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Augmented cerebral mitochondrial function and hippocampal ketone body metabolism in Db/db mice

Andersen, J.; Nissen, J.; Christensen, S.; Waagepetersen, H.

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Poster Sessions Monday/Tuesday
MTU01 Glia

MTU01-01

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

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

MTU01-02

The role of lysophosphatidic acid in the microglia-gliblastoma interaction

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

MTU01-03

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

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

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

MTU01-04

Functionalized lipidic nanocapsules internalization by oligodendrocytes in vitro

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

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

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

MTU01-05

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

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

MTU01-06

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

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

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

MTU01-07

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

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

MTU01-08

Noradrenergic modulation of cerebellar glial activity during nociception

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

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MTU01-09

Ischemic tolerance mediated by glia-neuron interactions

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

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

MTU01-10

The role of parenchymal cellular prion protein during glioblastoma progression

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

MTU01-11

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

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

MTU01-12

Mitotic defects in neural cells after aberrant nuclear entry of neurofibromin

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

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

MTU01-13

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

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

MTU01-14

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

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

MTU01-15

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

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

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

MTU01-16

Role of serotonergic signaling in regulation of astrocytes morphology

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

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

MTU01-17

The endoplasmic reticulum of astrocytes constitutes a dynamic glucose pool

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

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

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

MTU01-18

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

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

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

MTU01-19

Glutamine synthetase translational control in cerebellar bergmann glia cells

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

MTU01-20

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

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

MTU01-21

Trkb receptor mediates BDNF protection of astrocytes

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

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

MTU01-22

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

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

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

MTU01-23

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

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

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

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

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

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MTU01-24

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

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

MTU01-25

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

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

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

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

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

MTU01-26

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

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

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MTU01-27

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

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

MTU01-28

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

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

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

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

MTU01-29

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

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

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MTU01-30

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

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

MTU01-31

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

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

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MTU02 Gene Regulation and Genetics

MTU02-01

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

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

MTU02-02

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

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

MTU02-03

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

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

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

MTU02-04

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

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

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MTU02-05

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

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

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MTU02-06

Monoamine oxidase b gene polymorphisms revealed association with male ADHD probands

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

MTU02-07

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

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

MTU02-08

Microrna regulation in medial prefrontal cortex after nerve injury

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

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

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

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MTU02-09

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

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

MTU02-10

Characterization of natriuretic peptides and their receptors in the avian brain

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

MTU02-11

Elucidating the transcriptional regulation of NUR77 in neurons

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

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MTU02-12

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

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

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

MTU02-13

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

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

MTU02-14

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

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

MTU02-15

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

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Objective: Circulating microRNAs (miRNAs) are emerging disease biomarkers. However, comprehensive characterization of the serum miRNA profile in patients with traumatic brain injury (TBI) has rarely been reported. Our study aims to investigate the value of novel serum miRNAs for diagnosing TBI, especially mTBI, and further predicting therapeutic efficacy and clinical outcome.

Methods: A TaqMan Low Density Array was initiated to analyzed the expression of 754 serum miRNAs in two pooled samples from 15 severe traumatic brain injury (sTBI) (Glasgow Coma Scale [GCS] score $\leq$ 8) patients with unfavorable outcome and 15 normal controls. Markedly upregulated miRNAs in sTBI cases were subsequently

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

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

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

MTU02-16

Influence of placental allopregnanolone on adult brain transcriptome

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

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

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

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

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

MTU02-17

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

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

MTU02-18

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

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

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MTU03 Neuroinflammation

MTU03-01

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

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

MTU03-02

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

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

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

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

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

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

MTU03-03

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

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

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

MTU03-04

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

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

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

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

MTU03-05

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

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

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MTU03-06

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

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

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

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

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

MTU03-07

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

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

MTU03-08

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

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

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

MTU03-09

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

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Background: Emerging research indicates that physical activity can ameliorate chronic pain, but the underlying mechanisms are still obscure. We have shown previously that systemic inflammation induced in mice by intraperitoneal (i.p.) injection of lipopolysaccharide (LPS) caused mechanical hypersensitivity and activated satellite glial cells (SGCs) in dorsal root ganglia (DRG). LPS injection also caused a large increase in gap junction-mediated coupling among SGCs and also between neurons. In the present work we asked whether physical exercise can reduce the pain by reversing the changes in SGCs induced by LPS.
Results: We assessed pain with von Frey filaments and characterized SGCs in L4,5 DRG using dye injection, measured respo3nses to the pain mediator ATP by calcium imaging, and immunostained for the activation marker glial fibrillary acidic protein (GFAP). Seven days post-LPS, SGCs were activated, as evidenced by GFAP upregulation, and dye coupling among SGCs increased 3–4.5-fold. Sensitivity of SGCs to ATP increased 2-fold. Pain threshold decreased 3.5 fold. Injecting gap junction blockers i.p. reduced pain behavior in LPS-treated mice, indicating a role for SGCs in pain. The results suggest that SGC activation plays a role in pain mechanisms. LPS-injected mice were given 1 week of free wheel running, after which we characterized the changes in SGCs in DRG. Pain threshold increased back to control level, dye coupling among SGCs and neurons was restored to control level and sensitivity of SGCs to ATP was reduced 2-fold. GFAP levels decreased back to control level.
Conclusions: Physical exercise reverses SGC activation caused by systemic inflammation, which may explain the amelioration in pain in the systemic inflammation model by exercise.

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MTU03-10

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

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

MTU03-11

Role of microglial metabolism in perinatal neuroinflammation

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

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

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

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

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

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

MTU03-12

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

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

MTU03-13

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

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

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MTU03-14

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

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

MTU03-15

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

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

MTU03-16

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

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

MTU03-17

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

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

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

MTU03-18

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

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

MTU03-20

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

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

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MTU03-21

Parasympathetic cholinergic and peptidergic mechanisms of trigeminal pain

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

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MTU03-22

Activated perk pathway in the brainstem was induced by masseter inflammation

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

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

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

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

MTU03-23

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

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

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MTU03-24

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

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

MTU03-25

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

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

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

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

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

MTU03-26

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

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

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

MTU03-27

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

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

MTU03-28

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

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

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

MTU03-29

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

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

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

MTU03-30

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

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

MTU03-31

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

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

MTU03-32

Amyloid precursor protein modulates microglia and macrophage phenotype

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

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MTU04 Molecular Mechanism of Parkinson's Disease

MTU04-01

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

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

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

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

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

MTU04-02

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

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

MTU04-03

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

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

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MTU04-04

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

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

MTU04-05

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

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

MTU04-06

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

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

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

MTU04-07

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

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

MTU04-08

The subcellular localization of human tyrosine hydroxylase isoforms

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

MTU04-09

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

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

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

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

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

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

MTU04-10

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

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

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MTU04-11

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

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

MTU04-12

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

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

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

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MTU04-13

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

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

MTU04-14

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

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Objectives: Insulin resistance has been reported as a possible risk factor for Parkinson's disease (PD). Preclinical and clinical studies have suggested reduced insulin receptor expression and insulin resistance in PD brains. Recently, insulin resistance was found to be present in 62% of PD patients with dementia. In our previous study we found reduction in histone H3 acetylation could be a possible risk factor for insulin resistance induced PD pathology. Therefore, the present study was designed to explore the therapeutic potential of histone deacetylases inhibitor, Suberoylanilide hydroxamic acid (SAHA), in insulin resistance induced PD pathology.
Methods: High fat diet (HFD) feeding was used for induction of insulin resistance in animals. Male Wistar rats were subjected to a normal pellet diet or HFD for 8 weeks before they were infused with low dose of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle. The animals were then divided different groups. The treatment group received SAHA (25 and 50 mg/kg i.p.)/day for 14 days. Battery of behavioral parameters was performed to check the locomotor and gait abnormalities in rats. To delineate the molecular mechanisms, we study the changes in histone acetylation in striatum region.
Results: The animals subjected to HFD feeding followed by 6-OHDA infusion showed impaired locomotion and gait abnormalities. These rats also showed significant elevation in oxidative stress markers and neuronal damage along with significant reduction in histone H3 acetylation in striatal region. In contrast, rats treated with SAHA showed significant amelioration of locomotor and gait abnormalities along with reduced oxidative stress and neuronal damage. Treatment with HDAC inhibitor, SAHA results in significant elevation of histone H3 acetylation.
Conclusions: This is the first study exploring the role of histone acetylation/deacetylation in insulin resistance induced PD pathology. This study suggests the therapeutic potential of HDAC inhibitor, SAHA in ameliorating insulin resistance induced PD pathology. Future studies in this direction might possibly confirms the role of histone acetylation in PD associated with insulin resistance.

MTU04-15

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

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

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

MTU04-16

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

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

MTU04-17

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

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

MTU04-18

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

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

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

MTU04-19

The potential mechanism of salsolinol synthase induces apoptosis in PC12 cells

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

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

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

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MTU05 Neurological Dysfunction

MTU05-01

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

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Purpose: Docetaxel (DTX) is an anti-cancer drug which is used widely to treat different types of cancer, but it may cause several unwanted adverse effects. One of the most debilitating side effects is cognitive impairment. The purpose of this study is to examine the short-term cognitive impairment of DTX on rats with tumour by using Morris Water Maze (MWM).
Methods: Female Sprague Dawley rats were injected with LA7 cells into the mammary gland pad to produce the tumour. The rats were then divided into 3 groups; normal control without tumour (NC, n = 11), rats with tumour treated with DTX (single dose 5 mg/kg i.p.) (DTX, n = 11) and cancer control without DTX (CC, n = 11). A 2 day MWM protocol was used to assess the cognitive impairment. Hippocampus (responsible for short-memory) was examined for pro-inflammatory cytokine interleukin IL-1β, and oxidative stress markers thiobarbituric acid-reactive substances (TBAR) and reactive oxygen species (ROS).
Results: Only 24 h after DTX was injected, the escape latency to find the platform was increased significantly to 58.1 s compared to 8.6 s in NC and 24.7 s in CC, also the latency difference (first hidden platform - last visible platform) was increased significantly due to DTX to 59.7 s compared to 5.5 s in NC and 31.0 s in CC. Interleukin IL-1β concentration was increased significantly in DTX to 171.56 pg/mL compared to 23.30 pg/mL in NC and 11.67 pg/mL in CC. TBARS level was increased significantly in DTX to 2.7 folds compared to NC, while CC increased it to 1.56 folds, also ROS level was increased significantly in DTX to 2.45 folds while CC raise it to 1.40 folds compared to NC.
Conclusion: DTX significantly induce short-term cognitive impairment in rats with tumour.

MTU05-02

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

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

MTU05-03

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

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

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MTU05-04

Mitochondria pathway mediated neuroprotection by melatonin in valproic acid induced toxicity

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

MTU05-05

Direct modulatory effects of antiepileptic drugs on glycine receptors

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

MTU05-06

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

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

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MTU05-07

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

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

MTU05-08

Pathophysiological mechanism of Munc18-1 mutations in early infantile epilepsies

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

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

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

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

MTU05-09

Retrosplenial cortex modulates neuropathic pain via NMDA-glutamate receptors

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

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

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

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

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MTU05-10

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

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

We induced hydrocephalus in 12 three week-old Wistar rat pups by intracisternal injection of 0.05 mL of kaolin solution (250 mg/mL in sterile water) while 12 controls had sham injection. The hydrocephalic rats were divided into 3 groups consisting of 4 rats each which were sacrificed at 1, 4and 8 weeks post-induction of hydrocephalus along with their age-matched controls. Following sacrifice, half of the brain samples were stained with haematoxylin and eosin, cell counts were determined and data analysed using ANOVA at α0.05. The other half were processed for Transmission and Scanning Electron Microscopy (TEM and SEM) and the images analysed descriptively.

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

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

MTU05-11

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

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

MTU05-12

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

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

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

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

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

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

MTU05-13

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

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

MTU05-14

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

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

MTU05-15

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

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

Methods: C57BL/6 mice were sacrificed at 3 or 21 months of age, and their coritices were isolated and sonicated. Phospho-proteins and ubiquitinated-proteins were enriched by using PhosPro or Ubiquitinated protein enrichment kit. After phospho-proteins and ubiquitinated-proteins were labeled by iTRAQ, labeled peptides were analyzed by MALDI-TOF MS/MS.

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

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

MTU05-16

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

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

MTU05-17

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

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

MTU05-18

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

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

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

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MTU07 Synaptic Transmission

MTU07-01

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

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

MTU07-02

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

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Purpose: ELOngation of Very Long chain fatty acids-4 (ELOVL4) is an elongase responsible for biosynthesis of very long chain (VLC; ≥ C28) fatty acids, making VLC polyunsaturated fatty acids (VLC-PUFA) in retina and testes, and VLC saturated fatty acids (VLC-SFA) in skin and brain. Homozygous inheritance of the Stargardt (STGD3) mutation in ELOVL4 causes a CNS phenotype in humans, including seizures, intellectual disability, spastic quadriplegia, and death. We hypothesize that ELOVL4-synthesized VLC-SFA play an essential role in neural cell structure and function.

Methods: We generated a successful animal model for STGD3/STGD3 inheritance (−/−). ELOVL4 localization within the CNS was determined in wild type mice (+/+) using immunofluorescence (IF) microscopy. Hippocampal lipids were analyzed by mass spectrometry. Synaptic membrane fractionation was performed on baboon hippocampus for lipid analysis. Hippocampal slices from (+/+) and (−/−) mice were subjected to spontaneous multi-electrode array (MEA) recordings. Primary neuronal cultures from hippocampus of (+/+) and (−/−) mice were subjected to FM1-43 assessment of synaptic vesicle exocytosis rates.

