Metabolic impairments in neurons and astrocytes derived from human induced pluripotent stem cells of Alzheimer's disease patients

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Publication date:
2019

Document version
Publisher's PDF, also known as Version of record

Journal of Neurochemistry

Journal of Neurochemistry (2019), **150** (Suppl. 1), 73–161 doi: 10.1111/jnc.14776

Poster Sessions Monday/Tuesday

MTU01 Gene regulation & genetics (Session A)

MTU01-01

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

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

MTU01-02

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

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

MTU01-03

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

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

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

MTU01-04

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

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

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

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

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

MTU01-05

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

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Attention Deficit Hyperactivity Disorder (ADHD) is a common behavioral disorder. Several studies have tended to highlight the effects of antioxidants on regulation of ADHD. The present work investigated the effect of Gallic Acid (GA) and Ellagic Acid (EA) in a Trimethyltin Chloride-induced animal model of ADHD with insights from behavioral and genetic aspects. In this study, 60 pregnant Wistar rats were used in six groups (N=10 per group), namely Control (saline only), Sham (TMT 9 mg/kg), Ellagic Acid (TMT 9 mg/kg+ EA 10 mg/kg), and Gallic Acid (TMT9 mg/kg+ 50, 100, 200 mg/kg) groups. To induce ADHD, TMT was injected to animals on gestational day 15 (G15) intraperitoneally. Treated groups received EA and GA as gavage on G12 to G18. After delivery, 10 pups were selected out of each group to be studied. Then, on day 60 of delivery, Elevated Plus Maze, Open Field and T-Maze were conducted. Following, animals were terminated humanly and prefrontal cortex of their brains was collected for molecular studies followed by SYBER green qPCR to investigate the expression level of DRD4 and DRD5 genes. Behavioral findings revealed that EA and GA could significantly decrease hyperactivity, impulsivity and inattention in rats. The expression level of DRD4 and DRD5 were significantly down-regulated in the sham group as compared to the controls. In addition, EA and GA in all dosages caused an up-regulation of DRD4 and DRD5 expression, although these differences were not significant as compared to the sham group.

MTU01-06

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

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

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

MTU01-07

Hypoxia contributes to Alzheimer's disease by regulating CNTNAP2 gene

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Stroke is a risk factor for Alzheimer's disease (AD). Hypoxia is a major consequence of stroke. Contactin-associated protein-like 2 (CNTNAP2) functions as a presynaptic cell adhesion molecule, and plays important roles in neuronal network formation during development. CNTNAP2 rs802571 is a locus for susceptibility to late-onset AD, and abnormal CNTNAP2 expression has been found in the hippocampus of AD patients. However, it remains elusive how CNTNAP2 is regulated at the transcriptional level. In this study, we cloned a 2949 bp 5'-flanking region of the human CNTNAP2 gene. To investigate the activity of human CNTNAP2 gene promoter, thirteen deletion fragments of its 5'-flanking region were cloned into pGL3-Basic vector. Functional analyses showed that the transcriptional start site was located between -524 to -472 upstream of translational start site. The fragment -524 to -81 bp upstream of translational start site had the minimum promoter activity required for transcription. There were five 5'-RCGTG hypoxia-responsive elements (HRE) in the human CNTNAP2 gene promoter. Three putative functional HREs were located between -1322 to -1268 bp upstream of translational start site. The activity of CNTNAP2 promoter was increased to 270% when treated with hypoxia (1% O_2). Co-transfection with hypoxia-inducible factor 1 subunit α (HIF-1 α) expression plasmid significantly increased the CNTNAP2 promoter activity to 133%. This is the first study defining the promoter region of human CNTNAP2 gene. Our results demonstrated that CNTNAP2 is tightly regulated by hypoxia at the transcriptional level. Our study suggests a novel role of hypoxia in contributing to AD pathogenesis by regulating CNTNAP2 gene expression.

MTU02 Signal transduction & synaptic transmission (Session A)

MTU02-01

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

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Activity dependent bulk endocytosis (ADBE) is triggered during high neuronal activity in central neurons. It is a two-step process that generates synaptic vesicles from large bulk endosomes, which are directly invaginated from the presynaptic plasma membrane. The activity of glycogen synthase kinase 3 (GSK3) is essential for ADBE, however how this control is mediated is still incompletely understood. One GSK3 substrate that is present at the synapse is phosphatidylinositol 4-kinase IIa (PI4KIIα). Depletion of PI4KIIα using shRNA in cultured cerebellar granule neurons (CGNs) arrested ADBE. Delivery of exogenous PI4KIIα to these neurons fully restored ADBE, highlighting an important role of PI4KIIα. Interestingly, molecular replacement of endogenous PI4KIIα with a GSK3- phospho-mimetic mutant failed to rescue ADBE. However, kinase-dead and phospho-null mutants both fully restored ADBE, suggesting that GSK3-dependent phosphorylation of PI4KIIα negatively regulates this endocytosis mode. To determine potential phosphorylation-specific interactions, GST-PI4KIIα pull downs were performed. Mass spectrometry analysis revealed 5 presynaptic molecules that displayed an increased interaction with the phospho-mimetic PI4KIIα mutant, which were confirmed by Western blotting. Truncation and domain swop mutations revealed that mock phosphorylation of Ser-47 on PI4KIIα is critical in controlling these interactions. Intriguingly, individual depletion of two of these phospho-dependent interaction partners greatly reduced ADBE in CGNs. These results indicate a key role for PI4KIIα in ADBE and confirm the constitutively active GSK3 as a master regulator of this process. We propose that activity-dependent dephosphorylation of Ser-47 on PI4KIIα induces the release of key molecules which are crucial for the initiation of ADBE.

MTU02-02

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

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Interleukin-1β(IL-1β) is a pro-inflammatory cytokine of the immune system. While it contributes to various neuroinflammatory and neurodegenerative disease of the central nervous system (CNS), it also modulates several physiological functions. Thus, IL-1β is a neuromodulator in both dysfunctional and normal CNS. Epilepsy is a brain disorder hallmarked by excessive neuronal firing. IL-1β's role has been implicated in seizures, the defining feature of epilepsy. Of relevance to my research, we previously showed that the innate seizure threshold was lowered in mice in which IL-1β receptor was genetically disrupted. This provided compelling evidence to support the premise that constitutive IL-1β production in the normal brain influences neuronal excitability. However, cellular location and regulation of IL-1β release remain unknown. I hypothesized, i) unlike neuroinflammatory glial release, IL-1β is released constitutively by neurons and ii) P2X7R activation (a purinergic receptor implicated in the inflammatory release of IL-1β in periphery and in brain) will be necessary for physiological IL-1β release in CNS. This possibility was tested both *in vivo* and *in vitro*. *In vivo*, treatment with a selective antagonist of P2X7R, caused buildup of IL-1β in pyramidal neurons of CA3 region of hippocampus of mice brain. Using the convulsant agent, pentylenetetrazole, to model excessive neuronal excitation, I found that the seizure threshold was lowered in these mice relative to vehicle-treated controls in agreement with receptor knockout mice. Using P2X7R antagonism in primary hippocampal neuronal culture, similar IL-1β accumulation was found. The possible mechanism of constitutive release of IL-1β from neurons is under active investigation. Thus, constitutive IL-1β release from hippocampal neurons, which when restricted from release through P2X7R antagonism, appears to serve as modulated neuronal excitation. My research adds novel information regarding the physiological roles of IL-1β in CNS.

MTU02-03

Evoked release of soluble amyloid-beta species

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

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

MTU02-04

GM1 oligosaccharide is the active portion responsible for GM1 neuroprotective properties

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

MTU02-05

Neuroplastin-plasma membrane Ca^{2+} ATPases complexes: a new team regulating Ca^{2+} clearance, signaling, and synaptic plasticity

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Neuroplastins, type-1 transmembrane proteins with extracellular Ig-like domains, play a crucial role in synapse architecture and plasticity. We and others have shown that neuroplastins bind all four isoforms of the plasma membrane Ca^{2+} ATPase (PMCA) to regulate expression levels and Ca^{2+}-extruding activity of these pumps. Indeed, we have also shown that in neuroplastin-deficient neurons with altered long-term potentiation, the protein levels of PMCAs are reduced. A crucial Ca^{2+}-dependent downstream signaling pathway implicated in synaptic plasticity is via extracellular signal-regulated kinases (ERK). Thus, we hypothesize that neuroplastin-PMCA-modulated Ca^{2+} signals may regulate ERK activation during synaptic plasticity. Our preliminary results indicate that ERK phosphorylation and PMCA abundance are drastically altered in brain homogenates from neuroplastin-deficient mice. Using super resolution STED microscopy and FLIM/FRET-based biosensors, we monitor ERK activation and Ca^{2+} dynamics in synapses of cultured hippocampal neurons. We assessed the contribution of neuroplastin to PMCA levels and activity-dependent plastic mechanisms using lentivirus-driven overexpression and small extracellular peptides targeting neuroplastin and PMCA. We have observed that pharmacological inhibition of PMCA activity alters the normal ERK activation in electrically stimulated neurons. Also, we employ high-frequency stimulation protocols to study the link between neuroplastin/PMCA with ERK activation in synaptic junction fractions from hippocampal slices. Taken together, considering Ca^{2+}-dependent ERK activation as a prominent mechanism underlying activity-dependent synaptic plasticity, we propose neuroplastin-PMCA complexes as major regulators of synaptic Ca^{2+} clearance during synaptic plasticity.

MTU02-06

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

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Synaptotagmin-1 (syt1) is an essential synaptic vesicle protein that acts as the Ca^{2+}-sensor for fast, synchronous neurotransmitter release, with additional roles in other aspects of synaptic physiology. We have recently characterised the first known cases of human mutations in syt1, revealed through whole exome sequencing of 11 individuals with neurodevelopmental impairments not typical of any recognised disorder. 5 discrete *de novo* missense mutations have been identified in these individuals who exhibit a phenotypic spectrum of common symptoms including motor delay, intellectual disability, movement disorders and behavioural abnormalities. Given all human mutations cluster within the C2B domain, a Ca^{2+}-binding region crucial for syt1 function, we investigated whether these syt1 variants perturb the dynamics of neurotransmitter release. The homologous mutations were induced in pHluorin-tagged rat syt1 and expressed in cultured mouse hippocampal neurons, establishing that expression level and synaptic vesicle localisation of syt1 variants were equivalent to that of the WT protein, with the exception of one mutant ($p<0.01$, n=3-4). pHluorin imaging revealed that syt1 mutants slow the rate of evoked exocytosis in a dominant-negative manner ($p<0.05$, n=5-7), and notably this slowing was less pronounced for the variant presenting with a less severe clinical profile. These results suggest that

impairment of Ca^{2+}-dependent neurotransmitter release is likely an important pathophysiological mechanism underpinning this disorder and, together, our findings demonstrate that syt1 variants with mutation-specific impacts on protein functionality give rise to a distinct and recurrent neurodevelopmental disorder.

MTU02-07

Alpha 2 adrenergic receptor agonist guanabenz directly inhibits HCN channels

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

MTU02-08

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

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Most fast excitatory neurotransmission in the mammalian CNS is mediated by glutamate acting through ionotropic glutamate receptors (iGluRs). This receptor family includes the kainate receptors (KAR) consisting of the subunits GluK1-5, located both pre- and postsynaptically. KAR are believed to modulate the activity of neuronal networks by regulating neurotransmitter release. This modulatory role of KAR provides a therapeutic target for fine-tuning the balance of excitatory and inhibitory signaling. The physiological functions of KAR are incompletely understood, partially due to a lack of selective KAR pharmacological compounds. Extensive effort has thus been put into development of KAR selective antagonists but there still exists an unmet need for subunit-selective KAR ligands. 2,3-Quinoxalinediones were among the first antagonists applied in non-NMDA receptor research. Nonetheless, these initial compounds were not optimal for elucidating the functions of KAR as they are also antagonists of AMPA receptors. We have recently published on 2,3-quinoxalinediones substituted at the *N*1-, 6- and 7-positions which demonstrated GluK3 preference. Here, a new series of 2,3-quinoxalinedione analogues with substitutions in the 7-position have been characterized. One compound (EM17) showed high affinity at GluK3 (K_i = 78 nM) and was further characterized by patch clamp electrophysiology in HEK293 cells expressing GluK3 (K_B = 39 nM) and *in vivo* in the mouse tail flick test where it was efficacious as an analgesic. Finally, to understand the molecular interactions of this series of 2,3-quinoxalinediones we report a crystal structure of EM17 in complex with the GluK1 ligand-binding domain.

MTU02-09

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

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

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

MTU02-10

Inhibition of MMP-9 enhances cholinergic-induced synaptogenesis in hippocampal CA1 pyramidal neurons

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

MTU02-11

Subcellular localization of sphingosine 1-phosphate receptors in synapses of the mouse cortex

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Sphingosine 1-phosphate (S1P) has pleiotropic biological functions in the regulation of proliferation, survival, migration, inflammation or angiogenesis. S1P acts as intracellular second messenger, as well as extracellular receptor ligand via five G-protein coupled receptors (S1PR1-5). In the brain, S1P regulates neuronal proliferation or apoptosis, excitatory neurotransmission and neuroglia activation, and S1P metabolism alterations have been associated to neurodegenerative disorders. Interestingly, an agonist targeting S1PR1,3,4,5 (FTY720) shows neuroprotective properties through mechanisms that are not fully unveiled, but might include the control of neuroinflammation, vascular deterioration and synaptic dysfunction. The subcellular distribution of S1PRs in nerve terminals is hitherto unknown. The present study aimed at determining the synaptic localisation of S1PRs in the cortex of adult male mice. Synaptosomes were purified from mouse cortex homogenates using a sucrose density gradient centrifugation, and further fractioned into pre-, post- and extrasynaptic zones using a series of pH shifts that allow successive solubilisation of synaptic components (Phillips et al., Neuron 32:63, 2001). Western blot analysis of the obtained fractions, as well as total protein extracts from cortex revealed that S1PR1 is present in similar amounts in total extracts, synaptosomes and the extrasynaptic fraction, but absent from the pre- and postsynaptic fractions. S1PR2 was ubiquitously distributed, showing 3-fold higher levels in the presynaptic zone than the post- ($P<0.05$) and extrasynaptic ($P<0.05$) fractions. Similarly, S1PR4 was also distributed across all synaptic fractions but 4-fold more enriched presynaptically. S1PR3 and S1PR5 were not efficiently detected by immunoblotting in synaptosomes and synaptic fractions. Altogether, these results point towards S1PR2 and S1PR4 being particularly well poised to directly modulate synaptic transmission and plasticity upon S1P activation.

MTU02-12

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

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

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

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

MTU02-13

TGFb1-signaling importance in insulin-sensitive GLUT4 trafficking to membrane in the cortex of mice with acute liver failure

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Hepatic encephalopathy is a neuropsychiatric syndrome caused by liver failure (ALF) with multifactorial pathomechanism in which ammonia and neuroinflammation play a key role. TGF-b1, a pleiotropic cytokine controls different cell functions, among other glucose metabolism-dependent on insulin. TGF-b1 signaling is impaired in ALF mouse model. In the presented study the role of TGF-b1 signaling in insulin-sensitive glucose 4 transporter (Glut4) trafficking through PI3K/Akt/PKCz signaling pathway in cerebral cortex of ALF mice was measured. C57Bl6 mice with ALF induced by AOM injection (i.p.100 mg/kg), and mice with TGF-b1 neutralization (i.p.ab-TGF-b1, 1 mg/kg) were used. The redaction in TGF-b1 level by ~200% and ~100% in serum and cortex homogenates were observed. The expression of Insulin Receptor b and Insulin Receptor Substrate 1 were unaltered in both models. In addition, decreased expression of TGF-b Receptor 2 by ~15% in mice after neutralization of the cytokine in the absence of changes in ALF mice was observed. The subtle changes in the expression of p-PDK1 and p-Pi3K proteins were demonstrated in ALF brain homogenates, accompanied with reduction in p-PKCz by ~15% in mice after neutralization and decrease in p-AKT by ~50% in ALF mice were observed. In both groups the increase in Glut4 protein by ~100% and ~80% in the cytosolic fraction was observed, without changes in the membrane fraction. The results indicate that ALF alters brain glucose metabolism, however the mechanism of Glut4 translocation to the membrane in ALF mice might be not solely associated with TGF- b1 signaling. Particular mechanism requires additional research. Supported by the grant Prelludium10 2015/19/N/NZ5/02249.

MTU03 Neuroinflammation & neuroimmunology (Session A)

MTU03-01

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

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Background: The leaves of *Olax subscorpioidea* (0S) is a mainstay in the management of mental illness in folkloric medicine in Nigeria. Studies have shown its antidepressant, and anti-inflammatory properties in animals. However, with emanating evidences suggesting link between immuno-inflammatory pathways and depression, there is dearth of information linking or attributing its antidepressant effect to its anti-inflammatory mechanism. We thus evaluated antidepressant effect of n-butanol fraction (BF) of OS on LPS-induced depressive behaviours with respect to its action on inflammation.

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

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

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

MTU03-02

Ultrastructural study of mature and immature corpora amylacea in human brain

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

MTU03-03

Cytokine profile of patient with major depressive disorder

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

MTU03-04

Vascular and neurogenic rejuvenation in aging mice by modulation of ASM

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

MTU03-05

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

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

MTU03-06

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

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

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

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

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

MTU03-07

Combined administration of dopaminergic and nondopaminergic drugs reverses neuroinflammation in a rat model of parkinson's disease

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

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

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

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

MTU03-08

Systemic LPS induces vesicular co-expression of rage and LC3 in dopaminergic neurons of the substantia nigra

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The receptor for advanced glycation endproducts (RAGE) is a pattern-recognition receptor that triggers pro-inflammatory gene transcription mainly through NF-kB activation. Induction of RAGE is observed in several non-infectious pathologies with a pro-inflammatory characteristic, including diabetes, atherosclerosis, cancer and Alzheimer's disease. Disruption of autophagy flux and induction of macroautophagy are observed in α-synuclein mutants and are suggested to be a key step in Lewy's bodies formation. Here we used a rat model of long-term dopaminergic neurodegeneration induced by systemic LPS injection to evaluate the involvement of RAGE in α-synuclein and LC3-I/II expression and progression of dopaminergic cell death. Adult Wistar rats were subjected to systemic LPS injection (5 mg/kg, i.p.) and the substantia nigra was isolated at 6 and 10 months after injection for IF. RAGE, α-synuclein and LC3-I/II staining were all increased in dopaminergic neurons, although the number of TH-positive cells in the substantia nigra decreased in the course of 10 months. Co-localization of RAGE and LC3-I/II staining is observed in vesicles of TH-positive cells. Systemic (previous to LPS) or intranigral (2 months after LPS) injection of the RAGE inhibitor FPS-ZM1 inhibited RAGE and LC3-I/II expression, α-synuclein staining and preserved the number of TH-positive cells. These data indicate an important role for RAGE in dopaminergic neurodegeneration triggered by systemic inflammation, and also suggest that changes in the regulation of autophagic flux are a key step in the mechanism of neurodegeneration associated to RAGE. Financial support: CNPq, FAPERGS, CAPES and Propesq-UFRGS.

MTU03-09

Smoking mice: the effects of sub-chronic cigarette smoke exposure on microglia

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

MTU03-11

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

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

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

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

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

MTU03-12

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

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Complement pathway overactivation can lead to neuronal damage in a variety of neurological diseases. While Alzheimer's disease (AD) is characterized by both amyloid-beta plaques (amyloidosis) and Tau tangles, previous work examining complement has largely focused on amyloidosis models. We find that in mouse models of amyloidosis (PS2APP) or tauopathy (TauP301S), glial cells show increased expression of complement classical pathway components including C1q and the central component C3; however, complement proteins accumulate more extensively in TauP301S mice. Blocking complement function by knockout (KO) of C3 not only rescued the plaque-associated synapse loss in PS2APP mice, but also ameliorated neuron loss and brain atrophy in TauP301S mice. Neuroprotection in TauP301SxC3KO mice was accompanied by improvements in neurophysiological and behavioral measurements. We also find that C1q and C3 protein are elevated in AD patient brains, including at synapses. In AD patient CSF we find that levels and processing of C3 are increased and correlate with tau but not amyloid-beta. Together these results demonstrate that complement activation can contribute to neurodegeneration caused by tau pathology and suggest that blocking C3 function might be protective in AD and other tauopathies.

MTU03-13

Nω-NITRO-l-arginine methyl model of pre-eclampsia elicits differential IBA1 and EAAT1 expressions in brain

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

MTU03-14

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

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

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

MTU03-15

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

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

MTU03-16

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

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

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

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

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

MTU03-17

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

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

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

MTU03-18

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

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

MTU03-19

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

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

MTU03-20

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

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

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

MTU03-21

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

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

MTU03-22

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

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

MTU03-23

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

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

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

MTU03-24

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

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

MTU03-25

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

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

Methods: A series of FCs were synthesized (Gs_1-Gs_5) and docked at the sites of iNOS, COX-2 and NFκB. Acute toxicity of most active compound was carried as per OECD guidelines. In acute study, analgesic and anti-inflammatory activity of compounds were tested using acetic acid writhing, formalin induced nociception and carrageenan test in mice. In chronic studies, vincristine induced neuropathic pain was employed. Post mortem studies including biochemical analysis for inflammatory mediators and immunohistological examinations were performed.

Results: Docking studies on the active sites of COX-2, iNOS and NFκB indicated good binding of compound Gs_4 with appreciable docking score. Acute toxicity studies revealed largely unremarkable visceral organs including heart, liver and kidney. Pharmacological studies indicated significant analgesic effect and anti-inflammatory activity of different compounds with maximum activity of compound Gs_4. In neuropathic pain, marked reduction in pain behavior was observed in compound treated group. Compound Gs_4 also attenuated expression of COX-2, iNOS and NFkB, as well as inflammatory cytokines and oxidative stress.

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

MTU03-26

Esculetin ameliorates polyi:C-induced autism spectrum disorder in mice by impeding neuroinflammation and improving BDNF signaling

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

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

MTU03-27

DA attenuates LPS-induced cytokine expression by inhibiting the microtubule-dependent nuclear transport of NF-KB P65 in BV-2 cells

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

MTU03-28

Motor and synaptic deficit in 5-lipoxygenase knockout mice

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

MTU03-29

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

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

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

MTU03-30

Digoxin regulates oligodendrocyte number, function, and myelin structure

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

MTU04 Molecular basis of disease (Session A)

MTU04-01

Role of acetamide analogue in arthritic rat model

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

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

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

MTU04-02

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

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Peripheral neuropathy is a major adverse effect of paclitaxel chemotherapy. We have reported previously that paclitaxel increased TRPV1 sensitivity to repeated capsaicin application via TLR4-mediated mechanism. The results presented here describe the role of phosphatidylinositol 3-kinase (PI3K) in the paclitaxel-induced signaling between TLR4 and TRPV1. Neuropathy was induced by a single application of Paclitaxel (Mylan, 8 mg/kg, *i.p.*) in adult male mice C57BL/6. Mechanical allodynia was evaluated by paw withdrawal threshold measurement. Whole-cell patch-clamp recordings of miniature excitatory postsynaptic currents (mEPSC) were made from superficial dorsal horn spinal neurons. Paclitaxel-induced robust mechanical allodynia was prevented for up to eight days by PI3K antagonist wortmannin pretreatment *in vivo*. Both *in vitro* and *in vivo* paclitaxel treatments enhanced capsaicin-evoked responses recorded as an increased mEPSC frequency in dorsal horn neurons. Acute co-application of PI3K antagonist wortmannin or LY-294002 with paclitaxel attenuated this effect of paclitaxel. Acute *in vivo* paclitaxel administration also increased phosphorylation of Akt kinase, a marker of enhanced PI3K signaling, in rat L5 DRG neurons. Wortmannin pretreatment prevented this increase in pAkt expression. We showed that PI3K plays an important role in the early development and maintenance of mechanical allodynia and in the modulation of TRPV1 function after paclitaxel treatment. We suggest that inhibition of PI3K may help alleviate pathological pain in the paclitaxel-induced neuropathy. New data focused on paclitaxel-induced changes of inhibitory synaptic transmission will be presented on site. Grant Support: Czech Science Foundation 18-09853S, LQ1604 BIOCEV-FAR, BIOCEV CZ.1.05/1.1.00/02.0109, RVO:67985823, GAUK 734218.

MTU04-03

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

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Cobalt (Co) intoxication arising from occupational exposures and ion release from metal implants has been associated with neurological alterations such as cognitive decline, incoordination and depression. The present study evaluated the mechanisms of neuroprotection by Luteolin (100 mg/kg) and Gallic acid (120 mg/kg) in Wistar rats exposed to cobalt chloride (CoCl2) at 150 mg/kg for 7 consecutive days. Cognition, grip strength and motor coordination were assessed with the Morris water maze, hanging wire and Open Field tests, respectively. Rat's whole brain samples were processed for biochemical analyses of markers of oxidative damage and acetylcholinesterase activity. Immunohistochemistry was used to measure the immuno-reactivity of glial fibrillary acidic and calbindin D-28k proteins in brain tissues. Results indicate that CoCl2 induced neuro-behavioural deficits, specifically producing decreased exploratory activities, increased anxiety and significant reduction in hanging latency. Co-treatment with luteolin or gallic acid, however, restored these parameters to values near those of normal controls. Moreover, Luteolin and Gallic acid prevented CoCl2-induced increases in hydrogen peroxide, malondialdehyde and nitric oxide in the brain, while increasing the activities of acetylcholinesterase, glutathione S-transferase and superoxide dismutase. Furthermore, Luteolin or Gallic acid treatment produced increased astrocytic expression of glial fibrillary acidic protein (GFAP), with intense calbindin (CB) staining and pronounced dendrites in the Purkinje cells. Taken together, luteolin and/or gallic acid exerted protection against Co neurotoxicity by restoring Ca^{2+} homeostasis, acetylcholinesterase and antioxidant enzyme activities, while also inhibiting lipid peroxidation in the brain.

MTU04-04

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

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

Supported by RSF grant #19-74-30007.

MTU04-05

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

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

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

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

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

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

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881.**

Work in the authors labs is funded by the DFG (SFB779 to A.D., R.F, C.S., E.G.).

MTU04-06

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

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

MTU04-07

Regulation of KCNQ genes as a mechanism underlying epileptogenesis

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

MTU04-08

Ibogaine downregulates CREB1 and GRIA1 mRNA expression in the dorsal hippocampus

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

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

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

Ibogaine reduced CREB1 expression in dHPC relative to control ($p = 0.051$). A highly significant reduction in GRIA1 expression was found in dHPC of the ibogaine treatment group relative to saline control ($p = 0.003$). Differences in GRIA1 expression between dHPC and vHPC were highly significant. No significant differences in expression were found between withdrawal groups and combined morphine-ibogaine treatment.

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

MTU04-09

Beneficial effects of the regulation of miRNAs by dimethyl fumarate via NRF2 in tauopathies

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

MTU04-10

Radiation exposure induces acute trafficking of excitatory and inhibitory receptors in cultured hippocampal neurons

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

MTU04-11

A glucocorticoid receptor-dependent mechanism of bile acid action with therapeutic impact in polyglutamine disease

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Spinocerebellar ataxia type 3 (SCA3) is an autosomal dominant neurodegenerative disorder caused by a polyglutamine expansion within the protein ataxin-3, leading to protein dyshomeostasis and ultimately neuronal demise. Clinically, it is characterized by gait imbalance, with a mid-life onset. No directed treatments are currently available for this invariably fatal disease. In this work we tested bile acids as potential therapeutic agents for SCA3, since these molecules have been shown to be neuroprotective in other conditions. Using a *C. elegans* model of SCA3 we observed that tauroursodeoxycholic acid (TUDCA) was the most efficient bile acid in improving the animals' motor phenotype. A significant improvement was also observed in a pre-clinical trial using CMVMJD135 mice: chronically treated mice showed markedly improved performance motor behavior tests, reduced neuropathology and neuroinflammation markers. Using the *C. elegans* SCA3 model, we dissected the mechanism of action of this drug. Surprisingly, we observed that the effect of TUDCA was independent of its canonical nuclear receptor, the farnesoid X receptor (FXR), but fully dependent on the glucocorticoid receptor (GR). Moreover, GR protein levels were markedly decreased in the CMVMJD135 mouse model, and fully recovered upon acute treatment with TUDCA. Finally, and most importantly, we observed a decrease in GR levels in the pons, a highly disease-affected brain region, of SCA3 patients. In sum, we identified TUDCA, a drug with a high translational potential, as a contender compound for the treatment of SCA3, and propose a novel mechanism of action that could be of interest in the future, including for other neuromuscular disorders currently treated with glucocorticoids.

MTU04-12

Aberrant regulation of monoubiquitination via E3 ubiquitin ligase RNF20 confer Gbm cancer stem-like cells survival and maintenance

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

MTU04-13

Palmitate increases microglia-derived TNF-alpha levels and impairs hippocampal insulin signaling

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

MTU04-14

Neuropharmacological prospective of urena sinuata (borss) l

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Urena sinuata (Borss) L. is a wild shrubby plant with some folk medicinal use. To our knowledge, the biological importance of this plant has not been investigated yet. The present study aimed to determine the neuropharmacology, antinociceptive, anti-inflammatory and antipyretic effects of the chloroform extract of *Urena sinuata* leaves (CEUS) in rodents and to elucidate the possible mechanism of antinociception involved with its acute toxicity and phytochemical studies. Neuropharmacological activities of CEUS were conducted by hole cross, open field test, elevated plus-maze test and thiopental induced sleeping time test. For the analgesic activity of CEUS different methods like hot plate test, acetic acid induced test, formalin-induced test, tail immersion test and glutamate-induced nociception were used. Additionally, the possible mechanism of nociception is identified by cyclic guanosine monophosphate (cGMP) and ATP-sensitive K^+-channel pathway analysis. Carrageenan-induced rat paw edema and cotton pellet-induced granuloma test also were used to detect anti-inflammatory activity and brewer's yeast induced pyrexia test for antipyretic activity. The extract (200 and 400 mg/kg) was administered orally 60 min prior to subjection to the respective test. The results obtained demonstrated that CEUS produced significant ($p < 0.05$) neuropharmacological, anti-inflammatory and antipyretic activity with low or no toxicity. The extract also exerts antinociceptive response in all the chemical and thermal-induced nociception models. Furthermore, it involves cyclic guanosine monophosphate (cGMP) and ATP-sensitive K^+-channel pathway mediated antinociceptive effect. These data show for the first time that CEUS has significant neuropharmacological, anti-inflammatory and antipyretic effects which appear to be related to the inhibition of the glutamatergic system and rationalized the traditional use of the leaf in the treatment of different types of inflammation in intestines and bladder. Thus the leaves of *Urena sinuata* could be used in the treatment of several types of inflammation in intestines and bladder.

MTU04-15

Glast activity is modified by acute manganese exposure in bergmann glial cells

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

MTU04-16

The oligosaccharide portion of ganglioside GM1 as mitochondrial regulator

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

MTU04-17

Reductive reprogramming: a not-so-radical hypothesis of neurodegeneration

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Free radical-mediated oxidative stress, neuroinflammation, and excitotoxicity have long been hypothesized to contribute to the progression of Alzheimer's disease and other aging-related neurodegenerative disorders (NDD). Among these phenomena, the significance of oxidative stress and, more generally, redox perturbations, for NDD remain ill-defined and unsubstantiated. Here, I argue that (i) free radical-mediated oxidations of biomolecules can be dissociated from the progression of NDD, (ii) oxidative stress fails as a descriptor of cellular redox states under conditions relevant to disease, and (iii) aberrant upregulation of compensatory reducing activities in neural cells, resulting in reductive shifts in thiol-based redox potentials, may be an overlooked and paradoxical contributor to disease progression. In particular, I summarize evidence, from *in vitro* studies, which supports the view that reductive shifts in the extracellular space can occur in response to oxidant and inflammatory signals and that these have the potential to reduce putative regulatory disulfide bonds in exofacial domains of the N-methyl-D-aspartate (NMDA)-subtype of glutamate-gated receptors as well as other synaptic regulatory proteins, leading potentially to aberrant increases in neuronal excitability and, if sustained, excitotoxicity. Moreover, I provide data from my laboratory which establishes the presence in the brain, *in vivo*, of disulfide bonds in the NMDA receptor as well as other glutamate and non-glutamate-gated receptors. All of these are potential targets of reductive stress. This novel reductive reprogramming hypothesis of neurodegeneration provides an alternative view of redox perturbations in NDD and links these to both neuroinflammation and excitotoxicity.

MTU04-18

The role of monocarboxylate transporter-1 on cognitive deficits development during NAFLD

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

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

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

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

MTU04-19

Quantitative proteomic analyses of dynamic signalling events associated with neuronal death in excitotoxicity

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

MTU04-20

PPV-6 suppresses amyloid beta-induced cell cycle reentry in differentiated primary cortical neurons

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Amyloid beta-peptide (Aβ) is the main neurotoxic component of senile plaque, which is the pathological hallmark of Alzheimer's disease. In addition, Aβ is also known to trigger cell cycle reentry in post-mitotic neurons followed by cell death. Many studies have reported that polysaccharides from medicinal plants may carry therapeutic potential in AD. However, the mechanisms underlying polysaccharide-mediated inhibition of Aβ neurotoxicity is still unclear. Therefore, this study was designed to explore the neuroprotective mechanisms of polysaccharides extracted from a perennial vine (PPV-6). We hypothesized that PPV-6 may suppress cell cycle reentry and subsequent cell death induced by Aβ in the fully differentiated post-mitotic neurons. To test this hypothesis, post-mitotic primary cortical neurons were subjected to cotreatment with Aβ and PPV-6. Western blotting, immunocytochemistry, and flow cytometry were conducted to assess the extents of neuronal cell cycle reentry. MTT assay was performed to determine cell viability. Compared with Aβ alone, cell viability and morphology were recovered by cotreatment with PPV-6. Further, Aβ-induced upregulation of G1-phase markers including cyclin D1 and phosphorylated retinoblastoma protein (pRb), G2-phase marker such as proliferating cell nuclear antigen (PCNA), and mitotic marker histone H3 phosphorylated at Ser-10 were all reversed by PPV-6. Similar results were obtained with flow cytometry. Taken together, our finding indicated that the neuroprotective mechanisms of PPV-6 involve suppression of Aβ-induced neuronal cell cycle reentry and subsequent cell death.

MTU04-21

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

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

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

MTU04-22

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

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Neuro-inflammation, mitochondrial dysfunction, and impaired clearance of aggregate prone proteins are implicated in Parkinson's Disease (PD) pathogenesis; however, the mechanism by which inflammatory mediators increase the vulnerability of dopaminergic neurons to oxidative stress-induced apoptotic cell death remain poorly characterized. Therefore, the goal of the present study was to investigate the cell signaling events that underlie TWEAK induced apoptotic cell death using an *in vitro* dopaminergic cell culture model, N27 cells. Herein, we show that N27 cells express both TWEAK and its receptor Fn14 and that TWEAK-elicited dose dependent apoptotic cell death in N27 cells. Exposure of N27 cells to TWEAK evoked dissipation of mitochondrial membrane potential (MMP), suppression of GSH levels, activation of caspase-8 and 3 and concomitant up regulation of Phospho-Tau and Phospho-NF-kB P65 levels. Moreover, these changes were accompanied by the down regulation of Phospho-AKT, P-GSK3Beta (Ser9) and LC3 levels in TWEAK treated dopaminergic neuronal cells. Intriguingly, upregulation of TWEAK was evidenced in MPP^+ treated dopaminergic neuronal cells. Likewise TWEAK was upregulated in the SNpc of MPTP treated mice. Consistent with the role of TWEAK in the induction of oxidative stress response, pretreatment of N27 cells with varying concentration of quercetin, a bioflavanoid abrogated TWEAK-induced apoptotic cell death. In a similar fashion NFkB inhibitor, SN50 ameliorated TWEAK-induced loss of dopaminergic cell viability. Together, these data suggest that TWEAK exerts deleterious effects on dopaminergic neuronal survival via aberrant activation of NF-kB and impaired mitochondrial function in an oxidative stress dependent manner.

