study, being unique in its kind, is of great significance, hence showing the importance of the surrounding environment in health from childhood until adulthood, leading to the creation of new policies for toxicant usage control.



WTH11-01 | The sigma-1 receptor dynamics, monitored with super-resolution imaging microscopy

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The Sigma-1 receptor (Sig1R) is an endoplasmic reticulum (ER)-related membrane protein, which forms heteromers with other cellular proteins. As the mechanism of action and cellular motion of this chaperone protein is still a very intriguing question, the aim of the present study was to detect and analyze the intracellular dynamics of Sig1R in live cells using super-resolution imaging microscopy. The Sig1R-yellow fluorescent protein conjugate (Sig1R-YFP) was transfected together with fluorescent markers of cell organelles into human ovarian adenocarcinoma (SK-OV-3) cells using BacMam technology. The specific and high-affinity binding of prototypic Sig1R ligand [³H](+)-pentazocine was used as a special characteristic to control the functionality of Sig1R-YFP in SK-OV-3 cells. Imaging revealed that Sig1R-YFP is located mainly in the nuclear envelope and in both tubular and vesicular structures of the ER but was not detected in the plasma membrane, even after activation of Sig1R with agonists. The super-resolution radial fluctuations analysis (SRRF) of images obtained with a highly inclined and laminated optical sheet (HILO) fluorescence microscope indicated substantial overlap of Sig1R-YFP spots with endoplasmic reticulum marker KDEL-mRFP, slight overlap with the marker of mitochondria pmKate2-mito and no overlap with the markers of endosomes (mCherry-Endo-14), peroxisomes (mCherry-Peroxisomes-2), lysosomes (mCherry-lysosomes-20), or caveolae (Cav1-mRed). Activation of Sig1R with (+)-pentazocine caused a time-dependent decrease in the overlap between Sig1R-YFP and KDEL-mRFP, indicating that activation of Sig1R decreases its colocalization with the marker of vesicular ER but does not cause comprehensive translocations within the cells.

WTH12-01 | Modeling Prader-Willi syndrome using hypothalamic organoids from patient-derived induced pluripotent stem cells

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Prader-Willi Syndrome (PWS) is a genomic imprinting disorder caused by the loss of function of paternally expressed genes (PEGs) in the chromosome 15q11-13 region. Hyperphagia, hypogonadism, and short stature due to growth hormone deficiency are major clinical manifestations of PWS, and these phenotypes have been thought to be closely related to hypothalamic dysfunction. However, little is known about the cellular and molecular pathophysiology of PWS. Here, we established models of PWS using hypothalamic organoids from patient-derived

induced pluripotent stem cells (iPSCs). iPSC lines were generated from five PWS patients harboring different genetic backgrounds, such as deletion, maternal uniparental disomy, translocation, and imprinting defects. Optimizing the culture condition, we succeeded in generating RAX(+), NKX2.1(+), OTP(+) hypothalamic progenitor cells from those PWS-iPSCs as well as several hypothalamic neurons including proopiomelanocortin(+) cells, which are crucial for the regulation of feeding. Moreover, we focused on PEGs in chromosome 15q11-13, which are known to be highly expressed in the hypothalamus and related to PWS clinical symptoms. We found these PEGs, including *NDN*, *MAGEL2*, *MKRN3*, and *SNRPN*, were highly expressed in hypothalamic organoids from healthy control iPSCs, but not in PWS hypothalamic organoids. These data indicate that the PWS-iPSC-derived hypothalamic organoids recapitulate PWS phenotypes. To further characterize PWS phenotypes, we performed a single-cell RNA-sequencing analysis and found unprecedented abnormal astrogenesis in PWS hypothalamic organoids. Using these *in vitro* models, we are going to reveal the pathophysiological mechanisms of PWS.