Results: STGD3/STGD3 mice developed seizures at P19 followed by death at P21. Hippocampal lipidomic analysis of (+/+) mice confirmed the presence of 28:0/30:0 in sphingolipids. Membrane fractionation of baboon hippocampus revealed enrichment of 28:0/30:0, but not VLC-PUFA, in synaptic vesicle membranes. MEA recordings showed a significant increase in the amplitude and decrease in the inter-spike interval of action potentials in (−/−) vs (+/+) hippocampal slices. FM1-43 studies showed a significant increase in synaptic vesicle exocytosis rates in (−/−) vs (+/+) primary hippocampal neurons.

Conclusions: This is the first study to demonstrate that mutations in *Elovl4* cause a CNS phenotype in an animal model. These studies suggest a neuron-specific role for VLC-SFA in the regulation of pre-synaptic synaptic function by impacting the rate of synaptic vesicle release.

MTU07-03

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

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

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

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

MTU07-04

A new photochromic modulator of inhibitory CYS-loop receptor channels

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

MTU07-05

Adenosine A2A receptors stabilizes inhibitory synapses during development

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

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

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

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

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MTU07-06

In vivo photomodulation of GABA and glycine receptor channels

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

MTU07-07

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

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

MTU07-08

Calcium channel surface dynamic influences synaptic transmission

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The localization of voltage gated calcium channels (VGCCs) within the presynaptic active zone is critical for synaptic transmission. Using single particle tracking photoactivation localization microscopy (sptPALM) we localize VGCCs within active synapses and determine their surface dynamics in the membrane. Molecular interactions between active zone proteins, the C-terminus of VGCCs and vesicles have been shown to essentially regulate synaptic transmission. Whether interactions between VGCCs and scaffold proteins guide channel localization and mobility has been tested by using $Ca_v2.1$ C-terminal splice variants. Here, alternative splicing of exon 47 results in the expression of a shorter C-terminus (Δ47) lacking a variety of described protein-protein interactions.

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

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

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

MTU07-09

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

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

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

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

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

MTU07-10

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

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

MTU07-11

Physiological roles of glutamate secreted from VGLUT3-expressing neurons

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

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

MTU07-12

Novel pharmacological modulators of glycine receptors function

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

MTU07-13

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

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

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

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

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[1] Jane et al., (2009) Neuropharmacology, 56, 90–113

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MTU07-14

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

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

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

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

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

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MTU07-15

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

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

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

MTU07-16

Optical control of muscarinic acetylcholine receptors using photoswitchable bitopic ligands

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

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

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

MTU07-17

Glycine receptor subunits expressed in retina during rat development

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

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

MTU07-18

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

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

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

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MTU07-19

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

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

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

MTU07-20

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

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

MTU07-21

Probing subunit interfaces using genetically-encoded photocrosslinkers in glutamate receptors

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

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MTU08 Signal Transduction

MTU08-01

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

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

MTU08-02

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

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

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

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

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

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

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

MTU08-03

Serotonin receptor 5-HT7 mediated activation of CDK5

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

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

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

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

MTU08-04

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

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

MTU08-05

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

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

MTU08-06

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

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

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MTU08-07

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

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

MTU08-08

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

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

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MTU08-09

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

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

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

MTU08-10

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

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

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MTU08-11

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

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

MTU08-12

PAR1 activation induces calcium-dependent glutamate release from RPE cells

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

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

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MTU08-13

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

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

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

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

MTU08-14

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

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

MTU08-15

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

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

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

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

MTU08-16

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

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

MTU08-17

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

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

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MTU08-18

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

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

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

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

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

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

MTU08-19

Lipid raft integrity impacts cannabinoid receptor 1 signalling

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

MTU08-20

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

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

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MTU08-21

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

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

MTU08-22

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

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

MTU08-23

Mechanism of dopamine signaling for membrane excitability

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

MTU08-24

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

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

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

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

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

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

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

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

MTU08-25

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

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

MTU08-26

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

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

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MTU09 Neurogenesis and Cell Differentiation

MTU09-01

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

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Background: Kit receptor tyrosine kinase has been shown to regulate a wide range of biological functions in various cell lineages including pigment cells, hematopoietic cells, germ cells, neuronal cells of central nervous system (CNS). Kit mRNAs are expressed in cells affected by *Kit* locus mutants, however, in developing or adult brain which is one of the major tissues expressing Kit mRNA, no clear phenotype lead to the loss of specific cell types or functions was detected in the brain of *Kit* mutants.

Objective: To investigate the Kit function in neural lineage, we introduced a conditional loss of function mutation of *Kit* from a certain point of the developmental stage.

Methods: Transmembrane region of *Kit* was flanked by loxP to excise the *Kit* floxed allele by Cre recombinase. To take advantage of CNS specific induction of Cre, $Kit^{floxed/+}$ mice were crossed with *Sox1-Cre* mice expressing Cre directed by the neural lineage specific promoter sequence. The morphology, histology, gene expression, and the growth and differentiation of neural stem/precursor cells were analyzed.

Results: Expression of endogenous *Sox1* gene starts as early as E8.0 and *Kit* mRNA expression in their brain reduced into one half of the control in E10.5. The resultant $Kit^{floxed/+}$; *Sox1-Cre+* embryos showed significant reduction of the size of the forehead in E12.5, which generate a severe hypoplasia of CNS including brain, spine, and eye. This was accompanied by the increase of apoptotic cells in early embryonic brain and the gradual loss of self-renewal capacity of neural stem/precursor cells leading to the accelerated differentiation of neural stem cells to mature neural cells.

Conclusion: The phenotype appeared in $Kit^{floxed/+}$; *Sox1-Cre+* conditional Kit haploinsufficient embryos suggests that Kit expressed in developing CNS is functional signaling molecules necessary for proper brain development as the other cell lineages solely dependent for Kit in its certain developmental stage.

MTU09-02

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

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

MTU09-03

ENU mutagenesis screening to identify mutations that control corpus callosum development

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

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

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MTU09-04

Neurons on nanotopographies

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

MTU09-05

Obesity leads to impairment in neurogenesis and reduces brain size

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

MTU09-06

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

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

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

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

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

MTU09-07

Tau-dependent suppression of adult neurogenesis in the stressed hippocampus

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

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

MTU09-08

SIP1 controls dendritic arbor formation in the mammalian neocortex

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

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

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MTU09-09

Pyridoxine modulates neurogenesis by regulating CB1 cannabinoid receptor-interacting protein

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

MTU09-10

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

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

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

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MTU09-11

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

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

MTU09-12

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

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

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MTU09-13

GM1 neurotrophic properties are related to GM1 oligosaccharide - TRKA interaction

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

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

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

MTU09-14

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

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

MTU09-15

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

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

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

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

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MTU09-16

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

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

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

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

MTU09-17

ENU mutagenesis screening in mice identifies a mutation that causes microcephaly

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

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

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MTU09-18

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

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

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MTU09-19

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

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

MTU09-20

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

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

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

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

MTU09-21

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

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

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MTU09-22

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

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Theanine is an amino acid enriched in green tea by 2–3% with a chemical structure analogous to glutamine rather than glutamic acid. We have been studying pharmacological profiles of this green tea amino acid in neural progenitor cells, which are endowed to proliferate for self-replication and to differentiate into progeny lineages such as neuronal, astroglial and oligodendroglial cells, in embryonic, developing and adult rodent brains. Progenitor cells were exposed to theanine at different concentrations for determinations of the size of neurospheres composed of clustered proliferating cells and MTT reducing activity, followed by culture in the absence of theanine for immunocytochemical detection of the cells immunoreactive for MAP2 and GFAP among cells stained with Hoechst33342. In cultured neural progenitor cells from embryonic rat and mouse neocortex, theanine invariably promoted the formation of neurospheres and MTT reduction in a concentration-dependent manner at 1 to 100 μM, followed by facilitation of spontaneous differentiation into cells immunoreactive for MAP2 with a concomitant decreased number of GFAP-positive cells. In cultured progenitor cells from the hippocampus of adult nestin-GFP mice, theanine significantly increased the size of neurospheres. In murine embryonic carcinoma P19 cells exposed to theanine, similar facilitation was seen in proliferation and subsequent spontaneous neuronal differentiation. Upregulation was induced for the glutamine transporter *Slc38a1* transcript in rat and mouse progenitors exposed to theanine for 4 days, but not for 2 days, whereas theanine failed to further promote both proliferation and neuronal differentiation abilities already facilitated in P19 cells stably overexpressing *Slc38a1*. Significant alleviation was seen in cognition impairment scores measured by double-blinded physicians in healthy age-matched elderly people given capsules of green tea enriched of theanine than those given normal green tea capsules after daily oral intake for 7–12 consecutive months. We have made a dietary supplement product enriched of theanine as a chewable tablet for online sale to expect a beneficial support for the prophylaxis of particular cognition impairments.

MTU09-23

Regulation of proliferative activity by protease-activated receptor 1 in neural stem/progenitor cells generated after neuronal deg

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It is now clear that there is a continual turnover of the mammalian hippocampal dentate gyrus (DG) neurons throughout life even in adult. Various neurological injuries are widely recognized as promoting endogenous neurogenesis in DG. Thrombin-activated/protease-activated receptor-1 (PAR-1) is known to regulate proliferation of neural cells following brain injury including intracellular hemorrhage. Our previous studies demonstrated that the systemic treatment with trimethyltin chloride (TMT) causes the granule cell loss in the mouse DG, with being regenerated in the dentate granule cell after neuronal loss. To elucidate the roles of PAR-1 in neuroregeneration after neuronal degeneration, we evaluated the expression of PAR-1 in the newly generated cells following neurodegeneration in the DG of adult mouse. *In vivo* experiments, mice were given TMT to prepare hippocampal slices for immunohistochemical analysis using antibody against PAR-1 and nestin [neural stem/progenitor cells (NPCs) marker]. Cells positive for PAR-1 and nestin markedly increased in the DG on day 3–5 after TMT treatment. *In vitro* experiments, the exposure of NPCs derived from the DG after neuronal degeneration to thrombin significantly attenuated cell proliferation by bromodeoxyuridine incorporation assay. Our results suggest that PAR-1 has a critical role in proliferative activity inNPCs generated following neuronal degeneration in the DG.

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MTU10 Brain Bioenergetics

MTU10-01

Impaired mitochondrial and metabolic activity in neurons derived from human induced pluripotent stem cells of FTD patients

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Frontotemporal dementia (FTD) is the third most common form of primary degenerative dementia, accounting for up to 20% of young onset dementia. FTD is a neurodegenerative disorder characterized by cognitive impairment affecting the frontal and/or temporal lobes of the brain associated with progressive brain atrophy. Given that mitochondrial defects are commonly observed in neurodegenerative diseases, we investigated whether mitochondrial respiration and energy metabolism are affected in neurons derived from human induced pluripotent stem cells (hIPSC) obtained from FTD patients. With this aim, forebrain region-specific neurons were derived from FTD patients hiPSC lines expressing NESTIN, FOXG1, OTX2 and PAX6 mRNA. After subsequent maturation, glutamatergic cortical neurons expressing MAP2AB, TUJ1, TAU, VGLUT1, TBR1 and CTIP2 mRNA were obtained. Mitochondrial morphology and function were assessed using transmission electron microscopy and real-time monitoring of oxygen consumption via the Seahorse XFe96 Analyzer, respectively, in cultured hiPSC-derived neurons. The results were compared to CRISPR/Cas9-edited isogenic controls and neurons derived from an age-matched healthy subject. Ultrastructure analysis revealed mitochondria with poorly developed cristae as well as abnormal localization in the FTD patient-derived neurons. In line, oxygen consumption associated with mitochondrial activity was decreased in the FTD neurons. All of the observed phenotypes were reversed after targeted gene corrections in the isogenic controls. Our findings indicate that hiPSC-derived neurons from FTD patients display significant mitochondrial dysfunction that could potentially be targeted for treatment development.

MTU10-02

Augmented cerebral mitochondrial function and hippocampal ketone body metabolism in Db/db mice

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Aim: Type 2 diabetes mellitus (T2DM) is a risk factor for the development of Alzheimer's disease (AD). Hypometabolism of glucose have been observed in pre-symptomatic patients and animal models of AD, suggesting a fundamental pathological mechanism. The aim of this study is to elucidate cerebral metabolic consequences of T2DM to possibly reveal accelerating factors of T2DM on AD pathology.

Materials and methods: Db/db mice were used as a T2DM animal model at 16 weeks of age. Acutely isolated cerebral cortex and hippocampus slices of db/db mice were incubated in media containing [U-^{13}C]glucose or [U-^{13}C]β-hydroxybutyrate. Oxygen consumption and ATP synthesis rate of isolated whole-brain mitochondria were assessed by SeaHorseXFe96 and on-line luciferase based assay, respectively.

Results: Decreased ^{13}C enrichment from [U-^{13}C]glucose metabolism were observed in key metabolites in extracts of both cerebral cortical and hippocampal slices of the db/db mice. However, the glucose hypometabolism was more prominent in the cerebral cortex. Incubations with the ketone body [U-^{13}C]β-hydroxybutyrate showed increased ^{13}C labeling in citrate, glutamate and glutamine in hippocampal slices of db/db mice. These changes were absent in the cerebral cortex. Isolated whole-brain mitochondria from db/db mice, surprisingly displayed augmented oxygen consumption when stimulated with ADP, when pyruvate and malate were provided as substrates. This finding was supported by a significantly increased ATP production from isolated brain mitochondria of the db/db mice.