MTU04-23

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

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Stroke is the worldwide threat that causes death and disability in adults. The dynamic nature of mitochondria is related to cellular survival, growth and death. Previous literature have reported that ischemic stroke (IS) reduces mitochondrial respiration, enhances production of reactive oxygen species (ROS) and triggers apoptotic cell death, suggesting a prominent role of mitochondria in IS pathophysiology. The selected nutraceutical, PIP, has been reported to have anti- inflammatory and anti-oxidant properties. Pre-treatment of PIP has been found to be neuroprotective in IS. The present study emphasises on the possible neuroprotective role of PIP via mitochondria as therapeutic target for stroke treatment. After PIP administration (10 mg/kg b.wt. once daily, p.o. for 15 days), the male wistar rats underwent the tMCAO surgery. The right middle cerebral artery was occluded for 1 h followed by 23 h of reperfusion. Behavioural assessment was done after 24 h. The brain samples for analysis of mitochondrial impairment were extracted after behavioural assessment. The results showed that PIP significantly reduced the infarct volume, mitochondrial ROS and restored complexes activity, mitochondrial membrane potential and cytochrome c release, thereby, attenuated the mitochondrial wreckage. Taken together, our results are the first to demonstrate that PIP has the potential to constrict the mitochondrial dysfunction in IS.

MTU04-24

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

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Schizophrenia (SCZ) is a neuropsychiatric disorder, caused by genetic and environmental factors. Schizophrenic individuals exhibit cognitive deficits, positive (psychosis, hallucinations and delusions) and negative symptoms (depression, avolition and anhedonia), as well as reduced gray matter volume. Microanatomical analyses suggest that this gray matter reduction is due to diminished dendritic spine density, which likely happens as a result of exaggerated synaptic pruning. Recently, it has been shown that the classical complement cascade and CX3CL1/CX3CR1 pathway play a direct role in this process, triggering synaptic engulfment by microglia. In addition, astrocytes secrete cytokines capable of modulating the complement cascade. Taking into account that pre-natal infection acts as a risk factor for SCZ, this work aimed at analyzing complement components, proinflammatory cytokines and CX3CL1 production employing induced pluripotent stem cells (hIPSCs)-derived astrocytes from schizophrenic individuals after stimulation with TNF-α. The results demonstrate that TGF-β3 and IL-1β transcripts are altered in SCZ-derived astrocytes relative to healthy control-derived (HC) astrocytes. In contrast, C3, C4 and CX3CL1 show similar mRNA expression patterns in both groups. Finally, non-secreted CX3CL1 levels seems to be increased in SCZ astrocytes, suggesting problems in the release of the soluble form. Altogether, these results indicate that SCZ astrocytes produce immunomodulators in a distinct manner compared to HC astrocytes.

MTU04-25

A transposon-mediated somatic mutagenesis screen identifies new genes associated with malformations of cortical development

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

MTU04-26

Hypoxia or nicotine- which is worse on the infant brain? from neurotransmitters, growth factors, to apoptosis and microglia

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A respiratory hypoxic environment and cigarette smoke exposure around babies have long lasting effects on the brain such as decreased neuroprotection, decreased IQ, and long-term increased addictive behaviours (cigarette smoke exposure). Extensive studies of the effects of such exposures to the developing brain on the expression of neurotransmitters, receptors, growth factors, markers of apoptosis and microglia in the brain have been undertaken in our laboratory over the past decade and will be presented herein. Our results are from two brain tissue datasets: 1- infants who died suddenly and unexpectedly, and 2- piglet models of intermittent hypercapnic hypoxia (IHH) and postnatal nicotine exposure. Brain tissue was subjected to immunohistochemistry for apoptotic markers (caspase-3 & TUNEL), NMDA receptor 1, brain derived neurotrophic factor (BDNF) and its receptor TrkB, serotonin receptor 1A (5HT1A), pituitary adenylate cyclase activating polypeptide (PACAP) and its receptor PAC1, orexin, nicotinic acetylcholine receptors (nAChRs) and microglia. Staining was quantified and compared between exposures to control (non-exposures). We found that across the studies, the IHH exposure induced greater expressional changes than nicotine, and many changes equated between the piglet models and the infant findings when stratified for hypoxia related conditions of prone sleeping, bedsharing, and cigarette exposure, including the syndrome of Sudden Infant death (SIDS). These changes predominated in the brainstem medulla, a region containing nuclei of importance in cardiac and respiratory regulation.

MTU04-27

Sphingosine-1-phosphate signaling in stroke - a potential role for astrocytes

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

MTU04-28

Amyloid-beta alone induces changes in hippocampal GABAergic synapses

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

MTU04-29

An implantable microelectrode array for simultaneous *in vivo* recordings of glutamate, Gaba and neural activity

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Glutamate and γ-aminobutyric acid (GABA) are the most common neurotransmitters in the central nervous system. By exciting, inhibiting, and modulating neural elements and microcircuits, these chemicals critically regulate brain information processing and energy metabolism at different spatiotemporal scales. However, the exact relationship between the extracellular concentration of these molecules and emergence of specific patterns in neuronal ensemble activity remains elusive. Partly this is due to the fact that recording of the mean extracellular field potentials (mEFP) concurrently with a quantitative assessment of alterations in the concentration of such neurochemicals are currently unavailable. Here, we present a silicon-based implantable ultrafine microelectrode array (35 μm diameter) composed of several iridium-stabilized electrochemical and electrophysiological contacts. The electrophysiological electrodes have an average impedance of 0.5 MΩ at 1 kHz. The amperometric electrochemical channels, divided into two groups of glutamate- and GABA-responsive electrodes, show a sensitivity of 0.39 nA/μM for glutamate and 0.38 nA/μM on the adjacent channel for GABA. This novel multimodal microelectrode was used to simultaneously monitor extracellular glutamate and GABA concentrations, spikes, multi-unit neuronal activity (MUA) and local field potentials (LFP) in the lateral geniculate nucleus (LGN) of anaesthetized rats (n = 5). Retinal stimulation with flickering monochromatic light, emphasizing the simplest form of feedforward processing in thalamus, induced neuronal response patterns in LGN that were highly correlated with the temporal alterations in glutamate concentrations. GABA responses, while similar in profile to MUA and LFP recordings, were found to be event-selective, suggesting network-level processes. Our findings suggest that this multimodal method may greatly contribute into our understanding of microcircuit organization, by reducing the inherent ambiguity in the mEFP through neurotransmitter-release-tracking.

MTU04-30

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

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

MTU04-31

Pilot human study to define the impact of vascular and inflammatory risk factors in Alzheimer's disease

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

MTU04-32

Dysregulation of autophagy and stress granule-related proteins in stress-driven tau pathology

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Consistent with suggestions that lifetime stress may be an important AD precipitating factor, and knowing that imbalance in neuronal proteostasis associated with Tau misfolding and aggregation is a common feature between AD and other Tauopathies, we previously demonstrated that chronic stress and high glucocorticoid (GC) levels induce accumulation of aggregated Tau; however, the molecular mechanisms for such process remain elusive.Hereby, we monitor a novel interplay between RNA-binding proteins (RBPs) and autophagy has underlying mechanisms through which chronic stress and high GC levels impact on Tau proteostasis precipitating Tau aggregation. Using molecular, pharmacological and behavioral analysis, we demonstrate that chronic stress and high GC trigger an mTOR-dependent inhibition of autophagy, leading to accumulation of Tau and cell death in P301L-Tau expressing mice and cells. In parallel, we found that environmental stress and GC disturb cellular homeostasis and trigger insoluble accumulation of different RBPs, such as PABP, G3BP1, TIA-1 and FUS, shown to form Stress granules(SGs) and Tau aggregation. Interestingly, using an mTOR-driven pharmacological stimulation of autophagy (CCI-779) attenuated the GC-driven Tau and SG-related proteins accumulation, as well as the related cell death, suggesting a critical interaction between autophagy and SG response in chronic stress and GC driven neurodegeneration. Moreover, *in vivo*, this compound also reverted some of the previously observed behavioral deficits, acting as an anti-depressant and reverting short-term memory deficits.These studies provide novel insights into the RNA-protein intracellular signaling regulating the precipitating role of environmental stress and GC on Tau-driven brain pathology.

MTU04-33

Prenatal hypoxia-induced alterations are accompanied with malfunction of glutamatergic system in rat hippocampus

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

Scientific research was performed with involvement of the Research park of SPbU Observatory of Environmental Safety Center. The work was supported by RFBR grant no. 17-04-01118.

MTU04-34

Synapse formation and remodeling unveil a glutamatergic/GABAergic imbalance in hippocampal neurons in the VPA model of autism

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

MTU04-35

Short and long non-coding RNA interactions in trauma and fatty liver disease

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Trauma-related and metabolic impairments are notably linked, but the underlying mechanisms are incompletely understood. Here, we report that long non-coding RNAs and microRNAs (lncRNAs, miRs) might have co-evolved to ameliorate trauma, metabolic syndrome (MetS) and sepsis conditions. In diet-induced obese mice, antisense AM132 oligonucleotide suppression of the trauma-inducible miR-132 led to liver deregulation of MetS-associated miRs including miR-122-5p and miR-26a-5p, which were down-regulated in human fatty liver tissues. Both these miRs may be targeted by the lncRNA MIAT, known to be deregulated in MetS, myocardial and diabetic disorders. Supporting functional relevance, we found MIAT upregulation in human fatty liver samples. Next, we pursued miR-target associations in the web-available CAPSOD transcriptomic dataset of human liver and blood cell biopsies from sepsis and MetS patients compared to controls. Searching the 1,152 subjects' CAPSOD dataset identified the sepsis-related histocompatibility antigen HLA-DRA and the brain-expressed pseudogene PGOHUM-565 as targets of the primate-specific miR-608, compatible with the reduced risks of both sepsis and trauma in human carriers of single nucleotide polymorphisms interrupting miR-608-target interactions. Our findings support causal involvement of lncRNA-miR interactions in stress-related metabolic imbalances and predict that these interactions might have provided survival advantages to evolving primates in both contexts.

MTU04-36

Targeting FTD/ALS: udca prevents CHMP2B-intron5 induced neurodegeneration, revealing a novel drug target for dementia research

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Frontotemporal Dementia (FTD) is the second most prevalent form of early-onset dementia. The pathological mechanisms driving neuronal atrophy in FTD remain poorly understood and no therapeutic interventions exist. The FDA approved drug Ursodeoxycholic acid (UDCA), normally used to treat biliary cirrhosis, shows both cytoprotective and anti-apoptotic activity, however it's mechanism of action remains unknown. Mutations in the FTD gene CHMP2B ($CHMP2B^{Intron5}$) cause severe neurodegeration and apoptosis. Our data shows UDCA can alleviate these neurodegenerative phenotypes and provides novel insights into the mechanism of action of UDCA. Given that UDCA has just entered clinical trials in Europe for the treatment of Amytrophic Lateral Sclerosis (ALS) our data suggests that UDCA has a broader neuroprotective effect across the FTD/ALS spectrum.Our lab has previously characterised the effect of the mutant $CHM2B^{Intron5}$ protein in both Drosophila and mammalian neuron models. Ectopic expression of $CHMP2B^{Intron5}$ in cortical neurons causes dendritic collapse associated with autophagosome accumulation. Transgenic mouse models expressing $CHMP2B^{Intron5}$ globally or in forebrain neurons display neurodegeneration and behavioural deficits. Also prominent are accumulations in p62 and ubiquitin positive inclusions in neurons and glia, mirroring events in $CHMP2B^{Intron5}$ and other FTD/ALS (GRN, C9ORF72, MAPT) patient tissue. We show that UDCA alleviates apoptotic cascades, dendritic collapse and synaptic aberrations in both Drosophila and mammalian models of $CHMP2B^{Intron5}$ induced FTD. In addition, we identify a novel "orphan" receptor as a potential target of UDCA and demonstrate genetic manipulation of this receptor is sufficient to alter $CHMP2B^{Intron5}$ associated phenotypes. UDCA represents a compound with potential to be repurposed for the treatment of FTD associated with the $CHMP2B^{Intron5}$ disease causing mutation.

MTU04-37

Neurodevelopmental deficits in human isogenic fragile x syndrome neurons

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

MTU04-38

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

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

MTU04-39

Binding of ethanol in the C1 domain of presynaptic munc13-1: A molecular dynamics simulation study

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

MTU05 Brain development & cell differentiation (Session A)

MTU05-01

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

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Glial cells missing (*Gcm*) gene was discovered in *Drosophila* and thought to act as binary switch in determining the fate of neuron and glia. We reported that orthologs of *gcm* in mammals, *Gcm1* and *Gcm2*, were associated with Hes5 expression in neural precursor cells (NPCs) of early embryos with active demethylation of *Hes5* promoter (Hitoshi et al., 2011). To determine the differentiation fate of *Gcm1* or *Gcm2* overexpressed NPCs, we performed *in utero electroporation* (IUEP) to an embryonic brain at E14.5. Our results showed that *Gcm1* promotes differentiation of NPCs into GFAP and S100β positive astrocytes 72 hours after IUEP. *Gcm2* gene have polymorphism in the coding region between C57B6 and ICR mice, and IUEP of *Gcm2* from ICR promotes differentiation of NPCs into NeuN positive neurons, which were detected in the ventricular zone/subventricular zone rather than cortical plate. We could not observe any changes in the *Gcm2* gene from C57B6. These results suggest that *Gcm2* from ICR has stronger function than C57B6. To determine the association between these phenotypes and DNA demethylation, we performed *in vitro* assay using Neuro2a cells. To reveal the function of demethylation by *Gcm* genes, we performed sanger-bisulfite sequencing analysis. Since the *Gcm1*-overexpressing Neuro2a upregulated *Vegfa* expression, which have CpG islands in the first intron, we are analyzing it's methylation percentage. Because *Vegfa* intron 1 is already hypomethylated in the control, we could not detect changes by *Gcm1* overexpression. Now we are looking for other candidate genes in appropriate cell line and the best system to clarify the function of *Gcm1* and *Gcm2*.

MTU05-02

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

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Several studies indicate hypoxia as a key player in neuronal stem cell differentiation and proliferation. Low oxygen concentration increases the survival and proliferation rate of neuronal precursors as well as the differentiation to a dopaminergic phenotype when compared to normal oxygen concentrations. Hypoxia leads to the accumulation of the hypoxia inducible factor-1a (HIF-1a). As *in vitro* studies have shown, there is a correlation between its up regulation and the increase of neuronal markers. Therefore, HIF-1a emerges as a possible regulatory step of dopaminergic differentiation. This work aims to investigate the effects of the selective inhibition of HIF-1a on the differentiation induced by retinoic acid in human neuroblastoma cells from the SH-SY5Y lineage with the purpose of elucidating its role in the dopaminergic differentiation. siRNA reverse transfection was performed to inhibit the expression of HIF-1a and was followed by a 7 days differentiation protocol utilizing retinoic acid as a differentiation promoter. HIF-1a silencing efficiency was assessed by western blot and RT-qPCR and differentiation markers were analyzed by immunofluorescence and RT-qPCR. Neuron average length and total number of neurites per cell were assessed utilizing NeuronJ. Our results indicate that HIF-1a inhibition is capable of reducing the neuron-like phenotype, the immunocontent, and the expression of neuronal markers indicating the regulatory role of this transcription factor in neuronal differentiation.

MTU05-03

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

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

MTU05-04

ATOH1 requires primary cilia for the expansion of granule neuron progenitors by modulating centriolar satellites

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

MTU05-05

A *de novo* mutation of CEP170 leads to neuronal migration defects and human lissencephaly

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During cortical development, postmitotic neurons migrate along the radial fiber to organize the six-layered neocortex. In the process of neuronal migration, centrosome moves ahead of the nucleus into the leading process by the pulling forces of cytoplasmic dynein through the microtubule network. Defects in neuronal migration have been found to cause human brain disorder, lissencephaly (smooth brain). Here we identified a *de novo* mutation in the centrosomal protein *CEP170* in a lissencephaly patient. CEP170 is localized on the subdistal appendage of the mother centriole; however, its role in brain development and how the mutation caused lissencephaly are still unclear. To investigate the function of CEP170 in migrating neurons, we delivered CEP170 shRNA into the embryo mice by *in utero* electroporation to knock down CEP170 expression in progenitor cells. CEP170 dysfunction led to neuronal migration delay and presented the abnormal morphology in their leading process at postnatal day 6. The mutant CEP170 showed decreased localization to the centrosomes in culture cells. Immunoprecipitation also showed less interactions of CEP170 mutant with CCDC120 and CCDC68, two proteins that have been shown to recruit CEP170 to the subdistal appendage hierarchically. Since CEP170 has been shown to play a key role in microtubule organization, these results suggest that failure of CEP170 in centrosomal localization may lead to migration defect. Our findings reveal the role of CEP170 in cortical development and provide novel mechanisms of the pathogenesis of lissencephaly.

MTU05-06

Acute and chronic neurological consequences of neonatal zika virus infection in mice

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

MTU05-07

VPA treatment in neurosphere culture: an approach towards *in vitro* modelling of autism

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

MTU05-08

Temporal changes in the brain in neonatal hydrocephalic mice: structural and neurobehavioural findings

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

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

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

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

MTU05-09

A link between temporal competence and reprogramming

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

MTU05-10

Glial cells missing 1 promote cell differentiation and angiogenesis by growth factor expression

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

MTU05-11

Nigella sativa oil ameliorates the effects of early weaning on the cerebellum of wistar rats

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

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

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

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

MTU05-12

LIS1 alterations drive distinct epigenetic, post-transcriptional & chromatin accessibility modes to resolve lineage commitment

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

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

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

MTU05-13

The function of KLF5 gene in adult brain

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Klf5, one of the *Krüppel*-like factor (*Klf*) family genes, is an ortholog of Drosophila melanogaster gene *Krüppel*. It is a transcription factor involved in cellular differentiation or proliferation during mammalian development. *Klf5* deficient embryos are lethal at blastocyst stage showing defective trophectoderm development and implantation failure. Our previous data suggested that *Klf5* gene promotes the cell proliferation of neural precursor cells (NPCs) in the developing mice brain. In this study, we analyzed the function of *Klf5* in adult NPCs. Adult neural stem cells (NSCs) reside only in the subependymal zone (SEZ) and the subgranular zone of the dentate gyrus (DG). In these regions, the adult NSCs, which have the self-renewal and multipotent capabilities, are maintained in quiescent state. To investigate roles of *Klf5* in the adult brain, Nestin-Cre::CAGstopKlf5 mice were generated, in which neural precursor-specific recombination induces the *Klf5* overexpression. We perform a neurosphere assay using cells derived from the SEZ of *Klf5* overexpressing mice's brain. Contrary to our expectation, the number of neurosphere was decreased. We evaluated the incorporation of BrdU at DG and found that incorporated BrdU was decreased in Klf5 overexpressing mice's brain. *Klf5* overexpression mice showed small body weight and behavioral abnormality. Now, we are trying to evaluate the molecular mechanisms underlying these phenotypes.

MTU05-14

A hierarchy of beta-spectrins is required for maintenance, but not assembly, of axonal sodium channels clustering

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Highly-concentrated ion channels at axonal excitable domains including axon initial segments (AIS) and nodes of Ranvier are necessary to transmit action potentials. Cytoskeletal protein βIV-spectrin is proposed to stabilize voltage-gated sodium (Nav) channels at nodes, and βI-spectrin is reported to preserve this function after losing βIV-spectrin. However, patients carrying βIV-spectrin mutants showed epilepsy and intellectual disability, while their peripheral sensory functions were intact, suggesting βI-spectrin plays distinctive compensatory roles in the CNS and PNS. Furthermore, the necessity of β-spectrins for Nav channels clustering at axons are unknown. To determine the function of β1 and β4-spectrin in the nervous system, we generated mice lacking these proteins in the CNS and PNS neurons, respectively. Mice lacking both β1/β4-spectrin in PNS showed ataxia and impaired action potential conduction. With increasing age, there was progressive loss of nodal Nav channels. Losing β1-spectrin in the CNS showed normal AIS integrity while losing β4-spectrin reduced Nav channel intensity. Unexpectedly, β1-spectrin was only detected at the AIS of parvalbumin interneurons in β4-spectrin deficient mice. Mice lacking both β1/β4-spectrin showed severe seizures. These data suggest that β-spectrins are necessary to maintain Nav channels at axonal excitable domains. Furthermore, β4-spectrin is the primary stabilizer, while β1-spectrin performs secondary functions in a context-dependent manner.

MTU05-15

Cannabidivarin, capsaicin and the multiple fates of neural stem cells

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

MTU05-16

Analysis of TRPC5 expression in developing retina

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

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

MTU05-17

Cerebellar development and function in neonatal rats following intrauterine and postnatal exposure to caffeine

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

Pregnant rats received 50 or 100 mg/kg day of caffeine (CAF50, CAF100) by gavage throughout pregnancy and three weeks postnatal while the controls had sterile water. Post-delivery, the dams nursed their pups and after three weeks, the pups underwent neurobehavioral tests: cliff aversion for sensorimotor reflex development, negative geotaxis for motor coordination, and forearm grip strength test for muscular strength. By serial sacrifice from Day 19 to 21, morphological assessment of the cerebellum's development was assessed by measuring the external granular layer (EGL) thickness and the cellular density within the molecular layer.

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

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

MTU05-18

Cannabinoids, adenosine A2A receptors and postnatal neurogenesis

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

Acknowledgements: Supported by FCT, SFRH/BD/129710/2017 and iFCT, IF/01227/2015.

MTU05-19

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

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

MTU05-20

The role of NPRL2 and NPRL3 in neural development and disorders

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The neurodevelopmental disorder focal cortical dysplasia (FCD) is the most common cause of medically refractory epilepsy in children. Several genes have been identified to be involved in the pathogenesis of this disease, including NPRL2, NPRL3 and DEPDC5, the components of GATOR1 complex. GATOR1 complex acts as a negative regulator of mTORC1 in mTOR signaling pathway, which regulates cell growth, metabolism, autophagy, and proliferation. Although mutations in these genes have been reported to cause FCD and focal epilepsy, the functions of NPRL2/3 in neural development is still not fully understood. To investigate the roles of NPRL2/3 in cortical development, we delivered shRNA by *in utero* electroporation (IUE) to knock down NPRL2/3 in neural progenitors of mouse embryos. We found that NPRL2/3 knockdown during development caused neuronal migration delay. Furthermore, we observed morphological changes of dendritic spines in the NPRL2/3-knockdown neurons in postnatal mice. Meanwhile, we identified potential novel mutations on NPRL2 and NPRL3 in patients with focal epilepsy. To study whether these mutations may cause neuronal defects, we electroporated wild type or mutant NPRL2/3 into mouse embryonic neural progenitors. However, expression of these mutants did not cause apparent neural migration defects. Our study may help us understand the roles of NPRL2/3 in neuronal development and provide information for developing effective treatment to NRRL2/3-related neural developmental disorders.

MTU06 Bioenergetics & metabolism (Session A)

MTU06-01

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

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ATP-citrate lyase (ACLY) is the key enzyme generating cytosolic acetyl-CoA and oxaloacetate from citrate. Widely expressed in the brain, its role in healthy brain energy metabolism needs elucidation. We inhibited ACLY with BMS-303141 (3,5-Dichloro-2-hydroxy-N-(4-methoxy[1,1′-biphenyl]-3-yl)-benzene-sulfonamide)), at 1 μM (IC_{50}) and 10 μM applied to guinea pig cortical brain tissue slices that were incubated with 2 mM [2,3-^{13}C] pyruvate, 2 mM [3-^{13}C]lactate, or 0.25 mM [U-^{13}C]β-hydroxybutyrate (βOHB) for 60 minutes, or with 5 mM [1-^{13}C]glucose alone, or 0.5 mM [1,2-^{13}C]acetate and 5 mM [1-^{13}C]glucose for 90 minutes. The brain slices were extracted with methanol/chloroform, lyophilised and reconstituted in D_2O. ^{1}H, {^{13}C}-decoupled ^{1}, and {^{1}H}-decoupled ^{13}C NMR spectra were acquired from each sample (N = 4). The resultant isotopomers and metabolite pools were quantified. Inhibition of ACLY using 1.0 μM BMS-303141 with pyruvate, lactate, or βOHB as substrates resulted in increased incorporation of label into Krebs cycle intermediates and glycolytic by-products, showing that normal ACLY activity results in a significant efflux of label from the Krebs cycle. When [1-^{13}C] glucose was the sole substrate, total metabolite pools and net flux of ^{13}C into Krebs cycle intermediates and the glycolytic by-products lactate and alanine were significantly reduced. This effect was "rescued" by 0.5 mM [1,2-^{13}C]acetate, where 1.0 μM BMS-303141 resulted in increased incorporation of label from both glucose and acetate. BMS-303141 at 10 μM had limited further effects in all cases, suggesting that the ability of the system to accommodate further ACLY inhibition is limited. These results indicate that the impact of ACLY activity on Krebs cycle flux is significant, although it is currently not a part of most models of brain metabolism.

MTU06-02

Metabolic impairments in neurons and astrocytes derived from human induced pluripotent stem cells of Alzheimer's disease patients

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Metabolic impairments are one of the earliest cerebral pathogenic events in Alzheimer's disease (AD). In order to investigate the underlying mechanisms of these metabolic alterations, we studied cellular energy and amino acid metabolism in neurons and astrocytes derived from human induced pluripotent stem cells (hiPSC) obtained from AD patients and their respective CRISPR/Cas9 gene edited controls or age-matched controls. Cultures of hiPSC-derived neurons and astrocytes from AD patients and isogenic or aged-matched controls were incubated with ^{13}C-labeled energy substrates, enrichment in metabolites was determined using gas chromatography coupled to mass spectrometry, followed by metabolic mapping and Western blot analyses. Mitochondrial function was assessed via the Seahorse XFe96 Analyzer. AD neurons displayed changes in enzymes associated with glutamine and glutamate processing. Specifically, phosphatase-activated glutaminase (PAG) and aspartate amino transferase (AAT) were up regulated in AD neurons. The observed increase of PAG resulted in increased levels of glutamate converted from glutamine, which is expected to trigger excitotoxicity, one of the early pathological hallmarks in AD neurons. Similarly, increased levels of ATT resulted in increased conversion of aspartate to oxaloacetate fuelling the tricarboxylic acid cycle (TCA). Interestingly, a decreased mitochondrial respiratory function was observed in AD hiPSC-derived neurons, suggesting that an overactive TCA cycle might be a compensatory mechanism. The metabolic studies of astrocytes revealed increases in Lactate production, which could point towards a compensatory mechanism to counteract the increased glutamate levels secreted from AD neurons and thereby combatting excitotoxicity.

MTU06-03

Menadione-mediated WST 1 reduction as indicator for the metabolic potential of cultured astrocytes

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The water-soluble tetrazolium salt 1 (WST1) is frequently used as indicator of the metabolic potential of cultured cells and to determine cell viability. The presence of the membrane-permeable electron cycler menadione caused an almost linear increase in WST1 formazan with maximal WST1 reduction in the presence of 0.5 mM glucose that was not further stimulated by increasing glucose concentrations up to 10 mM after 30 min, while hardly any reaction was observed in the absence of glucose. Only the hexose mannose was able to fully replace glucose as substrate for WST1 formazan generation but not other sugars nor mitochondrial substrates. Intracellular menadione reduction is catalyzed by a cytosolic enzyme that uses efficiently both NADH and NADPH as electron donor, indicated by similar K_M- and v_{max}- values for both cofactors. Accordingly, application of WST1 and menadione to astrocytes led to rapid depletion of NADH and NADPH. The menadione-mediated WST1 reduction was highly sensitive towards dicoumarol as demonstrated for the enzyme in lysates by an inhibitor constant of approximately 2 nM and by the half-maximal inhibition of the cell-dependent WST1 reduction at around 45 nM dicoumarol. These data demonstrate that the NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1) is involved in cellular menadione reduction which in turn facilitates extracellular WST1 reduction. Interestingly, the amount of WST1 formazan generated matched well with the decrease observed in the lactate release, suggesting that electrons from glycolysis-derived NADH contribute to the menadione-

dependent WST1 reduction. In conclusion, the menadione-dependent WST1 reduction can be used as valuable tool to study the metabolism of cultured brain cells.

MTU06-04

NNT is required for brain mitochondrial redox balance and is highly expressed in nitric oxide synthase and serotoninergic neurons

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Nicotinamide nucleotide transhydrogenase (NNT) is a protein located in the inner mitochondrial membrane that catalyzes the reduction of $NADP^+$ at the expense of NADH oxidation coupled to inward proton translocation from the intermembrane space to the matrix. NNT is known to support NADPH-dependent processes such as peroxide detoxification and reductive biosynthesis. Considering the scarce knowledge about NNT roles in brain, we aimed at investigating NNT activity, distribution and contribution to brain physiology by using congenic mice carrying mutated *Nnt* alleles from the C57BL/6J strain ($Nnt^{-/-}$) or the wildtype *Nnt* ($Nnt^{+/+}$). Biochemical analyses of isolated brain mitochondria showed that the lack of NNT resulted in lower total $NADP^+$-reducing activity. In the absence of NNT, a higher mitochondrial H_2O_2 production was detected when the metabolism of respiratory substrates did not favor the flux through other mitochondrial NADPH sources or when the respiratory chain was inhibited. Concerning the spatial distribution of NNT in mouse brain, we observed a higher NNT expression and activity in the pons with increased NNT labeling of neurons in raphe and hindbrain nuclei. Most of the neurons exhibiting strong NNT labeling were also endowed with enzymes involved in biosynthetic pathways for 5-hydroxytryptamine and nitric oxide production, which require NADPH. Behavioral evaluations showed NNT absence is associated with impaired locomotor activity and depressive-like behavior in aged mice, but not in adults. These results indicate that NADPH from NNT activity has relevance for brain mitochondrial redox homeostasis and neurotransmission.

MTU06-05

Integration of micrornaome and metabolomics to dissect cerebral disease progression in X-linked adrenoleukodystrophy

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Introduction: X-linked adrenoleukodystrophy (X-ALD) is a progressive neurodegenerative disease caused by mutations in peroxisomal ABCD1 gene. X-ALD males develop fatal cerebral demyelinating disease (ALD) the mechanism for which remains unknown. We took a novel multi-omics approach of untargeted metabolomics and next generation sequencing (HiSeq) to find regulatory (microRNA) and active (metabolite) pathways underlying the fatal neuroinflammation in ALD.

Methods: Postmortem brain tissue from healthy controls and ALD patients were processed for microRNA (miRNA) and metabolite extraction and analysis. Data analysis was performed by "MetaboAnalyst 2.5" for GC-MS and Bioconductor for miRNA.

Results: Each measured miRNA and metabolite was screened using appropriate ANOVA models. Thresholds for significance were set to control the estimated false discovery rate, per platform, at 5%. We compared postmortem brain white matter of healthy controls (CTL) with normal looking area (NLA) and periphery of plaque/lesion (PLS) regions within the ALD brain white matter. Analysis of variance ($p < 0.05$) and Post-hoc t-tests identified nineteen miRNA and eleven metabolites that significantly differed across the three groups (control, NLA and PLS). Of the nineteen miRNA seventeen were increased (PLS > NLA > CTL) and two were decreased (CTL > NLA > PLS). Seven metabolites were upregulated (PLS > NLA > CTL) and four were downregulated (CTL > NLA > PLS). We calculated the Pearson's correlation coefficient between the expression of these nineteen miRNA and the metabolite intensities of eleven metabolites for putative links between the global gene expression modulators (miRNAs), and metabolites.

Conclusion: Our novel "transomic" modeling identifies, for the first time, integrated miRNA and metabolite pathways underlying demyelination in X-ALD.

MTU06-06

Axonal metabolic support and energy dynamics in active white matter tracts

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In white matter, axonal energy homeostasis critically depends on glial support. Failure in glial-mediated delivery of metabolic substrates into the axonal compartment results in axonal energy deficit and may anticipate the axonal degeneration described in several myelin disorders and neurodegenerative diseases. In mice, neuronal transgenic expression of an ATP-fluorescent sensor allowed us to visualize axonal energy content in acutely isolated optic nerves while simultaneously performing electrophysiological compound action potentials (cAP) recordings. The real-time monitoring of activity-dependent axonal ATP revealed a strong correlation between axonal energy metabolism and nerve conduction. Further on, to determine possible metabolic consequences of myelin defects we monitored ATP and cAP in $Plp1^{null/y}$ optic nerves. Genetic ablation of Plp1, encoding a myelin membrane protein, serves as a model of spastic paraplegia type-2, where an impaired axo-glia unit leads to secondary axonal loss. We found that the energy metabolism of myelinated axons of $Plp1^{null/y}$ optic nerves is perturbed long before the onset of clinical symptoms and major pathological changes. To understand further the role of oligodendroglia and myelin formation in the white matter energy balance, we focused on the metabolic properties of spinal cord sensory fibres *in vivo*, following a long-term FLIM analysis in a model of MS where we could determine the axonal metabolic changes induced by demyelination and remyelination. The parallel monitoring of axonal ATP and cAP is therefore a powerful tool to study white matter metabolism and metabolic support mechanisms under physiological conditions and in models of neurodegenerative disorders.

MTU06-07

The neuroprotective role of 5-methoxyindole-2-carboxylic acid in ischemic stroke injury in rat brain

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This presentation summarizes our findings that 5-methoxyindole-2-carboxylic acid (MICA) can serve as a chemical conditioning agent in neuroprotection against stroke injury and the corresponding underlying mechanisms. MICA is a reversible inhibitor of mitochondrial dihydrolipoamide dehydrogenase that is involved in bioenergetics and metabolism. We performed both MICA preconditioning and postconditioning in the rat brain using an ischemic stroke model. For preconditioning studies, MICA (200 mg/kg body weight) was mainly administered via diet intake for 4 weeks. For postconditioning studies, MICA (also 200 mg/kg body weight) was injected intraperitoneally at the onset of 24 h reperfusion following 1 h ischemia. Our results indicate that stroked animals treated with MICA either in preconditioning or postconditioning studies showed less brain infarction volume than that of vehicle-treated animals. Common protective mechanisms in both MICA preconditioning and postconditioning studies involve Nrf2 up-regulation of NQO1 expression, decreased oxidative stress, increased mitochondrial membrane integrity, and decreased cell death. Our findings demonstrate that MICA can produce an effective pre- and postconditioning effects in the ischemic brain of rat and the underlying mechanism likely involves preservation of mitochondrial function, upregulation of cellular antioxidative capacity, and attenuation of oxidative stress.

MTU07 Neuronal plasticity & behavior (Session A)

MTU07-01

Methyl jasmonate mitigates cognitive impairment and loss of neuronal dendritic spines in the brain of chronically stressed mice

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Morphological changes such as retraction of neuronal dendrites and spine loss have been identified as a common consequence of chronic stress on the brain. Methyl jasmonate (MJ) is a bioactive compound and naturally occurring anti-stress plant hormone previously reported with ameliorative effect against acute and chronic stress in mice. This study investigated the effect of MJ on cognitive impairment, dendritic spines, and density of mice subjected to unpredictable chronic mild stress (UCMS).

Mice were chronically stressed for 10 days and intraperitoneally treated with either vehicle or 50 mg/kg MJ. Thereafter, mice were assessed for freezing time in fear conditioning test as a measure of cognitive function. Dendritic morphology of brains using Golgi-Cox staining procedure followed by Sholl analysis and immunohistochemical expression of nrf2 and parvalbumin were also assessed.