WTH12-03 | Human choroid plexus-ventricle-cortical organoids model SARS-CoV-2 neuropathology in down syndrome and enable drug screening

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Clinical evidence suggests neurological complications in COVID-19 patients and that individuals with Down syndrome (DS) have high rates of mortality, yet SARS-CoV-2 implications in pathogenesis remain unclear largely due to the lack of *in-vitro* models recapitulating trisomy 21 neural pathology. Here, we used hPSC-derived neuroectodermal cells, the building blocks of the anterior body, to simultaneously generate choroid plexus (CP) that forms ventricles and cortical cells in organoids (CVCOs). CVCOs contain mature and functional CP and is in direct contact with cortical cells. The organoids exhibit notable similarities to native CP on the basis of their morphology, immunolabeling characteristics and gene expression patterns, and undergo functional maturation when cultured for 56 days. Large scale culture revealed reproducibility of the protocol independent of cell lines, clones or batches. CVCOs recapitulated key aspects of the trisomy 21 cortical pathology and identified defects in ciliogenesis and disorganized epithelial cells polarity of the developing CP. We further demonstrated that 28 days old CVCOs are capable of infection with SARS-CoV-2, CP within CVCOs served as viral "entry hubs", mediating viral spreading and infecting to cortical cells, while trisomy 21 cortical organoids showed little evidence of SARS-CoV-2 infection. Inhibition of TMPRSS2 activity, in trisomy 21 CVCOs, with FDA approved drugs prevented SARS-CoV-2 infectivity. Therefore, CVCOs recapitulate key features of developing forebrain structures observed *in vivo*, constitute a useful model for dissecting the role of CP in trisomy

21 forebrain development and SARS-CoV-2 entry and replication in trisomy 21 neural tissue as well as a suitable model for drug screening against SARS-CoV-2 pathology in trisomy 21.

WTH12-04 | Acentrosomal microtubule assembly and reactivation of quiescent neural stem cells

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The ability of stem cells to switch between quiescence and proliferation is crucial for tissue homeostasis and regeneration. Most neural stem cells (NSCs) that reside in mammalian adult brains are in a mitotically dormant, quiescent state but can exit from quiescence and become reactivated, in response to physiological stimuli such as the presence of nutrition and physical exercise. *Drosophila* NSCs, also known as neuroblasts, have emerged as a powerful model to study the mechanisms underlying NSC quiescence and reactivation in vivo. Quiescent NSCs are morphologically characterized by a primary cellular protrusion attached from their cell body and their reactivation depends on an evolutionarily conserved insulin/IGF signalling pathway. However, the structure and function of this protrusion are not well established. Here, we show that in quiescent NSCs, microtubules are predominantly acentsosomal and oriented plus-end-out towards the tip of the primary protrusion. We have identified Mini Spindles (MSPs)/XMAP215 as a key microtubule regulator in quiescent NSCs that governs NSC reactivation via regulating acentsosomal microtubule growth and orientation. We show that quiescent NSCs form membrane contact with the neuropil and E-cadherin, a cell adhesion molecule, localizes to these NSC-neuropil junctions. MSPs and a plus-end directed motor protein Kinesin-2 promote NSC cell cycle re-entry and target E-cadherin to NSC-neuropil contact during NSC reactivation. Therefore, the neuropil may function as a new niche controlling NSC reactivation, which may be a general paradigm in mammalian systems.

WTH13-01 | Dementia risk modifier TMEM106B increases with physiological aging and affects myelin lipid homeostasis in the hippocampus

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Aging is the greatest risk factor for the major forms of dementia, including Alzheimer's disease, frontotemporal dementia, and dementia

with Lewy bodies. Genetic evidence implicates altered lipid metabolism as a common mechanism sensitizing to various forms of dementia. This study aimed to identify changes in the hippocampal proteome with physiological aging, and to identify important regulators of hippocampal lipid metabolism.