Conclusion: Cerebral metabolism and energetics are affected in the db/db mouse. Hypometabolism of glucose is evident in the cerebral cortex and hippocampus. However, the hippocampus of db/db mice, exhibits augmented ketone body metabolism. Mitochondria isolated from the db/db brain showed a significant increase in respiration and ATP synthesis. The results suggest that the hypometabolism of glucose is the major deleterious effect of T2DM on brain energy metabolism. However, the increased ketone body utilization and augmented mitochondrial efficiency suggests compensatory mechanisms, possibly related to the glucose hypometabolism, in the diabetic brain.

MTU10-03

Compartmentalised signalling-metabolism coupling in brain cells - putative drug targets for neurological diseases?

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Faulty cellular signalling and metabolism is a hallmark of a number of brain diseases. In the field of cell biology it is becoming increasingly apparent that signalling pathways are compartmentalised in both space and time within the cell, and that an increased understanding of these signalling events may lead to the discovery of novel drug targets.

Compartmentalised coupling of cAMP and calcium signalling to brain cell metabolism has already been demonstrated implying a relevance to brain physiology and pathology.

In this poster presentation, we attempt to make the case that an appreciation of compartmentalised cellular signalling and metabolism is warranted to advance our understanding of cellular neuroscience, and to discover novel drug targets.

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MTU10-04

Comparison diffusion behavior of metabolites in brains of congenital portal systemic shunt and healthy mice *in vivo* at 14.1 t

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Diffusion-weighted ^{1}H MRS allows investigating the cellular compartmentalization of molecules in the living organs and may shed insight on alterations of cellular restrictions faced by metabolites in different cerebral abnormalities and diseases. The aim of this study was to determine whether diffusion behavior of metabolites in the congenital portal systemic shunt (PSS) mouse brain is different from ones of the healthy mouse *in vivo,* combining large diffusion weighting and ^{1}H MRS methods.

All experiments were performed on a 14.1T magnet using a home-built quadrature transceiver. Six adult PSS and six age-matched healthy (Ctrl) C57BL/6J mice have been prepared and anesthetized using isoflurane. ^{1}H-MRS data were acquired using localized diffusion-weighed STEAM-based spectroscopic pulse sequence (TE=16 ms and a mixing time of 113 ms), covering the b range from 0 to 45 ms/μm^2. Quantified data by LCModel were fitted using bi-exponential equation. Diffusion behavior of metabolites in the mouse brain was compared between PSS and Ctrl group.

The remarkable sensitivity and spectral resolution of localized short-echo ^{1}H MRS at 14 T allowed a precise measurement of the diffusion properties of metabolites in the brain of PSS and Ctrl mouse *in vivo* at very high diffusion weighting. The comparable diffusion properties of most investigated metabolites in the brain *in vivo* between PSS and Ctrl mice may indicate that unaltered barrier and cellular restriction dominate on the diffusion of metabolites in both group and therefore could support the hypothesis about the similarity of intracellular distribution space for these metabolites in PSS mice when compared to Ctrl mice. The slightly different diffusivity of Tau may be ascribed to possible cellular redistribution of Tau in PSS mice, however, it needs to be further explored.

MTU10-05

Intracisternal injection of [U-^{13}C]glucose for investigating brain metabolism in freely moving mice

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Purpose: To investigate brain metabolism using intracisternal delivery of [U-^{13}C]glucose, thus bypassing blood-brain barrier and avoiding effects of peripheral metabolism.

Methods: Mice (C57BL/6JRj, 8wo) were implanted a chronic cannula into cisterna magna. After recovery (24 h) an isosmolar 0.3M [U-^{13}C]glucose solution was infused using a microinjection pump. Animals were sacrificed by microwave irradiation. ^{13}C-labeling and metabolite amounts were determined using mass spectrometry and HPLC. Glycogen content was determined as glucose units after amyloglucosidase treatment.

Results: [U-^{13}C]Glucose injected at 2 μL/min (10 μL) resulted in fast label incorporation into brain lactate as well as glutamate and glutamine. Lactate labeling rapidly (within 10 min) decreased by about 50%, while enrichment in glutamate and glutamine kept increasing in the same time interval. Lactate was the only labeled compound recovered in cervical lymph nodes. [U-^{13}C]Glucose injected at 0.3 μL/min (4.5–18 μL) resulted in progressive rise of label incorporation into brain lactate, glutamate, glutamine, aspartate and GABA. Labeling of these compounds was significantly faster in awake than anesthetized animals. The absolute concentrations of glutamate and GABA were higher in the awake state whereas that of glutamine was lower (~20% changes). Brain glycogen was higher (+50%) during anesthesia and was negatively correlated with glutamate/GABA and positively correlated with glutamine.

Conclusions: Our results indicate that lactate is produced in excess of its utilization and rapidly leaves the brain, possibly through brain lymphatics. The rate of aerobic glycolysis is higher during wakefulness than anesthesia and so is the rate of transmitter synthesis, suggesting higher glutamatergic and GABAergic tone in awake animals. The correlations between brain glycogen content and glutamate/GABA and glutamine in different states indicate that glycogen synthesis/breakdown is modulated by brain activity and contributes as substrate to neurotransmitter synthesis, underlining its functional importance.

MTU10-06

Physiological roles of brain glycogen

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The role of brain glycogen has been traditionally associated with the preservation of neuronal function during energetically challenging states such as hypoxia, hypoglycaemia, ischemia, and seizures. Nevertheless, glycogenolysis also occurs in euglycaemia during an increase in neuronal activity, thus indicating that brain glycogen also supports neuronal function in non-pathological conditions. In order to address the physiological roles of glycogen in the brain, we generated a brain-specific glycogen synthase knockout mouse. These animals, that completely lack brain glycogen, show a significant deficit in learning capacity and in the activity-dependent changes in synaptic strength. Furthermore, they show greater susceptibility to hippocampal seizures and myoclonus following the administration of kainate and/or a brief train stimulation of Schaffer collaterals, which is in agreement with reports describing a relationship between brain glycogen and susceptibility to epilepsy. Within the brain, the presence of glycogen has been restricted mainly to astrocytes. Therefore, all physiologic roles of brain glycogen have been attributed exclusively to astrocytic glycogen. However our findings demonstrate the presence of an active glycogen metabolism also in neurons, which changes the current view of the role of glycogen in the brain. Taken together, our results reveal the relevant role played by glycogen in brain metabolism.

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MTU10-07

Heterogeneity of energy metabolism of astrocytes

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Astrocytes are a cell type in the brain which plays an important role in brain energy metabolism. However, astrocytes are most likely a heterogeneous population of cells as the environment and therefore the requirements for these cells are very different in different areas of the brain and – most likely – also at different positions within the same brain region. Therefore, we hypothesized that both basal and stimulated energy metabolism as well as functional properties of astrocytes might be substantially different in different brain regions reflecting these diverse environments and requirements. To address these questions we took advantage of genetically encoded, fluorescent sensors for metabolites and studied in cultured cells and in acutely isolated brain slices the dynamics of key metabolites including lactate, ATP, and the NAD^+/NADH-redox state. We identified distinct differences in basal energy metabolism as well as in the main regulatory mechanisms within populations of astrocytes but also between astrocytes located in different brain regions. These results support the hypothesis that metabolism of astrocytes is subject to cellular heterogeneity, which might also contribute to brain region specific vulnerability in disease states.

MTU10-08

Inhibition of soluble adenylyl cyclase (SAC) causes a robust increase in glycolysis in cultured astrocytes

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One of the rate limiting steps in glycolysis is catalyzed by the enzyme phosphofructokinase-1, which is allosterically activated by fructose 2,6-biphosphate (F2,6BP). The bifunctional enzyme phosphofructokinase 2/fructose-2,6-bisphosphatase (PFK2/FBPase-2) is responsible for the production and degradation of F2,6BP and thus involved in regulating glycolysis. The activity of PFK2/FBPase-2 is regulated by phosphorylation by cAMP dependent Protein Kinase A (PKA) which couples cAMP signaling and thereby the adenylyl cyclases to glycolytic activity. At present time, there is no knowledge on which role sAC has in regulating PFK2/FBPase-2 and thereby glycolysis.

This work characterizes the effect of inhibition of sAC on glycolytic activity and whether this change is mediated via a change in F2,6BP levels. Working with cultured astrocytes, C6 glioma cells and HEK cells we show that that inhibition of sAC causes a robust increase in glycolytic activity and glucose uptake. The ratio between PFK2 and FBPase-2 activity is investigated to determine if the increase in glycolytic activity is caused by an increase in the level of F2,6BP. The findings provide novel insight into the cAMP signaling mechanisms mediated by sAC and how these are involved in metabolic regulation.

A greater understanding of the pathways between cAMP formation and the endpoint, increased glycolytic activity in this case, can potentially provide new drug targets. In neurogenerative diseases such as Alzheimer's disease a decreased cerebral glycolytic activity has been observed in both patients and animal models of the disease. Thus, sAC could potentially become a drug target for treating Alzheimer's disease.

MTU10-09

Homocysteine affects the glucose and glutamate metabolism of human glioblastoma cells

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Homocysteine is an intermediate of S-adenosylmethionine cycle, which increased level in blood positively correlates with occurrence of several diseases including neurodegeneration. In addition to its neurotoxic effect, homocysteine is also capable to initiate death of human astrocytes. Since glia-neuronal cooperation is considered to be essential for sustaining neuronal energy metabolism and glutamate-glutamine cycle, we investigated the putative effect of homocysteine on the human glial cells to metabolize glucose and glutamate. The human glioblastoma cells, T98G, were used as a study model. The cells were incubated in medium supplemented with D,L-homocysteine in levels up to 0.1 mM for 4 or 28 h. After desired time of incubation, the concentrations of glucose, lactate and glutamate in the media were analyzed enzymatically. Short term incubation of human glioblastoma cells in the presence of homocysteine had no effect on their glucose, lactate and glutamate metabolism. Prolonged incubation for 28 h with D,L-homocysteine, even at the level of homocysteine 0.05 mM, has significantly stimulated the glucose and glutamate uptake. The rate of lactate released remained unchanged at all tested conditions. Our results show that prolonged presence of homocysteine at the levels reachable during hyperhomocysteinemia in human may affect the glial metabolism of glucose and glutamate. Since glucose is a major substrate for energy metabolism in brain parenchyma, increased glial uptake of glucose during hyperhomocysteinemia that is not accompanied by lactate release may lead to decreased availability of the fuel molecules for sustaining the energy needs of neurons. Furthermore, stimulated uptake of the glutamate into the glial cells could disturb the glutamatergic neurotransmission. Taking together, homocysteine could influence not only the physiological functions of neurons but also glial cells and such combine effect may play a role in etiopathogenesis of neurodegeneration associated with hyperhomocysteinemia.

MTU10-10

Beta-hydroxybutyrate metabolism and metabolic compartmentation in brain

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There is renewed interest in the use of the ketone body β-hydroxybutyrate (βOHB) to treat neurological disorders but its metabolism in brain still requires thorough characterisation. Here,

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we studied guinea pig cortical brain slices using increasing concentrations of [U-^{13}C]D-βOBH ([U-^{13}C]D-βOHB) in conjunction with [1-^{13}C]D-glucose under conditions of normo- and hypoglycaemia, as well as under high potassium (40 mmol/L K^+) depolarization in normo- and hypoglycaemic conditions. [U-^{13}C]D-βOHB at lower concentrations (0.25 and 1.25 mmol/L) was mostly metabolized in neurons and stimulated mitochondrial metabolism. In astrocytes it was incorporated into glutamine but did not stimulate metabolism in the cytosol. At higher concentrations (2.5 mmol/L) βOHB inhibited metabolism of [1-^{13}C]D-glucose and reduced total label incorporation and total metabolite pools. [U-^{13}C]D-βOHB could not substitute for glucose when glucose levels were reduced. Incorporation of label from [U-^{13}C]D-βOHB was decreased under depolarising conditions, showing that glucose was the preferred fuel under these circumstances. Unlike the case with labelled glucose, lactate and pyruvate, label from [U-^{13}C]D-βOHB was not used to make labelled acetate, suggesting that mitochondrial citrate made from [U-^{13}C]D-βOHB was not exported to the cytosol. Finally, inhibition of glutamine synthesis with MSO had no significant effect on incorporation of label from [U-^{13}C]D-βOHB into GABA C2,1 indicating that the majority of this GABA was synthesized *in situ* from [U-^{13}C]D-βOHB rather than from Gln C4,5 imported from astrocytes.

MTU10-11

Metabolism of mannose in cultured primary rat neurons

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Glucose is the main peripheral substrate for energy production in the brain. However, as other hexoses are also present in blood and cerebrospinal fluid, we have investigated whether neurons have the potential to metabolize, in addition to glucose, also the hexoses mannose, fructose or galactose. Incubation of primary cerebellar granule neurons in the absence of glucose or in the presence of fructose or galactose caused severe cell toxicity within 24 h, while the cells remained viable during incubation in the presence of either mannose or glucose. In addition, cultured neurons produced substantial and almost identical amounts of lactate after exposure to either glucose or mannose, while lactate production was low in the presence of fructose and hardly detectable during incubations without glucose or with galactose as carbon source. Determination of the K_M values of hexokinase in lysates of cultured neurons for the hexoses revealed values in the micromolar range for mannose (32 ± 2 μM) and glucose (59 ± 10 μM) and in the millimolar range for fructose (4.4 ± 2.3 mM), demonstrating that mannose is efficiently phosphorylated by neuronal hexokinase. Finally, cultured neurons contained reasonable specific activities of the enzymes phosphomannose isomerase, which is required for isomerization of the hexokinase product mannose-6-phosphate into the glycolysis intermediate fructose-6-phosphate. These data demonstrate that cultured cerebellar granule neurons have the potential and express the required enzymes to efficiently metabolize mannose, while galactose and fructose serve at best poorly as extracellular carbon sources for neurons.