Our results revealed that UCMS triggered a significant increase in freezing duration, a decrease in neuronal dendritic spine density, intersection, and length of hippocampal CA1, basolateral amygdala, and prefrontal cortex, with concomitantly increased nrf2 and decreased parvalbumin expressions. MJ significantly attenuated UCMS-induced cognitive dysfunction, alterations in dendritic arborization and protein expression, indicating less susceptibility to stressors and increased neuronal communication.

Due to the safety profile of MJ, it could have a therapeutic outcome in stress-induced cognitive deficits and neuronal alterations. We are currently investigating the molecular mechanisms underlying these effect using western blotting techniques for quantification of BDNF, HSP 70, CREB, and Glut1.

MTU07-02

The role of the orexin (hypocretin) system in context-induced relapse to alcohol seeking

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

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

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

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

MTU07-03

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

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

MTU07-04

Functional and molecular markers of vulnerability towards stress in a rat model of PTSD

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Stressful events represent a major risk factor for the development of neuropsychiatric disorders such as depression and PSTD. Although genetic vulnerability may represent a moderating factor, it is not clear what mechanisms address the individual responses towards pro-adaptive or maladaptive course. Here we aimed to investigate short/long-term cellular/molecular changes in the aftermath of acute stress to understand better the dynamics of the stress response and identify key factors that may trigger stress-related psychopathology. We adapted sucrose intake (SI) test to identify foot shock (FS)-stress-induced anhedonic phenotype. Baseline sucrose intake was established for 5 weeks in rats. Rats were randomly assigned to FS-stress/control groups and subjected to FS-stress. After 24 h in SI test, anhedonic animals (showing at least 25% within-subject decrease in SI) were classified as vulnerable (FS-V), while others as resilient (FS-R). We demonstrated that FS-stress increased basal glutamate release only in FS-V, while depolarization-evoked glutamate release was increased in both FS-V and FS-R. In Further, we observed significant increase in the nuclear expression of MR only in FS-R while GR expression remains unaltered. Recent studies have demonstrated multiple roles of miRNAs in governing functional and structural synaptic plasticity as well as in pathophysiology of psychiatric disorders. Currently we are investigating key miRNAs responsible for resilience and vulnerable response after single acute stress episode and their target genes at short- and long-term after stress. We have also investigated plasticity related protein like ERK1/2, CREB etc. expression and found increased nuclear translocation of pERK in FS-V rats. This study results will help to identify key molecules associated with stress-induced maladaptive (vulnerable) response and novel targets for therapy of stress-related neuropsychiatric disorders.

MTU07-05

Neonatal nicotine exposure primes midbrain neurons to a dopaminergic phenotype and increases adult drug consumption

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Nicotine is a psychoactive substance that induces addiction through neuroplasticity affecting the function of the Ventral Tegmental Area (VTA) and dopamine release in the reward circuitry. We have previously shown that altered neuronal activity can change dopamine (DA) expression both in the developing and adult brain. Here, we investigated the effect of neonatal nicotine (NN) exposure on DA plasticity of VTA neurons.

Osmotic pumps were implanted in lactating mice to deliver 2 mg nicotine/Kg/day to P2-P16 pups. Adult mice underwent nicotine and ethanol 2 bottle-choice test. Brains were processed for tyrosine hydroxylase and nuclear receptor related-1 protein (Nurr1) immunohistochemistry and *in situ* hybridization. Calcium spike activity was measured via *ex-vivo* calcium imaging. We overexpressed Nurr1 and altered neuronal activity (DREADDs) in non-DAergic VTA neurons using VGLUT2- & VGAT-cre mice.

NN exposure potentiated nicotine preference in adult (P90) mice, increased nicotine-induced responses, induced ectopic Nurr1 expression within VTA glutamatergic neurons. Adult nicotine exposure increased both the total number of DA neurons and DA co-expression with glutamate in the VTA of NN-exposed mice. Overexpression of Nurr1 induced an increase in nicotine preference when paired to DREADDs-mediated activity boost. Downregulation of Nurr1 in glutamatergic neurons revealed its expression is necessary for non-DAergic neurons to acquire a dopaminergic identity. These findings may provide a new critical link between developmental exposure to drugs of abuse and adult vulnerability to drug addiction.

This research was supported by the Tobacco-Related Disease Research Program Grant #271R-0020.

MTU07-06

Rauwolfia vomitoria AFZEL. root bark extract adversely affects behaviour and brain microstructures

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Rauwolfia vomitoria (RV) Afzel. is a medicinal plant of the Apocynaceae family, widely used locally in the management of psychiatry conditions. The plant is beneficial, especially when applied during diseased state, and as antipsychotics and antipyretics. Adverse effects have also been ascribed, especially to its constituents, reserpine and yohimbine. Thus, the present study evaluated the effect the root bark extract has on some behaviour and brain microstructures. Eighteen adult male Wistar rats of 220 g average body weight, were divided into three groups (n = 6); control (distilled water), 200 mg/kg and 400 mg/kg body weight of RV root bark extract. The administration was orally and lasted seven days. On day 8, the open field and Morris water mazes tests, as well as test for olfaction were carried out and the animals sacrificed. Their brains were processed for histology and immunoreactivity. Results showed that learning and memory were not affected, but there were freezing, sedation, and inhibition of locomotion and olfaction. The histology showed degenerative features in the olfactory bulb, hippocampus, dentate gyrus and cerebellum. Immunohistochemically, neuron specific enolase (NSE) expression was increased in the hippocampus and cerebellum of the 200 and 400 mg/kg RV groups; dentate gyrus of the 200 mg/kg RV group; and olfactory bulb of the 400 mg/kg RV group. NSE expression was decreased in the olfactory bulb of the 200 mg/kg RV groups and dentate gyrus of the 400 mg/kg RV group. Expressions of glial fibrillary acidic protein (GFAP) was increased in the olfactory bulb, hippocampus, dentate gyrus and cerebellum of the 200 mg/kg RV group, but decreased in the 400 mg/kg RV group, compared to the control. At these given doses, RV may be deleterious to the brain; in cognitive behaviour and microstructure, and these effects were dose dependent.

MTU07-07

Double hit of perinatal stress evokes depressive behavior and affects midbrain levels of dopamine, serotonin and their metabolites

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Perinatal stress is a susceptibility factor for the development of mental disorders, whose etiology is in possible changes in the integrity or functionality of neurobiological circuits. The aim of our study is to investigate the effects of double perinatal stress, consisting of the association of prenatal hypoxia ischemia and short maternal separation, on behavior depressive-like and levels of dopamine, serotonin and their metabolites in basal midbrain. Hypoxia Ischemia was induced by clamping the uterine arteries of pregnant rats for 45 minutes, on the 18th day of gestation. The same conditions were applied to form the SHAM group only without clamping the arteries. Maternal separation was performed from the first to the sixth day of birth. The anxiety-like and depressive-like behavior were analyzed by the open field, plus maze, forced swim and head shaking tests. Nociception response was evaluated by hot plate test. Analysis of the serotonin, dopamine and their metabolites contents in basal midbrain and prefrontal cortex were done by high performance liquid chromatography. Data were compared with a two-way ANOVA, expressed by mean ± SEM. Double perinatal stressed rats showed increase of depressive-like behavior and mesencephalic serotoninergic hypoactivity. These results are consistent with the hypothesis of increased vulnerability of the serotonergic system for perinatal stress.

This work was supported by FAPERJ grants.

MTU07-08

The role of kinesin 1 isoform, KIF5B, in dendritic spine plasticity

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Regulation and localization of plasticity-related proteins (PrPs) are important for proper synapse maturation and function. Kinesin 1 motor protein family is known to be important in carrying and distributing PrPs and mRNAs in dendrites. The three members of kinesin 1 family, namely KIF5A, B and C, were traditionally thought to be functionally redundant. However, previous studies found different phenotypes in knockout mouse models of each kinesin 1 family member. It therefore suggests that each kinesin 1 member has specific functions. In order to test this hypothesis, a KIF5B conditional knockout mouse model was generated, in which CRE recombinase expression was driven by CaMKII promoter to remove *kif5b* exon 2 endogenously. We used western blot and immunohistochemical staining to confirm the reduced protein level of KIF5B in the conditional knockout mouse model, while there was no significant effect on other isoforms of the kinesin 1 family. We also found that KIF5B conditional knockout mice had increased protein level of PSD95, NR2B, and GluR2. Next, we performed a series of behavioral tests to evaluate the cognitive functions of KIF5B conditional knockout mice. We found that they showed no significant different in anxiety-related behaviors, but they showed learning deficits in auditory-cued fear conditioning, novel object recognition test, three-chamber social interaction test, and Barnes maze. To investigate the cause of behavior deficit, we carried out chronic two-photon intravital imaging. We found that KIF5B conditional knockout mice showed an increase of dendritic spine turnover rate over a 7-day observation period. We will further investigate the role of KIF5B in learning-induced dendritic spine plasticity by *in vivo* imaging.

MTU07-09

Adult hippocampal neurogenesis impairment at pre-plaque stage in a transgenic rat model of Alzheimer's-like amyloid pathology

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The contribution of adult hippocampal neurogenesis (AHN) impairment on cognitive decline in early Alzheimer's disease (AD) remains poorly understood. This can be ascribed to the technical difficulties to measure AHN in post-mortem brains and patients. Furthermore, most animal models of AD exhibit an aggressive neuropathology at early age and harbor gene mutations and express transgenes that disrupts AHN by pathways not directly involved in AD pathology. To overcome some of these limitations, we studied AHN at pre-plaque stage (6-month-old) in hemizygous (Tg+/-) and homozygous (Tg+/+) McGill-R-Thy1-APP transgenic rats. This model exhibits a much less aggressive neuropathology that nevertheless is associated with a marked cognitive impairment from early age. Our results revealed that Tg+/+ rats showed a reduced number of PCNA+ cells, DCX+ immature neurons and BrdU+/NeuN+ colabed neurons in dorsal and ventral dentate gyrus. Moreover, dendritic arborization was less developed. AHN was not impaired in Tg+/- rats, although dendritic arborization was slightly decreased. On the other hand, both hemizygous and homozygous rats exhibited spatial memory impairments in the Morris water maze. These results suggest that: 1) AHN is dysregulated from the pre-plaque stage in homozygous rats; 2) AHN impairment is dependent on APP transgene copy numbers since hemizygous rats did not show it; 3) Dysregulation of AHN is not directly associated with spatial memory impairments since hemizygous rats exhibited spared neurogenesis despite showing spatial memory deficits. Funding: International Society for Neurochemistry CAEN Grant and Andalucía TECH-ICE (PG), and PICT-2015-0285 (LM).

MTU07-10

Selective long term memory impairment in transgenic McGill-R-Thy1-APP rat model of Alzheimer´s disease

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Memory impairment in early Alzheimer Disease (AD) is hypothesised to rely on the initial increase in soluble Aβ-oligomers, potent neurotoxins altering synaptic plasticity.

McGill-R-Thy1-APP Wistar-transgenic (Tg) rats, bearing human Amyloid Precursor Protein with Swedish and Indiana mutations of familial AD, offer a singular opportunity for testing learning and memory abilities at AD onset. Homozygous Tg rats show cognition deficits at 3 months; human Aβ accumulates intra-neuronally from first postnatal week and develops extracellular amyloid pathology by 6-9 months. Hemizygous Tg (He) show a more subtle phenotype and do not develop extracellular plaques even at 20 months.

13 month old He rats and their wild type litter-mates (WT) were left to freely explore an open field (OF) for 5 min and tested 24 hr later (long-term-memory, LTM); bi-dimensional exploration was quantified, being significantly lower in test than in training for both groups.

Rats were then trained in a two object recognition task (OR); both WT and He discriminated new vs. known object 1 hr later (short-term-memory, STM), while He rats did not show LTM.

They were then trained in an inhibitory avoidance (IA) to a mild foot-shock task. Latencies to go from an enlightened to a dark compartment where they get the shock, were recorded. Test latencies 24 hr later, were significantly higher than training latencies for WT, while remained unchanged for He.

Therefore, unlike WT, He evidenced deficits in memory formation for object discrimination and for an associative memory involving aversive and spatial components.

MTU07-11

Mesopontine cholinergic signaling influences stress responses affecting behaviour

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Pedunculopontine and laterodorsal tegmental nuclei (PPT/LDT) are heterogenous brainstem structures that contain cholinergic, glutamatergic and GABAergic neurons. Several neuropsychiatric disorders have been associated with degeneration of the cholinergic neurons in this brain region, however, the importance of PPT/LDT cholinergic signaling for cognitive and non-cognitive functions is poorly understood. Previous work suggested that PPT/LDT cholinergic neurons play a role in attention and other forms of higher-level cognition, however these studies used non-selective methods to kill cholinergic neurons. To test the role of acetylcholine in higher-level cognition, we selectively eliminated the vesicular acetylcholine transporter (VAChT) in the PPT/LDT to generate mice that have impaired cholinergic signaling without interfering with other brainstem cell types and co-transmitted chemicals. We tested these VAChT-deficient mice using conventional and touchscreen-based cognitive tasks and found that they had little to no impairments in many cognitive functions, including attention, yet failed to perform in the spatial and cued forms of the Morris water maze (MWM). Interestingly, spatial memory and visual spatial learning were intact in VAChT-mutants, but touchscreen performance was affected by a stressor and mice had altered corticosterone levels after the MWM. These results suggest that attention and many other cognitive functions are not affected by the loss of PPT/LDT cholinergic signaling, but an altered stress response can influence cognitive performance in aversive tasks.

MTU07-12

Enhancing adult neuroplasticity by epigenetic regulation of PV interneuron

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Parvalbumin-positive cells (PV), the major source of GABAergic inhibition in the brain, innervate hundreds of postsynaptic targets with multiple perisomatic synapses. They are important for the regulation of multiple cognitive functions and developmental cortical plasticity. Although PV cell function is being explored extensively, the mechanisms that control their development and plasticity have not been entirely resolved. Molecular mechanisms involved in synapse formation and strengthening include the regulation of specific subsets of genes by epigenetic modifications. Histone Deacetylase 2 (HDAC2) regulates excitatory synapse plasticity and memory formation. However, whether and how HDAC2 affects PV cell synapse development and brain plasticity is unknown. Here, we show that HDAC2 is expressed by PV neurons. In order to dissect its role in PV cell, we used the conditional KO mice (PV_Cre;HDAC2$^{lox/lox}$), which express Cre selectively in PV cells after P14. We found that adult PV_Cre;Hdac2$^{lox/lox}$ mice show enhanced fear memory extinction, along with a reduction of perineural nets (PNN) around PV cells somas in the prefrontal cortex and the basolateral amygdala as well as an increase in perisomatic PV synapse remodeling after fear extinction. Finally, the direct role of Hdac2 in PNN formation and PV cell synapse plasticity will be tested by viral overexpression of Hdac2 in prefrontal cortex of PV_Cre;Hdac2$^{lox/lox}$ mice. All together, our work supports the model in which PV cells and PNN play a pivotal role in brain plasticity and suggests that modulation of Hdac2, in combination with behavioral therapy can improve the treatment of post-traumatic stress disorder (PTSD).

MTU07-13

In silico characterization and functional analysis of non-synonymous polymorphisms present in gpm6a's extracellular coding regions

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Membrane glycoprotein M6a is mainly expressed in neurons of the central nervous system and it is involved in neuronal plasticity. M6a promotes neurite and axonal outgrowth, filopodia/spines formation and synaptogenesis in primary neuronal cultures and neuronal cell lines. Recently, altered expression or polymorphisms in the human M6a gene have been associated with neurological disorders such as schizophrenia, bipolar disorder, depression, claustrophobia and Alzheimer's disease. However, the molecular mechanisms underlying the development of such pathologies remain unknown. M6a, together with M6b and PLP/DM20, belongs to the tetraspan proteolipid protein family. According to its structure, we speculate that certain amino acids within M6a's extracellular loops mediate specific interactions with other proteins and those contribute to its function. Thus, out of more than a hundred submitted entries, we selected 13 non-synonymous SNPs from the NCBI dbSNP database located at the coding sequence of GPM6a's extracellular loops (EC1 and EC2). The selected nsSNPs had to be validated by frequency, cluster and/or 1000G. *In silico* analysis –using -Polyphen and I-mutant 2.0- predicted that all nsSNPs might decrease protein stability and have a moderate to strong functional damaging effect. In the case of SNPs located in the EC1, none of them modify M6a neuronal membrane distribution and topology, however, some of them rs375144137 (G69E), rs370813625 (T71P) and rs747244424 (T76I) impair M6a neurite extension in N2a cell line. We speculate that these variants blocked M6a´s neurite extension because of the drastic single amino acid substitution (non polar to acid or basic residue and polar to non polar) that might affect extracellular loops interactions.

MTU07-14

Adult neurogenesis in the paleognathous birds: the common ostrich (*struthio camelus*) and emu (*dromaius novaehollandiae*)

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Adult neurogenesis is a process occurring in varying magnitudes in different species ranging from invertebrates to vertebrates. We examined adult neurogenesis throughout the brains of the common ostrich (*Struthio camelus*) and emu (*Dromaius novaehollandiae*) using immunohistochemistry for the endogenous markers proliferating cell nuclear antigen, which labels proliferating cells, and doublecortin, which stains immature and migrating neurons. The distribution of PCNA and DCX labelled cells was widespread throughout the brain of both species. The highest density of cells immunoreactive to both markers was observed in the olfactory bulbs and the telencephalon, especially the subventricular zone of the lateral ventricle. The density of PCNA immunoreactive cells was less exuberant in the telencephalon of the emu compared to the common ostrich. Substantial numbers of PCNA immunoreactive cells were observed in the diencephalon and brainstem, but DCX immunoreactivity was weaker in these regions. PCNA and DCX immunoreactive cells were observed in moderate density in the cortical layers of the cerebellum of both species. Columns of migrating cells were observed at three distinct points extending from the lateral wall of the lateral ventricle into parenchyma of the telencephalon at rostral levels in both species. The distribution of putative proliferating cells and immature neurons in the brain of the common ostrich and the emu is widespread, far more so than in mammals, and compares with the neognathous birds, and suggests that brain plasticity and neuronal turnover is an important aspect of cognitive brain functions in these birds.

MTU07-15

Behavioral, cytoarchitectural, and neurochemical changes in the offspring of methylazoxymethanol treated in mice

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Methylazoxymethanol (MAM) treated pregnant rats at gestation day (GD) 17 has been shown to be a valuable animal model for schizophrenia. However, this model is still not established in mice models. To examine face, construct and predictive validities, we observed behavioral, cytoarchitectural, and neurochemical changes in the offspring of MAM treated mice. We found in contrast to a single injection of MAM to dams at GD 15, 16 or 17, its daily administration from GD 15 to 17 led to deficits in prepulse inhibition (PPI) of startle in the post-pubertal offspring. Moreover, we observed behavioral deficits such as increasing locomotor activity to NMDA antagonist MK-801, working memory and social interaction. These animals also showed a reduction of volume at the prefrontal cortex (PFC) and hippocampus, and neuroanatomical changes such as discontinuities and heterotopias in the hippocampus. Atypical antipsychotic drugs clozapine, risperidone, and aripiprazole, but not the typical drug haloperidol, reversed the deficit in PPI. Therefore, the treatment of pregnant mice with MAM during GD 15-17 MAM offers a new procedure to research neurobiological mechanisms involved in the pathogenesis of schizophrenia.

MTU07-16

Expression of molecular signatures during long-term memory consolidation, established by behavioural tagging model

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Memory is one of the most fundamental processes of brain and learning and memory are one of those frontier areas of neurobiology which attract us to investigate the intricacy of this process. Here we aimed to investigate the general mechanism of "Behavioural

Tagging" in long term memory (LTM) formation. Long term potentiation (LTP) is a form of synaptic plasticity and it is considered as a cellular model of learning and memory. One of the LTP specific PRPs, PKM-ζ, is required for the formation of LTM as well as for the maintenance of LTP. In our study, we have shown that for the consolidation of LTM, in addition to LTP-specific PRPs, synaptic tags are also required to interact with each other. In the present study, we investigated the involvement of LTP-specific PKM-ζ and learning tags within a critical time window, which are required for the formation of LTM without affecting STM. Behavioural tagging is an established model for the assessment of some forms of learning and memory. Despite being studied for LTM formation for many years, no studies investigated the role of PKM-ζ in Behavioural tagging model. Hence, by using these two different memories based tasks (Inhibitory avoidance and Novel object recognition tasks), we observed how PKM-ζ activated by exposing a novel arena after a weak training and led to the consolidation of memory. These findings thus show how the process of behavioural behavioural tagging activates LTP-specific PKM-ζ for the formation of LTM.

MTU07-17

Antinociceptive effect of diminazene aceturate, an angiotensin-converting enzyme 2 activator, in the mouse formalin test

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We have reported that intrathecal (i.t.) administration of angiotensin (Ang) II into mice produces a nociceptive behavior accompanied by the phosphorylation of p38 MAPK in the spinal cord, which was mediated through AT1 receptors. In addition, both the p38 MAPK phosphorylation and subsequent nociceptive behavior were attenuated by the i.t. co-administration of Ang (1-7), an N-terminal fragment of Ang II formed by angiotensin-converting enzeme 2 (ACE2), that acted via Mas receptors. However, the role of ACE2 on spinal nociceptive transmission remains unknown. Therefore, in the present study, we examined the effect of diminazene aceturate (DIZE), an ACE2 activator, on the formalin-induced biphasic nociceptive behavior. When administered i.t. 1 hr prior to the intraplantar injection of 2% formalin, DIZE dose-dependently attenuated the nociceptive behavior during the second phase, but not the first phase. The inhibitory effect of DIZE was prevented by i.t. administration of A779, a Mas receptor antagonist. The i.t. administration of DIZE also dose-dependently attenuated the Ang II-induced nociceptive behavior, which was prevented by i.t. administration of A779. Although phosphorylation of p38 MAPK was observed in the lumbar dorsal spinal cord after the injection of formalin or Ang II, these phosphorylation were attenuated by DIZE, which was inhibited by A779. In addition, DIZE significantly increased the ACE2 activity in the lumbar dorsal spinal cord. These results suggest that DIZE attenuates the formalin-induced nociceptive behavior during the second phase through the increase in Ang (1-7) generated from Ang II by the activation of ACE2 and subsequent inhibition of p38 MAPK phosphorylation via Mas receptors.

MTU07-18

Pain behavioural response in plasmodium berghei-induced malaria

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Several forms of pain are reported by patients with *Plasmodiumfalciparium* and are mostly described as one of the symptoms for malarial infection. Neural processes that influence pain perception in malaria-infected persons and its contributions to malaria related mortality are poorly understood. In the current studies, *Plasmodium berghei* (*Pb*) infected Swiss mice exhibited attenuated behavioural responses to noxious chemical without compromising the motor function. This parasite-induced analgesia improved with increase daily proliferation of erythrocyte stage *Pb* and it appeared to be synergistically mediated by opioidergic (via μ-opioid receptor) and serotoninergic (via 5HT2A receptor) system. Systemic intersection study among drugs of varying mechanisms capable of eliciting anti- or pro- nociception showed little or no contribution of pain system in malaria related death. Though there were mild morphological changes in brainstem cells and Nissl substance of infected mice, neural secretory activity (of serotonin, noradrenaline and ATPase) was preserved. These results indicated that animal model for malaria is not a potential model to unravel mechanism surrounding malaria parasite-induced pain reported in human. This however is the first report demonstrating that malaria parasite causes analgesic-like effects in mice.

MTU07-19

Lipid raft dynamics in adolescent brain: alcohol-stimulant co-use

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Co-use of alcohol and stimulants, including caffeine and amphetamine, is a growing concern, particularly among adolescents already prone to binge alcohol consumption. We are modeling chronic co-use of alcohol and stimulants in adolescent Long-Evans rats. Previous behavioral work in our lab has shown that chronic administration of caffeine or amphetamine with alcohol resulted in decreased severity of alcohol withdrawal symptoms. Alcohol withdrawal is considered a hyperglutamatergic state. Regulation of glutamate activity may occur in part through lipid raft structures. The goal of the present study was to characterize glutamatergic receptor components of lipid rafts as a way of determining if raft dynamics were a target for alcohol-stimulant interactions. Adolescent rats were fed ethanol, amphetamine, caffeine, or combinations of ethanol and amphetamine or caffeine as part of a liquid diet. Detergent-resistant membranes were isolated by ultracentrifugation and raft fractions identified by Western blotting with the raft marker flotillin. Higher (HBR) and lower (LBR) buoyancy raft fractions were identified. Compared to control rats fed liquid diet without drug, there was a 2-3 fold increase in the LBR fraction from rats consuming alcohol. Western blotting showed that this fraction was rich in NMDA-subtype of glutamate receptors which may account in part for upregulation of NMDA receptors during chronic alcohol consumption. Other components of glutamatergic transmission

(mGluR1 and 5; Homer) also appeared in raft fractions. When rats consumed ethanol along with caffeine, there was no increase in the LBR fraction. Similar results were obtained after co-consumption of ethanol and amphetamine. Thus, a change in lipid raft dynamics was correlated with alcohol withdrawal symptoms but not under conditions where stimulant co-consumption attenuated withdrawal severity. Lipid rafts may be a site wherein stimulants antagonize adaptive responses of the brain to chronic ethanol.

MTU07-20

Comorbidity between stress and cocaine: role of cofilin in nucleus accumbens during the acquisition of cocaine self-administration

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The development of addictive behavior is associated with functional and structural plasticity in the mesocorticolimbic pathway. Animal models have demonstrated that exposure to stress predisposes to developing substance use disorders. Our laboratory has shown that repeated stress alters the capacity of a subsequent cocaine injection to modulate dendritic spine morphology and actin dynamics. These findings demonstrate that the pharmacological inhibition of actin polymerization in the nucleus accumbens (NA) prevents stress cross-sensitization with cocaine and influences actin cytoskeleton remodeling in the NA. Thus, the main goal of this project is to evaluate the impact of the actin cytoskeleton in the changes underling the facilitatory influence of stress in the acquisition of cocaine self-administration (SA). For this purpose, we have generated a lentivirus containing a short hairpin RNA (shRNA) specific to cofilin, to inhibit its expression in NA, and explore its function during the acquisition of cocaine SA. Thus, Sprague dawley rats pre-exposed to chronic restraint stress, will be administered intra-accumbens with shRNA of cofilin, and later they will undergo surgery for implantation of catheters in the jugular vein one week before SA sessions. Our results reveal that the inhibition of cofilin prevents the stress-induced sensitization to cocaine and reverts the facilitation of the acquisition of cocaine self-administration induced by stress, suggesting that cofilin regulation is crucial for the stress-induced facilitation on the vulnerability to develop cocaine addiction.

MTU07-21

Outgrowth of filopodia is associated with intracellular trafficking of GPM6A

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Gpm6a is a neuronal membrane glycoprotein that functions in the processes of neuronal development and its overexpression leads to the extensive formation of filopodia. Neuropsychiatric disorders and chronic stress exposure have been linked to the alterations in Gpm6a expression levels or sequence. However, the mechanism of action of Gpm6a is not clearly understood. Previously, we identified K250, K255, and E258 in the C-terminal of Gpm6a as key functional residues for the formation of filopodia. Subsequent bioinformatic analysis revealed that K250, K255, and E258 are predicted as part of sorting signals of transmembrane proteins. Colocalization assay showed that deletion of the C-terminus diminishes the association of Gpm6a with clathrin in hippocampal neurons implying involvement of clathrin-mediated trafficking events. Moreover, using flow cytometry we found that subtitution of K250, K255, and E258 with alanine diminishes the amount of Gpm6a on cell surface and in case of K255 and E258 also leads to the lower amount of total expressed protein. Here using confocal microscopy we analyze the subcellular localization of the mutant forms of Gpm6a that fail to induce filopodia formation. K250A and E258A display increased intracellular accumulation of the protein and a preferential localization to Lamp1-positive structures. To determine if K250, K255 and E258 substitutions leads to an increased protein degradation we use flow cytometry to quantify the Gpm6a expression levels upon treatment with different protease inhibitors. The localization of Gpm6a mutants to LC3- and Calnexin- positive structures was also evaluated.

MTU07-22

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

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

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

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

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

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

MTU07-23

Mild ketogenic diet as promising approach for cognition enhancement: medium-chain triglyceride supplement improves memory in rats

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Ketogenic diet is known to be a viable approach for correction of brain disorders. The strict ketogenic diet is difficult to adhere to. Search for an intervention both beneficial and not too restrictive appears important. Intermittent mild ketosis by using medium-chain triglycerides (MCT: C8–C10) may be such intervention. The study was aimed at the investigation of effects of MCT-enriched diet on the memory of adult intact animals. Adult Wistar males kept on standard diet (SD) were tested in Y-maze, Open field, Object recognition. Then, 2 groups were formed: MCT (chow excluded for 6 h/day, MCT intragastric, 2 ml/kg) and SD control (water intragastric). After 2 weeks of diet, the tests were repeated, adding Morris water maze. Statistics: rm-ANOVA, *post hoc* Sidak; Student's, Mann-Whitney, $p < 0.05$. In the Y-maze, MCT group demonstrated better working memory: more spontaneous alternations compared to control. In the Open field, MCT animals showed decreased exploration than SD when normalized to pre-diet trials, indicating better memory of environment. No differences were found in the Object recognition. In the probe trial of Morris water maze, MCT animals spent more time in the target quadrant than SD group, indicating better spatial memory. MCT-enriched diet is shown to be a promising non-drug approach to improve cognitive functioning.

MTU07-24

Discovery of a key missing signaling between RHOA/RHO-kinase and ras underlying spine enlargement and LTP

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The small GTPase RhoA and its downstream effector Rho-kinase are considered as one of the key regulators in dendritic spine formation and synaptic plasticity. However, how RhoA/Rho-kinase signaling involved in modulating synaptic plasticity still remains unknown. We have recently developed a phosphoproteomic analysis method that uses affinity beads coated with 14-3-3 proteins to enrich phosphorylated proteins and established the kinase-associated neural phosphosignaling (KANPHOS) database that provides the phosphorylated sites identified by our phosphoproteomic approaches. Using the KANPHOS database, we identified SynGAP1, which is a synaptic Ras-GTPase activating protein, as a novel Rho-kinase substrate. In this study, we found that phosphorylation of SynGAP1 by Rho-Kinase increased its interaction with 14-3-3 but decreased with PSD-95, which is a major scaffolding protein in the postsynaptic densities of dendritic spines. SynGAP1 was dispersed from spines upon long-term potentiation (LTP) induction in cultured neurons, and this dispersion depends on phosphorylation of SynGAP1 by Rho-kinase. Moreover, we found that Rho-kinase increased Ras and ERK activity through phosphorylation of SynGAP1. Thus, the synaptic dispersion of SynGAP1 which phosphorylated by Rho-kinase during LTP represents may be a key signaling link element that transduces RhoA/Rho-kinase activity to Ras-ERK signaling-mediated spine enlargement, AMPA receptor (AMPAR) synaptic incorporation, and synaptic potentiation.

MTU08 Clinical studies, biomarkers & imaging (Session A)

MTU08-01

Sensitive and stable quantitation of endogenous oxytocin in mice using reduction/alkylation approach for elisa

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Oxytocin (OT) is a nonapeptide essential for the social brain. Several studies have reported the ability of plasma OT levels to be a neurological biomarker of autism, anxiety and other mental disorders. One of the main methods of OT determination - is immunosorbent assay (ELISA). Specificity of this method is criticized. Matrix interference and binding of OT with plasma albumins make data of direct determination doubtful. An established way to solve this problem – is a measurement of only the free OT fraction, employing solid phase extraction (SPE). However, SPE required a significant amount of plasma which made it difficult to use in research employing rodents.

Recently, Brandtzaeg group discovered reduction/alkylation followed by proteins precipitation (RAPPT) method that enables to save a sufficiently high amount of OT released from plasma proteins as well as provide a reduction of matrix interference. Importantly, the required volume of plasma is just 100 μl. This method was implemented for the Mass Spectrometry platform.

We aim to adopt this approach for ELISA. We have performed RAPPT prior to ELISA measurement using ICR mice plasma pool. OT levels after RAPTT were lower than in diluted plasma, but significantly higher compared with SPE samples. RAPPT samples demonstrate linearity within dilution, while samples after dilution only - not. Protein precipitation only leads to a dramatic loss of OT from the sample. This data indicated essential roles of RAPPT to release OT from binding with plasma albumins. Finally, we validated RAPPT sample treatment in comparison of plasma pools of ICR and CD38KO mice (a model with disrupted central OT release). Obtained data confirmed lower levels of OT in CD38KO plasma.

MTU08-02

Traumatic brain injury and risk of dementia: A meta-analysis of cohort studies using real-world data

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Introduction: Published epidemiological studies found positive association between TBI and dementia risk. However, there are studies which found no association. So, this study is aimed to assess the association between TBI and dementia risk.

Methods: Articles were retrieved from PubMed and Embase database by running the keywords related to TBI and dementia till February 2019. We included all the cohort studies which assessed dementia risk due to TBI. Newcastle-Ottawa scale was used to assess the study quality. The primary outcome of this study was to compute the pooled dementia risk due to the TBI. Secondary outcomes include dementia risk based on subgroups like dementia subtypes, geographic region, and sex. Statistical analysis was performed using Review Manager software.

Results: A total of sixteen articles qualified the inclusion criteria and comprised of 5,429,711 patients with mean age of 62.28 ± 11.4 years. Majority of the studies were of high quality. Pooled relative risk found that TBI significantly increased the dementia risk with a relative risk (RR) of 1.61 (95% CI: 1.39 – 1.86), $p < 0.00001$. Subgroup analysis also revealed significant Alzheimer's risk due to TBI with RR of 1.17 (95% CI: 1.12 – 1.22), $p < 0.00001$. Studies conducted in US (n = 6) and other parts of the world (n = 10) also found significant dementia risk due to TBI with RR of 1.54 (95% CI: 1.14 – 2.08), $p = 0.005$ and 1.67 (95% CI: 1.38 – 2.01), $p < 0.00001$. No significant association was observed with gender.

Conclusion: The finding of this study suggests that TBI is significantly associated with dementia as well as Alzheimer's risk.

MTU08-03

Imoportance of the existence of salive proteins for stress biomarkers founded by proteome after mental or physical stress loading

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Saliva is a useful sample non-invasively and repeatedly collected from body fluid. Our objective in the present study is to find salive biomarker proteins for mental and/or physical stress for quality of life. Quite recently, we investigated rat saliva marker proteins for mental or physical stress by proteome using rat stress models. The increased proteins by mental stress were subjected to liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). We detected the known enzymes and secretary proteins with MW od 20-70 KDa in rat saliva proteins. Furthermore, we analyzed the biomarkers for physical stress by proteome after tredmill runnning loading to rats. After the separation by SDS-PAGE, the increased proteins by physical stress were used for LC-MS/MS and comprehensive proteome analysis (isobaric Tags for Relative and Absolute Quantitation, iTRAQ). We might find biomarkers for mental and/or physical stress. In the present study, we discussed on the importance of the existence of saliva proteins as stress biomarkers of mental and/or physical stress loading to rats.