Post-mortem hippocampal tissue samples (CA1 region) from 76 cognitively- and neuropathologically-normal donors, aged 64–104, were used for data-independent proteomic analysis and targeted lipidomic profiling. The protein that was most significantly associated with age ($p_{\text{LinearRegression}} = 1 \times 10^{-6}$) was TMEM106B, a regulator of lysosomal and oligodendrocyte function. Genetic variants of TMEM106B (rs1990622) modulate the risk for Alzheimer's disease, frontotemporal dementia, Parkinson's disease, hippocampal sclerosis, and hypomyelinating leukodystrophy. The age-TMEM106B correlation was specific to TMEM106B rs1990622-A risk allele carriers, whereas individuals homozygous for the protective G allele showed no elevation of TMEM106B with age ($P_{\text{RiskAllele} \times \text{Age-Interaction}} = 0.038$). Lipidomic profiling revealed that TMEM106B risk allele carriers have diminished levels of myelin-enriched sphingolipids hexosylceramides (fold-change = -1.4 , $p_{\text{t-test}} = 0.002$) and sulfatides (fold-change = -1.5 , $p_{\text{t-test}} = 0.004$), with proteomic analysis showing a downregulation of myelin proteins MBP and PLP1. TMEM106B risk allele carriers simultaneously displayed an accumulation of polyunsaturated triglycerides and phospholipids, together with an elevation of cholesteryl-ester (22:6), strongly suggesting defective lipid catabolism and lipid droplet formation.

Our results provide the first evidence that TMEM106B protein abundance is increased with aging in the hippocampus of cognitively normal humans, and that this is dependent on the rs1990622-A risk allele. We also establish the TMEM106B risk allele as a regulator of lipid metabolism in the aging brain, and show that it is associated with myelin loss in cognitively normal individuals. We propose that TMEM106B and its genetic variants play a key role in modulating the early processes of neurodegeneration through its role in myelin maintenance.

WTH13-02 | Lipids as a novel biomarker for amyotrophic lateral sclerosis

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Diagnosis of Amyotrophic lateral sclerosis (ALS) can be a lengthy process, which is partially due to the lack of accurate biomarkers that are easily accessible. To date, blood-based biomarkers lack specificity and are unable to distinguish MND from other neurodegenerative disorders. It has increasingly been acknowledged that lipid metabolism plays a crucial role in ALS. Therefore, the aim of this study is to discover novel biomarkers that are specific to ALS, based on the blood lipidome of mutant SOD1 mice.



We performed targeted lipidomic analysis on plasma of SOD1^{G93A} and wildtype littermates at metabolomics Australia. Analysis was conducted pre-symptomatically at postnatal day (P) 60, symptomatic—P90 and end stage ~P150. Furthermore, we treated a separate group of mice with ambroxol, an inhibitor of a key enzyme in the glycosphingolipid pathway and a novel candidate for treating MND symptoms. Treatment started at P60 and blood was collected at P90 and end stage. We discovered that at P60, there is a distinct lipid dysregulation found in the blood of SOD1^{G93A} mice compared to their wildtype littermates. Next, we sought how lipids change with disease progression. Here, we compared targeted lipidomics of P60, P90 and ~P150 SOD1^{G93A} mice. We found that lipids have a dynamic abundance, changing with disease progression. Lastly, we show that ambroxol can significantly restructure lipid composition, possibly explaining extension in lifespan of mutant SOD1 mice. Together, we are the first to show that blood lipids have a strong potential to function as reliable biomarkers, which may improve diagnostic interval in MND.

WTH13-03 | Biophysical studies to understand GM1- α -synuclein interaction

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GM1 gangliosides are abundant in neurons of all animal species. Recent evidence is showing that GM1 deficiency can lead to failure of trophic plasma membrane signaling and to α -synuclein (α S) accumulation. Fibrillary α S aggregated is a Parkinson's Disease (PD) neurologic hallmark. Although the causes leading to α S aggregation are not clear, accumulating evidence shows peculiar interaction between GM1 and the protein. Interaction of the GM1 head group with α S, prevents protein conformational change necessary to protein aggregation and deposition. How GM1 exerts these functions is not clear, though a primary role of oligosaccharide portion (OligoGM1) is emerging.