MTU10-12

Rates oxidative metabolism in astrocytes and neurons are coupled to the glutamate-glutamine cycle in the tree shrew visual cortex

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Neuronal oxidative metabolism was shown to be coupled to the glutamate-glutamine cycle that represents glutamatergic neurotransmission. While metabolism in astrocytes is also stimulated by glutamatergic neurotransmission, it remains unclear whether the glutamate-glutamine cycle is coupled to glial oxidative metabolism. We took advantage of the columnar characteristics of the *Tupaia belangeri* primary visual cortex (V1) to measure metabolic changes induced by continuous stimulation of V1 using ^{13}C magnetic resonance spectroscopy (MRS) during infusion of [1,6-^{13}C]glucose *in vivo*.

Each animal under light isoflurane underwent the three MR modalities at 14.1T, namely blood oxygenation level-dependent functional magnetic resonance imaging (BOLD fMRI), ^{1}H and ^{13}C MRS localized in V1, either at rest (n = 4) or during stimulation (n = 5).

Visual stimulation resulted in a relatively large activated area in V1 that allowed localized MRS. Cortical brain activity resulted in a decrease in both brain glucose concentration (-17%; -0.34 μmol/g) and phosphocreatine/creatine ratio (−9%; −0.07) after 15 min of stimulation. At the individual level, close relationships between the neurotransmission rate (V_{NT}) and total cerebral metabolic rate of glucose oxidation ($CMR_{glc(ox)}$, R^2=0.68, P = 0.006), glial ($V_{TCA}{}^g$, R^2=0.66, P = 0.008) and neuronal ($V_{TCA}{}^n$, R^2=0.40, P = 0.066) oxidative metabolism were measured. At the group level, 20% increase in V_{NT} (+0.038 ± 0.042 μmol/g/min) resulted in a 24% ($\Delta V_{TCA}{}^g$=0.063 ± 0.057 μmol/g/min) and 12% ($\Delta V_{TCA}{}^n$=0.061 ± 0.032 μmol/g/min) increase in glial and neuronal TCA cycle activity, respectively, resulting in 14% increase in $CMR_{glc(ox)}$ (+0.058 ± 0.032 μmol/g/min).

We conclude that cortical brain activity resulted in a significant increase in cerebral metabolic rate of glucose, and an increase in both glial and neuronal oxidative metabolism. In both cells, the tricarboxylic acid cycle rate was correlated with the rate of the glutamate-glutamine cycle.

MTU10-13

Cortical neuronal glucose metabolism is impaired at mid stage in the hSOD1^{G93A} mouse model of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a multi-pathogenic disorder mainly characterized by the selective loss of motor neurons

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in brain and spinal cord. Although metabolic alterations have been reported in this disease, the specific biochemical changes in the energy producing pathways are unknown. We therefore investigated glucose metabolism in the superoxide dismutase ($hSOD1^{G93A}$) mouse model of ALS. Wild-type and $hSOD1^{G93A}$ mice (n = 11) at 17 weeks old were injected with 543 mg/kg [1-^{13}C] glucose and 504 mg/kg [1,2-^{13}C] acetate (i.p.) 15 minutes before sacrificed. Cerebral cortices were collected and extracted using methanol/chloroform. The labelled and unlabelled metabolites of amino acids were quantified using ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy and high performance liquid chromatography. Numerous metabolic alterations were found mainly related to neuronal glucose metabolism. Reductions in the amounts of glycolysis derived metabolites such as total and labelled lactate (by 28 and 54%) and total and labelled alanine (by 18 and 65%) respectively ($p < 0.05$) indicating impairments in glycolysis. Also, the incorporation of ^{13}C glucose via pyruvate dehydrogenase (PDH) enzyme into the 1st turn tricarboxylic acid (TCA) cycle metabolites such as [4-^{13}C] glutamate, [4-^{13}C] glutamine and [2-^{13}C] GABA was reduced by 42–54% ($p < 0.05$) showing less entry of glucose into the TCA cycle. The levels of some of the branched chain amino acids (BCAAs) such as isoleucine and leucine ($p < 0.05$) were reduced indicating a compensatory degradation of BCAAs mainly due to an increased energy demand and need for anaplerosis. There were minor changes in astrocytic metabolism including increased transfer of [1,2-^{13}C] acetate-derived glutamine towards the formation of GABA but no changes in the levels of [4,5-^{13}C] glutamate, [4,5-^{13}C] glutamine and [1,2-^{13}C] GABA or pyruvate carboxylase-derived metabolite levels. In conclusion, we found defective cortical neuronal glucose metabolism in the $hSOD1^{G93A}$ mouse model of ALS at symptomatic stage of the disease.

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MTU11 Neuroimmunology

MTU11-01

Role of ck2 in t-cell differentiation and autoimmunity

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CD4+ T cells are the major pathogenic cells in many autoimmune and inflammatory disorders, including Multiple Sclerosis (MS) and Experimental Autoimmune Encephalomyelitis (EAE), an animal model of MS. Th17 cells are important effector cells in the pathogenesis of MS, whereas regulatory T cells (Treg) are crucial in disease resolution. Current therapies for MS patients are incapable of stopping disease progression and are not curative. Thus, studies on the mechanisms of enhancing anti-inflammatory responses, such as enhancing trans-differentiation of pathogenic Th17 cells into beneficial Treg cells, may lead to new potential therapies for MS. Protein kinase CK2 (Casein Kinase II) is a constitutively active serine/threonine kinase composed of two catalytic subunits (alpha and/or alpha') and two regulatory beta subunits. CK2 is involved in the activation of multiple signaling pathways, including PI3K/AKT/mTOR and JAK/STAT, which are essential for the differentiation of CD4+ T cells. However, little is known about the specific function of CK2 in T cells, and the consequences of CK2 inhibition during CD4+ T cell differentiation and the pathogenesis of MS/EAE. Our data indicate that expression of the major catalytic subunit of CK2, CK2alpha, is induced in a time-dependent manner upon activation of CD4+ T cells both *in vitro* and *in vivo*. Utilizing a small molecule CK2 inhibitor CX-4945 (Silmitasertib), we find that inhibition of CK2 kinase activity significantly ameliorates the severity of EAE disease, which is correlated with regulation of Th17 and Treg cell frequencies. Treatment with CX-4945 inhibits the differentiation of Th17 cells, while promoting the differentiation of Tregs. Our preliminary results indicate that conditional deletion of CK2alpha in CD4+ T-cells inhibits Th17 differentiation and promotes Treg polarization, comparable to what was observed with CX-4945 treatment. Thus, we propose that CK2 kinase activity in CD4+ T cells correlates with the pathogenesis of MS/EAE by promoting inflammatory Th17 cell responses and suppressing anti-inflammatory Treg cell development, thereby affecting the ratio of these two important CD4+ T-cell subsets. Furthermore, inhibition of CK2 kinase activity may be a potential novel therapeutic treatment for MS patients.

MTU11-02

Generation of IL33 knockout mice and cell-based reporter assay for functional studies of IL-33 *in vitro* and *in vivo*

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Background: Interleukin-33 (IL-33), an IL-1 family cytokine, is a tissue-derived nucleus alarmin that drives inflammatory responses through binding to its receptor ST2. IL-33 is expressed by multiple tissues and released from nucleus upon tissue damage or inflammation.

Aims: In this study, we aimed to generate a cell-based functional reporter assay for screening of IL-33 activity. We also aimed to generate IL33 knockout mice for *in vivo* loss-of-function studies.

Methods and Results: We established the IL-33 reporter cells with stable expression of functional receptor complex for IL-33 (ST2L and IL-1RacP) and an AP-1/NF-kB/SEAP reporter cassette. The reporter cells secrete alkaline phosphates to the culture in response to IL-33 stimulation and the levels of the enzyme activity can be determined using colorimetric enzyme assay. Our results demonstrated the specificity of IL-33 in activation of ST2 signaling in the IL-33 reporter cells.

For generation of IL33 conditional deletion mice, the exon 5, exon 6 and exon 7 of the IL33 gene were flanked by two directional Lox-p sites. Germ line transmission and the establishment of IL33 floxed mice (IL33 $^{fl/fl}$) were confirmed by allele-specific genotyping and diagnostic restriction analysis of PCR products. The *IL33*$^{-/-}$ were further generated from *IL33* $^{fl/fl}$ mice by crossing the mice to C57BL/6-Tg(UBC-Cre) to remove the loxP-flanked exons. Deletion of targeted exons was confirmed using allele specific PCR and RNA-sequencing analysis. Immunofluorescent staining and ELISA analyses also confirmed the deficiency of IL-33 protein in *IL33*$^{-/-}$mice.

Conclusions: We have established a cell-based reporter assay for functional analysis of IL-33 activity. We also generated IL33$^{-/-}$ mice and IL33 $^{fl/fl}$ mice for conditional knockout studies. These materials will be useful for further investigation of the role of IL-33 in health and diseases.

MTU11-03

Curcumin reverses altered expression of GFAP astrocytes marker and hypercorticosolemia in depressed rats

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Astrocyte pathology has been consistently observed in Major Depressive disorders (MDD) and this could be seen in the altered expression of proteins for astrocyte markers such as the Glial Fibrillary Acid Protien (GFAP). Astrocyte deficit observed in MDD, astrocytes may become novel targets for antidepressant medication. The present study investigated the effect of curcumin and fluoxetine antidepressant drug on the expression of GFAP astrocyte marker and corticosterone (CORT) in depressed rats. Depression was induced in albino wistar rats using a modified Chronic Unpredictable Stress (CUS) method for 42 days. The rats were grouped into six groups of ten rats each. The Control received distilled water only. Depressed group received distilled water after CUS. Olive oil group received 0.8 ml/kg of virgin olive oil for 42 days after CUS. Curcumin group received 30 mg/kg of curcumin for 42 days after CUS. Fluoxetine group received 20 mg/kg of fluoxetine for 42 days after CUS. Fluoxetine+Curcumin group received 30 mg/kg of curcumin and 20 mg/kg of fluoxetine for 42 days after CUS. There was increased astrocyte pathology as indicated by the increased

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intensity in expression of GFAP astrocyte marker, hypertrophy of the astrocyte cell bodies and processes as well as increased scar formation in the Prefrontal cortex, dentate gyrus and CA3 region of the hippocampus of depressed rats compared to the control group. On the other hand, there was reduced intensity of GFAP expression in astrocyte cell bodies and processes in the Prefrontal cortex, dentate gyrus and CA3 region of the hippocampus of curcumin treated rats as well as the curcumin+fluoxetine treated group and in the control group. The CORT level was significantly increased in depressed rats compared to the control group whereas there was no significant difference observed in the curcumin treated group and other treatment groups. Hence, curcumin has shown to ameliorate the increased astrocyte pathology and hypercortisolemia seen inMDD.

MTU11-04

c-Abl regulates rotenone-induced inflammatory response via the activation of NLRP3 inflammasome

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Multiple evidences support the hypothesis that exposure to pesticides increases the risk of PD. Emerging evidence indicates that intracellular inflammasome complex namely NLRP3 complex is involved in the recognition and execution of host inflammatory response. Thus in the present study we investigated the hypothesis that NLRP3 inflammasome activation is linked to rotenone- induced microglial activation which is dependent upon a priming stimulus by a pathogen associated molecular pattern (PAMP) or damage associated molecular pattern (DAMP) respectively. We employed primary microglia, BV-2 microglial cell culture, and an *in vivo* endotoxin model of neurodegeneration to address the stated hypothesis. We found that LPS priming accelerated rotenone-induced NLRP3 inflammasome activation that was associated with the activation of caspase-1 and subsequent proteolytic processing and release of IL-18 and IL1β as well as the release of TNFα and IL-6 as assessed via WB analysis and Luminex multiplex technology. Mechanistic studies revealed c-Abl/PKCδ kinase signaling axis as a proximal signal that exacerbated rotenone-induced NLRP3 inflammasome activation, that is mediated via mitochondrial and autophagolysosomal system (ALS) dysfunction and accompanying downregulation of TFEB, a lysosomal transcription factor. Intriguingly, gene silencing and pharmacological inhibition of c-Abl attenuated NLRP3 inflammasome activation, mitochondrial and ALS dysfunction and PKCδ activation; while, c-Abl overexpression potentiated that response in LPS primed rotenone treated microglial cells. Furthermore, using an *in vivo* LPS model of neurodegeneration we showed that c-Abl upregulation positively correlated with NLRP3 inflammasome activation and accompanying sickness-like behavior. Our findings demonstrate for the first time that c-Abl/PKCδ signaling axis is a key regulator of NLRP3 inflammasome activation which is mediated partly via dysregulation of ALS and mitochondrial function during rotenone-induced microglial activation (supported by NS088206).