MTU08-04

Rage-associated serum markers along with motor and cognitive clinical parameters as predictors of parkinson's disease

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Parkinson's disease (PD) affects nearly 10 million people globally. The course of disease is highly variable and there are no established biomarkers with diagnostic value or predictive models. The receptor for advanced glycation end products (RAGE) is crucial in the propagation of inflammatory events, exerting a major role in neuroinflammation and dopaminergic denervation. We evaluated the correlation of inflammatory cytokines with RAGE agonists in serum, in parallel with cognitive (MoCA) and motor/non-motor (UPDRS) clinical parameters of PD. Blood samples were collected from 51 cases and 37 controls. Serum parameters were measured by Multiplex. PD-patients had increased concentration of serum HMGB1 and decreased IL-1β, TNF-α, IL-8, RANTES and IL-6. HMGB1 is correlated with other RAGE agonists, such as nitrotirosine, 4-HNE, CML along with S100B, but when analyzed together they do not predict the outcome. RANTES is negatively correlated to MoCA and TNF-α and together they show to be good predictors of PD. Although α-synuclein does not differ between control and PD, it is positively correlated to TNF-α in PD, and together they are factors that predict the disease. More parameters should be developed in order to discover RAGE-associated potential molecular markers that may aid in clinical diagnostic.

MTU08-05

Frontal theta asymmetry changes while watching emotional film clips and role of difference pair of frontal electrodes

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The brain hemispheric asymmetry has been hypothesized associated with emotional processing related emotional valence. Amygdala and prefrontal cortex are the important brain structures involved in emotional processing. Frontal alpha (8.5-13 Hz) asymmetry (FAA) has been received most attention to be a neuro-biomarker of emotion, but the brain asymmetry on other spectral frequencies, particularly in frontal theta (4.5-8 Hz) asymmetry (FTA), are needed to be clarified. This study aimed to investigate the FTA in response to emotional clips and the influence of difference pairs of frontal sites (F4/F3: medail-frontal vs. F8/F7: lateral-frontal). The participant were 10 healthy females (aged 20-32 years; M = 25.80, SD = 4.85). Two sets of emotional clips (4 clips per set) were used to elicit target emotional states (sadness, fear, happiness, and neutral). The FTA was calculated using the equation: ln[right]–ln [left] theta power. The results found the significantly decreased FTA at F4/F3 pair in response to negative and neutral clips when compared to resting-states. The significant reduction of FTA among types of emotional clips was obviously demonstrated at F4/F3 pair. However, only one pair of emotional clip (fear and happiness) can be revealed with FTA at F8/F7 pair. These findings indicated that FTA can be used for studying emotional response, especially with negative stimuli, and may be a valuable tool as a neuro-biomarker of emotion apart from the FAA. However, the selection of frontal electrode pairs is important. The FTA between medial-frontal electrodes seem to be sensitive with emotional response than the FTA between lateral-frontal sites.

MTU09 Neurodegeneration and mental health (Session A)

MTU09-01

Effects of garlic constituents in a rat model of reserpine-induced depression

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Commonly available chemical drugs used for nervous system disorders have various unfavorable side effects. Due to this, herbal medicines are being prescribed as an alternative therapy. In the present study, we have investigated the effect of antidepressant and neuroprotective effect of garlic extract against reserpine-induced depression in the rat model. Rats were divided into four groups: 1) control, 2) reserpine, 3), reserpine with garlic extract and 4) reserpine with fluoxetine. The forced swimming test was used to evaluate the antidepressant activity of garlic extract. The levels of antioxidant enzymes including SOD, GST and CAT and some metabolic enzymes such as LDH and MDH were significantly decreased in depressed rat brain. The levels of serotonin and acetylcholinesterase activity were also altered which suggested the abnormal dopamine cycle in the brain. A histological study shows significant malformations in brain parts. Reserpine-induced a significant increase in the immobility time of rats in the forced swimming test and treatment with garlic extract ameliorated the reserpine-induced changes. Reserpine-induced reduction in brain marker enzymes was improved using garlic extract. Also, it reduces the MDA level in the brain. The study suggests the antidepressant activity of garlic extract against the reserpine-induced depression.

MTU09-02

Potential effects of genistein on human amniotic mesenchymal stem cells for cholinergic neuronal differentiation

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Alzheimer's disease (AD) is an irreversible neurodegenerative disorder that worsens without receiving proper treatment. AD is widely known for causing dementia but it is also characterized by loss of cholinergic neurons and high production of reactive oxygen species (ROS). Mesenchymal stem cells (MSCs) became a recent area of interest as a treatment of AD, because under specific conditions MSCs can differentiate into cholinergic neurons. Previous studies have shown that pretreating amniotic fluid mesenchymal stem cells with an antioxidant called *N*-benzylcinnamide (PT-3) aided in preventing cell death during cholinergic neuronal induction. In the present study, human amniotic mesenchymal stem cells (hAMSCs) were retrieved with consent after full-term labor and prepared for cell culture (PSC5 cells). Additionally, phytoestrogens with antioxidative properties are also an upcoming candidate for the treatment of AD as low estrogen was found to be related to AD progression. Genistein (GEN), an isoflavone, binds to estrogen receptor α and even more estrogen receptor β (ERα and ERβ) and it is present in some legumes such as soy beans and red beans. GEN was found to be beneficial to the nervous system because of its estrogen-like properties but at the same time possessed low oncogenicity. Treatment of PSC5 cells with GEN for 48 h showed that it was able to protect cells from cell death via cell viability assay (MTT), and decreased the amount of ROS production measured by ROS assay.

MTU09-03

DY-9836 AS calmodulin inhibitor ameliorates cognition via inhibiting nitrosative stress and NLRP3 signaling in mice model of BCAS

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Vascular dementia (VaD) is a heterogeneous brain disorder or which there are no effective approved pharmacotherapy available. The aim is to elaborate the effect of calmodulin inhibitor, DY- 9836, and its loaded nano drug carrier system on cognitive impairment and gain a better understanding of the protective mechanisms in mice with bilateral carotid artery stenosis (BCAS). DY- 9836 (0.5 or 1 mg/kg) or DY-9836 (0.25 mg/kg)-encapsulated polysialic acid-octadecylamine (PSA-ODA) micelles (PSA- ODA/DY) were given to BCAS mice for 4 weeks. Administration of DY- 9836 or PSA-ODA/DY reduced escape latency in space exploration and working memory test compared with vehicle group. Vehicle treated mice showed reduced phospho- CaMKII (Thr286/287) levels in the hippocampus, whereas partially restored by DY- 9836 (1 mg/kg) or PSA- ODA/DY (0.25 mg/kg) treatment. In accordance with the pharmacological profile of DY- 9836 observed during behavioral studies, experimental molecular and biochemical markers induced by CAS, such as protein tyrosine nitration, Nod- like receptor protein 3 (NLRP3), caspase- 1, and interleukin- 1β, were reduced by DY- 9836 and PSA- ODA/DY treatment.This study discloses the novel therapeutic potential of DY-9836, and its encapsulated nanodrug delivery system significantly enhanced the cognitive function in mice model of Vascular dementia.

MTU09-04

Kolaviron mitigates rotenone-induced behavioural incompetence and nigrostriatal degeneration

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Parkinson's disease (PD) is the most prevalent movement disorder. Currently, therapies are palliative with associated irreversible behavioural incompetence. Here, we investigated the ability of kolaviron (KV), an anti-inflammatory agent, to rescue nigrostriatal damage and redo-inflammation in rats exposed to rotenone (ROT). Aged rats exposed to 11 days of rotenone intoxication were treated with KV either concurrently or for 18 days (7 days of pre-treatment prior 11-day concurrent ROT-KV treatment). ROT-exposed rats lost weight appreciably and travelled less distance with reduced speed, decline efficiency to maintain a straight path, enhanced freezing, increased immobile episodes and poor hole recognition. The motor incompetence was attributed to enhanced nigrostriatal degeneration and increased alpha synuclein formation. ROT resulted in reduced tyrosine hydroxylase (TH) intensity in substantia nigra (SNc; 50%) and striatum (75%), and depletion of SNc TH-positive cells (50%). ROT intoxication significantly elicited reactive species production, induction of striatal antioxidant system and damage to biomolecules. ROT increased COX-2 expression, myeloperoxidase activity and secretion of striatal inteleukine-6 (IL-6), IL-1β and tumour necrosis factor (TNF-α). KV treatment reversed the rotenone-associated locomotor impairment, exploratory deficits and motor/neuromuscular incompetence. KV-treated rats showed improved capacity to maintain efficient gait with minimal rigidity and enhanced coordination. KV pre-treatment preserved more than 70% striatal dopaminergic terminal and 75% SNc TH-positive neurons. KV significantly attenuated ROT-induced neurobiochemical imbalance, altered antioxidant defence system, reduced DJ-1 secretion, neuroinflammation and enhanced striatal infiltration of $CD45R^+$ cells. Taken together, kolaviron treatment mitigated the molecular processes and pathological features associated with PD via mechanisms related to its antioxidant and anti-inflammatory properties. Thus, kolaviron may be beneficial in the management of PD.

MTU09-05

Adenosine A1 and A2A receptors modulation by atorvastatin: neuroprotective and antidepressant-like effects

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Adenosinergic system is involved in key neuromodulatory processes. The purpose of this study was to evaluate the modulation of this system on neuroprotective and antidepressant-like effects of atorvastatin. Adults male *Swiss* mice received an acute administration of atorvastatin (0.1 or 0.01 mg/kg, p.o.). CHA (0.05 mg/kg, i.p, A_1R agonist) or DPCPX, (2 mg/kg, i.p. A_1R antagonist) was administered 30 min before atorvastatin. Animals received dipyridamole (0.1 μg/site, i.c.v, adenosine transport inhibitor) or SCH5621 (0.05 mg/kg, i.p., $A_{2A}R$ antagonist) 45 min after atorvastatin. Mice were subjected to tail suspension test (TST) and open field test (OFT), 1 h after Atorvastatin. For *ex vivo* evaluations mice received sub-effective atorvastatin for 7 days, once a day (10 mg/kg). Hippocampal slices were pre-incubated with DPCPX (250 nM) or SCH5621 (SCH, 100 nM) and subjected to glutamate toxicity protocol (10 mM) for 1 hour and cellular viability was evaluated. The coadministration of atorvastatin and CHA produced an additive effect, reducing the immobility time in TST. DPCPX administration prevented the immobility time reduction induced by an effective dose of atorvastatin. Similarly, atorvastatin antidepressant-like effect was blocked by SCH administration. To evaluate adenosine transport, dipyridamole was administrated but no alterations were observed in TST and OFT. In neuroprotective *ex vivo* evaluations, atorvastatin treatment prevented glutamate-induced cellular viability decrease, however, pre-incubation with DPCPX, or SCH prevented atorvastatin effect. This set of results suggests a dependence on A_1R and $A_{2A}R$ activation for the antidepressant-like and neuroprotective atorvastatin effects, as well as, a correlation between these mechanisms.

MTU09-06

Aging-induced neurodegeneration in relation to brain regional Aβ deposition, locomotor and cognitive function: role of carnosine

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Aging develops positive association of biological disability to the stress related physiological conditions to culminate into the risk of disease with the deterioration of different antioxidant system. Carnosine, an endogenous antioxidant dipeptide biomolecule present in different tissues including brain. The present study focuses on central amyloid beta (Aβ) and carnosine in relation to neurodegeneration, locomotor activity (LA) and cognitive function (CF) in young (4 months) and aged (18 and 24 months) male albino Wistar rats. Results revealed that aging significantly (1) enhanced brain regional Aβ deposition (as plaque) in the order of hippocampus > cerebral cortex > hypothalamus > pons-medulla, without its any existence in cerebellum in spite of having its (Aβ) highest level among the brain regions, (2) reduced (a) steady state level of carnosine and neuronal cell count of the brain regions studied and (b) LA and CF. Carnosine (2.0 μg/Kg/day, i.t for 21 consecutive days) attenuated this aging-induced (a) brain regional increase of Aβ levels and plaques along with their neuronal cell loss and reduction in endogenous carnosine level, (b) decrease in LA and CF towards the results that were observed in young rats. Thus it may be concluded that (a) aging-induced up regulation of brain regional Aβ in association with their reduction in carnosine content may be correlated with the neurodegeneration and down regulation of both LA and CF, (b) carnosine attenuated the above mentioned aging-induced changes in brain regulated behavior, possible by *in vivo* up regulation of antioxidant system.

Supported by DST-SERB, New Delhi, India and ICMR, New Delhi, India.

MTU09-07

The characterisation of oligodendrocytes derived from IPSC from als patients harbouring point mutations in the TDP-43 gene

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TDP-43 pathology is common to > 95% of ALS patients and has been identified in glia, including oligodendrocytes. Oligodendrocytes have two main roles in the brain, which are myelination and providing metabolic support and both are critical for maintaining neuronal health and function. The effect of TDP-43 pathology on oligodendrocyte function remains unknown. We derived oligodendrocytes from induced pluripotent stem cells (iPSC) from patients harbouring separate point mutations in the *TDP43* gene, namely G298S and M337V. We investigated the effect of these mutations on oligodendrocyte TDP-43 subcellular localization, cell morphology, and function. Using advanced CRISPR-Cas9 technology we generated an isogenic control line for comparison and also generated oligodendrocytes from an unrelated control. We assessed cellular development and morphology as well as metabolic capacity of diseased oligodendrocytes compared to controls using a 'disease in a dish' approach. For the first time, we demonstrated pathogenic TDP-43 protein mislocalization in iPSC-derived oligodendrocytes that was not present in the isogenic control or unrelated control. Despite this TDP-43 pathology, the oligodendrocytes did not have a developmental or morphological deficit and there was no effect of the *TDP43* mutations on the oligodendrocytes' metabolic capacity.

MTU09-08

Intranasal delivery of insulin for the restoration of memory signaling in Alzheimer disease

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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 like AD. Objective of study is to develop delivery-system to overcome BBB by employing novel, non-invasive approach via nasal route i.e. delivery of antibody appended Insulin encapsulated carrier, PEGylated nanoparticle coated with chitosan to facilitate nasal absorption for efficient transfer to brain. PEGylated-PLGA nanoparticles were prepared by modified Double Emulsification method and coated with chitosan by freeze drying. Characterization was done by FTIR, NMR and *in-vitro* parameters. *In-vivo* study comprised biodistribution in various organs and fluorescence microscopy, estimation of Anti-Aβ antibody, PET-Imaging of Brain, Hemolytic Toxicity studies, Histopathology of Nasal Mucosa and Brain with periodic Blood Glucose Level Monitoring. Degree of hemolysis showed PEGylated(PEG-NP's) and chitosan coated nanoparticles(cPEG-NP's) were less toxic. Blood glucose monitoring indicates reduction in blood glucose level in cPEG-NP's. Biodistribution assessment suggests nanoparticles showed maximum availability at olfactory bulb entrance. Chitosan coating increased CSF availability of drug even at initial period of administration. Uptake study shows intense fluorescence in brain revealing higher uptake of nanoparticles. These studies highlight possible biological significance of cPEG-NP's for delivery to brain. Results from various studies suggest nanoparticles are effective delivery system for targeted delivery of insulin in brain for extended period. Coating with chitosan elicits associated benefits in addition to prolonging uptake via intranasal route. This project may provide sound platform towards employment of this modified nanoparticulate carrier for brain delivery of proteins and peptides towards intranasal delivery of insulin for restoration of memory signaling in Alzheimer patients.

MTU09-09

Novel molecular-genetic probe for visualizing protein aggregation in neurodegenerative diseases by 3d electron microscopy

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We engineered a protein-fragment complementation assay for detecting and localizing protein aggregates associated with neurodegenerative diseases, including the homomeric aggregation of alpha-synuclein (αSyn) and tau proteins. This tool was built by bisection of miniSOG, a fluorescent flavoprotein derived from the light, oxygen, voltage (LOV)-2 domain of *Arabidopsis* phototropin. When brought together by interacting proteins, the fragments reconstitute a functional reporter that permits tagged protein complexes to be visualized by fluorescence light microscopy, and then by standard as well as "multicolor" electron microscopy (EM) via the photooxidation of 3-3'-diaminobenzidine and its derivatives. Unlike previous EM-compatible PCAs, the split-miniSOG fragments are relatively small, and display low affinity for each other, minimizing perturbation of the native dynamics of the tagged interacting partners. These experiments show the general utility of the system for detection of spatial organization of molecular complexes in mammalian cells at nanometer resolution. Using tagged monomers of αSyn, we carried out correlative analyses and observed the self-aggregation of the protein within neuronal cells for the first time. Applying 3D EM and electron tomography, we observed both straight and twisted αSyn filaments with a diameter ranging from 4 to 15 nm. In dendrites of expressing neurons, these aggregates were contained in membrane-limited organelles, fusing to the plasma membrane and suggesting release to the extracellular compartment. We further extended the split miniSOG proximity indicator/protein aggregation detection strategy to Tau proteins. This approach allows to clearly visualize how the aggregation occurs in the context of the intracellular milieu.

MTU09-10

Proteomic profiling of exosomes derived from brain microvascular endothelial cells under hypoxia: Potential role in remyelination

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Brain endothelium plays critical roles in the modulation of responses to injury, such as regulation of blood-brain barrier dynamics, neuroinflammatory processes, and reperfusion after stroke, and the molecular characterization of exosomes released by endothelial cells might enable the identification of adaptive signaling responses to stress. Here we profiled the protein content of brain microvascular endothelial cells (BMEC)-derived exosomes shed by primary BMEC harvested from adult rat brains that were subjected to 6 h of hypoxia followed by 18 h of recovery. We also charactrized exosomes released by BMEC cultured under normoxic conditions. Exosome molecular signatures and structure were confirmed by transmission electron microscopy, NanoTracking assays and the presence of exosome markers. Proteomic analyses were carried out with ESI-IMS-MS. We identified 262 high-quality hits with BMEC features such as molecules involved in focal adhesion, prostaglandin synthesis, and integrin signaling and molecular regulators that target recovery mechanisms after an injury to the CNS such as remyelination. We tested the potential of BMEC-derived exosomes to restore lisophosphatidylcholine-demyelinated corpus callosum in the rat, and we found that intracerebral injection of these extracellular vesicles improved the rate of remyelination as compared to non-treated animals. The proteomic characterization of extracellular vesicles released by BMEC under hypoxic stress enabled us to discover the participation of vascular endothelium in repairing processes like remyelination that could constitute new therapeutic targets to repair brain damage. This work was supported by DGAPA-PAPIIT grant IN226617.

MTU09-11

Agathisflavone binds to estrogen and retinoic receptors and drives remyelination in a demyelination-induced model

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Myelin dysfunction plays a significant role in the pathogenesis of various neurodegenerative diseases. Therefore, there is a critical need to develop more effective therapies for demyelinating disorders. Here, we have examined the protective effect of the flavonoid agathisflavone (FAB 5 and 10 μM) in the lysolecithin (LCT) model of demyelination, in mouse cerebellar slice organotypic culture. Treatment with LCT (0.5 mg/mL) resulted in significant demyelination, determined by loss of MBP immunostaining, and a significant increase in PLP1-DsRed+ mature oligodendrocytes and NG2 + oligodendrocyte progenitor cells (OPCs). Treatment with FAB significantly increased MBP immunostaining and the number and proliferation of OPCs. In addition, LCT induced astrogliosis (GFAP immunostaining) and microglial proliferation (IBA1 + Ki67 +), as well as a shift in microglia to a more inflammatory phenotype (M1/M2 ratio: CD16/32 + /CD206 +). These astroglial and microglial changes were reverted by FAB, which also reduced Tnfa, Il1b and Nos2 and increased Arg1, Tgfb and Acvr1b expression, as determined by RT-qPCR. Molecular docking analysis demonstrated intermolecular interactions between FAB and α/b-estrogen receptors (ERα/b), retinoic acid receptors (RAR), and α/γ retinoic X receptors (RXRα/γ), which are involved in myelination and neuronal survival. Furthermore, blockade of ERα reduced FAB-induced promotion of remyelination. Together these findings provide evidence that FAB has a significant protective effect against demyelination and implicates ER, RAR and RXR in these effects. Supported by CAPES/CNPq (SLC), BBSRC/MS (AMB), MS (FP).

MTU09-12

Hippocampus metabolic changes and memory impairment in mice under high fat-sucrose diet for 4 months are reversed by normal diet

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Type 2 diabetes (T2D) increases dementia risk through mechanisms that not fully understood. Brain metabolic dysregulation plays a role in T2D-induced memory dysfunction. We investigated the potential for reversibility of brain metabolic alterations and memory impairment in male and female C57BI6/J mice fed a high-fat and high-sucrose diet (HFHSD) that develop T2D. Age matched control mice were fed a 10%-fat diet (CD). Diabetic mice were exposed to HFHSD (60%-fat, plus 20% sucrose in drinking water) for 6 months. A group of mice under HDHSD for 4 months was reversed to CD for 2 months (RD). Metabolic profiles in hippocampus and cortex were measured longitudinally by magnetic resonance spectroscopy (MRS) at baseline, and after 4 and 6 months. Memory performance was assessed at 6 months using object relocation and novel object recognition tasks. HFHSD-fed mice developed overweight, glucose intolerance and insulin resistance, which recovered to control values after diet reversing (all $p < 0.01$, RD *vs*. HFHSD). HFHSD-fed mice showed poor memory performance in object recognition tasks (both $p < 0.001$ *vs.* CD), but not mice in RD. At 4 months of HFHSD, the hippocampal metabolic profile showed a prominent increment in taurine concentration ($p < 0.01$ *vs*. baseline). This change persisted at 6 months in HFHSD mice ($p < 0.01$ *vs*. baseline), but recovered to baseline levels in RD mice ($p < 0.05$ *vs*. HFHSD). Metabolic changes were not observed in cortex. We conclude that HFHSD leads to T2D and T2D-induced memory impairment, and increased hippocampal taurine concentration. T2D and brain alterations were all reversed upon dietary switch from HFHSD to a regular diet.

MTU09-13

Ascorbic acid augments nicotine neuromodulatory roles in transferrin-mediated cortico-hippocampal neuropathology in wistar rats

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Controlled activation of nAChRs in *invivo* model of neurodegeneration is a remarkable way of combatting molecular aberrations in Alzheimer's. Nicotine, being an allosteric modulator of these receptors, exacerbates production of reactive species thereby compromising its candidacy as a drug target for the management of Alzheimer's disease. We characterized the behavioral outcome and molecular fingerprints in transferrin-mediated neuroinflammation while exploring the potentials of nicotine in mitigating molecular aberrations in the presence of ascorbic acid,

Following due ethical approval, five groups (A-E) of Wistar rats (n = 8/group) were used for this study. Group A was treated with distilled water daily for 8 week. Transferrin-mediated neuroinflammation was achieved in groups B-E through daily oral infusion of 100 mg/kg of $AlCl_3$ for four weeks. Groups C-E were then post treated with ascorbic acid (100 mg/kg daily), nicotine (10 mg/kg daily) and nicotine (10 mg/kg daily) + ascorbic acid (100 mg/kg daily), for four weeks. Following behavioral assessments, prefrontal cortex (PFC) and hippocampus were prepared for biochemical analyses, histology and immunohistochemistry.

Nicotine+ascorbic acid significantly reversed reduction of/working memory, cognitive decline and special memory dysfunction. These correlated with nicotine-dependent modulation of TfP-1 expression that was complemented by significant reversal of neural oxidative and nitrosative stress by antioxidant properties of ascorbic acid. Furthermore, nicotine+ascorbic acid treatment regimen further inhibited neural dysfunction within PFC and hippocampus correlating with increased glucose-6-phosphate dehydrogenase. Nissl staining and immunohistochemical profiling of thin sections corroborated roles of nicotine+ascorbic acid in reversing AlCl3-induced neuropathology.

Summarily, we have showed the role of ascorbic acid in enhancing nicotine neuromodulatory activities in transferrin-mediated behavioral decline and molecular aberration in the frontal cortex and hippocampus of Wistar rats.

MTU09-14

Cholinergic regulation of plaque pathology in Alzheimer's disease knock-in mouse models

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Cholinergic deficiency is characteristic of many neurodegenerative disorders including Alzheimer's disease (AD). Decreased levels of the vesicular acetylcholine transporter (VAChT) have been detected in AD patients, and previous work suggested that cholinergic deficiency increase AD-like pathology in mouse models. In humans, plaque pathology has been linked to the loss of VAChT; however, whether changes in VAChT have a causal relationship with plaque accumulation is unknown. To study this aspect of AD, we crossed a humanized APP-knock-in mouse carrying 3 AD-associated mutations ($App^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$) with mice overexpressing VAChT using a BAC transgene. We analyzed the number and area populated by Aβ-plaques in the cortex, as well as Iba1 marker (for microglia) and GFAP (for reactive astrocytes). Our preliminary results show a significant decrease in the number of reactive astrocytes and the number of microglia associated with plaques at 2 months of age. Likewise, cortical plaque area was significantly decreased at 2 months, but not at 3 or 6 months. Remarkably, we observed a sharp decrease in the levels of VAChT in $App^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$-VAChT-BAC mice at 6 months, effectively reducing the overexpression of VAChT. Accordingly, $App^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice presented age-decreased VAChT levels at 3 and 6 months when compared to 2-months-old. Moreover, elimination of cortical VAChT increased the number of plaques in $App^{NL\text{-}F/NL\text{-}F}$ mice, a humanized model with less aggressive pathology. These results suggest a causal relationship between cholinergic tone and plaque accumulation in a humanized AD mouse model and that amyloid plaques can interfere with cholinergic tone by decreasing VAChT levels.

MTU09-15

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

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Globally, environmental and occupational exposures to heavy metals are an increasing health concern. Nickel (Ni) is one of such metals and has extensive industrial applications. Importantly there is no known physiological role for Ni in humans and other mammals. Brain damage has been severally implicated in Ni overexposure however, published reports are relatively limited. Here, we investigated specific neuronal susceptibility in a *C. elegans* model of acute nickel neurotoxicity. Wild-type *C. elegans* and worms expressing GFP in several neuronal subtypes were treated with $NiCl_2$ at the first larval (L1) stage. Our results show significantly increasing degeneration of cholinergic, dopaminergic and GABAergic neurons with increasing Ni concentration in worms expressing GFP for these neuronal subtypes. Also, significant functional changes in locomotion and basal slowing response assays reflected impaired cholinergic and dopaminergic neuronal function respectively. Interestingly, a significant effect on number of worms exhibiting shrinker phenotype indicated that function of D-type GABAergic neurons of *C elegans* may be specifically attenuated while the RME subset of GABAergic neurons is unaffected. GFP expression due to induction of glutathione S-transferase 4 (*gst-4*), a target of Nrf2 homolog *skn-1*, was increased in VP596 (Pgst-4::GFP; Pdop-3::RFP) worms highlighting increased SKN-1 activity and consequently, Ni-induced oxidative stress. RT-qPCR verified upregulation of this expression of *skn-1* immediately after exposure. These data suggest that developmental Ni exposure impairs cholinergic, dopaminergic and GABAergic neurotransmitter systems, probably via the generation of oxidative stress. Further studies are ongoing to unravel molecular mechanisms involved in Ni neurotoxicity using *C. elegans* model.

MTU09-16

Isolation and neuroprotective effect of ethyl acetate fraction of terminalia macroptera leaf

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Neurodegeneration is a process involved in both neuropathological conditions and brain ageing. It is known that brain pathology in the form of cerebrovascular and neurodegenerative disease is a leading cause of death all over the world. No effective treatment for these neurodegenerative diseases has been developed yet. Oxidative stress-mediated neurodegeneration is one of the key pathophysiological factors involved in these diseases. This study is aimed at investigating the neuroprotective effect of the ethylacetate fraction of *Terminalia macroptera* leaf and its isolates on SH-SY5Y neuronal cells. The ethylacetate fraction of *T. macroptera* leaf was subjected to solid phase extraction and preparative high-performance liquid chromatography (HPLC) to yield several polyphenolic compounds. We examine the neuroprotective effects of the ethylacetate fraction and the polyphenols isolated from it against hydrogen peroxide (H_2O_2)-induced cytotoxicity in SH-SY5Y cells. The results revealed that the ethylacetate fraction of *T. macroptera* showed the most favorable antioxidant activity in scavenging free radicals compared to the isolates such as Gallic acid, chebulagic acid, Quercetin-3-O-glucoside, chebulinic acid, vitexin, and ellagic acid. Component elucidation revealed that the ethylacetate fraction of *T. macroptera* is a rich source of phenolic compounds, especially flavonoids and tannins. The ethylacetate fraction of *T. macroptera* has the potential to be a novel neuroprotective agent to be considered in nutraceutical products for preventing oxidative-related disorders. Further investigation is necessary to verify the neuroprotective efficacy and mechanisms *in vivo*.

MTU09-17

Cnestis ferruginea ameliorates kainic acid-induced status epilepticus in rats: role of neuroinflammation and oxidative stress

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We have earlier reported anticonvulsant of *Cnestis ferruginea* root extract (CF), hence, this study aimed to investigate the mechanism underlying the protective effects of CF on kainic acid (KA)-induced excitotoxicity in the rat hippocampus. Intraperitoneal injection of KA (10 mg/kg) caused seizures and increased the expression of neurotoxic markers, immediate early genes such as cyclooxygenase 2 (COX-2), *cfos*, brain-derived neurotrophic factor (BDNF), and heat shock protein 70 (hsp70) and a delayed response gene (inducible nitric oxide synthase (iNOS)), which were measured at 6 and 72 h after KA injection, respectively, in the hippocampus. Pretreatment or post-treatment of mice with CF (400 mg/kg, p.o.) delayed the onset of KA-induced seizure as well as reduction in seizures score. KA increased c-Fos (transynaptic marker for neuronal activity) immunoreactivity in DG, CA1, and CA3 hippocampal regions which was attenuated by pre- and post-treatment of mice with CF. Moreover, CF treatments reduced KA-induced expression of COX-2, BDNF, and iNOS mRNA. Intraperitoneal injection of KA produced significant deficit in the antioxidant enzyme activities (GSH, superoxide dismutase (SOD) and catalase) when compared with the vehicle. However, pre- treatment and reversal with CF 400 mg/kg produced a significant enhancement of antioxidant enzyme activities suggestive of radical scavenging effect. Findings from this study showed that CF treatment suppresses KA-induced hippocampal injury through attenuation of excitotoxicity, neuroinflammation and enhancement of antioxidant defense mechanisms. Thus, suggest the beneficial effects of CF on the treatment of excitotoxicity-induced status epilepticus.

MTU09-18

Biochemical and behavioral evidence for neuromodulatory properties of ellagic acid against D-galactose neurotoxicity in mice

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Introduction: Ellagic Acid is a non-flavonoid polyphenolic compound found in different berries, mango, areca nut, walnut, green tea, and other fruits, food, and beverages as well as in wine. It has been reported to show different biological activities such as free radical scavenger, antidiabetic, antiangiogenic and antimelanogenic effects, and reduced heart infarction incidence and oxidative liver and kidney damage.

Objective: The aim of the present study was to investigate the protective effects of oral Ellagic acid against motor dysfunction, striatal oxidative stress and mitochondrial deficit induced by **D-galactose** in mice.

Methods: Male mice were divided into different treatment groups. D-galactose (100 mg/kg *s.c.*) was given for 42 days to induced neurodegeneration. Chronic (6 weeks) oral administration of Ellagic acid was given at three different doses (50, 100, 200 mg/kg) to find out neuroprotective effect. The neuroprotective effect was evaluated in term of behavioral (learning, memory and motor coordination) and biochemical (Lipid peroxidation, Nitrite (NO) level, AChE activity, Advanced glycation end products). Antioxidant enzyme estimations were also performed on, reduced glutathione (GSH), Superoxide dismutase (SOD), Catalase (CAT) activity. Mitochondrial Complex activity was performed on mice brain mitochondria for Complex-I (NADH dehydrogenase), Complex-II (succinate dehydrogenase activity) and Complex-III (MTT ability) activity.

Results: Chronic (6 weeks) oral administration of Ellagic acid at three different doses (50, 100, 200 mg/kg) was found to provide a significant neuroprotective effect in a dose-dependent manner in terms of reversing behavioral, biochemical, anti-oxidant and mitochondrial damage induced by D-galactose.

Conclusion: The results suggest that Ellagic acid has neuroprotective activity against D-galactose induced neurodegeneration. The present findings provide a rationale for the uses of Ellagic acid in neurodegenerative disorders.

MTU09-19

Neuroprotective effects of kynurenic acid analogue against secondary cascades of traumatic brain injury in mice

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Traumatic brain injury (TBI) is a major cause of fatality and disability across worldwide. Apart from, acute injury, secondary injury cascades such as oxidative stress, mitochondrial dysfunction, excitotoxicity, neuronal cell death etc. are detrimental factors for the life-long disability. Despite many efforts, limited progress has been made towards the development of pharmacological interventions to reduce the effects of TBI. The neuroprotective effects of Kynurenic acid are counterbalanced by 3-hydroxyanthranilic acid which is another neurotoxic metabolite of indoleamine-2,3-dioxygenase pathway. Here, we hypothesize that secondary injury cascade can be rescued by elevating neuroprotection *via* kynurenic acid. In current study, we had investigated the effect of kynurenic acid amide analogue (KAA; BBB permeable) against the secondary cascade after TBI in mice.Our data showed significant increase in mitochondrial dysfunction at 6 h and which persisted even after 72 h of injury. However, cell death of cortical neurons was evident after 24 hours post-injury. Animals (Swiss albino mice, 25-30 g) administered with Kynurenic acid amide analog (KAA, blood brain barrier permeable, NMDA receptor antagonist) (100, 200 and 400 mg/kg, i.p) 30 minutes after injury showed a dose dependent neuroprotective effect on mitochondrial dysfunction (complex-I, II & IV activities), oxidative stress. KAA also improved neuronal survival and neurological function significantly. KAA (100 mg/kg) were found effective at on long-term treatment (i.e. 72 hr to 21 days' time point). Overall,our data shows that KAA promotes neuroprotection against TBI-induced secondary cascade and improve neurological functions in mice model of TBI.

MTU09-20

Neuregulin 1 deficiency in dorsal root ganglia and dorsal roots in friedreich ataxia

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Friedreich ataxia (FA) is an autosomal recessive ataxia that in the vast majority of cases is due a homozygous guanine-adenine-adenine (GAA) trinucleotide repeat expansion in intron 1 of the frataxin gene. The complex pathological phenotype includes atrophy of the dentate nucleus, hypoplasia and inflammatory destruction of dorsal root ganglia (DRG), spinal cord hypoplasia and degeneration of dorsal spinal columns and dorsal spinocerebellar tracts, myelin deficiency in dorsal roots (DR) and sensory peripheral nerves, concentric cardiac hypertrophy or dilated cardiomyopathy, and destruction of pancreatic beta cells. Neuregulin 1 type III (NRG1 [III]) is a critical signaling protein for the myelination of dorsal roots and sensory peripheral nerves. Systematic immunohistochemical visualization of NRG1 confirmed paucity and smallness of NRG1-reactive DR axons, and a severe lack of myelination. NRG1 (III) signaling to Schwann cells occurs by binding to ErbB2-ErbB3 heteromers. An antibody to ErbB2 revealed abundant reaction product in DR of FA. Ventral spinal roots in FA displayed normal large NRG1 (III)-reactive axons, myelinated fibers, and abundant ErbB2 in Schwann cells. We conclude that lack of myelination in DR in FA is due to insufficient NRG1 (III) in DRG neurons and downstream fibers. The role of frataxin deficiency in this mechanism is unknown.

Supported by Friedreich's Ataxia Research Alliance.