The β -sheet-rich state of α S has long been associated with its pathological aggregation in PD due to genetic modification that flank a specific domain (34-KEGVLYVGSKTK-45). Structural studies have shown that this region of α S is an α -helical conformation and represents the ganglioside binding motif (GBM).

Aim of this project is to study the OligoGM1- α S interactions in order to understand the role of the oligoGM1 in prevention of α S aggregation.

Preliminary Small Angle X-ray Scattering (SAXS) experiments highlight that interaction between α S and GM1 induces an increase of the average area per molecule at the interface.

RT-QuIC *in vitro* assay demonstrated that OligoGM1 is able to prevent α S aggregation. By circular dichroism spectroscopy, we found that OligoGM1 do not induce any change in α S secondary structure. Preliminary computational results indicate that the interaction between the ligand and the protein residues K34 and K45 is not stable. In the docking pose the ligand Neu5Ac unit forms a salt bridge with K45 and lies in proximity of Y39 side chain but during simulation, OligoGM1 separates from α S.

NMR techniques were used to gain insights into the interaction mechanism and to support computational data with experimental evidences.

WTH15-01 | CNS-active reactivator of inhibited acetylcholinesterase provides neuroprotection in mice exposed to nerve agent

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Acutely toxic organophosphates (OP) readily cross the blood-brain barrier (BBB) and inhibit the enzyme acetylcholinesterase (AChE). Consequently, increased level and residence time of neurotransmitter acetylcholine leads to seizures and activation of glial cells. If not treated by reactivators of AChE activity and an antimuscarinic drug, OP poisoning causes hypoxia, vasodepression, and respiratory arrest, followed by death. Nevertheless, reactivators currently in use are quaternary compounds that do not cross the BBB and cannot restore the activity of synaptic AChE, leaving the brain vulnerable to long-term damage. We anticipate that the treatment with uncharged, but ionizable oximes that cross the BBB and reactivate OP-inhibited synaptic AChE will act protectively on the brain of mice exposed to a nerve agent. For that purpose, we have investigated the effect of centrally acting RS194B oxime in the brain of mice exposed to a nerve agent by comparing detected levels of specific proteins expressed in neuronal and glial cells in the brains of OP exposed mice with mice treated by oxime therapy and untreated control mice. An increased level of IBA-1 protein detected a microglial response, and astrogliosis due to the accumulation of GFAP protein was also evident in poisoned mice. Nevertheless, our results indicated that therapy with RS194B acted neuroprotective when compared to OP exposed mice, and mice treated with standard oxime 2-PAM, especially up to 1.5h after OP exposure. Moreover, in that period we observed that neuronal cell viability detected with NeuN immunoreactivity, was in the control range in mice treated with RS194B, unlike in OP exposed, and 2-PAM treated mice where NeuN levels were significantly below control. In conclusion, we can indicate the



survival of neurons and neuroprotection by RS194B therapy but further immunohistochemical studies are planned for confirmation.

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WTH15-02 | Novel NMDA-receptor antagonist reverses vanadium neurotoxicity in mice and mitochondrial damage in *Caenorhabditis elegans*