MTU11-05

S-guanylation of SNAP-25 by 8-nitro- cGMP attenuates the interaction of snare complex with complexin

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Introduction: 8-Nitroguanosine 3′,5′ -cyclic monophosphate (8-nitro-cGMP) is an unique second messenger, which is generated in the nitric oxide/reactive oxygen species signal. This molecule covalently binds to protein thiol groups, called protein S-guanylation, and alters protein functions. On the other hand, exocytosis is catalyzed by soluble N-ethylmaleimid-sensitive factor attachment protein receptor (SNARE) proteins and is modulated by some modulatory proteins. Complexin is a small protein that binds to SNARE complex with high affinity and modulates exocytosis. Previously we reported synaptosomal-associated protein 25 (SNAP-25) as a target protein of S-guanylation, however, the neurophysiological functions have not been clarified. Here, we investigated the role of S-guanylation of SNAP-25 by 8-nitro-cGMP in neurons.
Material and methods: The localization of 8-nitro-cGMP and S-guanylated proteins in the Wistar rat brain were confirmed by immunohistochemistry using each specific antibody. To confirm the effect of 8-nitro-cGMP for the interaction between SNARE complex and complexin, we performed pull-down assay using GST-tagged complexin, co-immunoprecipitation and blue native (BN)-PAGE followed by western blotting. SH-SY5Y neuroblastoma cells were transfected with FLAG-tagged SNAP-25 (wild-type and C90A) or V5-tagged complexin, treated with 8-nitro-cGMP, and then used as samples.
Results and discussion: The result of the immunohistochemistry revealed that the 8-nitro-cGMP and S-guanylated proteins were localized in neurons in the brain. Pull-down assay revealed that the amount of SNAP-25 pulled-down by GST-complexin was decreased by S-guanylation of cys90 in SNAP-25. We could obtain almost the same results from co-immunoprecipitation. Furthermore, BN-PAGE followed by western blotting revealed that the amount of V5-complexin detected in high molecular mass was decreased by 8-nitro-cGMP treatment. Our results suggest that S-guanylation of cys90 in SNAP-25 attenuates the interaction of SNARE complex, which would form a large oligomer, with complexin.

MTU11-06

Apoptosis and host-defense peptide cathelicidins determine different outcomes of bovine alpha-herpesviruses neuropathogenesis

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Alpha-herpesviruses are closely related viruses that cause neurological disease in humans and cattle. Bovine herpesvirus

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(BoHV) type 5 is an important cause of encephalitis in cattle. However, encephalitis by BoHV-1 occurs occasionally. It is unknown how the innate immune response contributes to their differences in neuropathogenesis. BoHV-5 specifically induced the expression of Toll-like receptors (TLRs) in bovine neural inflamed tissue (Marin *et al*., 2014). Antimicrobial cathelicidin peptides modulate TLR4 expression in epithelial cells although their function in the nervous system remains elusive. These innate factors together with specific BoHV-apoptotic potential could be determinant in the neuropathogenesis as they could promote or limit the inflammatory response. In this study, we determined apoptosis and the expression of cathelicidins in the bovine nervous system during BoHV-1 and 5 acute infections. Calves were inoculated with BoHV-1 Cooper or BoHV-5 97/613 strains ($10^{6.3}$ $TCID_{50}$) or inert culture medium (control). At 6 days post-infection (dpi), different regions of central nervous system (CNS) and trigeminal ganglion (TG) were collected for immunocytochemical detection of cleaved caspase 3 and messenger gene determination of bovine cathelicidins BMAP27 and BMAP28 (RT-qPCR). At 6 dpi, caspase 3-apoptotic neurons were detected in the TG of BoHV-1-infected calves, whereas fewer numbers of caspase 3 positive neurons were observed in BoHV-5-infected calves. Cathelicidins expression was up- and down-regulated in CNS from BoHV-1- and BoHV-5-infected calves, respectively. BMAP27 was noticeably up-regulated in TG from BoHV-1-infected calves, while BMAP28 was only detected in BoHV-5-infected calves. Our findings suggest that modulation of apoptosis and cathelicidin expression orchestrate the final inflammatory outcomes of alpha-herpesviruses infection of the nervous system. Inhibition of both factors might be responsible for neurological lesions in BoHV-5 infection. Further research will be required to determine the role of cathelicidins during infectious diseases of the CNS.

MTU11-07

Potential link between C5a receptor and mood disorders in mouse exposed to experimental malaria *in utero*

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In Africa, a large number of pregnancies are exposed to *Plasmodium falciparum* infection during pregnancy. The in-utero environment extremely influence childhood neurodevelopment and behaviour. The complement 5a receptor is linked to several disease conditions. However, the influence of *Plasmodium berghei* during pregnancy on maternal complement 5a receptor and subsequently on fetal behaviour is unknown. Pregnant mice were intra-peritoneal infected on gestational day 13 with $1.02x10^5$ infected red blood cells. Infected red blood cells used in this experimental infection were obtained from *in vivo* passage of *P. berghei* in mice when the percentage of iRBCs reached approximately 10-20%. A section of pregnant mice (both infected and uninfected) were allowed to deliver and the progenies monitored up to postnatal day 42 when depression-like behaviour was evaluated using tail suspension test model. The other pregnant mice were subjected to cardiac puncture on gestational day 19 for C5a receptor estimation using Elisa assay. We show that pregnant mice infected with *P. berghei* had elevated C5a receptor compared with uninfected pregnant females. We also show that *P. berghei*-exposed offspring presented a depressive like behaviour compared to unexposed controls. Our results demonstrates a pathogenic role of complement 5a receptor signaling and its possible role in mediating depression which is linked to *Plasmodium berghei* infection during pregnancy.

MTU11-08

Neonatal proinflammatory treatment affects the cognitive functions development and brain neuroplasticity-related gene expression

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Pro-inflammatory cytokines such as IL-1β, IL-6, and TNFα are the main mediators of the neuro-immune interactions and are known to affect learning and memory. Pro-inflammatory cytokines elevation during neonatal period is associated with high risk of neuropsychiatric symptoms in later life. However, their effects on the maturation of brain functions during early postnatal development are not completely defined.

We have investigated the effects of the treatment with IL-1β or bacterial mimetic lipopolysaccharide (LPS, pro-inflammatory cytokine inducer) during the 3rd week of life, which corresponds to human perinatal period in terms of the CNS development, on the development of behavior and brain gene expression in Wistar male rats. Early-life IL-1β injections impaired working memory, while LPS enhanced anxiety. Long-term memory in active avoidance and Morris water maze paradigms was affected in adult rats both after neonatal IL-1β and LPS treatment, whereas in adolescent animals, exploratory behavior and locomotor activity was changed. IL-1β treated animals had long-lasting changes in the D2 dopamine receptor, FGF2, TIMP1 and MMP9 mRNA expression in the medial prefrontal cortex and hippocampus in the region- and task-dependent manner. The treatment of rats with LPS during early life induced the short-term and long-term changes in the expression of AMPA и NMDA glutamate receptor subunits, TIMP1 and MMP9 genes in the hippocampus and medial prefrontal cortex.

The impairments induced by the elevation of pro-inflammatory cytokine level during the early postnatal period may be associated with the development of psycho-neurological decline of young and adult patients with attention deficit hyperactivity disorder or other cognitive dysfunctions, and the mechanisms of that may be explained by dysregulation of brain neuroplasticity-related gene expression. Supported by RFBR projects 17-04-02116 A, 16-34-00873 mol_a, 16-34-00316 mol_a.

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MTU11-09

Neurotrophin-3 modulates microglial phenotype in the traumatized CNS

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Over two-thirds of spinal cord injury (SCI) patients have anatomical preservation at the injury site, yet this preserved tissue is typically completely or partially dysfunctional. Therefore, to provide improved function in these patients, strategies are needed that enhance the function of the remaining connections by enabling the inherent plasticity of the CNS. We found an unanticipated interplay between a neurotrophin and cellular immune processes that may provide a means to promote plasticity after SCI. Moreover, we have evidence that this interplay may be exploited to induce neuroplasticity in *chronic* SCI. Our earlier work showed that unilateral viral-vector mediated over-expression of Neurotrophin-3 (NT-3) in lumbar motoneurons induced axon growth from the contralateral corticospinal tract (CST) towards the source of the NT-3. Subsequently, we showed that there is an immune component to the NT-3-induced axonal sprouting and that microglia and T cells are likely involved. When the host animals were rendered immunocompetent by pharmaceutical or genetic manipulations the NT-3 did not induce sprouting. Grafting normal T cells into immunocompetent rats enabled NT-3-induced axonal sprouting. Recent data show that microglia and macrophage have the NT-3 receptor TrkC and that NT-3 re-programs inflammatory macrophages and microglia to a more pro-regeneration phenotype. These experiments suggest that future therapeutic strategies based upon manipulation of microglia may promote enhanced neuroplasticity and increase functional recovery in patients with chronic SCI. Supported by the Mission Connect a project of TIRR Foundation, The Dana and Christopher Reeve Foundation and the NIH-NINDS.

MTU11-10

Inorganic arsenic mediated disturbance in microglial glutathione metabolism leads to bystander death of immature neurons

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Low level environmental arsenic exposure has toxic effects on neurons leading to cognitive dysfunction. Inspite of this fact, microglial role in arsenic mediated neurotoxicity remain elusive. In this study, arsenic -exposed microglial (N9) culture supernatant induces bystander death of neuro-2a (N2a) cells, which resembles developing neurons; contrarily, arsenic-exposed N2a culture supernatant did not affect N9 viability. These results indicated that microglia create toxic environment for N2a in presence of arsenic. Screening for involvement of toxic factors like reactive oxygen species (ROS), nitric oxide (NO), interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) secreted from N9 cells in bystander N2a death is found to be negative. Arsenic exposure induces GSH synthesis in N9 cells by uptake of cystine from culture medium through cystine/glutamate exchanger (xCT) resulting in lower cystine and higher glutamate concentration compared to control culture supernatant. Cystine/glutamate imbalance is also enhanced by increased xCT expression in Nrf2 dependent manner in microglia. Bystander death of N2a is rescued by supplementation of 200 μM cystine to arsenic-exposed N9 culture supernatant. Simultaneous exposure of excessive glutamate and arsenic compromises N2a viability which is again rescued by cystine addition. Therefore, microglia executes bystander N2a death simultaneously by reduction of extracellular cystine concentration and competitive inhibition of cystine transport due to high extracellular glutamate levels. *Ex-vivo* microglia from gestationally arsenic exposed mice releases excessive glutamate and reduces cystine levels in culture supernatant as compared to control and n-acetyl cysteine co-treated group. Immunofluorescence staining of brain cryosections from treated group showed more apoptotic immature neurons. Interestingly, TUNEL fluoresecence did not merged with microglia specific Iba1. Finally bystander death of primary immature neuronal cultures using primary microglia arsenic treated culture supernatant is also confirmed. Collectively, xCT is indispensable for survival of immature neurons both *in-vitro* and *in-vivo* in presence of arsenic and microglia.

MTU11-11

One-two punch, combination of immune tolerance and myelin repair therapy to effectively target disease course and severity in MS

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The CNS autoimmune disease Multiple Sclerosis (MS) is characterized by demyelination and neurodegeneration. Available FDA-approved disease modifying therapies are global immunosuppressants and have limited efficiency. We have developed a novel method of inducing immune tolerance to selectively regulate known immune responses without compromising the entire adaptive immune system. We have demonstrated an effective means of ameliorating disease in a mouse model of MS through tolerance induction in autoreactive T cells using i.v. infusion of nanoparticles coupled with or encapsulating myelin peptides (Ag-PLG) that effectively reduces disease burden in relapsing-remitting (RR-EAE) and chronic-progressive (C-EAE) mouse models of experimental autoimmune encephalomyelitis. This works to prevent disease induction, but more importantly can stop disease progression in mice treated following the initial clinical episode resulting in antigen-specific blockade of disease relapses. At present, there are no available therapies marketed for myelin repair in MS. The objectives of the study were to prevent disease progression as well as to promote CNS repair and neuroprotection. We tested an FDA approved cardiac glycoside (Na^+/K^+ ATPase) and uncovered it promoted an increase in the oligodendrocyte cell lineage *in vitro* and *in vivo*, in the non-T cell-mediated Cuprizone model of demyelination/remyelination promoted a quicker restoration of myelin integrity, and improved clinical score throughout the autoreactive Th1/Th17 driven C57BL/6 Chronic EAE time course. Additionally, we tested the hypothesis that to effectively target disease course and severity in MS, regulated by autoimmunity and neurodegeneration, a combination of selective immune regulation and myelin repair therapy is required. Combination therapy using Ag-PLG immunoregulatory therapy and the cardiac glycoside completely

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ameliorated clinical disease severity. Findings from these studies may not only prove a rapid and safe therapeutic strategy for EAE reversal, but will pave the way for future clinical studies in MS undertaking this combinatorial therapeutic approach.

MTU11-12

Role of MECP2 in neuroimmune interactions

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Rett Syndrome is an autism spectrum disorder (ASD) caused by mutations in Methyl Cytosine Binding Protein 2 (MeCP2) and mouse models of Rett have been widely used for studying ASDs. The main goal of our project is to use this monogenic model of ASD, in order to evaluate the role of altered immunity in the pathogenesis of this disorder. To this end we first evaluated the autoimmune response in the context of the experimental autoimmune encephalomyelitis (EAE). Male MeCP2 WT and MT mice, were immunized with MOG $_{35\text{-}55}$ peptide, scored daily for EAE symptoms and sacrificed at 12 dpi (acute stage) or at 30 dpi (chronic stage). When WT-EAE animals and MT-EAE animals were compared, we found that MeCP2 MT mice showed an accelerated onset of the disease and more severe clinical scores. Coronal sections of spinal cord were subjected to IHC to analyze the level of expression of Iba1 (microglia) and to assess CNS lymphocyte infiltration during EAE. MeCP2 MT animals showed increased levels of infiltrating cells and microgliosis compared to WT mice; this observation correlated with the individual clinical score reached by each animal. To determine the response of immune cells, we re-stimulated spleen mononuclear cells derived from MeCP2 WT and MT mice with MOG peptide *in vitro*. Proliferation index and cytokine production was assessed. We observed increased proliferation index in all EAE animals compared to CFA in response to MOG stimulus, with no significant differences between MT and WT MeCP2 mice. Nevertheless, when the cytokine response was analyzed, MT-EAE group showed increased IFN-gamma levels in response to MOG in comparison with WT-EAE animals. Our results showed a more severe neuroinflammation in the absence of MeCP2, suggesting that Mecp2 has an active role in regulating the immune response and maintaining the neuroimmune homeostasis.