MTU09-21

Lead aggravates the diabetic-induced neurodegeneration and neuro-protecting effect of C. carandas

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Diabetes, an unresolved metabolic disorder and lead contamination are prevalent problems in contemporary society. Previously, reported suggest that either diabetes or lead exposure resulted in neurodegeneration in male rats. The aim of this study was to evaluate whether diabetic rats exposed to lead demonstrate a higher degree of neurotoxicity, inflammation and neurodegeneration when compared with lead-exposed control rats. And the neuroprotective, anti-inflammatory and antioxidant activity of *C. carandas* constituents were also evaluated. Diabetes was induced by injecting a single dose of streptozotocin (40 mg/kg body weight). Control and diabetic rats were exposed to lead through oral gavage for a period of 21 days and assessed for neuro-inflammatory markers and oxidative endpoints. Significant reduction in brain antioxidant enzyme activity, membrane proteins and ion channels including Acetylcholinesterase, Na^+-K^+ ATPase levels and glutathione levels were observed in diabetic rats with an elevation in levels of superoxide anions, hydrogen peroxides, lipid peroxidation. Mild histopathological malformations were observed in the brain of the diabetic rats. TNF-α, IL-6 transcripts and nuclear factor-κB expression were increased in the diabetic rat brain. Similar oxidative and neurotoxicity was observed in lead-exposed control rats. Further, lead-exposed diabetic rats showed additional deterioration in hippocampal and inflammation end points and noteworthy elevation in oxidative toxicity suggesting that treatment with lead exacerbates neurotoxicity in streptozotocin-induced diabetic rats. The ameliorative efficacy of the aqueous extract of C. Carandas was analysed in diabetic rat exposed to lead. The study shows the significant neuroprotective and anti-inflammatory activity of the extract.

MTU09-22

HSP90 co-chaperone stress inducible phosphoprotein-1 is necessary for chaperone activity and neuronal resilience during aging

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Stress inducible phosphoprotein 1 (STI1) is a co-chaperone of the Hsp70-Hsp90 machinery and can be secreted by cells such as astrocytes. In the extracellular space, STI1 interaction with prion protein (PrP^C) results in pro-survival signaling and neurotrophic effects. Deletion of STI1 in mice is lethal and STI1 haplo-sufficient neurons are less resilient to stress. Recent *in vitro* work has implicated Hsp90 in regulating cellular senescence, a phenotype seen in aging. It remains unknown how reduced STI1 levels affects chaperone machinery function *in vivo* and cellular resilience during aging. To overcome the difficulty of studying STI1 *in vivo* due to embryonic lethality of STI1 KO mice, we generated a mouse line with a hypomorphic *Stip1* allele that produces a partially functional protein (reduced by 80%). These mutant mice have a significant decrease in Hsp90 client proteins and a subset of Hsp90 co-chaperones, suggesting that STI1 acts as a major regulatory node. Moreover, they present age-dependent hippocampal neuronal loss, and consequent memory deficits in the Morris water maze. To determine whether STI1 is required for normal aging in a cell autonomous or non-cell autonomous way we are currently generating neuronal and astrocyte selective STI1 mutant mice. Preliminary results from aged mice with STI1 knocked down in astrocytes revealed no memory impairment or hippocampal neuronal loss. Overall, our work will test whether STI1 is important for maintaining brain cell viability during aging and how its function in the chaperone machinery may maintain proteostasis.

MTU09-23

Physical exercise during pregnancy prevents cognitive impairment induced by amyloidβ in adult offspring rats

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Alzheimer's disease (AD) is the main aging-associated neurodegenerative disorder and is characterized by mitochondrial dysfunction, oxidative stress, synaptic failure, and cognitive decline. It has been a challenge to find disease course-modifying treatments. However, several studies demonstrated that regular physical activity and exercise are capable of promoting brain health by improving the cognitive function. Maternal lifestyle, including regular exercise during pregnancy, has also been shown to influence fetal development and disease susceptibility in adulthood through fetal metabolism programming. Here, we investigated the potential neuroprotective role of regular maternal swimming, before and during pregnancy, against amyloid-β neurotoxicity in the adult offspring. Behavioral and neurochemical analyses were performed 14 days after male offspring received a single, bilateral, intracerebroventricular (icv) injection of amyloid-β oligomers (AβOs). AβOs-injected rats of the sedentary maternal group exhibited learning and memory deficits, along with reduced synaptophysin, brain-derived neurotrophic factor (BDNF) levels, and alterations of mitochondrial function. Strikingly, the offspring of the sedentary maternal group had AβOs-induced behavioral alterations that were prevented by maternal exercise. This effect was accompanied by preventing the alteration of synaptophysin levels in the offspring of exercised dams. Additionally, offspring of the maternal exercise group exhibited an augmentation of functional mitochondria, as indicated by increases in mitochondrial mass and membrane potential, α-ketoglutarate dehydrogenase, and cytochrome c oxidase enzymes activities. Moreover, maternal exercise during pregnancy induced long-lasting modulation of fusion and fission proteins, Mfn1 and Drp1, respectively. Overall, our data demonstrates a potential protective effect of exercise during pregnancy against AβOs-induced neurotoxicity in the adult offspring brain, by mitigating the neurodegenerative process triggered by Alzheimer-associated AβOs through programming the brain metabolism.

MTU09-24

Effect of melatonin on methamphetamine (meth)induced alteration of app cleaving enzymes related to Alzheimer's disease

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Methamphetamine (METH) is an addictive drug, which has been found to cause neurotoxicity to central nervous system through multiple mechanisms. METH abusers are likely to develop Alzheimer's disease (AD) and suffer cognitive disabilities as well as alterations in brain chemistry as the pathological cascade observed in AD brains, however, the underlying mechanism of METH-induced Alzheimer's disease remains unknown. Melatonin, hormone is mainly produced by pineal gland plays critical rule in physiological function and brain protective effect. In this study we aimed to investigate the effect of METH on amyloid precursor protein (APP) cleaving enzymes and its upstream pathway in Alzheimer's disease pathway and to determine the protective effect of melatonin on METH-induced Alzheimer's disease. SH-SY5Y cell lines and Male *Wistar* rats are used to investigate the effect of METH on APP cleaving enzymes and upstream pathway likes GSK3-β. Our results showed that METH significantly increased the β- and γ-secretase, amyloidogenic protein marker. On the contrary METH decreased the α-secretase, non-amyloidogenic biomarker protein. These effects of METH are prevented by pretreatment with melatonin. The result in this study suggested that METH induced the production of amyloid peptide and melatonin exerted it protective effect on METH-induced Alzheimer's disease pathway.

MTU09-25

Assessment of the mechanism of actions of bacopa floribunda on amyloid beta 1-42-induced Alzheimer's disease in male wistar rats

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This study aimed at assessing the effects of *Bacopa Floribunda* (BF) leaves' extracts on hippocampal changes Amyloid beta 1-42 (Aβ)-induced Alzheimer's disease. A single bilateral dose (4 μg/μl/site) of Aβ (1-42) was injected into the lateral ventricles using a stereotaxic apparatus while BF (200 mg/kg) was given orally for 21 days. Forty eight (48) adult male Wistar rats (170-220 g) were randomly divided into eight groups (n = 6). Group A received intracerebroventricular (ICV) injection of normal saline, Group B received ICV injection of Aβ alone, Groups C and D received only Ethanolic and Aqueous extracts of BF respectively, Groups E and F were post-treated with Ethanolic and Aqueous extracts of BF respectively after receiving ICV injection of Aβ, Groups G and H were pretreated with Ethanolic and Aqueous extracts of BF before the Aβ ICV injection. Rats were subjected to Y-maze and Novel Object Recognition tests and sacrificed by cervical dislocation. Twenty-four hours after the last administration, rats were sacrificed and brain tissues excised. The hippocampus was removed and some were assayed for the levels of glutamate, acetylcholinesterase, Na^+ - k^+ ATPase activities and Amyloid beta deposition using ELISA kits, while the rest were processed for histology using Hematoxylin & Eosin (H&E) and nissl body stain. Data were analyzed using One-way ANOVA followed by a post-hoc test and expressed as Mean ± SEM. Results showed that BF was able to reverse some perturbations caused by Aβ (1-42)-induced Alzheimer's disease as evident in changes in the hippocampal levels of acetylcholinesterase, Na^+ - k^+ ATPase and glutamate in the different treatment groups.

MTU09-27

Ameliorative potentials of bryophyllum pinnatum on kianic acid induced temporal lobe epilepsy in models

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Bryophyllum pinnatum is reportedly used in managing epilepsy in African folk medicine, albeit without significant empirical or experimental evidences. Effects of aqueous leaf extract of *bryophyllum pinnatum* (AEBP) on the kainic acid induced epileptic activities in the temporal lobes was studied using Wistar rats. Twenty-five (n = 25) adult male Wistars (average weight = 135 g) which were randomly divided into 5 groups. Group A was the control group, and only fed *ad libitum*; Group B was administered Kainic acid only to induce epilepsy; Group C was first administered kainic acid to induce epilepsy and thereafter *bryophyllum pinnatum* (300 mg/kg/day) to observe its ameliorative properties; Group D was administered ketogenic diet (1 kcal/ml/day) after inducing epilepsy with kainic acid and Group E was administered the anti-epileptic carbamazepine (100 mg/kg/day) following initial administration of kainic acid to induce epilepsy. Epilepsy was induced with 10 mg/kg of kainic acid in every instance and confirmed and observed using the Racine scale. After treatment for 21 days, behavioural observations on memory and cognition were carried out using the Barnes mazes. Animals were sacrificed, and the temporal lobe cortex and hippocampus tissues were processed for histological and immunohistochemistry studies using the hematoxylin and eosin, Nissl stain and glia acidic fibrillary acid proteins techniques. Neurotransmitters- glutamate, serotonin and dopamine activities were assayed in brain homogenates. Histological and histochemical evidences on neurons and astrocyte morphologies and special distribution, astrocyte reactions, cortical histological integrity, neurotransmitters activities showed that *bryophyllum pinnatum* had potentials to ameliorate epilepsy and the effects with significant relative to carbamazepine and ketogenic diet. It also significantly ameliorated behavioural aberrations attributable to epilepsy [$p \leq 0.05$]. This plant's aniti-epilepsy potentials should be explored further.

MTU09-28

Clofibrate, A PPAR-α agonist mitigated sodium fluoride-induced neuro-inflammation, oxidative stress and motor incoordination

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Fluoride is an environmental contaminant that is present in air, water and soil. It is commonly added in minute quantity to drinking water, toothpaste, and mouth rinses to prevent tooth decay. Epidemiological findings have demonstrated that exposure to fluoride induced neurodevelopmental toxicity, developmental neurotoxicity and motor disorders. The neuroprotective effect of clofibrate, a Peroxisome Proliferator-Activated Receptor alpha (PPAR-α) agonist was investigated in the present study. Forty-male Wistar rats were used for this study and were randomly grouped into ten rats per group as control, sodium fluoride (NaF) alone (300 ppm), NaF plus Clofibrate (250 mg/kg) and sodium fluoride plus Lisinopril (10 mg/kg), respectively, for seven days. Sodium fluoride was administered in drinking water while Clofibrate and Lisinopril was administered by oral gavage. Markers of neuronal inflammation and oxidative stress, acetylcholinesterase (AchE) activity and neurobehavioral (Hanging wire and Open Field) tests were performed. Immunohistochemistry was performed on brain tissues and were probed with Glial fibrillary acidic protein (GFAP), Ionized calcium binding adaptor molecule 1 (Iba1) and cerebellar Ca^{2+} binding protein calbindin D-28k (CB). The results showed that NaF significantly increased makers of oxidative stress, neuro-inflammation and inhibited AchE activity. Immuno-staining revealed reactive astrocytes, microgliosis, loss of dendritic spines and arborisation in Purkinje cells in rats administered only NaF. Neurobehavioral results showed that co-treatment of NaF with Clofibrate improved muscular strength, locomotion, reduced anxiety as well as significant reduction in astrocytic count. Altogether, co-treatment of NaF with either Clofibrate or Lisinopril demonstrated neuroprotective effect by mitigating neuronal inflammation, oxidative and motor incoordination. Hence, Clofibrate is a novel drug candidate against neurodegeneration and motor disorders.

MTU09-29

Norvaline, a novel Alzheimer's disease-modifying agent

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Alzheimer's disease (AD) is an irredeemable chronic neurodegenerative disorder and the predominant cause of dementia. The disease progression is associated with amyloid plaques' deposition and neurofibrillary tangles' formation in the brain, yet clinical dementia is the end stage of the enduring pathology. Recent evidence points to severe characteristic metabolic dysfunction as a leading cause and hallmark of AD that is apparent decades prior to the disease manifestation. State-of-the-art metabolomics studies prove that complex arginine and branched-chain amino acids (BCAAs) metabolism disturbances accompany AD. Lower plasma valine levels are associated with accelerated cognitive decline, and, conversely, an increase in valine concentration is associated with reduced risk of AD.

We administered an arginase inhibitor norvaline, which is an uncommon non-proteinogenic BCAA and a valine's isoform, chronically to a mouse model of AD. A set of immunohistochemistry, proteomics, and transcriptomics assays was applied to evaluate the neuroprotective effect of the substance and identify the biological pathways activated by the treatment.

The results verify that norvaline reverses the cognitive decline in the AD mice. The neuroprotective effect is associated with significantly reduced hippocampal arginase levels and diminished amyloidosis. Moreover, the treatment moderates the rate of Tau protein phosphorylation, alleviates microgliosis and apoptosis. Additionally, we disclose the treatment-associated increase in the hippocampal expression levels of synaptic plasticity-related proteins, expression levels of cytosolic branched-chain amino acid aminotransferase, and an activation of several, involved in cell survival and neuroplasticity, biological pathways.

The data suggest that norvaline is a potent arginase inhibitor and modulator of glutamate metabolism. The substance possesses various modes of action, which improve the symptoms of AD and even interfere with its pathogenesis. Therefore, norvaline presents a promising neuroprotective molecule with manifold biological potentials that might be tailored for the treatment of a range of neurodegenerative disorders.

MTU09-30

Antibody-based therapeutic approach to target TDP-43 proteinopathy

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TAR DNA-binding protein 43 (TDP-43) is a DNA/RNA binding protein mainly localized in the nucleus of cells. In pathological conditions, TDP-43 mislocalizes and aggregates in neuronal cytoplasm forming hyperphosphorylated, fragmented and ubiquitinated inclusions which impair its physiological functions. This condition is called TDP-43 proteinopathy and can be primarily observed in amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD) cases. TDP-43 has two RNA-recognition domains (RRM1 and RRM2) both involved in relevant aspects of the protein function. Different studies had highlighted the involvement of the RRM1domain in TDP-43 proteinopathy. Through the RRM1 domain, indeed, TDP-43 can interact with p65, the main subunit of NF-KB, inducing a hyperactivation of the factor which ultimately lead to increased neuroinflammation and neuron toxicity. Moreover, the RRM1 domain is a sensitive target for detrimental effects, such as oxidation and misfolding, that eventually induce TDP-43 proteinopathy. We generated a monoclonal antibody against the RRM1 domain of TDP-43 with the aim of reducing pathological events mediated by this protein portion. Viral-mediated delivery into the CNS of a single chain antibody, derived from the antigen binding fragment of the monoclonal antibody, in mutant TDP-43 mice was found to reduce cognitive and motor impairments as well as to decrease TDP-43 cytoplasmic mislocalization, aggregation and neuroinflammation. These observations support the feasibility of an immunotherapeutic approach to mitigate TDP-43 pathology in ALS and FTD.

MTU09-31

Pioglitazone reversed hippocampal insulin resistance in an amyloid-beta fibrils induced animal model of Alzheimer's disease

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Backround: Complications of Alzheimer's disease (AD) have made the development of its therapeutic intervention quite a challenging task. Numerous studies have supported the hypothesis that central insulin resistance plays a significant role in AD. Serine phosphorylation of Insulin Receptor Substrate-1 (IRS-1) has been found to be a contributing factor in neuronal insulin resistance. Pioglitazone (PIO) is a peroxisome proliferator-activated receptor gamma (PPAR-γ) activator, known for its significant antidiabetic functions, has also demonstrated neuroprotective actions.

Methods: In the present study, AD was induced by i.c.v administration of Amyloid-β (1-42) fibrils in Wistar rats. After 7 days of recovery, rats were treated with 10 mg/kg and 20 mg/kg of PIO orally for 28 days. Behavioral analysis was done in the last week of our experimental study. On the 36th day, rats were sacrificed and their hippocampus was separated from the whole brain, then homogenized and stored for biochemical estimations.

Results: PIO significantly reversed the cognitive and memory impairment, as assessed by the Morris water maze test, in Aβ (1-42) fibrils infused Wistar rats. PIO also significantly attenuated Aβ (1-42) level, IRS-S307 activity, GSK-3β activity, TNF-α level, AChE level, nitrite level and oxidative stress in the hippocampus. Histopathological evaluation, done through H&E and Congo red staining, also demonstrated neuroprotective and anti-amyloidogenic effects of PIO in the hippocampus.

Discussions: Our study concludes the protective action of pioglitazone against hippocampal insulin resistance and Alzheimer's disease complications, supporting the potential role of hippocampal insulin resistance targeting against the AD.

MTU09-32

Stress-induced inhibition of 82-KDA choline acetyltransferase nuclear translocation

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Decreased function of basal forebrain cholinergic neurons (BFCNs) in Alzheimer's disease (AD) causes cognitive deficits. Choline acetyltransferase (ChAT) is a key enzyme in cholinergic neurons that synthesizes acetylcholine (ACh). The M-ChAT transcript of human ChAT gene encodes both cytoplasmic 69- and nuclear 82-kDa isoforms of ChAT. We reported that expression of 82-kDa ChAT in neural cell nuclei alters expression of several genes, including some involved in regulation of APP metabolism. Necropsy human brain shows nuclear localization of 82-kDa ChAT in cholinergic neurons, with subcellular distribution changing in aging and mild cognitive impairment and AD with reduced levels of 82-kDa ChAT in nucleus and increased levels in cytoplasm. Being a cysteine-rich protein, 82-kDa ChAT is susceptible to cellular and oxidative stress. Our studies reveal that exposure of SH-SY5Y neural cells to beta-amyloid or oxidative stress reduces 82-kDa ChAT levels in nucleus. We have also characterized homodimerization of 82-kDa ChAT through bimolecular fluorescence complementation assay. Our results show nuclear localization of 82-kDa ChAT homodimers in control neural cells, whereas stressed cells exhibit perinuclear aggregate formation, which may lead to the observed decrease in nuclear localization. Due to primate-specific expression of 82-kDa ChAT and inaccessibility of human brain neurons, we produced BFCNs from human induced pluripotent stem cells (hiPSCs) as a model. We verified differentiation of iPSCs to BFCNs by monitoring expression of cholinergic phenotypic markers and functional parameters, such as ChAT, choline uptake and ACh synthesis. Importantly, 82-kDa ChAT is expressed in these BFCNs and located in nuclei. Taken together, we have demonstrated a possible mechanism for modulation of nuclear translocation of 82-kDa ChAT in stressed cells and developed a model for study of 82-kDa ChAT in human neurons.

MTU09-33

Cassia tora reverses Aβ1-42 aggregation *in vitro* and conveys multiple neuroprotective effects in aluminium-induced ad rats

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Alzheimer's disease is a progressive neurodegenerative disorder characterized by the presence of neuritic plaques and neurofibrillary tangles and its multifactorial nature calls for multi-target-directed approaches for therapeutic treatment. This study is firstly aimed at determining the effects of Cassia tora methanolic fraction (MECT) on Aβ1-42 aggregation *in vitro*. Secondly, it evaluates the effects on aluminium-induced neurobehavioral and neuropathological changes *in vivo* in rats. MECT was prepared and tested for its ability to prevent and/or reverse Aβ1-42 aggregation by measuring thioflavin-T fluorescence and by transmission electron microscopy. For *in vivo* experiments, AlCl3 was administered orally for 60 consecutive days in the absence or in the presence of MECT. Behavioural were employed for neurobehavioral assessment. Then, biochemical assays measuring acetylcholinesterase activity and oxidative stress as well as examination of the expression levels of pro-inflammatory cytokines and BDNF in the hippocampus and frontal cortex were performed. Finally, histopathological assessment of neuronal health in the CA1 and CA3 regions of the hippocampus by cresyl violet staining was carried out. MECT inhibits Aβ1-42 aggregation from monomers and oligomers and disintegrates pre-formed Aβ1-42 fibrils. Moreover, MECT dose-dependently improves the cognitive and behavioural impairments observed in aluminium-treated rats. Furthermore, the extract alleviates AChE hyperactivity as well as oxidative stress and inflammation observed in the hippocampus and the cerebral cortex of aluminium-treated animals Finally, MECT precludes aluminium-induced neuronal collapse. Our study reveals that the methanolic extract of Cassia tora is able to prevent most of the AD-related events and therefore stands as a promising mild and natural anti-AD multi-target compound.

MTU09-34

Rage inhibition reduced neuroinflammation and dopaminergic neurodegeneration in a long-term response to LPS systemic inflammation

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Neuroinflammation is one of the major contributors to the progressive loss of dopaminergic (DA) neurons in Parkinson's disease (PD). The receptor for advanced glycation endproducts (RAGE) has been demonstrated as an important mediator of neurodegeneration triggered by inflammation. In this study, we investigated the effect of RAGE inhibition in a long-term response to systemic inflammation in Wistar rats induced by a single dose of lipopolysaccharide (LPS, 5 mg/kg, i.p.). The multimodal RAGE blocker, FPS-ZM1, was administrated to selectively inhibit RAGE either intraperitoneally (1 mg/kg, i.p.) one hour before LPS injection or intracranially (40 μg per rat, i.n.) two months after LPS injection. Immunostaining of Iba-1 and GFAP demonstrated that LPS modulates microglia and astrocyte in the substantia nigra (SN) 15 days, 30 days, 6 months and 10 months after injection. By contrast, 10 months after LPS injection both FPS-ZM1 administrations reduced glial activation, suggesting that RAGE mediates the neuroinflammation resulted from systemic stimulus. In addition, immunostaining of RAGE and TH demonstrated a progressive increase of RAGE in the SN, which is accompanied by DA neurons loss. FPS-ZM1-induced RAGE inhibition also reduced DA neurons loss, showing a neuroprotective effect of FPS-ZM1 against LPS insult. Overall, our results indicate RAGE as mediator of neuroinflammation and DA neurodegeneration triggered by LPS systemic inflammation. Therefore, RAGE inhibition has a potential application in neuroprotective therapies for PD and associated disorders.

MTU09-35

Anthranilate sulfonamides attenuate oxidative stress in human neuronal cells

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Oxidative stress is associated with neuronal damage which is considered to be a risk factor for pathogenesis and development of neurodegenerative diseases (NDs) including Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and others. Thus, antioxidant therapy is a plausible strategy to delay NDs progression. Recently, anthranilic acid and sulfonamides have been reported to possess anti-inflammatory and antimicrobial activities. However, the underlying molecular mechanisms of anthranilic sulfonamide hybrids against oxidative damage have not been fully elucidated. In this study, hybrids of anthranilic sulfonamide incorporating benzenesulfonyl chlorides and anthranilic acid were synthesized, and neuroprotection properties of the compounds against H_2O_2-induced oxidative stress in neuronal cells were also investigated by using MTT assay, carboxy-H_2DCFDA assay, flow cytometry, and western blotting. Both cell viability and reactive oxygen species (ROS) assays showed that pretreatment with anthranilate sulfonamides effectively attenuated H_2O_2-stimulated cytotoxicity and ROS production. Surprisingly, these synthesized compounds promoted antiapoptotic protein (BCL-2) and triggered Sirtuin (SIRT1) signaling pathways in human neuronal cells. Moreover, the binding interaction of anthranilate sulfonamides to SIRT1 protein targets is elucidated and characterized by an *in silico* molecular docking. Taken together, anthranilate sulfonamides might act as sirtuin-activating compounds which are novel therapeutic candidates for NDs.

MTU09-36

Chlorogenic acid protects against MPTP induced neurotoxicity in parkinsonian mice model via its anti-apoptotic activity

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Parkinson's disease (PD) being one of the most common neurodegenerative disease is primarily caused by the degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta of the midbrain. Studies have been conducted in various models including, cell cultures, animal models along with post-mortem brain studies on human bodies to reveal the role of apoptosis in the neuronal cell death in PD. The studies have proven to determine treatment for symptomatic relief but till date, no cure has been identified for PD. Although in the past few years, studies have been correlating neuroprotective effects of chlorogenic acid (CGA) to neurodegenerative diseases and thus, various PD models have been established to study its treatment therapy. With a similar objective, we aimed to study the effect of CGA, a natural polyphenolic compound, in MPTP-induced mice model of PD. On CGA supplementation, significant motor coordination and the antioxidant defense has been observed in contrast with MPTP-injected mice. Furthermore, the improved tyrosine hydroxylase expression inside the nigrostriatal region in CGA-treated mice supports the neuroprotective effect of CGA. Here, 1-methyl-4-phenylpyridinium (MPP^+) ion insult in the DA neurons were protected by CGA by reversing aberrant expression of apoptotic markers (Bcl-2, Bax, and Caspase-3). After CGA supplementation, the activity of pAkt1 was promoted, which has further inhibited the apoptosis of DA neurons. In Real-time PCR analysis, CGA treatment after MPTP intoxication showed reduced expression of IL-1β, IL-2, and IL-6; the pro-inflammatory cytokines. These studies have concluded that against MPTP-intoxication, CGA has offered the neuroprotective effect through its anti-apoptotic activity.

MTU09-37

Role of glutamate dependent signalling pathways during Alzheimer disease and diabetes

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Worldwide, epidemiological findings have revealed that the prevalence of Alzheimer Disease (AD) is more prominent in Diabetes Mellitus (DM) affected individuals. The relationship between neurodegeneration and metabolic disorder is still unclear, which makes them a public health concern. The present study was designed to investigate the role of glutamate dependent signaling pathways during AD and DM. Female Wistar rats weighing 180-200 g were divided into three groups viz: Normal control, Aβ (1-42) treated (AD model) and Streptozotocin treated (DM model). All the treatments were continued for a total duration of one month. A significant decline in the learning and memory was observed in AD and DM animals when compared to the normal control animals. The levels of neurotransmitters γ-aminobutyric acid and glutamate were also found to be significantly increased in the cerebrum and cerebellum of AD and DM animals in comparison to controls. On the contrary, activities of proteins regulating glutamate metabolism viz: glutamate synthetase, glutamate alpha decarboxylase and glutamate dehydrogenase were found to be significantly decreased in brain of AD and DM animals as compared to controls. The protein expression of glycogen synthase kinase-3, glial fibrillary acidic protein and amyloid precursor protein were also elevated in brain samples of AD and DM animals in comparison to control group. The brain sections of diabetic animals showed similar alterations in the neurohistoarchitecture as that of AD brain sections. Hence, the present study reveals that glutamate dependent signalling pathways plays a vital role in diabetes induced Alzheimer Disease.

MTU09-38

Neurotoxic implications of rotenone induced alpha-synuclein conformers

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Genetic mutations and environmental factors are believed to initiate the pathogenesis and aggregation of α-synuclein (α-syn), linked to Parkinson's disease (PD) and other synucleinopathies. However, initiation of α-syn unfolding, structural alterations and aggregation upon environment exposure events are still obscure. Here, we studied rotenone-α-syn interactions as rotenone; a pesticide is known to induce the α-syn aggregation. We also characterized the initial events in α-syn misfolding. The study of aggregation kinetics was done with Thioflavin T assay, Circular dichroism and Transmission electron microscopy suggested that rotenone increased the rates of aggregation with initial structural loss and fluctuations at amino acid level in α-syn and form seeds with distinct structural morphology. For rotenone induced α-syn conformers we further performed cytotoxicity studies using MTT assay, AnnexinV apoptosis assay and found them cytotoxic for neuronal cells. The mechanistic study through DCFDA Assay and JC1 assay has been shown that these rotenone exposed α-syn seeds did not cause the oxidative stress but depolarized the mitochondrial membrane potential which results in cell death. In brief we found that rotenone induced α-syn aggregation by affecting the initiation of misfolding events and these aggregated species cause cellular toxicity by altering the mitochondrial membrane potential. Implication of these findings suggests that may be environmental exposure also leads to seeds formation which are cytotoxic and remain dormant till further exposure or favourable conditions and have capability to induce the disease at later stages.

MTU09-39

Nickel-induced neurodegeneration in the hippocampus, striatum and cortex; an ultrastructural insight, and the role of caspase-3 an

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Human overexposure to nickel (Ni) emanating from the increasing application of Ni compounds in modern technology is a major public health concern. Nickel has been shown to be teratogenic, immunotoxic, genotoxic and carcinogenic. The current knowledge on Ni neurotoxicity is still relatively limited. We have previously demonstrated that Ni treatment alters cognitive and locomotor behaviors, induces oxidative stress and neurodegeneration in brains of rats. In this study, we examine the ultrastructural changes to neurons in the hippocampus, striatum and cortex of the brain following Ni treatment, as well as attempt to delineate the roles for caspase-3 and α-synuclein in Ni-induced neurodegeneration. Rats were treated with either saline, 10 or 20 mg/kg of nickel chloride for 4 weeks via oral gavage. Electron microscopy analysis revealed ultrastructural alterations in neurons of the hippocampus, striatum and cortex following Ni treatment. Mitochondria structural integrity within neurons were markedly compromised. We also detected elevated caspase-3 activity in hippocampus and striatum, as well as verexpression of α-synuclein in the cortex following Ni treatment. Our study demonstrates that mitochondria are a key target in Ni-induced neurodegeneration. Additionally, we implicate apoptotic pathway via caspase-3 action as the executioner and perturbation of α-synuclein expression in Ni-induced neurodegeneration.

MTU09-40

The role of tau phosphorylation at the AT8 pathological site in brain development

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Tau is a microtubule (MT)–associated protein, which stabilizes MTs in axons of neurons, contributing the structural support of neuronal network in healthy brains. In contrast, in Alzheimer's disease (AD) brains, tau is phosphorylated around 40 sites and forms aggregates called neurofibrillary tangles, which are supposed to cause neurodegeneration. Among many pathological phosphorylation sites, the AT8 site is particularly interesting because the site used for diagnosis of AD but also is phosphorylated during early brain development. However, it is not completely understood yet how the AT8 reactivity is generated in AD brain and contributes to AD development. We reported that the AT8 site was highly phosphorylated in fetal mice brains, and dephosphorylated at around postnatal day 14 when the neuronal circuit is established. We found hypothyroidism delayed not only brain development but also dephosphorylation at the AT8 site, indicated their direct relationship. Here, we examined localization of phosphorylated tau and non-phosphorylated tau at AT8 site in neurons and found that AT8 phospho-tau was present highly in the cell body and AIS region and less in distal axons. In contrast, non-phosphorylated tau at AT8 site was absent in AIS region and found highly in distal axon. We introduced wild-type (WT) human tau or AT8 site mutants, either Ala or Glu, in tau-knockdown neurons and found that 2E and WT, but not 2A human tau induced axon extension. Thus, axon development requires tau phosphorylated at AT8 site. This is the physiological role of the AT8 phosphorylation in tau, but would also provide important information on AD development.

MTU09-41

Neuroecotoxicology: effects of environmental heavy metal exposure on the brain of african giant rats

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Introduction: Increased exploitation of minerals has led to pollution of environments. Information on brain effects of such exposure is limited. Due to its exploratory activities, the African giant rat(*Cricetomys gambianus*) provides a unique model for ecotoxicological research to determine levels of animal and human exposure to different environmental pollutants. The aim of the present study is to unravel neuropathological features of this animal sampled from agro-ecological zones of Nigeria.

Materials and methods: With ethical approval, the animals were collected in the field in three Nigerian regions according to previously determined data on heavy metal exposure: mangrove forest (high vanadium, selenium); woodland savanna (high lead, selenium, zinc); rain forest (low levels of heavy metals). Immunofluorescence and Immunohistochemical analyses were conducted, focusing on different neuronal cell types sensitive to oxidative stress.

Results: Interesting results were obtained concerning orexin-A and melanin concentrating neurons of lateral hypothalamus and dopaminergic neurons of substantia nigra pars compacta (SNC). Stereological cell counts of tyrosine hydroxylase cells showed a significant loss (-41.8%) of SNC dopaminergic neurons in the animals exposed to vanadium (mangrove), and (-50.7%) in those exposed to lead (woodland savanna), compared to those from rain forest zone. Similarly, a significant loss (-39.9% and -40.8% respectively) of parvalbumin-containing interneurons in the cingulate cortex has been documented in same animal groups compared to those of rain forest.

Conclusion: These perhaps are the first "neuroecotoxicological" findings in distinct neuronal cell groups. The implications of these findings are highly relevant for human population living in these areas, not only in Nigeria but also in similarly polluted areas elsewhere in the world.

MTU09-42

Muscarinic acetylcholine receptors in alcohol use disorder

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Despite the large socioeconomic burden of alcohol use disorders (AUD), therapeutic treatment options are limited. Basic neuroscience has identified a number of targets that contribute to alcohol reward craving and relapse; however few of these successfully translate to human populations. AUDs are characterised by a transition to compulsive alcohol seeking, which is hypothesized to involve a shift from ventral to dorsal striatum. In addition, a medial to lateral shift in the dorsal striatum is implicated in the transition from goal-directed to habitual alcohol seeking. Muscarinic acetylcholine receptors (mAChRs) are potential targets for AUD treatment as they are expressed within the mesocorticolimbic reward system, including dense expression in the dorsal striatum. Here they modulate dopamine and glutamate release, which may regulate reward processing. To assess the role of mAChRs in AUD, we first conducted genome-wide RNA sequencing in the caudate/putamen of 10 human alcoholics and 10 healthy controls and concurrently examined mAChR expression in the corresponding regions in rat (dorsolateral and dorsomedial striatum) following chronic alcohol consumption/withdrawal using qPCR. Next we examined the role of select mAChR subtypes in alcohol consumption and seeking using selective allosteric modulators. Finally, we probed the role of specific mAChR subtypes in the dorsal striatum in alcohol consumption and seeking. Collectively, our data show that mAChRs are potential novel target pharmacotherapies for the treatment of AUD.

MTU09-43

Alteration of dopaminergic behaviors in a parkinson's disease model through P2x4R modulation by ivermectin

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Dopamine is a key neurotransmitter within the brain that plays a role in the mesolimbic pathway (associated with reward-based behavior) and the nigrostriatal pathway (which is associated with motor control and reward-based cognition). The ability to modify dopaminergic activities within these pathways provides a potential target for the treatment of dopaminergic disorders. Ivermectin (IVM), a P2X4 receptor (P2X4R) positive modulator, has been shown to reduce alcohol consumption in mice and alter L-Dopa induced rotational behavior in the 6-hydroxydopamine (6-OHDA) model. P2X4R knockout mice showed reduced rotational behavior changes (+/- IVM) further implicating P2X4Rs and IVM. To further investigate the effects of IVM on L-Dopa enhancement, I tested additional behaviors linked to the striatum of the medial forebrain bundle in unilaterally lesioned mice via 6-OHDA stereotaxic injection. Following lesion confirmation (via amphetamine [5 mg/kg] challenge) mice were subjected to a battery of behavioral tests to evaluate motor coordination, anhedonistic behavior, learning and memory. IVM (5 mg/kg, I.P.) was administered 8 hours prior to L-Dopa injection (5 mg/kg S.C.) and mice were observed while performing on the rotarod, sucrose preference test or novel object recognition test. L-Dopa and IVM+L-Dopa altered performance on rotarod tests and novel object recognition tests. We found IVM + L-Dopa was able to alter significantly motor coordination and produced a trend in altering learning and memory and anhedonistic behavior in a Parkinsonian mouse model. Overall, these initial findings further illustrate IVM's potential importance as a potential adjunct therapy for Parkinson's disease.