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Various NMDA-receptor antagonists have been investigated for their therapeutic potential in Alzheimer's disease with memantine shown to be safe, with relative efficacy. There is, however, a need to develop novel drugs to counter tolerance, with better efficacy in ameliorating neurodegeneration. We have shown neurodegeneration in different models of vanadium-exposed mice. This study was designed to evaluate and ascertain the potency of a novel NMDA-receptor antagonists (Compound C) to ameliorate neurodegeneration and mitochondrial damage in vanadium-exposed mice and *Caenorhabditis elegans* respectively. Five dams were used in this study; with eight pups per group ($n = 8$ pups) randomly assigned to five test groups, intraperitoneally treated for 6 months. Group A received sterile water (control), Group B received vanadium (3mg/kg sodium metavanadate), Group C received vanadium and Compound C (100mg/kg) simultaneously, Group D was withdrawn from vanadium treatment after 5 months and administered sterile water to the 6th month and Group E was administered with Compound C alone. The same groupings were replicated in the worms, *C. elegans* to evaluate the anti-oxidative properties of Compound C in the mitochondria of Vanadium exposed worms. Assessment of pathologies and neurodegeneration in different brain regions of mice was done to test the ameliorative effects of Compound C using different immunohistochemical markers and targeted *in vivo* fluorescence analyses of mitochondria-dense pharyngeal tissue in *C. elegans*. Vanadium exposure in mice resulted in reduced myelin expression, microgliosis, pyknosis of neuronal cells, cell loss and destruction of apical dendrites with greater percentage of cytoplasmic vacuolations and morphological alterations. In addition, there was decreased mitochondrial content in the pharyngeal tissue of *C. elegans*. Treatment of vanadium-exposed mice and *C. elegans* with Compound C resulted in the preservation of cellular integrity in the same anatomical regions and restoration of mitochondrial content and vitality in the mice and *C. elegans* respectively.

WTH15-03 | Pro-maturing effect on OPCs driven by transferrin-loaded extracellular vesicles

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Extracellular vesicles (EVs) are lipid bilayer-bound vesicles released by cells into the extracellular environment. EVs are involved in diverse cellular functions, playing a major role in cell-to-cell communication in both physiological and pathological scenarios. Therefore, EVs are powerful mediators of information and constitute a promising therapeutic strategy. Oligodendrocytes (OLs) are specialized myelinating glial cells developed from oligodendrocyte precursor cells (OPCs) in the central nervous system. While demyelination results from damage to OLs, remyelination failure has been mainly attributed to poor OPC differentiation. Glycoprotein transferrin (Tf) plays a critical role in iron homeostasis but also has pro-differentiating effects on OLs *in vivo* and *in vitro*. Previous studies by our group demonstrated a remyelinating effect of apotransferrin (aTf) in animal models of cuprizone-induced demyelination and hypoxia/ischemia, as the transcranial and intranasal administration of aTf reduced white matter damage, neuronal loss, and astrogliosis in different brain regions. In previous work, we isolated and successfully loaded plasma EVs (EVTf) through aTf binding to its receptor, contained in the membrane of EVs. In the present work, we show that Tf-loaded EVs enter OPCs in a time-dependent manner. This process is mainly mediated by clathrin-caveolin endocytosis, which requires EV surface proteins and glycans for their internalization. EVs internalized by OPCs co-localized with intracellular lysosome marker Lamp1. EVTf demonstrated a pro-maturation effect on primary OPC cultures and promote their differentiation into myelinating OLs, as evidenced by an increase in MBP⁺ cells and a concomitant decrease in PDGFR α ⁺ cells. These results were also verified through MBP immunoblots analysis. Finally, studies using primary OPC-microglia co-cultures demonstrated that EVs are internalized by OPCs and foster their maturation even in the presence of activated microglia. Our results demonstrate that EVTf has access to the OPC inducing its maturation and, therefore, might have the potential of being tested as a remyelinating agent.

WTH15-04 | Inhibition of bromodomain and extraterminal proteins attenuates microglial phagocytosis

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Dysregulation of microglial phagocytosis may have detrimental consequences and significantly contribute to the pathomechanism of neurodevelopmental and neurodegenerative disorders. Therefore, a better understanding of mechanisms controlling glial phagocytic