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MTU12 Cellular Mechanism of Alzheimer's Disease

MTU12-01

Effects of presenilin-1 mutations in mitochondrial dynamics

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Early stage of Alzheimer's disease reveals mitochondrial deficit and dysfunction. Mitochondrial dysfunction in Alzheimer's disease causes synaptic alteration, imbalance of lipid homeostasis, calcium homeostasis, and lack of ATP production. Familial Alzheimer's disease-linked Presenilin-1, catalytic subunit of γ-secretase, mutations cause early onset Alzheimer's disease. All mutation types have different pathological mechanism and ultimately break down cellular homeostasis. Our research shows more details about relationship between Presenilin-1 mutations (PS1A431E, PS1E280A, PS1H163R, PS1M146V, PS1ΔE9) and mitochondrial dysfunctions. All of PS1 mutants-expressing cells exhibited mitochondrial dysfunctions and reduced levels of proteins involved in mitochondrial dynamics without alteration of total mitochondrial biogenesis.

MTU12-02

Protective effect of periodic dietary restriction on behavior and hippocampal deficits in a mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative pathology associated with progressive decline in cognition and brain functions. Accompanying amyloid β (AB) deposition, astrocytes and microglia lose their neuroprotective functions and induce pathways that amplify inflammation. Dietary restriction (DR) has been shown to decelerate the aging process and reduce the impact of age-associated diseases, probably modulating oxidative and inflammatory status, regulating autophagy and inducing cell protection.

Our objectives were to evaluate neuroprotective effects of DR in a model of familial AD and to parallelize *in vivo* results using an *in vitro* model of nutrient restriction on glial cells exposed to AB. We established a model of periodic DR in control and PDAPP-J20 transgenic mice. Daily food consumption was restricted to 60% for 5 days/week every one week for a total of 6 weeks.

At 8 months of age, cognitive deficits and anxious-like behavior were found in *ad libitum* fed transgenic mice and were prevented by DR. In parallel, hippocampal neurogenesis was decreased in transgenic mice under *ad libitum* diet whereas transgenic mice under DR showed a neurogenic status similar to controls. *In vitro* experiments were done on C6 astroglial cells exposed to AB with and without nutrient restriction (FBS 2% vs. 10% in RPMI). Serum deprivation and AB induced autophagy. Subsequently, conditioned media (CM) from C6 were used to stimulate BV2 microglia. Microglial NFkappaB nuclear translocation was increased when exposed to CM from C6 cells with ABeta but not from C6 cells exposed to AB and serum restriction. Our results suggest neuroprotective effects of nutrient restriction in the context of AD, with glial activation and autophagy as potentially involved pathways.

MTU12-03

Inhibition of DRP1 ameliorates mitochondrial fission and and cognitive impairment in Alzheimer's disease model

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Excessive mitochondrial fission is a prominent early event, and contributes to mitochondrial dysfunction, synaptic failure and neuronal cell death in the progression of Alzheimer's disease (AD). In the present study, we examine the role of Drp1, a key regulator of mitochondrial fragmentation, in mitochondrial and synaptic dysfunction-induced by Aβ, and AD-like neuropathology and cognitive functions in AD mice. Our results demonstrate that the inhibition of Drp1 alleviates mitochondrial fragmentation, loss of mitochondrial membrane potential, ROS production, and ATP reduction in neurons treated with Aβ oligomers. An inhibitor of Drp1 also significantly restores Ab-mediated depression of synaptic vesicle exocytosis. Furthermore, Drp1 inhibition significantly improves learning and memory, synaptic density, and prevents mitochondrial fission, lipid peroxidation, BACE1 expression and Ab deposition in an AD mouse model. These results provide evidence that Drp1 plays an important role in Aβ-mediated and AD-related neuropathology, and in cognitive function in an AD animal model. Thus, inhibiting excessive Drp1-mediated mitochondrial fission may be an efficient therapeutic avenue for AD.

MTU12-04

Untargeted 1 h-NMR spectrometry to detect central and systemic metabolic changes in a rat model of early Alzheimer's disease

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Characterization and comprehension of the Alzheimer's Disease (AD) presymptomatic stage would help to better understand disease progression, develop early detection methods and improve treatment. Our main goal was to perform untargeted NMR metabolomics in hemizygous McGill-R-Thy1-APP (TG±) rats which compiles several biochemical and neuropathological characteristics detected in presymptomatic human AD brain. Experiments were performed in male TG+/- (n = 8) and non-transgenic littermate (WT) (n = 8)

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rats of 9 months of age. CSF, hippocampus, and plasma were collected to study changes at central and systemic levels. Hippocampus were homogenized in 80% methanol to extract soluble metabolites. Differences between TG+/- and WT were analyzed by 1D 1H-NMR, whereas metabolite identification was performed by 2D NMR and confirmed by spiking with standard compounds. Experiments were carried out on a Bruker Avance II spectrometer operating at 600.3 MHz. Only few metabolites changed between TG+/- and WT rats in each sample type (n = 2 in CSF, n = 1 in plasma, and n = 3 in hippocampus). In plasma, lactate levels were higher in TG+/- than WT ($p < 0.07$), resembling the already described increase in presymptomatic AD patients. By contrast to what was described using MRI in dorsal hippocampus of homozygous McGill-R-Thy1-APP rat, which mimic late stages of AD, we did not find significant decrements in the N-acetylaspartate, GABA, and Glutamate levels in TG+/- as compared to WT. However, we did observe increased levels of NAD/H ($p < 0.01$) and decreased levels of nicotinamide ($p < 0.01$). These two metabolites are part of the NAD^+ salvage pathway, which is essential to maintain the NAD^+ pool. Our results suggest that a rise in NAD^+ synthesis, probably to ameliorates mitochondiral disfunction, and/or an impairment in NAD^+ conssumig enzimes activity like sirtuins, affecting epigenetic and metabolic processes, are taking place in the brain at early stages of AD.

MTU12-05

Amyloidogenic processing of β-amyloid precursor protein promotes ferroptosis: implications for Alzheimer's disease

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Intraneuronal iron imbalance is a predominant catalyst for reactive oxygen species production, particularly within iron accumulating neurodegenerative diseases such as Alzheimer's disease (AD). In AD, amyloid precursor protein (APP) has historically been associated with amyloid-β (Aβ) derived neurotoxicity, but we recently discovered that APP also has a role in neuronal iron homeostasis by, in part, promoting iron efflux through cell surface stabilization of the iron pore ferroportin. Detailed cell surface characterization confirms that the location of ferroportin on the neuron surface is increased upon iron incubation and is dependent upon APP. Altering the proteolytic processing of APP at the cell surface by suppressing secretase expression or activity, expression of APP carrying familial AD mutations or disrupting lipid rafts, causes consequential changes in neuronal iron homeostasis. Enhancing the amyloidogenic pathway of APP processing leads to intracellular iron accumulation and contributes to oxidative stress and toxicity. Iron induced toxicity caused by increased amyoidogenic processing of APP appears to be mediated by the ferroptosis inhibitor ferrostatin-1. With increased amyloidogenic processing of APP being a major contributor to sporadic AD, these studies increase our understanding as to why iron accumulation and increased susceptibility to reactive oxygen species neurotoxicity are prevalent with the disease.

MTU12-06

Imbalance in BDNF and probdnf levels in serum and cerebroespinal fluid in untreated Alzheimer's disease patients

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Brain-derived neurotrophic factor (BDNF) is essential for the survival and differentiation of neurons, and it's considered a key target in the pathophysiology of various neurodegenerative diseases. There are not full consensuses regarding the serum levels of BDNF in Alzheimer's Disease (AD). In this study, we measured serum and CSF BDNF levels in patients with AD newly diagnosed without treatment with drugs that can upregulate the expression of BDNF. BDNF serum concentrations were lower in AD with depression follow to AD without depression, controls with depression and finally controls without depression. Apathy, MMSE, semantic verbal fluency and several depression scales also correlated with lower levels of BDNF. Contrarily to BDNF, pro-BDNF induce apoptosis through its interaction with p75NTR and its co-receptor, sortilin. In CSF the ratio proBDNF/mBDNF is increased compared to controls. In the hippocampus of human AD samples, proBDNF is modified by AGE/ALEs preventing its processing to the mature form and thus, increase the pathogenicity of the proform.

MTU12-07

The role of olfactory dysfunction in the pathogenesis of Alzheimer disease

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Alzheimer disease (AD) is a chronic disorder that affects millions of individuals worldwide. Olfactory dysfunction is a common symptom of several neurological disorders including AD. Studying the mechanisms underlying the olfactory dysfunction may lead to the discovery of potential biomarkers and/or treatments for neurodegenerative diseases. Objective: to determine if olfactory dysfunction predicts future cognitive impairment and to characterize the olfactory system in a murine model expressing a genetic factor of AD. For the human study, quantitative olfactory tests have been

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done on 93 subjects from the NuAge cohort accepting to participate in the ORCA secondary study. The t-MMSE was used to assess cognition status, and an olfactory self-report collected. In a separate cohort, olfactory cortical volume was calculated using MRI from healthy old adults and AD individuals. Based on the self-report, 81% of our participants claimed to not suffer from any problem with olfaction. However, based on the UPSIT, 94% show olfactory dysfunction. We also detected a significant decrease in olfactory cortical volume in AD compared to controls. Murine study: Preliminary data demonstrate there is a significant decrease in expression of the proform of caspase-9, caspase-3 and the caspase substrate STK3, in the olfactory bulb of mice expressing human APOE4 compared with controls. The data also suggest that Iba-1 is increased in the olfactory bulb of APOE4 mice compared to wild type. The activation of caspase-3 may be the cause of the decreased levels of STK3 through caspase cleavage and may play role in the inflammation observed.

MTU12-08

The role of copper in ubiquitin-dependent protein degradation in Alzheimer's disease

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Disruption to copper homeostasis is a feature of Alzheimer's disease (AD). Recently it was discovered that copper reduces Amyloid Precursor Protein (APP) endocytosis from the plasma membrane and promotes its ubiquitination. The importance of this finding is underlined by studies that indicate that endocytosis is a key step in amyloidogenic processing of APP to form neurotoxic amyloid beta (Aβ) peptides. Ubiquitin plays a fundamental signalling role in proteasome-mediated protein degradation, endocytic protein sorting and targeting membrane proteins to lysosomes for degradation via autophagy. Our HYPOTHESIS is that APP amyloidogenic processing is modulated by copper-responsive ubiquitination of APP, signalling it towards a degradative pathway rather than an endocytic pathway where it encounters the enzymes responsible for Aβ generation, namely β- and γ-secretase.

SPECIFIC AIMS:: Aim 1: To determine the role of Cu-responsive ubiquitination of APP on its localization and degradation in cultured mouse neurons.

Aim 2: To compare Cu-responsive ubiquitination of APP in differentiated neurons that have been re-programmed from healthy and AD patient human fibroblasts using induced pluripotent stem cells (iPSCs).

Aim 3: To determine if mutations that cause familial AD affect Cu-responsive ubiquitination of APP using cultured mouse and human fibroblasts.

Aim 4: To identify novel Cu-responsive ubiquitin targets in AD-affected and healthy control fibroblasts using an 'ubiquitin-omics' approach.

We propose that copper is a physiological co-factor for the ubiquitination of APP, a neuroprotective mechanism that reduces the level of amyloidogenic processing.

MTU12-09

The relationship between the amyloid precursor protein and tar DNA binding protein 43 (TDP-43): links to Alzheimer's disease

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An increasing body of literature supports a role for TAR DNA binding protein 43 (TDP-43) in the pathogenesis of Alzheimer's disease (AD). Recent studies have founds TDP-43 inclusions present in the brains of substantial numbers of AD patients, with prevalence reported as between 19 and 57%. Other researchers have suggested that TDP-43 inclusions are correlated with greater disease severity as measured by Braak staging. In addition, TDP-43 has been suggested to modulate the expression and proteolytic processing of amyloid precursor protein (APP). In this study, co-immunoprecipitation and immunocytochemistry were used in SH-SY5Y cells and control induced pluripotent stem cell (iPSC)-derived human neurons to assess the possible direct interaction between the predominantly nuclear TDP-43 and the nuclear fragment of APP, namely AICD. We were unable to show any co-immunoprecipitation between TDP-43 and AICD. Furthermore, AICD and TDP-43 showed opposing nuclear localisation in SH-SY5Y cells and iPSC-derived neurons, suggesting a lack of direct interaction between TDP-43 and AICD. APP isoforms were subsequently over-expressed in SH-SY5Y cells, and TDP-43 expression was monitored by immunoblotting. Although no change was observed, preliminary data generated by targeting APP with siRNA suggest that APP may regulate TDP-43 expression in an indirect manner. In addition, TDP-43 over-expression was preliminarily shown to modulate the expression of the APP holoprotein and the β-secretase, BACE1. Taken together, these findings suggest that the correlation of the presence of TDP-43 inclusions with the severity of AD may derive, at least in part, from the ability of TDP-43 to regulate the expression of APP and BACE1. Furthermore, our data suggest that, in the case of APP and TDP-43, the relationship may be bidirectional. An improved understanding of the contribution of TDP-43 to the pathogenesis of AD could reveal novel therapeutic avenues.

MTU12-10

Lupeol isolated from betula alnoides inhibit phophodiesterase and ameliorates streptozotocin induced cognitive impairment and NEU

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Objective: To investigate the therapeutic potential of lupeol (isolated from *Betula alnoides)* in STZ-induced experimental dementia of Alzheimer's type in rats.