MTU10 Intracellular trafficking & proteostasis (Session A)

MTU10-01

Axonal trafficking of L1CAM in cortical neurons

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Introduction: Neurons are highly polarized cells with two distinct compartments, somato-dendritic and axonal. The maintenance of this polarity depends mainly on axonal proteome. There is increasing evidence supporting that axons and dendrites can autonomously synthesize and export transmembrane (TM) proteins through a local secretory pathway. Although in CNS axons it has been well described the presence of endoplasmic reticulum (ER), the presence or functionality of a Golgi-like structure has been poorly described. Recent evidence showing axonal proteins with immature N-glycosylation in the neuronal surface suggests a Golgi-independent route. Here we study L1-cell adhesion molecule (L1CAM), an axonal TM protein that contributes to the outgrowth and pathfinding of the growth cone, and which delivery is not fully understood. We hypothesize that the trafficking of L1CAM from axonal ER to plasma membrane (PM) is Golgi-independent in cortical neurons axons.

Methods: We isolated the axonal compartment of cultured embryonic cortical neurons (E18) using microfluidic chambers. To synchronize ER export of L1CAM, we used an ER retention/release system based on FM4/DD. Disruption of ER to Golgi trafficking was achieved with Golgicide-A (GCA) and Brefeldin-A (BFA). WB analysis and glycosidases treatment was performed to study L1CAM N-glycosylation.

Results: L1CAM was locally exported from the axonal ER to PM. Axonal trafficking was resistant to GCA, but sensitive to BFA. There is a differential N-glycosylation profile in axonal and somato-dendritic compartments.

Conclusions: L1CAM can be locally exported in cortical axons but a BFA-resistant Golgi trafficking is necessary. Our results suggest that there is a contribution of Golgi-like structures in local local delivery of L1CAM to axonal PM.

MTU11 Glial cells (Session A)

MTU11-01

Oligodendrocyte progenitor cell diversity in the healthy brain

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Oligodendrocyte Progenitor Cells (OPCs) make up 2-8% of the adult brain and remain proliferative throughout life. Historically, a majority of the OPC field has focused on the their role as a progenitor pool for mature oligodendrocytes, but recent literature has begun to explore the idea that the population of OPCs maintained in the adult brain encompasses multiple diverse subpopulations that may have roles beyond that of producing oligodendrocytes. OPCs have been shown to maintain neuronal health in the hypothalamus and play an integral role in both depression and a mouse model of multiple sclerosis. Further elucidation of both the molecular and functional diversity of OPCs will potentially reveal novel functions for this cell type and provide evidence for the production of new therapeutics for diseases in which OPCs may be implicated. To address the question of overall molecular diversity of OPCs in the adult brain, we have performed single-cell sequencing of PDGFRα-reporter positive cells from the brains of adult mice. Based on unbiased clustering, we have preliminarily identified 4 populations of OPCs. Go Term analysis of these clusters reveals significant enrichment of genes related to a variety of functions, including regulation of dendrite development, cytokine-mediated signaling pathways, and regulation of response to oxidative stress. Ongoing work includes validation of OPC clusters using immunofluorescent techniques to detect transcripts of genes specifically enriched in each OPC cluster. This work provides an overview of the transcriptional state of adult OPCs during homeostasis, as well as a foundation for future investigation into novel functions of OPCs. Identification of subpopulations of OPCs may have important implications for diseases in which OPCs may be playing a significant role.

MTU11-02

Lysosomal function and dysfunction in astrocytes

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During neurodegenerative diseases (NDs), astrocytes become reactive and engulf and degrade dead cells as well as protein aggregates *via* the lysosomal pathway. As a consequence, lysosomal impairment in these cells interferes with astrocyte function and contributes to the onset and the disease progression of NDs. In PD, astrocytes participate to the clearance of neuronal-released a-syn toxic species. LRRK2 is a kinase that impinges on the lysosomal pathway in different tissues (PNAS 2014, BBRC2016) and alteration of LRRK2 kinase activity in neurons is associated with PD (Front Mol Neurosci 2017, J Neurochem 2015). Of note, LRRK2 is highly expressed in glial cells and mutated LRRK2 might impact on astrocyte functionality. My research aims to define pathological implications of mutated LRRK2 in the phagycotysis/lysosomal pathways in astrocytes and imply whether targeted therapies focused on restoring astrocytes degradative capacity might be a route for drug intervention in PD.

MTU11-03

Myelin breakdown favors mycobacterium leprae survival in schwann cells

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Leprosy neuropathy is a chronic disorder caused by the infection of the peripheral nerve by the intracellular pathogen mycobacterium leprae (M leprae). Schwann cells are remarkable in supporting Mycobacterium leprae persistence in the nerve. While M leprae binding to myelinated Schwann cells has been implicated in demyelinating phenotype, what exactly role M leprae plays during myelin breakdown and clearance are not entirely clear. Here, we provided strong evidence of close interaction of M leprae and degenerating myelin profiles *in vitro* and *in vivo*. We also observed accelerated myelin breakdown and clearance in infected Schwann cells that was accompanied by reduced expression of myelin-related genes *in vitro* and *in vivo*. Furthermore, this increased myelin breakdown was associated with the upregulation of autophagic myelin destruction in Schwann cells *in vitro* and in nerve biopsies. Finally, when we blocked myelin degradation by pharmacological inhibition of JNK/c-Jun pathway in Schwann cells, we drastically reduced M leprae viability in the host cell. Overall, these results provided novel evidence of the ability of M leprae in advancing myelin breakdown to benefit its persistence intracellulary in Schwann cells.

MTU11-04

DAAM2 antagonizes VHL to modulate oligodendrocyte differentiation and remyelination after white matter injury

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White matter injury (WMI), or loss of myelinating oligodendrocytes (OLs) results in devastating neurological disorders, such as hypoxic ischemic encephalopathy in neonates and multiple

sclerosis in adults. Despite the robust regenerative capacity of OLs, myelin repair under pathological conditions have been unsuccessful. Indeed, lesions of patients are found populated with stalled OL precursors, highlighting the need of deciphering inhibitory cues. Previously, we identified a novel gene Daam2 that suppresses OL differentiation and WMI repair. Meanwhile, Yuen et al. reported that HIF arrests OL maturation. Upon Daam2 overexpression during tumorigenesis, we observed a significant reduction of VHL, a well-known ubiquitin-ligase targeting HIF, leading to the hypothesis that Daam2 suppresses OL differentiation and WMI repair by antagonizing VHL functions. Using genetic mouse models and OL cultures, we discovered a functional antagonizing relationship between Daam2 and VHL during development, which is conserved in lysolecithin-induced demyelination and hypoxia-induced hypomyelination mouse models. Lastly, Daam2 promotes VHL ubiquitin-proteasomal degradation under the regulation of targeting E3 ligase, the direct manipulation of which is sufficient to alter OL differentiation *in vitro*. Importantly, human expression data indicates promising therapeutic prospects. Together, we propose Daam2-VHL as a novel regulatory mechanism for OL differentiation and a potential therapeutic target for WMI.

MTU11-05

NG2 glia are vulnerable at breaches of the blood brain barrier during secondary degeneration following neurotrauma

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Blood brain barrier disruption accompanies secondary degeneration, both adjacent to and remote from a primary injury, and leads to increased neuroinflammation and damage. NG2 + glia are an integral component of the blood brain barrier, and encompass both pericytes and oligodendrocyte precursor cells. However it is not yet known if oxidative damage to NG2 + glia occurs to a greater degree at sites of blood brain barrier breach than sites where the barrier is intact, thereby perhaps contributing to secondary degeneration. Here we use the partial optic nerve transection model of secondary degeneration in adult female rats and semi-quantify 8-hydroxy deoxyguanosine (8OHdG) immunoreactivity as an indicator of oxidative damage to DNA in NG2 + glia and glial fibrillary acidic protein (GFAP) + astrocytes, together with Immunoglobulin G immunoreactivity as an indicator of blood brain barrier breach. 8OHdG immunoreactivity was increased 1 day after injury in both NG2 + glia and GFAP+ astrocytes surrounding blood vessels ($p \leq 0.001$). However, only in NG2 + glia surrounding vessels, was 8OHdG immunoreactivity higher at sites of blood brain barrier breach than where the barrier was intact ($p \leq 0.01$). Ethynyldeoxyuridine labelling of proliferating cells demonstrated that the percentage of proliferating NG2 + cells around RECA+ blood vessels was increased after injury ($p \leq 0.05$), whereas the percentage of proliferating RECA+ endothelial cells did not increase at this time point. Thus, NG2 + glia may be particularly vulnerable to oxidative damage at sites of blood brain barrier breach, associated with a proliferative response.

MTU11-06

Effects the remyelination-promoting antibody rHIgM22 on sphingolipid metabolism in primary cultured glial cells

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Recombinant human IgM22 (rHIgM22) binds to myelin and oligodendrocytes (OLs) and promotes remyelination in mouse models of multiple sclerosis. Literature suggests that rHIgM22 recruits a multimolecular complex formed by Lyn, integrin $\alpha v\beta 3$ and PDGFRα, triggering Lyn activation and promoting oligodendrocyte precursor cells (OPCs) survival and proliferation. However, its exact mechanism of action remains to be elucidated.

We have shown the involvement of different sphingolipids in rHIgM22 binding at the cell surface, suggesting that reorganization of lipid membrane microenvironment might be relevant in its biological activity.

Thus, we assessed the effect of a 24 hours, single dose treatment with rHIgM22 on sphingolipid metabolism in cultured rat mixed glial cells (MGC), OPCs and OLs. The treatment had no significant effects on the lipid pattern of MGC. However, in OPCs and OLs it determined an increase in the levels of gangliosides GD3 and GM3, both known for their ability to interact with and modulate the activity of different growth factor receptors.

In addition, rHIgM22 determined a reduced activity of the acid sphingomyelinase (ASMase), with a consequent reduction of ceramide (Cer) generation. Ceramide generated by the action of ASMase represents an important pro-apoptotic signal, but also potent regulator for the organization of sphingolipid-rich signaling platforms. Remarkably, genetic deficiency or pharmacological inhibition of ASMase effectively protect against demyelination and other detrimental effects in MS models.

Altogether, our results support the notion that rHIgM22 protective effects might be mediated by alterations of lipid-dependent membrane organization and/or signalling in different cell types present in the nice of MS lesions.

MTU11-07

In vivo activation of microglial Gi signalling using chemogenetics

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Microglia, the immune cells of the central nervous system, survey their surroundings and respond to external stimuli to maintain homeostasis in the brain. To do this, microglia express an array of receptors that allow them to receive and respond to signals from neighboring cells. Many of these receptors are G protein-coupled receptors, which regulate a variety of microglial functions through different signalling pathways. Gi receptors have been shown, for example, to modulate microglial phagocytosis and

chemotaxis. We have generated mice expressing Gi (hM4Di) Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) selectively in microglia. These mutated muscarinic receptors no longer respond to their endogenous ligand acetylcholine, but they can be activated by clozapine-N-oxide (CNO) and other compounds at doses that are inert at other receptors. These mice therefore allow for the selective activation of microglia Gi signalling *in vivo* without simultaneously affecting receptors on neurons or astrocytes. Activation of microglial Gi DREADD by CNO initiates Gi intracellular signalling pathways in DREADD-expressing microglia. Remarkably, activation of Gi signalling, via CNO injection, does not affect baseline behavior. Furthermore, chronic activation of microglial Gi signalling in mice does not appear to alter the expression of pro-inflammatory cytokines in the brains of LPS-injected mice. Lastly, phagocytic activity of primary microglia is not affected by activation of this pathway. Taken together, our results suggest that specific activation of Gi signalling in microglia is possible and appears to have no negative consequences in healthy mice.

MTU11-08

Dissecting the role of DAAM2 during astrocyte development and associated disease

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Astrocytes play a vital role in CNS physiology including synaptogenesis and formation of functional blood-brain-barrier. Consequently, astrocytes are associated with numerous neurological disorders and malignancies. However, the molecular mechanisms that control astrocyte development, diversity, and dysfunction remain poorly defined. Development of cell lineages follows a sequential series of differentiative steps, culminating with the differentiation of lineage-specific progenitors into mature populations that execute specific physiological functions. While the paradigm of stepwise lineage differentiation is much appreciated in neuronal and oligodendrocyte lineages, the intermediate steps of astrocyte lineage remain unclear. Therefore, unraveling mechanisms regulating astrocyte development and homeostasis will provide critical insight into the pathology and treatment of multiple neurological disorders. While we discovered previously that Daam2 suppresses oligodendrocyte differentiation during development and repair, how Daam2 operates during astrocyte development remains completely unknown. Here, we found that astrocyte-specific loss of Daam2 results in abnormal astrocyte maturation and alterations in synaptogenesis followed by the aberrant neuronal activity. Additionally, loss of Daam2 enhanced inflammatory responses in photothrombotic stroke model, suggesting critical functions of Daam2 in astrocyte development and tissue repair. To decipher how Daam2 suppresses astrocyte maturation, we performed screening and identified NBCe1, Na^+/HCO_3^- cotransporter as an inverse functional regulator of Daam2 in astrocyte development. Together, these studies elucidate the mechanistic link between Daam2 and its associated genes during astrocyte development and may provide new, tractable pathways of undefined intermediated astrocyte lineage in CNS development as well as associated injury repair.

MTU11-09

Acute toxicity after uptake of copper oxide nanoparticles in glial cells

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Copper oxide nanoparticles (CuO-NPs) have been known for their high cell toxic potential. However, interferences by copper ions in CuO-NP preparations have been a hurdle in the investigation of specific nanoparticle-mediated toxicity. In order to distinguish between the adverse effects exhibited by CuO-NPs and ionic copper that was present in the CuO-NP preparation, we have applied the membrane-impermeable copper chelator bathocuproine disulfonate (BCS) in a molar ratio of 20% of total copper, in order to chelate the ionic copper extracellularly released from the CuO-NPs. Physicochemical characterization of CuO-NPs revealed that the presence of BCS did not alter their size or surface charge. Application of CuO-NPs induced a time-, concentration- and temperature-dependent copper accumulation and severely compromised cell viability in C6 glioma cells and primary astrocytes. These consequences were not altered in the presence of BCS, while the tremendous copper accumulation and severe toxicity found upon application of ionic copper was prevented. The observed impairment of cell viability correlated well with the increase in the specific cellular copper content for both types of copper species applied and was only observed for conditions where the specific cellular copper contents exceeded 30 nmol copper per mg protein. The copper-induced toxicity to glial cells was accompanied by an increase in the generation of reactive oxygen species, which was partially prevented by BCS in copper ion-treated glial cells. In conclusion, the application of BCS allows to clearly distinguish between adverse effects caused by extracellular CuO-NPs and copper ions, and demonstrates that intact CuO-NPs are taken up and impair the viability of glial cells.

MTU11-10

Potentiation of adult oligodendrogenesis as a candidate target for ms therapy

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Oligodendrocytes, the myelinating cells of the Central Nervous System (CNS), are generated upon differentiation of oligodendrocyte precursor cells (OPCs), which possess proliferative and migratory capabilities and are present in the CNS postnatally. Under pathological conditions such as in Multiple Sclerosis (MS), there is a depletion of oligodendrocytes, but OPCs present in the brain parenchyma or derived from subventricular zone (SVZ) neural stem cells (NSCs) can differentiate, migrate and partially remyelinate the lesioned areas. Herein, we aimed at characterizing the MS mouse model, experimental autoimmune encephalomyelitis (EAE), as well as the process of adult oligodendrogenesis. Behavioural tests were performed to evaluate motor function. Cellular differentiation was assessed by immunohistochemistry for bromodeoxyuridine (BrdU) colocalization with oligodendrocytic markers in brain regions of interest. Western blot and ELISA assays were used for myelin

protein levels and inflammatory cytokine quantification. Results for EAE model characterization suggested that motor impairment is proportional to the clinical score. Moreover, an increase in the levels of the pro-inflammatory cytokine TNFα ($n = 5$, $p < 0.01$), and a tendency for increased IL-1β were observed in EAE mice. Importantly, a tendency for increased BrdU+ cells in the SVZ, corpus callosum (CC) and cerebral cortex (CT) was observed, accompanied by a significant increase in NG2 + BrdU+ cells in the CC of EAE mice ($n = 3$, $p < 0.05$), hinting at the migration of precursor cells from the SVZ to the CC. Altogether, this work allowed the characterization of the oligodendrogenesis process and of the EAE model throughout time, supporting future studies involving the modulation of adult oligodendrogenesis as a putative therapy for MS.

MTU11-11

Age-related changes in astrocytes contribute to synapse loss and dysfunction

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Aging is associated with synaptic deficit and cognitive decline. Several evidences have shown the role of astrocytes in synaptic function during brain development and diseases, although there is still a lack of evidence on their involvement in age-related cognitive decline. Here we investigated the phenotype and function of astrocytes in aging. We used hippocampal tissue from young (2-3 months) and aged (~21 months) C57Bl/6 mice for *in vivo* analysis. We also established an *in vitro* model for astrocyte senescence, in which cultures were maintained for 30-35 days *in vitro* (DIV) (senescent astrocytes) or 7-10 DIV (control astrocytes). We observed a ~90% reduction in synaptic density in the hippocampal dentate gyrus of aged mice. The GFAP immunostaining revealed astrocyte hypertrophy and decreased levels of synaptogenic factors in dentate gyrus of aged mice. Senescent astrocytes cultures showed a reactive phenotype, based on LCN2 immunostaining. The ACM from these cells presented a decreased capacity to support neurite outgrowth and synaptogenesis on neuronal cultures, possibly due to a significant reduction in synaptogenic factors expression and secretion. Our results point to a key role of changes in astrocyte phenotype and function to the age-related synaptic loss and dysfunction. The protocols of this study were approved by the Committee for Animal Research of the Federal University of Rio de Janeiro and the University Medical Center Utrecht. **Support:** CNPq, CAPES, FAPERJ, Ministério da Saúde, ZonMW Memorabel.

MTU11-12

Altered myelinic nanochannel integrity modulates ALS disease progression in SOD1 mutant mice

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Amyotrophic lateral sclerosis (ALS) is a highly debilitating and fatal disease characterized by the progressive loss of motor neurons. The mechanisms leading to disrupted oligodendroglial support of motor neurons in ALS are poorly understood. To investigate this, we first confirmed that selective removal of oligodendroglial mutant SOD1-G37R expression delays disease onset, prolongs survival, and improves motor performance. Given the recently discovered novel role of oligodendrocytes in supplying nutrients to neurons via a system of nanometer-wide cytoplasmic channels, we utilized immuno electron microscopy and found that mutant SOD1 is present within paranodal loops and the inner periaxonal tongue. This raises the intriguing possibility that SOD1 aggregates within these nanochannels could perturb the motor-driven transport of transporter proteins (i.e. MCT1) within oligodendrocytes and disrupt the free diffusion of nutrients from the oligodendrocyte to the motor neuron. To investigate the role of perturbed myelinic nanochannel integrity as a potential mechanism leading to impaired oligodendroglial metabolic support of motor neurons in ALS, we crossed mutant SOD1-G93A mice with mice lacking expression of CNP, a protein that keeps myelinic nanochannels open by preventing excessive myelin membrane compaction. Double SOD1-G93A and CNP^{null} mutants show reduced survival and worsened neurological scores. Decreased frequency of myelinic nanochannels in CNP^{null} mice could accelerate ALS disease progression in double mutants by further limiting the transport of nutrients from the oligodendroglial compartment to the axonal compartment. These data provide novel insights into the mechanisms leading to impaired oligodendroglial support of motor neurons in ALS.

MTU11-13

The innate capacity of MS oligodendrocytes to produce efficient myelinating oligodendrocytes

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Multiple Sclerosis (MS) a neuroinflammatory demyelinating disease, could result either from extrinsic activation of the immune system or a process initiated intrinsically by primary cytodegeneration of neuroglia. Whether failed remyelination in MS results from the incapacity of the MS oligodendroglia to function properly, or from environmental cues is still at debate. MS oligodendrocytes are not easily accessible. The induced pluripotent technology constitutes a powerful tool to generate MS oligodendroglia. We showed that transplantation of iPS-derived oligodendrocytes in adult

demyelination condition results in excellent integration and functional remyelination of host axons. Here, we ask whether MS oligodendrocytes exhibit a primary blockade or behave as efficiently as the healthy control cells.

We transplanted Human iPS-oligodendroglia from RRMS patients and their siblings in the developing brain and spinal cord of Shiverer:Rag$^{2-/-}$ mice and sacrificed mice at 4, 8, 12, 16 and 20 weeks-post-transplantation to evaluate their fate and functional properties as myelin-forming cells.

Data showed that MS oligodendroglia survive, vastly distribute/migrate over time and differentiate efficiently in myelin-forming cells generating compact myelin within the murine brain and spinal cord as efficiently as healthy controls. Transcallosal conduction velocities were significantly delayed in non-grafted shiverer mice compared to the wild-type mice but rescued in the grafted mice.

Our data suggest the innate capacity of MS cells to produce efficient myelinating oligodendrocytes. The chimeric mouse-human glial network could be a target as a preclinical model for drug screening of promyelinating compounds for personalized therapy in MS. SM is beneficiary of an ECTRIMS fellowship. Support by the International Progressive MS Alliance Grant #: PA-1604-08492.

MTU11-14

Function of microglia and the exosomes content are influenced by the origin of cells

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Microglia cells are key players of the cross-talk between the nervous and immune systems. In present study we used a combination of proteomic and systemic biology analyses to demonstrate that neonatal microglial cells derived from cortex and spinal cord expressed different phenotypes upon the physiological or pathological conditions. Primary cells were isolated from neonatal rat cortex and spinal cord. For proteomic analyses of microglia cells, the proteins were extracted with RIPA buffer and processed using FASP and nanoHPLC-MS/MS analyses. Functional analyses, including neurite outgrowth and glioma proliferation analysis in 3D spheroid cultures, were performed to test biological activity of cortex microglia exosomes compared with spinal cord microglia exosomes. The results highlight variability in protein production on both cellular and exosome levels. Bioinformatics data reveal for proteins extracted from cortex microglia anti-inflammatory and neurogenesis/tumorigenesis characteristics, while for proteins isolated from spinal cord microglia involvement in the inflammatory response. *In vitro* assays indicate that the microglia located at different CNS areas reveal differential biological functions through released vesicles. While exosomes from both microglia sources enhanced growth of DRGs axons, only the spinal microglia vesicles significantly attenuated glioma proliferation. The results show that exosomes produced by two different sources of microglia do not have the same pattern nor the same biological functions. Thus, microglia function is dependent on its cellular microenvironment which conditions its phenotype. Supported by APVV 15-0613, ERANET Axon Repair, INSERM, SIRIC-ONCOLille Grant-DGOS-Inserm 6041aa.

MTU11-16

Role of coronin-1a in human fetal brain derived astrocyte physiology and activation in HIV-1 neuropathogenesis

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One of the major challenges of neuroAIDS field is lack of apt model to study HIV-1 neuropathogenesis. This prompted us to develop a well-characterized Human Fetal Brain-derived Neural Precursor cell (hNPCs) culture system. We maintain hNPCs as multipotent stem cells and differentiate neurons and astrocytes for our studies. In HIV/AIDS, astrocytes cause indirect damage to neuron that culminates into neurocognitive deficits. Cellular and molecular mechanisms for astrocyte activation are unclear, hence requires extensive explorations. Recent reports using rat model system, suggest coronin-1a is important for cognitive abilities. Coronin-1a, an actin-binding protein, is associated with important cellular processes such as cell migration, phagocytosis, morphogenesis, cellular trafficking, cytokinesis etc. However, role of Coronin-1a in astrocyte physiology is underexplored. Using hNPCs model, we attempted to investigate the role of Coronin-1a in modulation of astrocytic function by neurotoxic HIV-1 protein Tat. Our studies reveal that HIV-1 Tat can modulate Coronin1A expression in astrocytes. Interestingly, knockdown of coronin-1a resulted in altered physiological features, such as decreased calcium flux, altered PLCy1, and ERK1/2 phosphorylation patterns in ATP stimulated astrocytes. Having observed its role in Calcium signaling, we were curious if it also contributes to HIV-1 Tat-induced astroglial activation. Knockdown of this protein alleviates the HIV-1 Tat-induced astrocyte activation marked by measuring the levels of Glial fibrillary acidic protein (GFAP), cytokine, and glutamate release. These results provide novel insights into the field of neuroAIDS by identifying important roles of Coronin-1a in modulation of astrocyte physiology and pathophysiology.

Research Fellowship to HSP by CSIR, New Delhi and financial support from NBRC core funds to PS is greatly acknowledged.

MTU11-17

Treatment of experimental allergic encephalomyelitis (EAE) by the metabotropic receptor agonist chpg, reduces disease progression

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Previous studies demonstrated that a metabotropic glutamate receptor (mGluR) agonist, ACPD (1-amino-1,3-dicarboxycyclopentane), is able to reverse deficits in brain-derived neurotrophic factor (BDNF) and myelin protein levels following injury in a cuprizone model of demyelination. Group I mGluRs (mGluR1 and mGluR5) were localized to astrocytes and effects of ACPD were found to be dependent upon the production of BDNF by these cells. Recent

work indicates that similar effects are elicited by an intraperitoneal injection of the Group I mGluR agonist, CHPG (2-chloro-5-hydroxyphenylglycine). In this study, we tested whether CHPG could reverse clinical signs in EAE mice immunized with myelin oligodendrocyte glycoprotein (MOG). CHPG (20 mg/kg, injected every other day) delayed MOG-induced EAE, and ameliorated clinical signs when treatment was initiated after mice developed hindlimb paralysis. This effect was accompanied by reversal in the loss of BDNF and myelin proteins in the lumbar spinal cord. Moreover, preliminary data revealed increased colocalization of mGluR5 with GFAP+ astrocytes within lesioned sites, with rare colocalization with Iba1 + activated microglia, or CD11b+ microglia and macrophages, suggesting that astrocytes or microglia may be targets of CHPG action. In contrast, no colocalization of the receptor with CD4 + T-helper cells, CD45R+ B-cells, or Ly-6 g+ neutrophils was observed, suggesting that peripheral immune cells do not express mGluR5 at early or late stages of disease. Future studies will be performed to define the cellular mechanisms underlying the effects of CHPG on glial cells and continue to explore the potential of metabotropic agonists as targets for treating demyelinating diseases. Supp. NMSS RG4257B4/1 and NIH RO1 NS036647.

MTU11-19

Regulation of microglial aktivity by Gq-DREADD mediated signalling

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G protein coupled receptors (GPCR) are widely expressed across different cell types in the brain. They can act through Gq, Gs or Gi signaling which have been shown to regulate several functions in microglial cells. Microglia are the resident immune cells of the central nervous system. They have an important role responding to injury, infections and removing damaged cells and cross talk to neurons via a number of GPCRs. How activation of Gq-mediated signaling in microglia regulate some of its critical functions *in vivo* is poorly understood, because neurotransmitter receptors are located in all different cell types in the brain. To address this question, we generated a microglia Gq-DREADD (Designer Receptors Exclusively Activated by Designer Drugs; hM3Dq) mouse line. hM3Dq is a mutated muscarinic receptors type 3 that no longer responds to acetylcholine, but is activated by clozapine-N-oxide (CNO) and similar compounds. We confirmed in this mouse line that hM3Dq is expressed only in microglia and that CNO increases intracellular calcium concentrations only in Gq DREADD microglia, indicating Gq pathway activation. Treatment with CNO also increased phagocytosis of fluorospheres by Gq DREADD microglia. *In vivo,* chronic activation of hM3Dq by CNO (ip) does not affect baseline behaviour, however it decreased LPS-induced sickness behaviour and the upregulation of inflammatory cytokines mRNA in the brain. Our results show that hM3Dq-specific microglia mice can be a useful tool to understand how microglia GPCR-signaling modulates its activity *in vivo*.

MTU11-20

Deletion of glial ABCA1 causes glaucoma-like optic neuropathy

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Glaucoma is second leading cause of blindness worldwide which is characterized by damages or degeneration of retinal ganglion cells (RGCs). Although an elevated intraocular pressure (IOP) is the main risk factor, it has become apparent that many other factors are involved in the etiology of glaucoma. Among genetic risk factors, single nucleotide polymorphism (SNP) of *ABCA1* gene has been identified as the risk for glaucoma from large scale genome wide association studies (GWAS). However, its pathogenic mechanisms are totally unclear. To clarify this, we raised three issues to be revealed. First, it is unclear whether or not ABCA1 affects IOP. Second, it is undermined which of gain-of-neurotoxicity or loss-of-function of ABCA1 causes glaucoma. Third, it is unknown which type of cells contributes to glaucoma. We analyzed conventional ABCA1 knockout (KO) mice and found that IOP was not changed. We also found that ABCA1 was highly enriched in astrocytes of ocular tissues. To further elucidate the role of astrocytic ABCA1, we generated astrocyte-specific ABCA1 knockout (cKO) mice. The cKO mice showed significant increase in the number of apoptotic RGCs and reduction in visual function at middle-age (12 months old). Taken together, our data showed that (1) ABCA1 has no impact on IOP; (2) loss-of-function of ABCA1 is involved in glaucoma; and (3) ABCA1 in glial cells contributes to pathogenesis of glaucoma.

MTU11-21

Studying the role of dark microglia in early postnatal development in CX3CR1-deficient mice

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Dark microglia (DM), a recently discovered microglial phenotype, has been associated with ultrastructural alterations caused by oxidative stress, as well as down-regulation of microglial homeostatic proteins like CX3CR1.These cells were shown to be abundant in mouse models of psychiatric and degenerative diseases, as well as in normal post-natal development. DM were observed in brain regions like the hippocampus, were associated with an upregulation of cd11b and appear to be involved in synaptic modulation. Although DM can be identified by electron microscopy due to their electron dense cyto- and nucleoplasm, the impossibility to study these cells with other techniques has left many questions unanswered. Our goal is to identify a marker that labels these cells selectively, in order to analyze their molecular signature, regional density, localization, morphology and ultrastructure in a myriad of pathologies. The putative DM marker's specificity was assessed

using correlative immunocytochemical light and electron microscopy. Furthermore, series of brain sections providing a non-biased representation of the brain of young male and female CX3CR1-deficient mice (postnatal day 14 and 21), a model where synaptic dysfunction can be seen, were imaged using a slide scanner to reveal their distribution. Our results revealed that DM are found in unexpected regions in the grey (e.g. striatum) and white matters (e.g. *arbor vitae* of the cerebellum). Moreover, we found that only some DM were positive for our marker, suggesting that multiple DM subpopulations co-exist. Their close association with myelinated axons in the white matter suggest a new potential role for these cells. Further studies will be carried out to investigate DM's role in the white matter of young mice.

MTU11-22

Menadione induces rapid radical formation and MRP1-mediated gssg export in rat astrocytes

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Menadione (2-methyl-1,4-naphtoquinone) is a derivative of vitamin K and takes part in redox cycling, thereby generating reactive oxygen species (ROS) in cells. In brain, astrocytes defend themselves and also neighbouring cells against xenobiotics and toxins using an efficient antioxidant defence system. To test for adverse consequences of menadione on brain cells, primary astrocyte cultures were treated with menadione for up to 6 h. Concentrations of up to 30 μM menadione did not affect viability or cellular glutathione redox state. In contrast, 100 μM menadione caused a quick impairment in lactate release and a delayed increase in extracellular lactate dehydrogenase activity, demonstrating metabolic impairment and loss in membrane integrity, respectively. Already within 5 min after exposure, 100 μM menadione caused formation and cellular accumulation of glutathione disulfide (GSSG) which was accompanied by an increased ROS-staining, clearly indicating oxidative stress. The intracellular GSSG accumulation was followed by an export of GSSG that was prevented by MK571, an inhibitor of the multidrug resistance protein 1 (Mrp1). In glucose-deprived cells glutathione oxidation and ROS formation were already observed for lower concentrations of menadione compared to glucose-fed cells, most likely due to a lack in NADPH regeneration by the pentose phosphate pathway. Co-incubation of astrocytes with dicoumarol, an inhibitor of the menadione-reducing enzyme NAD(P)H: quinone acceptor oxidoreductase 1 (NQO1), did not prevent menadione-induced ROS formation nor GSSG accumulation. These data demonstrate that in primary astrocytes menadione rapidly induced NQO1-independent ROS production and GSH oxidation to GSSG which is followed by a Mrp1-mediated export of GSSG.

MTU11-23

Autotaxin, a regulator of oligodendrocyte differentiation during remyelination

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Autotaxin (ATX), also known as ectonucleotide pyrophosphatase/phosphodiesterase 2(ENPP2) or phosphodiesterase-Iα (PD-Iα)/ATX, is a secreted glycoprotein primarily known for its enzymatic lysophospholipase D (lysoPLD) activity, which generates the lipid signaling molecule lysophosphatidic acid (LPA). LPA, in turn, exerts its functions through activation of a family of G protein-coupled receptors (GPCRs), the so-called LPA receptors. In our own studies, we identified ATX as a protein that is released by cells of the oligodendrocyte (OLG) lineage and functions via two mechanisms to drive OLG differentiation. Initially, we uncovered that ATX, via its C-terminally located modulator of oligodendrocyte remodeling and focal adhesion organization (MORFO) domain, promotes the establishment of a complex and expanded process network by post-migratory, premyelinating OLGs. More recently, we focused on ATX's lysoPLD activity and found that the ATX-LPA axis promotes the expression of genes well-known to be associated with the earlier stages of OLG differentiation via, at least in part, the modulation of histone deacetylation. Studies undertaken in the developing zebrafish substantiated a critical role of ATX in regulating OLG differentiation during development. Interestingly, there is evidence for reduced levels of ATX in the central nervous system (CNS) parenchyma in the major demyelinating disease in humans, Multiple Sclerosis. Here, we show that, similarly, ATX levels are reduced during toxin-induced demyelination. In addition, we present, data that support a role of OLG-derived ATX in regulating OLG differentiation not only during development but also after toxin-induced demyelination.

MTU11-24

Comparison of molecular signatures of OLIG2-lineage astrocyte and GFAP-positive astrocyte using laser microdissection

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Our lineage tracing study using Olig2^{CreER}; Rosa-CAG-LSL-eNpHR3.0-EYFP (Olig2 ^{CreER}; YFP) transgenic mice revealed that a subpopulation of Olig2-lineage mature astrocytes (Olig2-astrocytes) distributed widely but unevenly in the adult brain; regions rich in Olig2-astrocytes tended to lack GFAP-positive astrocytes (GFAP-astrocytes). Even within a single brain nucleus, Olig2-astrocytes and GFAP-astrocytes occupied mutually exclusive territories. External globus pallidus (GPe) is one of the representative nuclei. Interestingly, brain nuclei rich in Olig2-astrocytes tended to strongly express GABA-transporter 3 (GAT-3) in astrocytes and vesicular GABA transporter (vGAT) in neurons, suggesting that Olig2-lineage astrocytes may be involved specifically in inhibitory neuronal transmission by forming tripartite synapses.

To compare molecular signatures of the two kinds of astrocytes, we applied laser microdissection to the GPe in combination with

immunohistochemistry. The method enabled us to differentially collect two types of astrocytes from a single section of the GPe, where the territories of Olig2- and GFAP-astrocytes were intermingled. Feeding tamoxifen-containing chow for 1 week to adult Olig2^{CreER}; YFP mice successfully enhanced recombination and subsequent YFP fluorescence. Brain sections were then labeled with anti-GFAP antibody and Alexa 594-labeled secondary antibody. We dissected out YFP-expressing cells with bushy morphologies (Olig2-astrocyte) and Alexa 594-labeled star-like cells (GFAP-astrocyte) from single sections. mRNAs from each type of astrocytes were isolated and subjected to molecular comparison using qPCR. Consistent with our previous report, Olig2-astrocytes expressed lower GFAP mRNA than GFAP-astrocytes. The RT-qPCR analyses further showed that Olig2-astrocytes expressed higher level of GAT-3 gene than GFAP-astrocytes. These results strongly suggest that Olig2-astrocytes constitute a distinct subpopulation of astrocytes subsidiary to inhibitory GABAergic transmission.