activity may provide novel therapeutical targets. Our study aimed to analyze, whether bromodomain and extraterminal (BET) proteins, the readers of histone acetylation code, have a role in the regulation of microglial phagocytosis. Therefore, we used both pharmacological (JQ1) and genetic (siRNA) approaches for BETs' inhibition in mouse microglial BV2 cells. Our results demonstrated that neither JQ1 nor siRNA treatment has cytotoxic effects as well as does not affect the BV2 proliferation and mobility. By using fluorescent beads-based assay and fluorescently-labeled amyloid-beta peptides we demonstrated that JQ1 significantly attenuated the phagocytic activity of microglial cells. Gene silencing experiments revealed that all three BET isoforms (Brd2, Brd3, Brd4) contribute to the regulation of phagocytosis, however, the Brd3 seems to be the least involved, since Brd2 and Brd4 knock-down brought better effects. The mechanism of BET- dependent alterations in phagocytic activity is likely related to changes in transcription, thus we performed the analysis of the mRNA level of 84 phagocytosis-related genes using the gene expression array. It was demonstrated that in JQ1-pretreated BV2 cells the expression of 14 genes (e.g., Siglec1, Sirpb1a, Cd36, Clec7a, Itgam, Tlr3) was significantly inhibited. Our results demonstrated that BET proteins control the microglial phagocytosis, therefore the inhibition of BET proteins may offer a new strategy of mitigation of the overactive microglial activity.

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WTH15-05 | INVESTIGATING OREXIN INVOLVEMENT IN STRESS-INDUCED BINGE EATING IN FEMALE MICE

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Stress and negative affect (e.g., sadness, anger, loneliness) are known to trigger overeating, particularly in women. This form of maladaptive eating behavior, commonly referred to as "emotional eating", is associated with binge eating and higher risk of obesity. The neural mechanisms that underpin this form of dysregulated eating are yet to be elucidated but likely implicate neuronal substrates involved in both homeostatic and hedonic feeding. The orexin (hypocretin) system has been previously implicated in reward, stress and feeding. Thus, we aim to investigate the role of the orexin system in stress-induced binge eating in females. Mice were subjected to a protocol that employed a mild psychological stressor and intermittent access to a highly palatable food reward to induce binge eating in mice. Mice exposed to a frustrative stressor consume significantly more of the food reward compared to control mice. The impact of systemic administration of the orexin 1 receptor antagonist SB-334867 (15 or 20mg/kg, sc) on stress-induced binge eating is currently being assessed and preliminary data from the

first cohort of mice will be presented. Furthermore, brain slices from the lateral hypothalamic area of stress binge and control mice were processed for Fos and orexin immunostaining. Early analysis is indicative of a trend toward significant neuronal activation of orexin neurons as a result of stress-induced binge eating; however, full quantification of immunohistochemical data is ongoing. We hypothesize that orexin neurons will be significantly activated as a result of stress-induced binge eating as compared to control and that systemic blockade of orexin 1 receptors will ameliorate this behavior, thus implicating orexin signaling at orexin 1 receptors in stress-driven maladaptive eating.

WTH15-06 | Probing Caffeine administration as a medical management for hydrocephalus; an experimental study

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Hydrocephalus is currently managed by CSF diversion from the cerebral ventricles to other body sites, but this is complicated by obstruction and infection in young infants, thus adding to morbidity and mortality. Studies have reported caffeine to be a pleiotropic neuroprotective drug in the developing brain due to its antioxidant, anti-inflammatory and anti-apoptotic properties, with improved white matter microstructural development. In this study, we investigate the use of caffeine administration as a possible means of pharmacological management for hydrocephalus.

Sixty eight 3 day-old mice pups from eight dams were divided into three groups: hydrocephalus was induced in the pups in two groups by intracisternal injection of sterile kaolin suspension and their dams were given either caffeine (60mg/kg by gavage) or water daily for 21 days, the dams in the control group had water; the pups received caffeine via lactation. Developmental neurobehavioral tests were performed until Day 21, when the pups were sacrificed. Their brains were removed and processed for H&E, Cresyl, and Golgi staining; both quantitative and qualitative analyses were then carried out. Improved motor activities and reflexes were observed in the caffeine-treated pups, although weight gain was reduced in them. Caffeine administration was associated with reduced cell death and increased dendritic arborization of the neurons in the sensorimotor cortex and striatum.

Caffeine administration appears to have promise as an adjunct in hydrocephalus management and its use needs to be further explored.