Material and methods: Lupeol was isolated from the *BA* and its phosphodiesterase (PDE) inhibitory activity was assessed through *in-vitro* PDE inhibitory assay. STZ was administered intracerebroventrically (ICV) on day 1 and 3 (3 mg/kg, ICV bilaterally) in rats. lupeol was administered (25, 50 and 100 mg/kg/day p.o.) 1 hr following 1st STZ infusion upto 21st day. Morris water maze (MWM), locomotor activity and object recognition task (ORT) were used to assess behavioral changes in rats. On 22nd day, animals were sacrificed and hippocampus were isolated for biochemicals

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(acetylcholinesterase (AChE), lipid peroxidase (LPO), reduced glutathione (GSH and nitrite), neuroinflamatory (Tumor necrosis factor alpha (TNF-α), Interleukin 1 beta (IL-1β), and IL-6), neurotransmitters (NTs) analysis (dopamine, norepinephrine, serotonin, 3,4-Dihydroxyphenylacetic acid (DOPAC), Homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) and cyclic nucleotide (cAMP and cGMP) estimation. Histological estimation of the brain slides were carried out using Hematoxylin and eosin staining.

Results: STZ infusion significantly impaired memory as observed in MWM and ORT, increased oxidative stress (LPO, nitrite, AChE) and decreased enzyme (GSH), increased pro-inflammatory levels (TNF-α, IL-6 and IL-1β), altered NTs level (DA, NE, 5-HT and their metabolites) and decreased cyclic nucleotide levels. Lupeol showed potent PDE inhibitory activity in *in-vitro* PDE inhibitory assay and post treatment of lupeol (25, 50 and 100 mg/kg) significantly restore STZ induced behavioral, biochemical, NTs abnormalities and cyclic nucleotide levels in rat brain. Histological examination of lupeol treated brain show improvement in neuronal flora as compare to STZ treated rats.

Conclusion: The findings of the present study suggests that lupeol inhibit PDEs, reduced neuroinflammation via acting through multiple mechanisms and would be a used as a target molecule to curb cognitive decline associated with neurodegenerative disorders such as AD.

MTU12-11

Human TP53 ARG72PRO SNP dictates neuronal susceptibility to Aß-neurotoxicity upon CDK5-induced P53 stabilization in mitochondria

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Alzheimer's disease (AD) is a complex multifactorial disease in which neural death occurs predominantly by apoptosis. The p53 tumour suppressor protein functions as a key regulator of cell apoptosis and has been described to accumulate in affected brain areas from AD patients. However, the role of p53 in AD is controversial. This protein naturally occurs in humans in two functional variants with single nucleotide polymorphism (SNP) resulting in Arg or Pro at residue 72 that modulates the apoptotic activity of the p53 protein. Our objective was to evaluate the impact of the *Tp53 Arg72Pro* SNP on amyloid-ß (Aß)-induced neurotoxicity. Cortical primary neurons from humanized *Tp53 Arg72Pro* knock-in mice were treated with 10 mM Aß25-35 oligomers for 24 h and protein expression levels, mitochondrial function and apoptosis were evaluated. In some experiments, neurons were lipotransfected with siRNA against cyclin dependent kinase-5 (Cdk5) or plasmids containing apoe2, e3 and e4 variants. We found that Aß triggered Cdk5-induced p53 phosphorylation and stabilization in both Arg^{72}-p53 and Pro^{72}-p53-expessing neurons. However, neurons carrying the polymorphic variant Arg^{72}-p53 were more susceptible to Aß-induced mitochondrial dysfunction and apoptosis than neurons with the Pro^{72}-p53 variant. Moreover, Arg^{72}-p53 promoted p53 translocation to mitochondria after Aß treatment, whereas this effect was not found in Pro^{72}-p53 neurons. Then, the Arg^{72}-p53 variant promotes p53 accumulation in the mitochondria and increases neuronal vulnerability to Aß-induced neurotoxicity. Finally, the expression of apoe4, the well-known major risk factor for AD, in both neurons abrogated this effect, and Pro^{72}-p53 neurons became as vulnerable as Arg^{72}-p53 to Aß-induced neurotoxicity. In conclusion, the *Tp53 Arg72Pro* polymorphism modulates neuronal susceptibility to Aß toxicity and determines damage extent. These results make this SNP a possible biomarker of genetic risk and progression for AD. This study was funded by ISCIII grants –PI15/00473, RD16/0019/0018-, European Union -686009- and FEDER.

MTU12-12

Complement C3 and C3aR receptor promote tau pathology and Alzheimer`s disease

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Background: Besides the pathological hallmarks of β-amyloid (Aβ) plaques and neurofibrillary tangles (NFTs), increasing evidence suggests that neuroinflammation plays a significant role in AD pathogenesis. The complement pathway is a key regulator of innate immunity. Recent findings from our laboratory showed that the central complement component C3 is upregulated in astrocytes of AD patients and mouse models. Moreover, elevated astroglial C3 can modulate Aβ dynamics and pathology and reduce dendritic morphology and synaptic function through microglial and neuronal C3a receptor (C3aR), respectively. Giving the importance of the C3-C3aR axis in the CNS, we are interested in understanding whether the complement pathway also impacts the NFT pathology.

Methods and Results: First, we analyzed the expression patterns of complement pathway genes in AD, MCI and non-cognitive impaired brain samples obtained from the ROS-MAP cohorts and revealed a strong upregulation of complement pathway genes that is highly correlated with Braak staging and cognitive decline. Prominent complement upregulation was also observed in a PS19 mouse model of tauopathy in response to NFT pathology. Next, to study the functional effects of the C3-C3aR pathway on reactive gliosis and NFT pathology we manipulated C3aR expression in PS19 animals. AAV-driven neuronal overexpression of C3aR exacerbated tau/NFT pathology in PS19 mice. Conversely, knocking out C3aR in PS19 mice resulted in significant reduction of inflammation, phospho-tau levels and associated pathology and improved behavioral deficits of PS19 animals. Finally, we utilized RNA sequencing and reverse-phase proteomics (RPPA) to identify C3aR downstream signaling pathways that promote such effects and identified the Jak-Stat3 pathway as a potent regulator of reactive gliosis and tau pathology in PS19 mice downstream of C3aR.

Conclusions: Our data shows that C3-C3aR activation promotes reactive gliosis and tau pathology in PS19 mice through the activation of the Jak-Stat3 pathway and indicates that blocking C3aR can be therapeutically beneficial for AD treatment.

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MTU12-13

The distribution and function of neuronal PSER727-stat3 is modify by mediators released by astrocytes in response to aβos

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Amyloid-beta oligomers (AβOs) have been found in Alzheimer's disease (AD) brains and there is vast evidence that supports a role of AβOS, which would trigger synapse failure and memory impairment. Astrocytes respond to AβOs through a process called reactive astrogliosis, which generates reactive oxygen/nitrogen species (ROS/RNS) and inflammatory cytokines that affect surrounding neurons. Stat3 is a crucial transcription factor involved in maintenance and function of nervous system and its deregulation has been implicated in AD. Growth factors induce serine-727 phosphorylation and this modification is associated with modulation of transcriptional activity of Stat3. The main goal in this work is determine if hippocampal neuronal Stat3 is affected by reactive astrocytes.

Methods: Primary hippocampal neuron and astrocytes cultures were used. Changes in pSerStat3 distribution were detected by immunocytochemistry. The oxidative tone and ROS production were evaluated by redox cytochemistry and Hyper strategy. Protein and mRNA levels were determined by Western blotting and qPCR, respectively.

Results: We found that AβOs induced no changes in protein, mRNA Stat3 levels and pSerStat3 cellular distribution, in neurons. However, AβOs treatment in mixed neuron-astrocytes cultures induced notorious neuronal pSerStat3 redistribution. Reactive astrocytes increased the expression of pro-inflammatory cytokines, and astrocyte-conditioned media treated with AβOs (ACM-AβOs) increased the neuronal oxidative tone. ACM-AβOs induced a decrease of Stat3 target genes (survival and antioxidant response) and an increase of pro-apoptotic Bax/Bcl2 ratio.

Conclusion: We propose that in hippocampal neurons, pSerStat3 is a sensor for stressor astrocyte-produced induced by AβOs activation.

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MTU12-14

Role of DRP1 in ad pathogenesis

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Alzheimer's disease (AD) is an age-related disorder characterized by deposition of neurotoxic form of beta-amyloid (Aβ) and degeneration of neurons. In the brain, accumulation of Aβ in the mitochondrial compartment has a responsible role in impairing mitochondrial physiological functions. Impaired regulation of mitochondrial dynamics, which shifts the balance towards fission, is associated with neuronal death in age-related neurodegenerative diseases, such as AD. We investigated the effect of inhibition of mitochondrial fission protein Drp1 (dynamin-related protein 1) by Mdivi-1 in AD mice and neuronal cells. Mdivi-1 reduced reactive oxygen species, oxidative stress level, BACE1 level, and amyloid plaques. The treatment of Mdivi-1 also improved memory and cognitive functions in AD mouse models. Mdivi-1 treatment delayed Aβ-mediated mitochondrial fragmentation and oxidative stress in neurons. These results indicate that inhibition of mitochondrial fission can be a therapeutic approach for AD.

MTU12-15

Critical roles of cathepsin b in Alzheimer's disease-like phenotypes following chronic systemic exposure to porphyromonas gingival

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A number of clinical and experimental studies have revealed a strong association between periodontitis and accelerated cognitive decline in Alzheimer's disease (AD), however, the precise mechanism of their association remains unclear. In this study, we found that chronic systemic exposure to lipopolysaccharide derived from Pg (PgLPS; 1 mg/kg, daily, i.p.) for five consecutive weeks induced learning and memory deficits with the intracellular accumulation of Aβ in neurons in the middle-aged (12 months old) wild-type (WT) mice, but not in young (2 months old) WT or middle-aged CatB-deficient ($CatB^{-/-}$) mice. PgLPS significantly increased the expression of cathepsin B (CatB), a typical cysteine lysosomal protease, in both microglia and neurons in middle-aged WT mice, while increased expression of mature IL-1β and TLR2 was restricted to microglia in the hippocampus of middle-aged WT mice, but not in that of the middle-aged $CatB^{-/-}$ ones. In *in vitro* studies, PgLPS (1 μg/ml) stimulation upregulated the mean mRNA expression of IL-1β, TLR2 and downregulated the protein levels of IκBα in the cultured microglia. These PgLPS-induced responses were significantly inhibited by pharmacological or genetic inhibition of CatB.

Furthermore, the mean mRNA expression of APP and CatB were significantly increased in the primary cultured hippocampal neurons after treatment with conditioned medium from PgLPS-treated WT, but not from $CatB^{-/-}$, primary cultured microglia (MCM). Taken together, these findings indicate that chronic systemic exposure to PgLPS induces AD-like phenotypes, including microglia-mediated neuroinflammation, intracellular Aβ accumulation in neurons and impairment of the learning and memory functions in the middle-aged mice in a CatB-dependent manner.

We conclude that CatB plays a critical role in the initiation and exacerbation of neuroinflammation following chronic systemic exposure to *Pg*, leading to induce AD-like phenotypes. Therefore, CatB can be a potential therapeutic target for AD.

MTU12-16

HMGB1 promotes autophagy and increase amyloid-β clearance in Alzheimer disease models

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The accumulation of amyloid-β (Aβ) neuritic plaques and intracellular tau protein tangles are key pathological characteristics

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of Alzheimer's disease (AD). There is evidence that the autophagosome-lysosomal degradation is a central role in AD, and disturbing the processing of autophagy lead to Aβ accumulation and provoke AD pathology. Previous studies showed that high-mobility group box 1 (HMGB1) promotes mitochondrial dysfunction–triggered striatal neurodegeneration via autophagy activation. In this study, we focused on whether the HMBG1 could regulate autolysosome formation and clearance of Aβ in AD models. We administrated HMGB1 inhibitor Glycyrrhizin in hippocampus with Aβ-treated mice by stereotaxic injection, then analyzed changes in expression of autophagic protein and phospho-c-Jun amino-terminal kinases (p-JNK). p-JNK and autophagic marker LC3-II were significantly reduced by the HMGB1 inhibitor glycyrrhizin. Glycyrrhizin also significantly inhibited survival of hippocampal neurons with Aβ-treatment. Moreover, neuronal death was replicated by exposing hippocampal neurons in culture to Aβ. Furthermore, to elucidate the role and mechanism of HMGB1 in clearance of Aβ, we investigated the impact of HMGB1 on autophagy activation and autolysosome formation *in vitro*. shRNA-HMGB1 inhibited cellular viability and reduced LC3-II expression compared with cells treated with Aβ only. Immunofluorescence revealed that HMGB1-shRNA in Aβ-treated neuronal cells increased the number of LC3-II-positive autophagosomes that were colocalized with the lysosomal marker. p-JNK expression was significantly reduced by shRNA knockdown of HMGB1, an effect that was reversed by exogenously increased expression of HMGB1. Furthermore, HMGB1-shRNA markedly reduced Aβ immunoreactivity colocalized within lysosomes and increased intracellular Aβ levels compared with Aβ-treated cells. These results suggested that HMBG1 regulated autolysosome formation and clearance of Aβ in AD models, and exerted a neuroprotective effect through modulation of Aβ clearance in Aβ-treated neuronal cells. HMGB1 would be a future strategy for AD treatment via autophagy pathway for clearance of Aβ in AD.