MTU11-25

Parallel S1P receptor signalling synergise to induce neuroprotective signalling

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Sphingosine 1-phosphate (S1P) is an essential lipid metabolite with potent vasculoprotective and neuroprotective properties. S1P signals through its own family of five G-protein coupled receptors, $S1P_1$-$S1P_5$, activating intracellular pathways that regulate proliferation, differentiation, and survival. The multiple sclerosis drug Fingolimod is a potent S1P receptor agonist that causes lymphopenia. However, recent research has also established direct neuroprotective properties of Fingolimod on astrocytes in multiple neurodegenerative paradigms including Alzheimer's and Parkinson's disease. In this study, we show S1P upregulates brain-derived neurotrophic factor (BDNF), leukaemia inhibitory factor (LIF), platelet-derived growth factor B (PDGFB), and heparin-binding EGF-like growth factor (HBEGF) in astrocytes but not neurons, and S1P is a much more potent inducer than Fingolimod. Accordingly, in an *in vitro* model of neuronal excitotoxic cell death, S1P significantly attenuates apoptosis whilst Fingolimod does not. Specific antagonists of $S1P_1$ and $S1P_2$ both inhibited neurotrophic gene induction in response to S1P, indicating simultaneous activation of both receptors is required. Phosphoproteomic analysis, siRNA, and Western blotting showed that $S1P_2$ signals through Ga13, RhoA, Jun and Yap to drive neurotrophic gene expression. Fingolimod does not activate $S1P_2$, explaining why it does not promote significant neurotrophic gene expression in astrocytes. Supplementing Fingolimod with a constitutively active G13 boosts expression of neurotrophic factors. These results demonstrate that S1P utilises dual signalling pathways from independent receptors to maximise neurotrophic gene expression and protection against excitotoxicity.

MTU11-26

Striatin-3 is a novel glial RAC1 effector

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During development, Schwann cells undergo extensive cytoskeletal reorganization as they insert cytoplasmic extensions into axon bundles to sort, ensheath, and myelinate axons. Similarly, following peripheral nerve injury there is extensive actin polymerization around Schmidt-Lantermann incisures as Schwann cells differentiate into a repair phenotype. Both processes are regulated by Rac1. Our lab previously demonstrated that Rac1 activation during development is driven by engagement of β1 integrin with laminins and is essential for radial sorting. Therefore, we performed a proteomic screen to look for novel Rac1 effectors in peripheral nerves and identified striatin-3 (Strn3) as a candidate. Initial *in vitro* data suggests that Strn3 knockdown in SCs decreases their ability to adhere to various substrates including axons and reduces proliferation. We are developing a mouse model with ablation of Strn3 specifically in Schwann cells along with a Strn1/3 double Schwann cell knockout. Additionally, we have found that Strn3 may be highly expressed in axons at nodes of Ranvier and in oligodendrocytes. Therefore, we are also developing mouse models with specific ablation of Strn3 in neurons or oligodendrocytes to characterize the functional significance of Strn3 in axons and myelinating glia of the central nervous system.

MTU11-27

Presentation of acute motor deficit and subsequent recovery following internal capsule demyelination in mice

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS characterized by progressive remyelination failure and accumulated motor disability. However, whether remyelination promotes motor recovery remains unclear. Here, we compared the effect of experimental demyelination with focal ischemia induction in the internal capsule (IC), a white matter region associated with motor impairment in MS and stroke, on motor behavior in mice. First, to induce the demyelination, we injected lysolecithin (LPC) into right side IC using stereotaxic technique. At 7 days post lesion (dpl), demyelination was observed in the IC by immunofluorescence staining and FluoroMyelin. Next, to examine whether this model affects motor function, we performed adhesive tape removal test, cylinder test, wire hang test and ladder walking test. IC-demyelinated mice reduced motor function in mice at 7 dpl. Further, we demonstrated that demyelination of right IC significantly impaired both left forelimb and left hindlimb motor function.

Moreover, these mice exhibited motor deficit until 14dpl, but regained motor function by 28dpl, corresponding with reduced inflammation, decreased axonal dystrophy, and increased oligodendrocytes in lesions. By contrast, injection of endothelin-1 (ET1) into the IC, which is known to induce white matter infarct, displayed lasting motor deficit, which is accompanied by persistent inflammation and axonal dystrophy, and reduced oligodendrocytes in lesions. These results demonstrate that IC demyelination induces acute motor deficit and subsequent motor recovery through remyelination, and suggest that inflammation resolution and the restoration of axonal integrity may be required for successful remyelination and motor recovery. Therefore, IC demyelination is a tractable model for assessing the influence of remyelination on motor behavior, and may be used to complement future drug screens for the identification of compounds for promoting remyelination.

MTU11-28

Guanosine and guanine can differently modulate sumoylation in rat cortical astrocytes

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SUMOylation is a posttranslational protein modification that can participate in endogenous protective mechanisms, and defective SUMOylation is observed in a diverse array of neurological disorders. Guanosine and its metabolite guanine, two endogenous molecules from the purinergic system, also play physiological and protective roles in different disorders. Here we investigated whether guanosine and/or guanine can modulate SUMOylation. Primary cortical astrocytes (14 days *in vitro*) were treated with guanosine and guanine (1, 10, 100, 300 or 500 μM) for 1 h and 6 h. Data from Western blotting labelling were obtained from the whole membrane signal always divided by its respective loading control (GAPDH). One-way ANOVA followed by Neuman-Keuls *post hoc* test was used to analyze the data. Global SUMO-2/3-ylation increased two-fold in astrocytes treated for 1 h with guanosine (10 - 500 μM, $n = 4$). Conversely, guanine (at 500 μM, $n = 4$) decreased global SUMO-2/3-ylation in astrocytes treated for 1 h. SUMO-2/3 levels go back to control levels in astrocytes treated with guanosine (10 - 500 μM, $n = 4$) for 6 h, however treatment with guanine (at 500 μM, $n = 4$) for 6 h caused a significant increase in SUMO-2/3 levels. To our knowledge this is the first report where molecules from the purinergic system are shown to be modulating SUMOylation. These are promising results that raise several questions concerning the functional consequences of this modulation for cellular protection.

MTU11-29

Ionotropic mechanism of NMDA receptor-mediated calcium fluxes in cultured mouse astrocytes: is it modulated by neurons?

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It is well established that activation of NMDA receptors (NMDAR) in cultured rodent astrocytes triggers calcium fluxes. In rat astrocytes the fluxes involve calcium derived from both extracellular and intracellular compartments, reflecting ionotropic or metabotropic mechanisms, respectively. Here we analyzed the two mechanisms in cultured mouse astrocytes and the role of neuronderived factors in the process. Cultured mouse cortical astrocytes were treated with 100 lM NMDA and changes in the intracellular calcium concentration ([Ca2+]i) were measured with the fluorescent calcium indicator, Fluo-3-AM. The NMDA-dependent fluxes were absent in the presence of a NMDAR channel blocker MK-801 and in cultures with silenced NMDAR subunit GluN1 (siGluN1), but were not altered by incubation with blockers of: i) IP3 receptor (xestospongin C), ii) ryanodine receptor (ryanodine) and iii) mGluR5 (MPEP). The results indicate that the mechanism by which NMDAR in mouse astrocytes exclusively mobilize extracellular, but not intracellular calcium, reflecting ionotropic mechanism. The ionotropic nature of the fluxes was not altered by preincubation of astrocytes with a medium derived from cultured neurons, indicating that neuron-derived soluble factors are neutral to the process. Evaluation of the calcium fluxes in astrocytes co-cultured with neurons are under way to assess whether direct cell contact between astrocytes and neurons plays a role in establishing the nature of astrocytic calcium fluxes. Supported by National Science Centre of the Republic of Poland (NCN), grant no 2017/27/N/NZ3/02819.

MTU12 Neuron-glia interactions (Session A)

MTU12-01

DTI found structural altercations induced by long-term optogenetics stimulation of striatal medium spiny neurons

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Striatal medium spiny neurons (MSNs) control motor function. Hyper- or hypo-activity of MSNs coincides with basal ganglia-related movement disorders. Based on the assumption that lasting alterations in neuronal activity lead to structural changes in the brain, understanding these structural alterations may be used to infer MSN functional abnormalities. To infer MSN function from structural data, understanding how long-lasting alterations in MSN activity affect brain morphology is essential. To address this question, we conducted a proof-of-concept study using mice that express channelrhodopsin-2 (ChR2) only in the MSNs. We utilized ex vivo diffusion tensor imaging (DTI) for comprehensive visualization of structural alterations. One-week optogenetic stimulation was conducted to the hemispheral dorsal striatum (dStr) of mice which express a ChR2-YFP in MSNs. As a result, a rotation behavior induced by optogenetics stimulation to the MSNs was impaired after one-week stimulation. The stimulation decreased fractional anisotropy (FA), reflecting structural alterations of axons and myelin, in the ipsilateral dStr, motor cortex (M), and substantia nigra reticular (SNr), compared with the contralateral side. Histological approach using a super-resolution microscopy showed the smaller diameters of YFP positive axons and dendrites of MSNs. In addition, the diameters of myelin proteolipid protein (PLP) positive axons were smaller and PLP positive myelination was thinner in dStr, M, and SNr. These structural changes were approved by observation with an electron microscopy and were highly correlated with the DTI-FA change. These results indicated that the long-term activation of dStr MSN, resulting in motor dysfunction, altered structures of MSNs and myelinated neurons of the MSN-related circuit. This combinatorial study provides a useful tool to understand the causal relationship with functional and structural alterations.

MTU12-02

Involvement of the gut microbiome-brain microglia axis in a maternal high-fat diet mouse model of neurodevelopmental disorders

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A high-fat diet (HFD) during pregnancy and/or nurturing is associated with an increased risk of neurodevelopmental disorders in the offspring. Previous results in mice revealed that maternal HFD (mHFD) leads to the development of behavioral abnormalities, including stereotypic and repetitive movements, caused by a gut dysbiosis, which promoted altered neuronal network activation. The gut microbiome may influence brain development by altering microglia, the immune cells that regulate the formation of neuronal circuits. However, these effects on microglia and their consequences on the brain and behavior remain largely undetermined. We hypothesized that a dysbiosis of the gut microbiota caused by a mHFD could alter microglial function leading to an imbalance of excitatory/inhibitory neuronal input relevant to known behavioral deficits. To test this hypothesis, female mice received a HFD (rich in saturated and unsaturated fats) for 4 weeks before breeding until weaning of their litter. Offspring's behavior was assessed during adulthood, which identified increased repetitive movements. Another cohort of animals was sacrificed at 30 days of life to collect brain and gut tissues. The microbiome will be analyzed by sequencing of 16S RNA. To assess changes in neuronal circuits and their interactions with microglia, a triple immunostaining will be performed to label excitatory synapses (Vglut1/Homer1) or inhibitory synapses (vGAT/gephyrin) in addition to microglia (IBA1) among the hippocampus and amygdala, two regions involved in the glutamatergic circuit. This project will help to understand the link between gut dysbiosis caused by mHFD and the pathogenesis involving microglia and excitatory/inhibitory imbalance of neurodevelopmental disorders.

MTU12-03

Microglia contribute to the loss of inhibitory synapses in chronic toxoplasma gondii infection

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Schizophrenia is a complex and heterogeneous neurological disorder associated with debilitating cognitive impairment, acquisition of positive symptoms (hallucination and psychosis), and loss of behaviors that are normally present in healthy individuals (apathy and social withdrawal). Evidence from both human patients and rodent models suggest that schizophrenia-associated behaviors result from alterations in the assembly and function of inhibitory synapses, including inhibitory axo-somatic synapses. In addition to genetic causes (such as those examined with genetic mouse models) environmental factors can both increase the risk of schizophrenia and alter inhibitory circuit function in the brain. One such environmental factor is infection with *Toxoplasma gondii*, an intracellular protozoan parasite that infects over one-third of the human population worldwide. We previously discovered abnormalities in inhibitory synapse organization and function in chronically *Toxoplasma*-infected brains. Here, we sought to test whether chronic infection specifically alters axo-somatic inhibitory synapses. We performed ultrastructural analysis of inhibitory axo-somatic synapses in the CA1 region of mouse hippocampus and in layer V of cerebral cortex using Serial Block Face Scanning Electron Microscopy. In parasite-infected brains we discovered a significant reduction of inhibitory axo-somatic synapses in CA1 and neocortex. Interestingly, we observed a dramatic ensheathment of neuronal somas in these regions by microglia-like cells in *Toxoplasma*-infected brains. These findings were further corroborated with *in situ* hybridization for *Syt1* (a marker for neuronal somas) coupled with immunohistochemistry to visualize phagocytic microglia. Thus, we

not only identified a significant reduction in axo-somatic synapses in parasite-infected brains, but our data suggests a role for microglia in inhibitory synapse loss.

MTU12-04

Quetiapine reverses the malfunctions in the behaviour and the neuron-microglia protein systems of prenatally LPS-treated offspring

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The risk of developing schizophrenia appears to be a serious threat for an adult offspring of mothers exposed to prenatal insults during pregnancy. The immunological aspect seems to indicate the importance of systems controlling neuron-microglia interactions, including chemokines and clusters of differentiation. The study was designed to examine: 1) an influence of the changes induced by the prenatal treatment with lipopolysaccharide (LPS) on the behaviour and protein levels of CX3CL1, CX3CR1, CD200, CD200R in the hippocampus and the frontal cortex of adult offspring rats; 2) an impact of 14-day-treatment with quetiapine on above-mentioned aspects. Every other day from the 7th day of pregnancy, rats were injected with LPS. 3-month-old male offspring were subjected to the behavioural examination (the PPI test). Afterwards, rats were treated with quetiapine for 14 days. The PPI test was performed again and animals were sacrificed to dissect hippocampi and frontal cortices. CX3CL1, CX3CR1, CD200 and CD200R levels were measured using ELISA assays. The results of the PPI test showed disturbances in the prepulse inhibition in LPS adult offspring. These changes were normalized after 14-day injections of quetiapine. Prenatal administration of LPS disrupted homeostasis of CX3CL1-CX3CR1 and CD200-CD200R systems in the examined brain areas and the treatment with quetiapine had a normalizing effect on part of these aspects. Prenatal exposure to lipopolysaccharide causes schizophrenia-related changes in adult offspring rats. The behavioural alternations are followed by disturbances in the protein systems. The antipsychotic drug – quetiapine exerts a positive effect on examined aspects. Nevertheless, the matter raised in the presented summary requires further research. Funding: grant no. 2015/19/B/NZ7/02394, NCN, Poland.

MTU12-05

Alpha-synuclein oligomers enhance astrocyte-induced synapse formation through TGF-β1 signaling in parkinson's disease model

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Parkinson's disease (PD) is characterized by selective death of dopaminergic neurons in the substantia nigra, nigrostriatal pathway degeneration, increase of glutamatergic synapses in the striatum and aggregation of a-Synuclein. Evidence suggests that oligomeric species of a-Synuclein (aSO) are the genuine neurotoxins of PD. Whereas several studies support the direct neurotoxic effects of aSO on neurons, their effects on astrocytes have not been directly addressed. Astrocytes are essential to several steps of synapse formation and function, including secretion of synaptogenic factors, control of synaptic elimination and stabilization, secretion of neuro-gliomodulators, and modulation of extracellular ions and neurotransmitters levels in the synaptic cleft. Here, we showed that aSO induce astrocyte reactivity and enhanced the synaptogenic capacity of human and murine astrocytes by increasing the levels of the known synaptogenic molecule, transforming growth factor beta 1 (TGF-β1). Moreover, intracerebroventricular injection of aSO in mice increased the number of astrocytes, the density of excitatory synapses, as well as TGF-β1 levels in the caudate-putamen of injected animals. Inhibition of TGF-b1 signaling impaired the effect of the astrocyte conditioned medium on glutamatergic synapses *in vitro* and striatal synapse formation *in vivo*; whereas addition of TGF-b1 protected dopaminergic neurons against synapse loss triggered by aSO. Together, our data suggest that aSO have important effects on astrocytic functions, and describe TGF-β1 as a new endogenous astrocyte-derived molecule involved in the increase of striatal glutamatergic synaptic density present in early stages of PD.

MTU12-06

Unraveling the role of SUMOylation in peripheral myelination

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The mechanisms that govern myelination in the peripheral nervous system are not completely understood. Post-translational modifications of proteins are necessary for peripheral myelination, and malfunction of these pathways leads to neuropathies. Whether SUMOylation, covalent attachment of Small Ubiquitin-like Modifier (SUMO) proteins to the substrate, is involved in the formation of myelin and/or in the pathophysiology of peripheral neuropathies is not known. We have recently identified SUMO2 as a novel protein that may be involved in the early interaction between axons and Schwann cells. In the pseudopod system, Schwann cells are cultured on a porous surface, and stimulated to extend pseudopods towards a neuronal membrane preparation. One of the proteins found in the proteome of Schwann cell pseudopods is SUMO2, along with 10 immediate neighbors in the interactome. Using a prediction algorithm, we evaluated which of the proteins found in the pseudopods have SUMO interaction motifs (SIMs) and/or SUMOylation sites. Interestingly over 85% of those proteins have either SIMs or SUMOylation sites: 67% of proteins may be SUMOylable, 58% of the proteins may interact with SUMO through SIMs. Proteins involved in cytoskeleton organization represent the major category of enriched proteins bearing SIMs and/or SUMOylation sites, and proteins related to regulation of cellular component organization are the most significantly enriched in pseudopods. Pharmacological modulation of SUMOylation and gene targeting of SUMO proteins affect myelination *in vitro*. Our current research is focused on the role of SUMOylation, its substrates, and SUMO2 in Schwann cell function and peripheral myelination. The discovery of SUMO targets will contribute to understanding the mechanisms of PNS development and demyelinating pathologies.

MTU12-07

Circuit-specialized transcriptional control of astrocytes contributes to learning and memory

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Astrocytes are abundant in the brain with diverse functions that contribute to brain homeostasis. However, whether astrocytes in different regions of the brain exhibit specialized characteristics is largely unknown. Here, in order to understand region-specific transcriptional regulation of astrocytes, we knocked out NFIA, a transcription factor pivotal to inducing gliogenesis, in four different regions of the adult mouse brain. We found that NFIA regulated great amount of genes in the hippocampus but only minimal amount of genes in other brain regions. Loss of NFIA in adult astrocytes resulted in aberrant morphology with fewer, shorter processes in the hippocampus. NFIA-deficient hippocampal astrocytes had reduced proximity to neurons, impaired detection of neurotransmitters, and reduced intracellular calcium activity. These astrocytic defects were associated with impaired long-term potentiation (LTP) and learning/memory deficits in astrocytic NFIA knockout mice. Our findings have identified the first region-specific transcriptional mechanism that regulates adult astrocyte morphology and function, and provide further insight into the contribution of astrocytes to learning and memory.

MTU12-08

New neurons reach and regenerate stroke-injured brain tissue by clearing a path through glia

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New neurons are continuously generated by the neural stem cells in the ventricular-subventricular zone (V-SVZ) even in the adult brain. After brain injury, the immature new neurons (neuroblasts) migrate toward the lesion. However, the ability of the mammalian brain to regenerate neuronal circuits for functional recovery is quite limited. By time-lapse imaging and immunohistochemistry using a mouse model for ischemic stroke, we showed that neuroblast migration is restricted by astrocytes, a major population of glial cells, activated in response to tissue damage in and around the lesion. To migrate through the meshwork of the astrocytic processes, the neuroblasts secrete a diffusible protein Slit1 to disrupt the actin cytoskeleton in reactive astrocytes that express its receptor, Robo2. By enhancing the Slit1-Robo2 signaling, V-SVZ-derived neuroblasts transplanted into the post-stroke brain could migrate closer to the lesion. Some of these cells matured into neurons possessing morphological and electrophysiological properties of the striatal projection neurons lost by stroke. They were efficiently integrated into the neuronal circuit, resulting in functional recovery in the post-stroke mice. These results suggest that the positioning of new neurons is critical for functional neuronal regeneration in stem/progenitor cell-based therapies for brain injury.

MTU12-09

The role of astrocytes in memory: focus on pattern separation

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Pattern separation (PS), a cognitive function thought to depend on adult born neurons (ABNs) in the dentate gyrus (DG), involves the transformation of representations of distinct features into unique, less overlapping representations, helping to minimize memory interference. As it has recently been shown that astrocytes play a leading role in helping ABNs to integrate and function in DG networks, we hypothesized that astrocytes themselves could play a role in regulating PS. We manipulate astrocytes by expressing excitatory DREADDs specifically on astrocytes by crossing $Glast^{creERT2}$-mice with floxed hM3-Gq-DREADD-mice. We surgically implant cannulas into the DG of each mouse, allowing us to directly inject clozapine-n-oxide (CNO), or vehicle. We confirm the expression and co-localization of DREADDs on astrocytes, and a lack of DREADDs expression on ABNs or mature neurons. We also validated the functionality of the DREADDs by performing in vitro experiments with astrocytic cultures from these animals and measuring Ca^{+} levels after CNO induction. We have two main approaches for the study of PS: Spontaneous Location Recognition (SLR); and the touchscreen-based Location Discrimination (LD) task. These tests are the same ones used to reveal a role for ABNs in PS. We found that activation by CNO of the excitatory Gq-DREADDs on the astrocytes of the DG region improved PS performance in both the SLR and LD tasks.

The finding that selective astrocyte manipulation can robustly and selectively improve memory function provides compelling evidence for the importance of astrocytes in cognition, and introduces astrocytes as a novel target for intervention in neurodegenerative and neuropsychiatric diseases affecting memory.

MTU12-10

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

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

MTU12-11

Volume electron microscopy of the white matter in the hereditary demyelinating disease model

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The connections of neurons are dependent on axons, long neurites which are enriched in the white matter. Many of the axons are ensheathed in the white matter by myelin, and the myelin ensheathment divides axons into structurally and functionally distinct domains. Myelin supports salutatory conduction and maintenance of axonal integrity. Volume electron microscopic imaging of the white matter has recently started providing structural information for the better understanding of physiology and pathology of the white matter. For example, the distribution and morphology of organelles in axons were changed by demyelination in the white matter, and it was suggested that such alterations are beneficial for axonal functions and survival. Among them, mitochondria associated membranes (MAM), physical connections between mitochondria and endoplasmic reticulum, are critical for cellular functions such as Ca^{2+} signaling, lipid transport. Disruption of the connection has been implicated in mitochondrial dysfunction, which has been proposed as a major contributor of axonal degeneration in diseases of myelin. However, the changes and roles of these juxtapositions are still unclear in demyelinated axons. In this study, we investigated three-dimensional ultrastructural changes of axonal MAM in chronic demyelination, using the serial block-face scanning electron microscopy and a mouse model of chronic demyelination caused by extra-copies of proteolipid protein. The results suggest that modulations of MAM as well as mitochondria are caused by chronic loss of myelin, and that such organelle changes are involved in the pathophysiology of hereditary myelin diseases.

MTU12-12

NG2 GLIA-specific KIR4.1 knockout as a tool to understand the impact of neuron-glia synaptic signaling

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NG2 glia in grey matter receives direct synaptic input from glutamatergic and GABAergic neurons. During development, NG2 glia upregulate Kir4.1 channels. To test if Kir currents regulate the efficiency of synaptic activation of NG2 glia, we used NG2-CreERT2 knock-in mice to selectively ablate the Kir4.1 gene upon tamoxifen administration.

Mutant NG2 glia, deficient of Kir currents, displayed a more positive resting potential and increased membrane resistance in comparison to control NG2 glia. Monitoring responses upon Schaffer collateral stimulation revealed similar EPSC amplitudes in Kir-deficient NG2 glia compared to control cells. Moreover, mEPSP amplitudes were enhanced and the time constant of voltage decay was prolonged in Kir4.1 deficient glial cells. To investigate the impact of Kir4.1 deletion in NG2 glia on neural signaling, field potentials were recorded in the hippocampus after stimulation of Schaffer collaterals. Long term potentiation (LTP), induced by theta-burst stimulation, was significantly impaired in the hippocampal CA1 region of mice with NG2 glia-targeted Kir4.1-deficiency. Despite impaired LTP, Kir4.1-deficient mice showed increased novelty preference in the object location recognition test, and improved new partner preference in the partner recognition test. In the hippocampus, NG2 glia-targeted deletion of the Kir4.1 gene entailed an upregulation of MBP protein 8 weeks after tamoxifen injection. These findings show that Kir4.1 channels in NG2 glial cells regulate their excitability, influence myelination and are important for proper hippocampal synaptic plasticity and behavior.

MTU12-13

Muscarinic acetylcholine receptors regulate the expression of KIR4.1 channels and BDNF in cultured mouse astrocytes

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Astrocytes are the most abundant glial cells and regulate neuronal excitability by maintaining ion homeostasis, metabolizing neurotransmitters and secreting neuroactive substances. Recent evidence illustrates that astrocytic brain-derived neurotrophic factor (BDNF) expression is specifically modulated by inwardly rectifying potassium (Kir) channel subunit Kir4.1 channel, which mediate the spatial potassium buffering function of astrocytes (Front. Mol. Neurosci., 10, 408, 2017; Int. J. Mol. Sci., 19, 3313, 2018). In the present study, we investigated the effects of acetylcholinergic agents on mRNA expression of Kir4.1 and BDNF in primary cultured mouse astrocytes in order to explore the neural factors influencing on the Kir4.1-BDNF system. Treatment of astrocytes with acetylcholine (ACh, 3-30 μM) significantly inhibited Kir4.1 expression and increased BDNF expression in a concentration-related manner. Both inhibition of Kir4.1 and enhancement of BDNF expression by ACh (10 μM) were antagonized by muscarinic ACh receptor antagonist atropine (3 μM), however, the nicotinic ACh receptor antagonist mecamylamine (30 μM) showed no effects. In addition, inhibition of Kir4.1 expression by ACh was significantly antagonized by the selective muscarinic ACh M_1 antagonist pirenzepine (10 μM), which also inhibited acetylcholine-enhanced BDNF expression. The present results strongly suggest that ACh inhibits Kir4.1 channel expression and increases BDNF expression via activation of muscarinic ACh M_1 receptor in astrocytes.

MTU12-14

Anti-inflammatory effect of carbon monoxide on the neuron-microglia communication

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Microglia, the 'resident immunocompetent cells' of the central nervous system (CNS), are key players in innate immunity and tissue homeostasis. However, dysfunctional microglia contributes heavily to the creation of a toxic inflammatory milieu, a common driving force for the pathophysiology of several CNS disorders. Thus, strategies have been postulated to tackle exacerbated tissue inflammation by modulation of microglial function. Carbon monoxide (CO) is an endogenous gaseous molecule, produced by the degradation of free haem. Long considered a catabolic waste product, it has now emerged as a player in neurobiology, having shown neuroprotective properties in *in vivo* and *in vitro* models. We aimed at studying CO as a modulator of microglial reactivity, focusing on its communication with neurons by limiting inflammation and consequently providing neuroprotection. For this, we used a BV2 microglia-CAD neuron cell line conditioned media protocol. Treating LPS-activated BV2 microglia with CO limited expression and secretion of inflammatory cytokines (TNF-alpha, NO). Neurons subsequently challenged with inflammatory media displayed high cell death and dysfunction levels. This is partially reverted whenever microglia is pre-treated with CO, indicating the gas promotes neuroprotection in a non-cell autonomous mode. Likewise, CO stimulated the expression of neuronal glycoprotein CD200 and its microglial receptor CD200R1 *via* PPAR-γ transcription factor. The CD200-CD200R1 axis is involved in the fine regulation of microglial function, and we are elucidating how CO-driven modulation of this tight contact alters cell function, fate and homeostasis. Altogether, this is a stepping stone to understand CO's impact on novel cell-cell regulatory mechanisms.

MTU12-15

Retinal inputs signal through astrocytes to recruit interneurons into mouse visual thalamus

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The lateral geniculate complex includes a number of retinorecipient thalamic nuclei that play important roles in receiving, processing, and relaying image- and non-image forming visual information. Recent studies have revealed that neonatal innervation of these regions by retinal ganglion cell axons play an instructive role in the postnatal development and maturation of thalamic circuits. For example, surgical or genetic removal of retinal inputs at birth impairs the recruitment of local GABAergic interneurons into visual thalamus. Here we sought to identify the mechanisms that underlie retinal input-dependent interneuron migration into visual thalamus. Focusing on this, we first explored transcriptomic changes in neonatal mouse visual thalamus lacking retinal input. Using microarray analysis and *in situ* hybridization (ISH), we discovered that the expression of Fibroblast Growth Factor 15 (FGF15) in visual thalamus is dependent upon the retinal inputs. To test whether FGF15 was required for interneuron recruitment into visual thalamus, we examined thalamic development in *Fgf15*$^{-/-}$ mutant mice. In these mutants, we observed a significant reduction in GABAergic interneurons both by ISH and by crossing these mutant mice to *Gad67-GFP* reporter mice. We hypothesized that retinal inputs induced FGF15 expression in retinorecipient neurons. To test this, we performed ISH to detect *Fgf15* mRNA in reporter mice that label distinct populations of cells in visual thalamus. Surprisingly, our data revealed that FGF15 is generated by thalamic astrocytes and not neurons, suggesting a novel role for astrocytes in thalamic development. Taken together, these results suggest the existence of novel axon-glia-neuron signaling pathway that underlies subcortical visual circuit formation.

MTU12-16

High fat diet promotes cognitive impairment, neuroinflammation and decreased hippocampal plasticity: role of microglial exosomes

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Western dietary habits including high fat foods are increasingly represented in juvenile populations, and constitute one of the factors that affect brain health, potentially leading to long lasting effects. The aim of this study was to assess the impact of an early exposure to a high fat diet (HFD) on mouse hippocampal plasticity. C57BL/6J male mice were exposed to HFD for 6 weeks since weaning. Glucose and IL1β levels were found to be higher in serum of HFD mice, without overweight. In the hippocampus, neuroinflammation was evidenced by Iba1+ cells reactivity and increased expression of TNFα and IL1β in HFD group, which also exhibited a strongly reduced neurogenic capability: decreased Ki67+ cells and immature DCX+ neurons in the SGZ of the dentate gyrus. We also found a reduced proportion of mature Dil-labeled dendritic spines from CA1 neurons and diminished levels of the scaffold protein Shank2, suggesting a defective connectivity. Moreover, HFD mice exhibited spatial memory alterations in the novel object location recognition test. To study whether microglia could be mediating HFD-associated neuronal changes, primary microglia was incubated with palmitate, a saturated fatty acid present in HFD. Palmitate induced a proinflammatory profile as shown by secreted cytokine levels and exosome-like extracellular vesicles that were able to induce an immature dendritic spine phenotype in primary GFP+ hippocampal neurons, in line with the *in vivo* findings. These results provide novel data concerning microglia-neuron communication and highlight that fat excess during an early period of life could negatively impact on hippocampal plasticity in a neuroinflammatory context, where microglia-derived exosomes could be directly implicated.

MTU12-17

Astrocytic insulin-like growth factor-1 protects neurons against excitotoxicity

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Background: Exogenous insulin like growth factor-1 (IGF-I) is known to be neuroprotective in animal models with brain insults, while it can also cause hyperexcitability in rodents. In this regard, the role of endogenous IGF-1 in brain responses to brain insults like excitotoxicity, a common pathology in brain injuries remains elucidated. Here, we investigated the potential role of cell-specific endogenous IGF-I in the kainic acid (KA) -induced degeneration of the neurons.

Methods: KA was given to primary cultured cortical neurons and co-cultured astrocytes were added as a supportive system. We evaluated the cell proliferation rate, IGF-1 level in different groups and applied the PCR-Chip assay to explore the downstream of IGF-1. In addition, we applied the viral transfer of astrocytic IGF-1 to rodents treated with KA and assessed the associated molecular marker and behavioural outcomes in these rodents.

Results: We found KA induced increased cell death and hyper-phosphorylated tau in neurons; co-cultured astrocytes could prevent these pathologies, and this rescuing effect was abrogated with blockade of the astrocytic IGF-1 with AG1024 (IGF-1R inhibitor). PCR-Chip assay identified that astrocytic IGF-1 could decrease the p-GSK-3 at Thy 216 in neurons treated with KA and this effect was abrogated with AG1024 as well. In addition, in vivo study showed that gene transfer of astrocytic IGF-1 decreased p-tau and cognitive dysfunction in KA mice.

Conclusion: Our results show astrocytic IGF-1 exhibit neuroprotective properties in neurodegenerative processes in the CNS.

MTU13 Lipids (Session A)

MTU13-01

Identification of the antigen recognized in vitro by RHIGM22, a remyelination-promoting human monoclonal antibody

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Recombinant human IgM22 (rHIgM22) binds to myelin and oligodendrocytes (OLs), and promotes remyelination in models of multiple sclerosis. rHIgM22 preferentially reacts with sulfatide-positive (O^{4+}) OLs, and its binding is abolished in brain slices from Cst (-/-) mice, suggesting its binding requires the presence of a product of cerebroside sulfotransferase. However, literature suggests that cell populations lacking sulfatide expression, such as microglia and oligodendrocyte precursor cells, are responsive to rHIgM22, thus the identity of the antigen recognized by this antibody remains to be elucidated.

We tested the binding of rHIgM22 to purified lipids and lipid extracts from various sources using TLC immunostaining and surface plasmon resonance (SPR) with lipid monolayers. Our results show that IgM22 binds to sulfatide and lysosulfatide *in vitro*, while it does not bind to other myelin sphingolipids. In addition, rHIgM22 also reacts with phosphatidylinositol, phosphatidylserine and phosphatidic acid, present in lipid extracts from various sources, including CST ko mice brains, mixed glial cultures, isolated astrocytes and microglia.

These data suggest that sulfatide at the OLs surface might be important for the binding of rHIgM22 to these cells. On the other hand, its ability to bind some glycerophospholipids could explain the biological responses elicited by rHIgM22 in cells lacking sulfatide expression. The *in vitro* reactivity of rHIgM22 suggests that binding of rHIgM22 to intact cells might require a complex molecular arrangement, and, in particular, sulfatide and other membrane lipids might be part of the functional rHIgM22 antigen localized at the cell surface.

MTU13-02

Gender-specific changes to sphingolipid metabolism may sensitise the aging brain to neurodegeneration and Alzheimer's disease

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The major risk factors associated with Alzheimer's disease (AD) are age and inheritance of the ε4 allele of the *APOE* gene, which encodes the lipid transporter protein, Apolipoprotein E (ApoE). This suggests a key involvement of lipid transport and metabolism in AD. Sphingolipids, are a class of lipids that exhibit alterations at the prodromal stages of AD, in both brain tissue and serum. Our study investigated sphingolipids as a function of age and APOE genotype in neurologically normal subjects, aged 65 and over. Lipids were quantified from the hippocampus of post-mortem tissue (n = 80) using mass spectrometry. Significant changes to sphingolipids were observed as a function of age, and were gender-specific. Females had a pronounced decline in the sphingosine-1-phosphate:sphingosine ratio ($p = 0.0020$). In contrast, males exhibited increases in ceramides, sulfatide and sphingomyelin ($p < 0.005$). No association between lipids and *APOE* genotype was identified. Previous literature has demonstrated AD is associated with a decline in cerebral glucose utilisation, potentially caused by a loss of insulin receptors at synaptic membranes of the cerebral cortex and hippocampus. Ceramide is a pro-apoptotic lipid implicated as a driver of of insulin resistance in metabolic tissue, whereas S1P is associated with increased glucose-stimulated insulin secretion. In the age of precision medicine, there is a need to discern whether risk factors and etiology of AD differs between genders. Our results establish gender-specific differences in sphingolipid metabolism in the aging human brain, which may contribute to a pro-neurodegenerative phenotype.