WTH15-07 | Loss of PKC δ in sensory neurons promotes hyperalgesia through VEGF/VEGFR1 and NGF/TrkA in osteoarthritis induced rodent model

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Objectives: Osteoarthritis (OA) is a prevalent, complex, and conventional age-associated degenerative joint disease in which fundamental changes to articular cartilage are the pathophysiological hallmark. Here, we concluded whether protein kinase C δ (PKC δ), absence in peripheral sensory neurons (DRG), induced pain perception and explored its molecular mechanism.

Methods: OA was induced by partial medial meniscectomy (PMM) surgery in 11 weeks old PKC δ deletion ($^{PKC\delta\text{ fl/fl}; ROSA\text{ CreERT}2}$), a gene specifically knockout of PKC δ in sensory neurons ($^{PKC\delta\text{ fl/fl}; Nav1.8\text{ CreERT}2}$) transgenic mice. After 12 weeks of PMM surgery, did histopathology of the knee joint and molecular mechanism of pain-related behavior in mice by immunostaining of mice's knee cartilage, synovium, and DRG samples.

Results: In the PMM-induced OA model, PKC δ deletion mice prevent cartilage degeneration and induce OA-associated hyperalgesia. But in, sensory neuron-specific deletion of PKC δ mice did not protect the cartilage protection and induced OA-related hyperalgesia. Increased pain perception from knee synovium through the N-terminal of peripheral neuron fibers to sensory neurons in DGR by nerve growth factor (NGF)/tropomyosin receptor kinase (TrkA) and vascular endothelial growth factor (VEGF)/vascular endothelial growth factor receptor1(VEGFR1) axis.

Conclusions: Increased the distribution of synovium sensory neurons in the joint synovium and amplification of NGF/TrkA and VEGF/VEGFR1 signaling cause OA associated pain independently cartilage prevention. This OA-related pain targeting sensory neuron-specific PKC δ is essential for developing the therapeutic drug to treat pain-related symptoms in patients with OA. Our novel results also provide a first-time resolution of the roles of PKC δ in distinct compartments of the OA joint.

WTH15-08 | Berberine loaded MCM-41 mesoporous silica nanoparticles affected cell viability and apoptosis, and improved mitochondrial health

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Berberine (BBR) loaded Mobil Composition of Matter-41 (MCM-41) mesoporous silica nanoparticles (MSNs) were prepared using a modified Stober approach. The synthesized MSNs-BBR were evaluated for particle size, morphology, polydispersity index, zeta potential, drug loading, entrapment efficiency, and in vitro drug release. Spectral analysis of the

formulation was carried out using IR spectroscopy, powder X-ray diffraction analysis, Raman spectroscopy analysis, X-ray photoelectron spectroscopy analysis, and thermogravimetric analysis. Brunauer-Emmett-Teller analysis was done to determine specific surface area. Computational models were constructed for the molecular dynamics simulation study. A small PDI value indicated good dispersion homogeneity of the nanoparticles. The size of the prepared MSN-BBR was in the range of 80-100 nm. XRD and SEAD analysis indicate the amorphous nature of the material. The percentage of BBR loading and entrapment efficiency in MSNs-BBR were found to be $28.16 \pm 2.5\%$ and $75.21 \pm 1.55\%$, respectively. The zeta potential value of MCM-41 ($-36.86 \pm 1.1\text{mV}$) was attributed to the presence of silanol groups on the silica surface. AFM results suggested an increased surface roughness of MSNs-BBR due to the bumps associated with the surface drug. TGA analysis confirmed BBR loading in MSNs. The drug release was delayed release up to 72h in the selected dissolution media and followed a simple diffusion or quasi-diffusion-controlled drug release mechanism. A molecular dynamics simulation study confirmed the diffusion process of the entrapped drug molecules. A dose-dependent proliferation of SH-SY-5Y cells was recorded. Phase-contrast microscopic images revealed an increase in apoptotic cells and a decrease in viable cells. MSNs-BBR treated SH-SY-5Y cells stained with DAPI showed nuclear apoptotic bodies and fragmented cell nuclei. Flow cytometric analysis showed an increased red to green ratio depicting improved mitochondrial health and prevention of apoptotic process and restoration of cellular viability in MSNs-BBR treated SH-SY-5Y cells.

WTH15-09 | Quantification of striatal neuropeptide Y-expressing interneurons and their modulation by nicotinic acetylcholine receptors

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The striatum is the main input nucleus of the basal ganglia and it plays a crucial role in behavior, learning and motivation. In terms of cellular composition, GABAergic interneurons represent about 4% of the total number of striatal neurons and current literature describes about 10 different types of these neurons. Neuropeptide Y-expressing (NPY+) interneurons represent one such type of striatal interneurons. Electrophysiological studies suggest that NPY+ interneurons form synaptic connections with striatal cholinergic interneurons and they are activated by beta2-containing nicotinic acetylcholine receptors (beta2* nAChRs). There is still relatively little information about the function of NPY+ interneurons in the striatum. Namely, it is not known what is the behavioral significance of this small population and to what extent it is controlled by striatal acetylcholine. In the present project, we quantified the NPY+ interneurons in the dorsal striatum using stereology in mice expressing GFP under the control of NPY promoter. Then, we used FISH to



determine the expression of beta2* nAChRs by NPY+ interneurons and we used CRISPR to abolish the function of these receptors specifically in striatal NPY+ interneurons. Finally, a battery of behavioral tests was used to determine the effect on striatal-based behavior. The stereological quantification of NPY+ interneurons showed approximately 12 thousand positive cells per dorsal striatum and a decreasing density gradient of these cells along the mediolateral axis. Surprisingly, the FISH analysis showed little colocalization of NPY and beta2 RNA probe with less than 10 % of NPY+ neurons expressing beta2* nAChRs. In addition, the majority of beta2-expressing NPY+ neurons were located in the lateral part of the dorsal striatum. In line with our FISH data, the behavioral tests showed no effect of the CRISPR-induced beta2 deletion in NPY+ neurons on striatal-based behavior.

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WTH15-10 | The impact of physical exercise on brain microcirculation, astrocytes, and microglia in a model of cerebral hypoperfusion

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Background: Brain circulation disorders such as chronic cerebral hypoperfusion have been associated with a decline in cognitive

function during the development of dementia. Currently, physical exercise has been proposed as an effective intervention to promote brain function improvement. However, the neuroprotective effects of early physical exercise on the astrocyte communication with the microcirculation and the microglial activation in a chronic cerebral hypoperfusion model are still unclear. The aim of this study was to investigate the impact of early intervention with physical exercise on cognition, brain microcirculatory, and inflammatory parameters in an experimental model of chronic cerebral hypoperfusion induced by permanent bilateral occlusion of the common carotid arteries (2VO).

Methods: Wistar rats aged 12 weeks were divided into four groups: Sham-sedentary group, Sham-exercised group, 2VO-sedentary group, and 2VO-exercised group. The early intervention with physical exercise started 3 days after 2VO or Sham surgery. After 12 weeks of exercise (or not for the sedentary group) the brain functional capillary density and endothelial-leukocyte interactions were evaluated by intravital microscopy; cognitive function was evaluated by open-field test; astrocyte coverage of the capillaries, microglial activation, and structural capillary density was evaluated by immunohistochemistry.

Results: Early moderate physical exercise was able to normalize functional capillary density and reduce leukocyte rolling in the brain of animals with 2VO. In addition, physical exercise improved astrocytes coverage in blood vessels of the cerebral cortex and hippocampus, decreased microglial activation in the hippocampus, and improved cognitive function.

Conclusions: Microcirculatory and inflammatory changes in the brain appear to be involved in triggering a cognitive decline in animals with chronic cerebral ischemia. Therefore, early intervention with physical exercise may represent a preventive approach to neurodegeneration caused by cerebral hypoperfusion.

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