MTU13-03

Correlation of plasma omega fatty acid index with alpha power during working memory in acute mild traumatic brain injury

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We explored the relationship between alpha power during working memory (WM) processing and the plasma levels of n-3/n-6 polyunsaturated fatty acids (PUFA) in acute mTBI to understand mechanisms and to discover biomarkers of injury. Brain challenges using 0-back and 2-back WM tests were administered within 5 days post-injury with simultaneous electroencephalography (EEG) from 21-head sensors. Plasma unesterified and esterified PUFAs were quantified using gas chromatography/mass spectrometry. The proportions of n-3, n-6, the n-3/n-6 ratio, and the n-3 index were determined and correlated with alpha power for all six brain regions. Esterified n-6 PUFAs negatively correlated with 0-back alpha wave power in the frontal, left temporal, and occipital brain regions while unesterified n-3 negatively correlated with the central brain region of mTBI participants. Esterified n-3/n-6 ratio positively correlated with 0-back alpha power of all six brain regions while esterified n-6 negatively correlated with 2-back alpha power of all brain regions. In contrast, the esterified n-3/n-6 ratio positively correlated with 2-back alpha power in all six brain regions. N-3 and n-6 fatty acids did not correlate with alpha power in the controls. Our studies reveal brain region-specific correlations of 0-back and 2-back alpha frequency with plasma PUFAs in acute mTBI, but not in controls, suggesting that changes in plasma omega-3/6 profile underlie abnormal brain functions during WM challenge at the early phase of mTBI. Therefore, restoring the n-3 PUFA index may enhance prognosis by normalizing alpha power disturbance in mTBI.

MTU13-04

Neuroprotective sphingosine 1-phoshate is essential for amyloid formation, oligodendrocyte survival and cognitive function in AD

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Sphingosine 1-phosphate (S1P) is a potent vasculo- and neuroprotective signalling lipid that promotes neurotrophic growth factor expression and pre-synaptic acetylcholine and glutamate release. S1P is synthesized primarily by sphingosine kinase 2 (SphK2) in the brain. We recently demonstrated pronounced loss of S1P, and SphK2 activity, early in Alzheimer's disease (AD) pathogenesis. Using human female hippocampal tissue samples from neuropathologically normal donors, we recently showed that S1P levels decline with age ($r = -0.5$, $p = 0.002$), leading us to speculate that loss of S1P sensitizes to AD development. To test whether SphK2 deficiency synergises with amyloid beta (Aβ) in promoting AD, SphK2 knockout (SphK2-/-) mice were crossed to the J20 mouse model of familial AD amyloidosis.

Suprisingly, SphK2 deficiency reduced Aβ content, plaque burden and reactive astrocyte immunoreactivity in J20 mice. Reduced Aβ was associated with significant improvements in hypersynchronous activity and cross-frequency coupling measured by hippocampal electroencephalography. Despite reduced amyloid burden, SphK2-deficient J20 mice exhibited severe hypomyelination in the hippocampus and cortex, hippocampal atrophy and significant deficits in the Y-maze and social novelty memory tests, when compared to the J20 or SphK2-/- strains.

In summary, endogenous S1P, synthesized by SphK2, is reduced with ageing and AD pathogenesis, yet required for Aβ formation. However, memory deficits and myelin loss and hippocampal volume in J20 mice were exacerbated on a SphK2-/- background, indicating that age-dependent SphK2 depletion promotes neurodegeneration and urging consideration of oligodendrocyte attenuation as a potential enforcer of neurodegeneration in AD.

MTU13-05

Evidence that human glioma cells form pregnenolone via a CYP11A1-independent pathway

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The term "neurosteroids" refers to steroid hormones, such as pregnenolone, synthesized and acting in brain. In peripheral steroidogenic tissues, pregnenolone is formed from cholesterol by the cytochrome P450 11A1 enzyme. This conversion involves two hydroxylations at C22 and C20 of cholesterol followed by cleavage of the side chain between C20-22 to form pregnenolone and isocaproic aldehyde. Although pregnenolone is found in the brain, protein expression of CYP11A1 has been difficult to detect. We found extremely low levels of *CYP11A1* mRNA in human glioma cells but no protein expression. Therefore, we investigated the ability of human glioma cells to synthesize pregnenolone in a CYP11A1-independent manner. Unlike testicular and adrenal cortical cells, treatment of glioma cells with the CYP11A1 inhibitor DL-aminoglutethimide or the non-specific CYP inhibitor ketoconazole did not inhibit pregnenolone production by glioma cells. Pregnenolone synthesis can be increased with addition of the substrates 22R-, 22S-, and 20α-hydroxycholesterols suggesting the involvement of a desmolase activity although the enhanced pregnenolone formation was not blocked by DL-aminoglutethimide or ketoconazole. These data suggest that glioma cells can produce pregnenolone independently of CYP11A1 activity. CYP enzymes are monooxygenases generating free radicals, so we examined whether this alternative pathway involves reactive oxygen species (ROS). Although high doses of DL-aminoglutethimide and ketoconazole increased pregnenolone production by glioma cells, only ketoconazole increased cellular ROS. Addition of the antioxidant Trolox, an analog of vitamin E, did not change pregnenolone production despite blocking ketoconazole's effect on increasing cellular ROS. Treatment with hydrogen peroxide also did not have a significant effect on pregnenolone production. Taken together these results suggest that human glioma cells form pregnenolone via a CYP11A1- and ROS-independent pathway.

MTU13-06

Hexa-associated GM2 gangliosidosis in a family of wild boars

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Gangliosidosis are inherited lysosomal storage disorders caused by defective activity of a lysosomal hydrolase required for ganglioside catabolism, resulting in the intra-lysosomal accumulation of undegraded metabolites. The molecular mechanisms linking the lysosomal accumulation to the pathology are still obscure. We report on a novel form of GM2 gangliosidosis in wild boar (*Sus scrofa*). Three littermate wild boards, from a free ranging farm, presented neurological signs (dysmetria, ataxia, quadriplegia and lateral decubitus) at 6 months of age. Viral, bacterial and toxicological analysis were performed to exclude possible exogenous causes of symptoms. Animals were euthanized at approximately one year of age. Necropsy revealed in all affected animals reduced consistency of cerebral and cerebellar parenchyma. Histology revealed enlarged foamy neurons, with diffusely severely vacuolated cytoplasm in brain, cerebellum, spinal cord, peripheral ganglia and retina. EM revealed the presence in neurons of numerous lysosomes, filled by membranous material. Biochemical studies revealed the presence of an elevate amount of GM2 ganglioside, confirming the diagnosis of GM2 gangliosidosis. In addition, genetic analysis revealed the presence of a recessively inherited missense variant (p.Arg499Cys) in the *hexosaminidase subunit alpha* (*HEXA)* gene located within the GH20 hexosaminidase superfamily domain of the encoded protein. In man and other species, pathogenic HEXA variants are known to be associated with the disease. In conclusion, this HEXA-associated form of GM2 gangliosidosis, described for the first time in wild boars, is thus very similar to human disease.

MTU14 Other topics (Session A)

MTU14-01

Expression of FMRFamide and GFSKLYFamide peptides in holothuria scabra: Implication for the neuroendocrine system

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Neuropeptides are key mediators of physiological processes in animals and a considerable amount of information has been accumulated on their diversity and functions across phyla. FMRFamide-related peptides are a large collection of neuropeptides found in invertebrates and vertebrates. They are identified by the possession of a C-terminal -RFamide amino acid sequence and are often coded for by multiple genes. Echinoderms are of phylogenetic importance to chordates in that they are deuterostomes. The sea cucumber, *Holothuria scabra* is a high-premium tropical echinoderm species that is overexploited globally and may be in imminent danger of extinction in some areas. Conservation of this species and its amenability to culture is however hampered by our limited knowledge of its biology, including the neurohormonal system. In this study, FMRFamide peptide, a cardioactive neuropeptide first isolated in a mollusk and GFSKLYFamide peptide, an Echinoderm SALMFamide were investigated for their presence in *H. scabra*. We used indirect immunofluorescence technique with confocal microscope and dot immunoblot assay utilizing polyclonal antisera raised against FMRFamide and GFSKLYFamide peptides. Both FMRFamide- and GFSKLYFamide-immunoreactivity were demonstrated to be widely distributed in *H. scabra* tissues, such as the radial nerve cord, body wall, intestines and coelomic fluid. This underscores the potential physiological roles of these peptides, which might be working as neurotransmitters and neuromodulators in this species.

MTU14-02

Promoting endogenous photoreceptor regeneration in the mammalian retina

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Regenerating the retina using endogenous stem cells is a promising therapy for vision restoration. While fish Müller glial cells (MG) can regenerate the retina, this natural ability was lost in mammals. Recently, however, some genetic manipulations in mouse MG were found to trigger neuron production, but it remains unknown whether MG can generate cone photoreceptors, which are essential for high acuity vision. Interestingly, MG have a similar gene expression profile to late-stage retinal progenitors, and our previous work identified temporal identity factors that can reprogram late progenitors to produce early-born cones. We hence hypothesized that these factors might reprogram MG into cone-producing progenitors. We co-electroporated Cre-dependent constructs into GlastCre^{ERT};RosaYFP$^{fl/fl}$ retinal explants, which express Cre^{ERT} specifically in MG, allowing expression of genes of interest and cell lineage tracing with the YFP reporter. Of the 21 combinations tested, one was able to reprogram MG into immature cones. MG-derived cells migrated to where cones normally reside, downregulated glial markers, started expressing the cone marker RxRg and adopted a cone-like morphology. These factors were also sufficient to reprogram MG to immature cones *in vivo* in the adult mouse retina, and into more mature cones under certain culture conditions. It remains to be determined whether these cells are functional, but this work suggests that stimulating cone production from endogenous glia might represent a new therapeutic opportunity for retinal degeneration.

MTU14-03

Inner hair cell and neuron degeneration contribute to hearing loss in a DFNA2-like mouse model

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DFNA2 is a progressive deafness caused by mutations in the voltage-activated potassium channel KCNQ4. Hearing loss develops with age from a mild increase in hearing threshold to profound deafness. The first phase starts around 10-15 years old, progressing to the last phase by the age of 70. Studies using transgenic mice for *Kcnq4* expressed in a mixed background demonstrated the implication of outer hair cells (OHCs) at the initial phase. However, it could not explain the last phase mechanisms of the disease. Genetic backgrounds are known to influence disease expressivity. To unmask the cause of profound deafness phenotype, we backcrossed *Kcnq4* knock-out allele to the inbred strain C3H/HeJ and investigated inner and outer hair cell and spiral ganglion neuron (SGN) degeneration across lifespan. In addition to the already reported OHC death, C3H/HeJ strain also exhibited inner hair cell (IHC) and SGN death. We tracked the spatiotemporal survival of cochlear cells by plotting cytocochleograms and neuronal counts at different ages. Cell loss progressed from basal to apical turns with age for both hair cells. Interestingly, the time-course of cell degeneration was different for each cell-type. While for OHCs it was already present by week 3, IHC and neuronal loss started 30 weeks later. We established that OHC loss kinetics slowed down from basal to apical regions correlating with KCNQ4 expression pattern determined in wild-type mice. Our findings indicate that KCNQ4 plays differential roles in each cochlear cell-type impacting in their survival ability. IHC and SGN neuron death generates severe hearing loss that could be associated to the last phase of DFNA2.

MTU14-04

Copper uptake and toxicity in cerebellar granular neurons after application of copper ions or copper oxide nanoparticles

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Copper is essential for brain cells as cofactor of various enzymes. Nonetheless, disturbances of copper homeostasis are known to cause oxidative stress and neurological disorders. However, there is only little known on copper uptake and metabolism in neurons. To investigate copper uptake in neurons we have used cerebellar granule neuron-rich primary cultures as model system. The low basal copper content increased strongly after application of copper chloride in a time and concentration-dependent manner, reaching values of up to 40 nmol copper per mg protein after exposure to 100 μM copper chloride for 30 min. Exposure of neurons to copper chloride in the presence of ascorbate increased cellular copper contents more than 7 times which was accompanied by severe toxicity. Correlation of cellular copper contents with cell viability revealed that specificcopper values of above 30 nmol copper per mg protein were accompanied with a strong loss in cell viability. Copper accumulation and copper-induced toxicity was prevented by the application of the copper chelators bathocuproine disulfonate and tetrathiomolybdate, whereas the application zinc ions, known copper transport competitors, did not lower neuronal copper uptake. Comparison of neuronal copper accumulation after application of copper chloride or copper oxide nanoparticles showed that cellular copper contents after incubation with 100 μM copper chloride was 33 % lower than after incubation with the same concentration of copper oxide nanoparticles. These results demonstrate that copper uptake in neurons is prevented by copper chelators and accelerated by ascorbate which leads to an imbalance of cellular copper homeostasis that severely damages neurons. Moreover, copper accumulation was also observed after application of copper oxide nanoparticles but the mechanisms involved in the uptake need to be further elucidated.

MTU14-05

Targeting mitochondrial dynamics by environmental toxicant bisphenol-A in the rat hippocampus

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This study summarized the neuronal demise following Bisphenol-A (BPA) exposure linked impaired mitochondrial biogenesis and dynamics in rat brain hippocampus. Mitochondria are known for their multiple essential cellular functions beyond ATP production/ energy transduction, impacting most of the areas of cell biology and related mitochondrial medicines in brain. Most of the proteins that help in mitochondria biogenesis (formation of healthy mitochondria) and regulate their dynamics (fission/fusion) are encoded in nucleus. BPA is a known xenoestrogen, found in consumable plastics and causes neuronal apoptosis after chronic exposure in the rat hippocampus and linked cognitive deficits. Any pathogenesis in mitochondria is responsible for impaired mitochondrial dynamics and biogenesis, which is directly associated with cognitive impairment. In this study, we have investigated the gene expression and protein levels of parental factors of mitochondrial biogenesis (PGC1α, TFAM etc.) and dynamics (DRP-1, MFN-1/2) after BPA exposure in the adult rat hippocampus and in NSC derived neuronal culture. Our result showed reduced protein levels of PGC1α, TFAM, while imbalanced dynamics showed through excessive fission protein DRP-1. Beside this, our study also investigates the regulatory circuitry and inter-linked events involved in both mitochondrial dynamics and biogenesis. With this, our work holds promising in understanding the role of BPA induced mitochondrial pathophysiology that may give new therapeutic targets to neurodegenerative disorders.

MTU14-06

Metabolic profiles of the synthetic cannabinoid, athpinaca, in human liver microsomes with isomeric discrimination

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The illegal use of synthetic cannabinoids (SCs) has become a serious problem worldwide. To promote further research of SCs, precise discrimination of SCs and their metabolites from their isomers and derivatives in human biological specimens is needed. Here, we aimed to develop a method to specifically detect SCs and their structural isomers and to investigate SC metabolic profiles in human liver microsomes (HLMs). ATHPINACA isomer 1 and 2 were incubated with HLMs at designated time points up to 3 hrs. LCMS-IT-TOF data from resulting samples were analyzed by ESI positive/negative mode. The product ion spectra of parent SCs obtained from the protonated molecules revealed a clear difference between the two isomers, likely due to the stability of the resulting adamantyl cation—although chromatography showed similar retention times for both parent compounds. Both parent SCs were quickly metabolized with half-lives of approximately five min. 14 metabolites in isomer 1 and 12 metabolites in isomer 2 were annotated. The major metabolizes were di-hydroxylated isomer 1 and mono-hydroxylated isomer 2, suggesting that hydroxylation of the adamantyl moiety is likely the major metabolic pathway of ATHPINACA isomers. This is the first report to characterize the metabolism of ATHPINACA and its adamantyl positional isomer. The information about differences in the product ions of the parent compounds or their major metabolites is useful for discriminating between the two isomers in forensic cases and pharmacokinetic/ pharmacodynamic studies.

MTU14-07

L-theanine inhibits the proliferation of neural cell lines via an L-glutamine transporter SLC38A1

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L-Theanine (g-glutamylethylamide) is an amino acid contained in green tea leaves with structural analogy to glutamine and is suggested to be taken up into cells mediated by a glutamine transporter Slc38a1. Recently, the oral intake of L-theanine is expected to suppress anxiety, sleep disturbance, and cognitive impairment. Some reports showed that L-theanine possesses anti-cancer activities against some cancers. Here, it was investigated whether L-theanine inhibits cell proliferation and its mechanism is via Slc38a1. The cell proliferation rate was measured by MTT method, and the cell viability was measured by propidium iodide staining. Expression of the amino acid transporter was carried out by real time RT-PCR method. L-Theanine inhibited cell proliferation in mouse motor neuron cell line (NSC-34), mouse neuroblastoma cell line (Neuro 2A) and human neuroblastoma cell line (SH-SY5Y) in a concentration- and time-dependent manner. However, it had little effect in human brain glioblastoma cell line (U-251 MG), mouse astrocyte cell line (C8-D1A), mouse brain endothelial cell line (bEnd3) and human umbilical vein endothelial cells (HUVEC). There was a positive correlation between the L-theanine-dependent inhibition of cell proliferation and the expression level of Slc38a1 mRNA (r^2 = ~0.66). Therefore, it was suggested that in these neural cell lines, the suppressive effect on cell proliferation was caused by L-theanine which was taken up into the cells via Slc38a1.

MTU14-08

Agrin as a presynaptic differentiation inducer and its proteolytic regulation

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Agrin, a heparan sulfate proteoglycan molecule, has been well studied for its critical role in promoting acetylcholine receptor (AChR) clustering during postsynaptic differentiation at the neuromuscular junction (NMJ). A previous study also suggested that agrin regulates growth cone guidance in spinal neuron cultures; however, the functional roles of agrin and its proteolytic regulation in presynaptic differentiation remain unclear. Matrix metalloproteinases (MMPs) play critical functions in the remodeling of extracellular matrix (ECM) proteins to control cell migration and motility. Interestingly, membrane-type 1 MMP (MT1-MMP) and secreted MMP-3 have previously been shown to extracellularly cleave agrin that affect synaptic structures. Using *Xenopus* primary cultures, this study aims to investigate how agrin and its proteolytic regulation by MMP activity spatio-temporally modulate presynaptic differentiation at developing NMJs. Firstly, local application of agrin spatially induces the clustering of mitochondria and synaptic vesicles, two well-known presynaptic markers, along the neurites. The findings indicated that agrin is an effective presynaptic differentiation inducer. Secondly, pharmacological inhibition of MMP activity or reduced expression of MT1-MMP significantly inhibited agrin-induced presynaptic differentiation. We next observed that agrin is spatially enriched at nerve-muscle contact sites, which is coupled with ECM degradation and nerve-induced AChR clustering in nerve-muscle co-cultures. Since agrin is usually secreted globally along the neurites, localization of agrin at synaptic sites inferred that MMP proteolytic activity cleaves agrin that is present in extra-synaptic regions. Lastly, MMP inhibitors or morpholino-mediated MT1-MMP knockdown also inhibited agrin deposition and the formation of nerve-induced AChR clusters at synaptic sites. Taken together, our results demonstrate a previously unappreciated role of agrin in presynaptic differentiation and the regulatory role of MT1-MMP in this process.

MTU14-09

Estrogen-deficiency induced cognitive impairment: role of HB-EGF/EGFR signaling in autophagy and neuronal apoptosis in hippocampus

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Estrogen deficiency in post-menopausal condition promotes hippocampal neuronal apoptosis and learning-memory impairment. However, the underlying mechanism remains uninvestigated. Since excessive autophagy promotes the self-digestion of cells while growth factor signaling promotes cell survival pathway. So, we hypothesized their participation in estrogen deficiency-induced learning-memory impairment, and for this, the ovariectomized (OVX) rat model was used. We observed that estrogen deficiency induces autophagy in hippocampal neurons marked by increased LC3-II, Beclin-1, ATG7, ATG5/12 and autophagosomes along with decreased p62 levels. In addition, autophagy regulators p-AKT/AKT, p-mTOR/mTOR and p-ULK1/ULK1 were down-regulated in OVX rats. Further, we observed mitochondrial localization within the autophagosomes along with decreased VDAC and COXIV levels in OVX rats indicating mitochondrial loss during estrogen deficiency. On investigating up-stream effectors molecules, we identified down-regulated HB-EGF, along with decreased EGFR activation in OVX rats suggesting its role during estrogen deficiency. We observed that 17β-estradiol or HB-EGF treatment restores autophagy markers, autophagy regulators as well as mitochondrial loss. We finally correlated our observation with neuronal apoptosis and learning-memory impairment. We observed that HB-EGF or autophagy inhibitor, 3-MA treatment not only inhibited OVX-induced apoptosis of hippocampal neurons but also restored learning-memory performances, assessed through Y-Maze and Passive avoidance tasks. Thus, our study highlighted the involvement of HB-EGF/EGFR signaling in mitochondria-associated autophagy in estrogen deficiency which leads to neuronal apoptosis and learning-memory impairment in estrogen-deficient females.

MTU14-10

Characterization of vascular changes in the regenerating optic nerve

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The vascular and the nervous systems are highly branched networks that are functionally and physically interdependent during development. Vascular patterning and neural wiring share some guidance cues and receptors. Most recently this relationship has also been investigated in the peripheral nervous system (PNS) regeneration. In the PNS, nerves and blood vessels often run in parallel and endothelial cells play an important role on guiding the formation of the bands of Bügner and serving as a scaffold for regrowing axons. Here, we used the optic nerve crush (ONC) model with a combinatorial treatment that stimulates retinal ganglion cells (RGCs) growth after injury. Our results show that in control animals that didn't receive treatment for regeneration, there was a 2-fold change in the total number of blood vessels 7 days after crush, compared to a unlesioned nerve. Moreover, there were more blood vessels at 0,5 mm after the crush site than in distal parts of the nerve. The combination of treatments promoted extensive regeneration of RGCs and increase in survival. Interestingly, the increased regeneration throughout the nerve is not followed by an increase in the number of blood vessels. There is no difference in the total number of blood vessels 2 weeks after crush between animals that received the combined treatment for regeneration and control animals. This study provides interesting insights into role of blood vessels in the regeneration of an adult CNS` tissue.

MTU14-12

Effects of prenatal ischemic hypoxia on cardiovascular risk and rat behavior

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Perinatal stress has been associated with increased susceptibility to affective disorders. Hypertension is a chronic disease that fundamentally compromises the balance of vasodilatory and vasoconstricting mechanisms, causing damage to the organs irrigated by them. There are enough evidences showing a relation between mental and cardiovascular diseases, suggesting that depression could be a risk factor to acute myocardial infarction and cardiovascular mortality. In the present study, we investigated whether there is an association between prenatal hypoxia ischemia, depression and late cardiovascular disease. Ischemic hypoxia was induced by clamping the uterine arteries of pregnant rats (Wistar) for 45 minutes, on the 18th day of gestation. Same conditions were applied to the pregnant rats to form the SHAM group, only without the uterine arteries clamping. Analyzes of serotonin, dopamine and their metabolites contents in basal midbrain were performed by the High- performance liquid chromatography technique. Morphometric analyses was also studied in aortas and hearts tissues. Oxidative stress was studied by the balance between anti-oxidants enzymes and the damage in proteins by carbonylation and immunohistochemetry for 8-isoprostane. The animals were also submitted to systemic arterial pressure measurement using the noninvasive method of caudal plethysmography. The mesenteric artery was isolated to study the performance of the vascular reactivity induced by vasoconstrictors and vasodilators. Data were statistically analyzed using one-way ANOVA and student t test. These results are consistent with the hypothesis of increased vulnerability of the serotonergic system for perinatal stress and the physiological changes in the cardiovascular system before hypertension is established. Although not hypertensive, hypoxic animals are more likely to develop hypertension at older ages than control groups.

This work was supported by CNPq, CAPES and FAPERJ grants.

MTU14-13

Cellular and molecular mechanism of bisphenol-a (BPA) mediated effect(s) on protein quality control in the rat hippocampus

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Widespread uses of plastic products containing BPA, well known xenoestrogen, have hazardous health impact. In present study, we have investigated the effect(s) of BPA on neurogenesis and protein quality control in rat brain hippocampus. For experimental examination Wistar rats were orally administered BPA (40 & 400 μg/kg body weight) during gestation and postnatal periods. Expression of genes and protein levels were analysed by qRT-PCR and western blotting, respectively. To study proteins localization, immunohistochemical and ultrastructural studies were performed by immunofluorescence and transmission electron microscopy studies *in vitro* and *in vivo.* Results suggested that exposure of BPA in neural stem cells (NSCs) culture derived from rat brain hippocampus showed reduced proliferation and differential potential. Furthermore, we have observed that BPA exposure induces generation of autophagic flux as a protective response in neuronal cells. Electron microscopy analysis revealed that BPA exposure induced generation of autophagosomes and autolysosomes. Moreover gene and protein expression analysis depicts an altered protein quality control in the BPA exposed rat brain hippocampus. Our finding suggests that BPA exposure not only decreases NSCs proliferation and neuronal differentiation, but also increases neurodegeneration, autophagy & apoptosis both *in-vitro* and *in-vivo*. Therefore altered protein quality control might be responsible for BPA induced defects in cognitive function in the rat brain.

MTU14-14

Quercetin modulates neuronal activity of the RAT arcuate nucleus

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Quercetin, an important flavonoid found in fruits and vegetables, possesses antioxidant, anti-inflammatory, and anti-obesity activities. However, there is no evidence to suggest anti-obesity effects of quercetin through central effects, especially for the hypothalamic brain regions that play an importance role in regulation of energy balance. Hence,This study investigated the effects of quercetin on neuronal activity in the hypothalamic food intake regulating areas including the arcuate nucleus (ARC), the ventromedial hypothalamus (VMH), and the dorsomedial of hypothalamus (DMH) and also investigated the effects of quercetin on activity of neuropeptide (NPY) neuron of the ARC in male Wistar rats. Rats orally received vehicle and quercetin (100, 200, 400 mg/ml/kg) for 30, 60, 90 and 120 min. Brains were fixed and sectioned. Free-floating sections were subjected to Fos, NPY and Fos/NPY immunohistochemical staining. The highest number of Fos+ neurons/area were found in the ARC in the rats treated with 100 mg/ml/kg of quercetin for 120 min. In the ARC, there was significantly more number of Fos+ neurons/area in quercetin treated group than vehicle-treated group. Number of Fos+ neurons/area in the VMH in quercetin treated group was significantly lower than in vehicle-treated group. Number of Fos+ neurons/area in the DMH were not different between quercetin and vehicle treatments. Fos+/NPY+ neurons/area, and the ratio and percentage of Fos+/NPY+ neurons to total number of NPY+ neurons in the ARC induced by quercetin were significantly lower than vehicle-treated group. The results suggested that quercetin may involve in the regulation of food intake and energy homeostasis by activate neurons in the ARC and inactivate neurons in the VMH. Quercetin may exert anti-obesity effect by inactivating NPY/AGRP neurons and activate POMC/CART neurons in the ARC.

MTU14-15

Curcumin inhibits bisphenol-a (BPA) mediated rat hippocampal de-myelination via notch signaling

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Xenoestrogen BPA is component of plastic materials such as feeding bottles and food cans. Previously, we have shown adverse effects of BPA on myelination. The aim of this study is to understand mechanistic action of curcumin against BPA induced alterations in hippocampal myelination. BPA (40 μg/kg b.w, *peroral*) and curcumin (20 mg/kg b.w, *intraperitoneal*) were administered to rats from postnatal day (PND) 21-90. Curcumin treatment after BPA exposure improved immunoreactivity of fluoromyelin, $Olig2^+/MBP^+$ and MBP^+/NF^+. It also significantly increased number and size of oligospheres, augmented number of $A2B5^+/PCNA^+$ (proliferation), $MBP^+/CNPase^+$ (differentiation), β-III $tubulin^+/MBP^+$ (myelination) cells and up-regulated expression and levels of myelin protein. We studied the effect of curcumin on canonical Notch pathway, which is essential for maintenance of oligodendrocyte progenitors (OPCs). *In-silico* studies predicted interaction of BPA and curcumin with Notch1, Hes1 and Mib1. Curcumin treated BPA exposed groups exhibited significantly enhanced gene expression and protein levels of Notch pathway markers. Notch pathway inhibition *via* Notch1 siRNA and DAPT resulted in further significant decline in number of OPCs in BPA exposed group as compared to BPA alone group. OPCs number was not ameliorated after curcumin treatment in Notch inhibited groups indicating notch mediated neuroprotective action of curcumin. Curcumin treatment in BPA exposed group significantly improved learning and memory, ultrastructural architecture and myelin sheath thickness. Results highlight the significance of curcumin as potential therapeutics against xenobiotics induced neurotoxicity.

MTU14-16

The unfolded protein response (UPR) is upregulated in several important regions of the sids brain

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The unfolded protein response (UPR) is linked to multiple neurodegenerative diseases such as Alzheimers' and Parkinsons', and has also been implicated in a subset of SIDS infants. Recent work in our laboratory identified an increase in phosphorylated protein kinase R (PKR)-like ER kinase (p-PERK) - a key component of one arm of the UPR - in the hypothalamus of SIDS infants ($p<0.000$) (Hunt et al., 2015). We aimed to determine whether p-PERK is similarly increased in other brain regions of SIDS babies including the brainstem and cerebellum, as well as any potential links to SIDS risk factors of 'prone sleeping', 'upper respiratory tract infection (URTI)', 'bed-sharing' and 'parental smoking'. The immunohistochemical expression of p-PERK was studied in the brainstem pons, medulla and cerebellum of SIDS (n=28) compared to non-SIDS (n=12) infants. The p-PERK positive neuron percentage was significantly increased in the cuneate nucleus ($p=0.035$) in SIDS compared to non-SIDS cases, and the inferior olivary nucleus (ION) and locus coerulus (LC) showed a trend towards the increase ($p=0.057$, $p=0.084$ respectively). Analysis for the risk factors showed changes attributed to bed-sharing only, with a significant increase in the purkinje cell layer of the cerebellum ($p=0.025$) and the dorsal raphe nuclei of the pons ($p=0.048$), as well as a trend towards the increase in the LC ($p=0.073$) and the ION ($p=0.066$) of bed-sharing infants. These results indicate that a certain subset of SIDS infants are experiencing an upregulation of the UPR via the p-PERK pathway, and that bed-sharing plays a contributing role.

MTU14-17

Dopaminergic neuroregeneration in the diencephalon of 6-OHDA-lesioned adult zebrafish-based parkinson's disease model

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Studies on endogenous neuroregeneration using mammalian-based Parkinson's disease (PD) models are often hampered with futile axogenesis. The emergence of zebrafish with remarkable neuroregeneration capacity may address these limitations. This study established a 6-OHDA-lesioned adult zebrafish-based PD model and investigated the neuroregenerative processes of diencephalic dopaminergic neurons (DpN). 25 mg/kg of 6-OHDA were intracerebroventricularly administered into the diencephalon of adult zebrafish (*Danio rerio*). Immnunoflurorescence was conducted to quantify tyrosine hydroxylase immunoreactive (TH-ir; indicating DpN) and Bromodeoxyuridine-immunoreactive (BrdU-ir; indicating proliferative cells) at brain regions of interest [olfactory bulb (OB), telencephalon, diencephalon]. To elucidate neurodifferentiation activity, Foxa2 and Nurr1 differential gene expressions at different regions were enumerated using qPCR. At day 3 post-lesion, diencephalic TH-ir cell count revealed >85% DpN significantly ($p<0.05$) ablated when compared to intact fish. Whereas, at day-30 post-lesion TH-ir cell count increased significantly ($p<0.05$) than day-3 post-lesion; but exhibited no significant difference with intact. Cellular proliferation demonstrated a transient yet significant ($p<0.05$) decrease and then increase in OB (-55%; +114%) and telencephalon (-73%; +194%) at day 5 and 7 post-lesion, but BrdU-ir cell count in diencephalon remained unchanged at all time points. Conversely, significant ($p<0.05$) decline and gradual increase of Foxa2 (-44% at day 3 and 9 post-lesion) and Nurr1 (-46% at day 3, 9 and -65% at day 14 post-lesion) were observed in the diencephalon; whilst no significant changes ($p>0.05$) of both differential markers were discerned in OB and telencephalon. These findings warrant further investigations to harness these potentials and apply towards future human DpN regenerative studies.

MTU14-18

New modulators of the capsaicine receptor TRPV1 in fermented foods

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TRPV1 (transient receptor potential vanilloid subfamily, member 1), similar to other TRP channels, is a putative six-transmembrane-spanning protein with a pore region localized between transmembrane segments 5 and 6. TRPV1 is a non-selective cation channel with a preference for calcium that is directly activated by noxious temperature (43 °C) or capsaicin. Generally speaking, capsaicin-sensitive neurons are bipolar neurons with unmyelinated axons (C-fibres) and somata in sensory (dorsal root and trigeminal) ganglia. Of note, a subset of sensory neurons with thin myelinated axons (Aδ fibres) is also capsaicin sensitive. TRPV1 is thought to mediate the phenomenon of peripheral sensitization that involves a reduction in the threshold of activation and an increase in the responsiveness of the peripheral termini of nociceptors. TRPV1 has been reported to have anti-obesity effect by sweating, neuropathic pain curing effect by desensitization, the protective effect against neural cells, and headache inhibition. It was reported that polyamines, by virtue of their cationic charge, can regulate the activity of TRPV1. To investigate new substances acting on TRPV1, we examined the effect of ingredients in fermented food on it. The fermented food contained various amines. We focused the amines contained in Japanese sake. Agmatine and 2-phenylethylamine showed TRPV1 agonist activity and these EC_{50} were 250 μM and 14.2 μM, respectively. At that time, the EC_{50} of capsaicin was 3.7 μM. Tyramine, isoamylamine, and putrescine did not show any activity against TRPV1. Lactic acid, phthalic acid, and crotonic acid potentiated TRPV1. It was indicated that the substances existing in sake modulate TRPV1 activity. To investigate the mechanism of action of these substances, we will examine about electrophysiological activities in detail and perform behavioral experiments by animals.

MTU14-19

Effect of repeated transcranial magnetic stimulation on opioid status in fibromyalgia patients by sucrose induced analgesia

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Fibromyalgia (FM), is a chronic pain syndrome involving altered pain modulation system and no effective treatment yet. The endogenous opioids are one of the important regulators of Gonadotropin Releasing hormone(GnRH) in the hypothalamus. There was an indirect estimation of opioid levels by estimating plasma LH level. Opioids are known to exert an inhibitory control on gonadotropic releasing hormone (GnRH) neurons. The GnRH neurons in turn control the release of LH and FSH. Endogenous opioid system was assessed in FM patients before and after treatment with Repeated Transcranial Magnetic Stimulation (rTMS) and compared along with subjective symptoms. Series of blood samples were collected from the FM patients (n = 86) and healthy Controls (n = 90) before (2 ml each 10 min earlier) and after (at 0, 5, 10, 15 and 20-min intervals) the participant was provided 25% freshly prepared sucrose solution to drink. The patients were given rTMS as treatment for 4 weeks (5 days per week/20 days). Again, blood samples were collected after the treatment and pre and post treatment data were compared. LH was estimated in blood by Electrochemiluminescence immunoassay methods. The basal LH concentration (lg/dl) in controls was 6.1 2.85 which decreased to 5 min post-sucrose ingestion. It gradually decreased significantly at 5,10,15 and 20 min (5.6 2.58, 5.3 2.52, 5.1 2.39, 4.8 2.34 respectively). LH concentration in FM before rTMS remained unaltered from the basal level (4.5 2.12) through 20 min post sucrose ingestion (4.3 2.13, 4.1 2.52, 4.2 2.06. 4.1 2.11) while after rTMS there was significant decrease in LH level after sucrose ingestion, indicating effects of rTMS in FM patients. rTMS elicited a sustained beneficial effect in FM patients.