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MTU13 Therapeutic Approaches of Parkinson's Disease

MTU13-02

PNR2B mediates the role of FYN kinase in levodopa induced dyskinesia in a mouse model of Parkinson's disease

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Levodopa (L-DOPA) induced dyskinesia (LID) is one of the undesired side effects of Parkinson's disease (PD) treatment. To reduce the development of LID, without affecting the positive restorative effect of dopamine stimulation, is one of the greatest challenge in this area. We have previously explored the pathway Pleiotrophin/RPTP ζ/b/Fyn at the postsynaptic density complex and found that Fyn-KO mice developed less LID than WT littermates. Fyn kinase modulates the N-methyl D-aspartate (NMDA) receptor through phosphorylation of the NR2B subunit and this could explain the role of Fyn in LID. The main goal of this work is to demonstrate the direct relation between Fyn and NMDA receptor in a paradigm of dyskinesia. We lesioned Fyn-KO and WT mice with 6-hydroxydopamine (6-OHDA) and treated them daily with L-DOPA to model LID. Postmortem dopaminergic denervation was confirmed by immunodetection of tyrosine hydroxylase in the substantia nigra pars compacta. Several molecular markers, in particular the amount and phosphorylation status of NR2B subunit of NMDA, were determined in the striatum by Western blot. As expected, in WT mice we found upregulated the transcription factor ΔFosB and ERK phosphorylation, both previously reported markers of LID, while Fyn-KO mice showed a significant reduction of LID accompanied by a downregulation of ΔFosB and NR2B phosphorylation (pNR2B). In conclusion, pNR2B is downregulated in dyskinetic Fyn-KO mice, what can explain a reduced NMDA signaling and therefore the observed reduced dyskinesia. In this sense, Fyn would be an attractive target to modify the NMDA signaling and a promising treatment to modulate LID without affecting the therapeutic efficacy of L-DOPA in PD.

MTU13-03

Brain preconditioning reprograms the inflammatory response to neuronal damage in rat model of Parkinson's disease

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Background: Neuroinflammation is considered as both cause and consequence of neuronal injuries and has a key role in the pathogenesis of neuronal disorders such as Parkinson's disease (PD). Based on this, modulation of inflammation in the brain can be offered as one of the therapeutic approach to control neuronal damage in PD. Recent studies have indicated that Lipopolysaccharide (LPS) preconditioning could be used as a potential alternative strategy to attenuate, postpone or even cure the deficits of neuronal disorders. The purpose of this study was to study the effect of the brain preconditioning with single low dose of LPS on inflammatory profile in 6-OHDA model of PD.

Methods: To clarify the neuroprotective effect of brain LPS preconditioning on inflammatory profile in PD rats, motor coordination and balance were evaluated using rotarod in all animals. In addition, the effect of the LPS preconditioning on neuronal damage were observed in the substantia nigra by using nissl staining. Finally, gene and protein expressions associated with different cascades induced by LPS preconditioning including TLR4 and inflammatory signaling pathways were analyzed using RT-PCR and Western Blot.

Results: Based on the behavioral assessments, preconditioned animals performed significantly better on the behavioral performance in 6-OHDA rat model of PD. Furthermore, neuroprotective effect of brain LPS preconditioning were consistent with the histological observation. In addition, our result showed that the brain LPS preconditioning reduced inflammatory response in the brain of PD rat by enhancing NFκB inhibitors (e.g. SHIP1 and TOLLIP) as well as anti-inflammatory cytokines. These findings were confirmed by western blot analysis.

Conclusion: Generally, reduction in inflammatory response through the brain LPS preconditioning may contribute to the induction of tolerance to neuronal damage in PD. This neuroprotection parallel the reprogramming strategy that leads to the synthesis of new markers to change molecular response against brain lesions. Altogether, our findings demonstrate that LPS preconditioning has a therapeutic effect on the modulation of neuroinflammation and this could suggests a promising therapeutic strategy for various neuronal disorders such as PD.

MTU13-04

Addressing the mechanism of priming of dopamine receptors and its effect on drug-induced dyskinesia

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The gold standard treatment for Parkinson's disease is still the use of L-DOPA, which after prolonged use induces severe motor complications known as L-DOPA-induced dyskinesia (LID). In contrast, D2 agonists are frequently used in clinical practice because its low propensity to induce dyskinesia. *Priming* is defined as the behavioral and molecular sensitization occurring after the first exposure to L-DOPA or a full DA agonist. Priming is not necessarily associated with dyskinesia but once it has occurred, lower doses of L-DOPA or dopamine agonists are enough to induce dyskinesia. Priming has been pharmacologically studied but the underlying molecular mechanism is not well understood. The aim of

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this work is to understand the mechanisms of priming and evaluate the effect of D1/D2 receptor stimulation on subsequent DA agonist responses. C57BL/6 mice injected with 6-OHDA received a dyskinetogenic dose of L-DOPA or saline to induce priming, and then were treated with the D2R agonist Quinpirole. We compared Quinpirole-induced dyskinesia after increasing doses vs prolonged treatment with the maximum dose of Quinpirole, and observed higher dyskinesia scores in primed vs non-primed animals. Immunohistochemistry of striatal FosB and cFos, showed increased levels of both dyskinesia markers in primed mice compared to saline pre-treated. Furthermore, we analyzed selective cFos expression in D1R expressing cells, using bacterial artificial chromosome transgenic mice expressing tdTomato in striatal projection neurons that express D1 receptor. We reproduced L-DOPA priming and Quinpirole administration protocol, and observed significant cFos immunoreactivity in D1R expressing neurons in primed mice but was almost insignificant in non-primed mice. These results confirm once again the importance of behavioral sensitization and increased dyskinesia marker expression after D1/D2 receptor stimulation. Furthermore, they demonstrate that D2 receptor stimulation has a great impact onto D1R expressing cells only after priming and shows the important role of D2 receptor in dyskinesia development, even though current concepts emphasize the role of D1 receptor as the major player in dyskinesia induction.

MTU13-05

IGF-1 gene therapy in early parkinson′s disease

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Backgound: Insulin-like growth factor-1 (IGF-1) is an endogenous peptide transported across the blood brain barrier that is protective in several brain injury models, including in an animal model of Parkinson's disease (PD).

Objectives: To determine in an experimental model of the neuropathology if IGF-1 gene therapy could: 1) improve the cognitive dysfunctions and 2) induce changes in the neuronal activity of the affected brain areas.

Methods: Male Wistar rats were bilaterally injected in CPu with either the neurotoxic 6-hydroxydopamine (6OHDA rats), or vehicle (SHAM rats) as controls. Then they were divided into 6 experimental groups according to the gene therapy with adenovirus in hippocampus: G1) SHAM (vehicle-vehicle), G2)6OHDA (neurotoxic-vehicle), G3) SHAM-RAd-DS-Red, G4) SHAM-RAd-IGF-1, G5) 6OHDA-RAd-DS-Red, G6) 6OHDA-RAd-IGF-1. At 3 weeks post lesion with 6OHDA and injection with adenovirus the animals were tested for spatial memory with Y-maze test and for locomotor activity. At the end of the study the rats were perfused, the brains fixed and immunohistochemistry performed for TH and IGF-1. All data were compared by 2-way ANOVA ($p < 0.05$ considered as statistically significant).

Results: 6OHDA causes cognitive deficits in G2 compare to G1 ($p > 0.05$) indicated by a decreased in spontaneous alternation percentage. This effect could not be attributed to decreased motor activity, because the number of arm entries was not significantly changed and neither the number of cm performed after amphetamine administration. This effect was partially reverted with IGF-1 overexpression in G6 respected to G5 ($p > 0.05$). There were no significant changes in G2 respect G5 and in G1 respected to G3 and G4. Preliminary results showed that IGF-1 gene therapy induce an increase in TH expression in the nigrostriatal pathway.

Conclusions: our results suggest that IGF-1 could be an important neuroprotective molecule against neurodegeneration. Its effect on neuronal activity could explain in part the improvement in the cognitive symptoms that we observed in this animal model of PD.

MTU13-06

TRKB agonist provide neuroprotection via attenuating neuroinflammatory cascade and regulator of g-protein signaling 4 (RGS4)

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Parkinson disease is (PD) a debilitating motor disorder affected million populations worldwide. The objective of our study was to investigate the effect of TrkB agonist; 7,8-dihydroxyflavone (7,8-DHF) on 6-hydroxydopamine (6-OHDA) induced inflammation cascade in Neuro-2a (N2a) cells and MPTP-induced neuroinflammatory cascade connecting the link with RGS4 in striatum region of male swiss albino mice (weight 25–30 g). The Neuro-2a (N2a) cells treated with 6-OHDA of various concentrations (1.5–100 μM) for 24 hrs and the cytotoxicity (6-OHDA, 25 μM) was prevented by 7,8-dihydroxyflavone (3, 6 and 12 μM) evaluated by MTT and LDH assay. The neuroinflammatory markers such as NF-κB and Cox-2 protein expression were markedly upregulated by 6-OHDA treatment which further reduced with 7,8-DHF cotreatment. Further, animal studies, MPTP (30 mg/kg, i.p. for 5 days i.e. 10th day to 14th day) were conducted for 28 days study period. The 7,8-DHF (10 mg/kg) treatment for 28 days mitigated symptoms in MPTP-treated animals induced motor deficits as evaluated on rotarod test, open field test, and grip strength test. The biochemical oxi-donitrosative stress markers such as lipid peroxidation, nitric oxide level and reduced glutathione (GSH) level, proinflammatory cytokines (TNF-α, IL-1β) level in striatum and substantia nigra were found to be high in MPTP-treated animals. Furthermore, the real-time PCR for the genes (iNOS, COX-2, NF-κB, Nrf2, PARP-1, RGS4) and western blot for the protein expression studies were found to be altered in the striatum (NF-κB, Nrf2, PARP-1, RGS4) and substantia nigra (NF-κB, Nrf2) of animals which got reversed by the 7,8-DHF treatment. Hence, 7,8-dihydroxyflavone significantly ameliorated the induced neuroinflammatory cascade in N2a cells and animals as well as downregulated the RGS4 activation in striatum region of mice model through its potential antioxidant activity. Thus, TrκB agonist may be the futuristic non-dopaminergic candidate to treat Parkinson's disease progression.

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MTU13-07

Pathological α-synuclein transmission initiated by binding lymphocyte-activation gene 3

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Parkinson's disease (PD) is the second most common neurodegenerative disorder causing serious movement disability and cognitive impairment in those afflicted. Pathologically, PD is characterized by the accumulation of α-synuclein (α-syn) in Lewy bodies and neurites. There is dopamine neuron degeneration in the substantia nigra pars compacta which causes many of the major symptoms of PD. Emerging evidence indicates that the pathogenesis of PD may be due to cell-to-cell transmission of misfolded preformed fibrils (PFF) of α-syn. The mechanism by which α-syn PFF spreads from neuron to neuron is not known.

Here, we show that LAG3 (lymphocyte-activation gene 3) binds α-syn PFF with high affinity (dissociation constant = 77 nanomolar), whereas the α-syn monomer exhibited minimal binding. Tau-biotin PFF, β-amyloid-biotin oligomer, and β-amyloid-biotin PFF do not bind to LAG3, indicating that LAG3 is specific for α-syn PFF. α-Syn-biotin PFF binding to LAG3 initiated α-syn PFF endocytosis, transmission, and toxicity. Neuron-to-neuron transmission of pathologic α-synuclein and the accompanying pathology and neurotoxicity is substantially attenuated by deletion of LAG3 or by antibodies to LAG3. Lack of LAG3 substantially delayed α-syn PFF–induced loss of dopamine neurons, as well as biochemical and behavioral deficits *in vivo*. The identification of LAG3 as a receptor that binds α-syn PFF provides a target for developing therapeutics designed to slow the progression of PD and related α-synucleinopathies.

MTU13-08

Antidyskinetic effect of acute guanosine administration in reserpinized mice

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Dyskinesia is characterized as involuntary movements that affect several body parts. Several neurological disorders can exhibit this symptom, such as Parkinson's disease. Dyskinesia can be induced by the alkaloid reserpine that acts as an inhibitor of vesicular monoamine transporter (VMAT-2). The consequent monoamine neurotransmitters depletion induces hypolocomotion, muscle rigidity and involuntary movements. Guanosine (GUO), an endogenous nucleoside, has been evidenced as a neuroprotective agent, although the exact mechanism of GUO action is not fully characterized. This study evaluated the therapeutic potential of GUO as an antidyskinetic agent in mice treated with reserpine (1 mg/kg, *subcutaneously,* every other day). GUO (7.5 mg/kg p.o.) was administered 24 h after the last reserpine injection and 20 min before behavioral test. GUO prevented the increase of orofacial dyskinesia induced by reserpine. Additionally, the antidyskinetic effect of GUO was abolished by prior administration of the A_1 adenosine receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 0.75 mg/kg). Reserpinized mice also showed a cataleptic state when evaluated in the bar test. Likewise, this behavior was prevented by GUO. Interestingly, DPCPX also abolished the anti-cataleptic effect of GUO, besides presenting an anti-cataleptic effect *per se*. Reserpine increased cells damage and reactive oxygen species (ROS) levels in the striatum of treated mice. GUO was effective in reducing the increase of ROS levels, but it did not alter cells damage induced by reserpine. This study shows for the first time an antidyskinetic effect of GUO and its effect of modulating motor and neurochemical impairments induced by reserpine.

MTU13-09

Sphingosine 1-phosphate receptors modulators decrease neuroinflammation and prevent Parkinson's disease symptoms in mptp mice

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Sphingosine-1-phosphate receptors (S1PR) may be an attractive molecular target to the treatment of neurodegenerative diseases. For instance, Fingolimod (FTY720), an immunomodulator that acts on S1PR, has been documented to display neuroprotective effects in multiple sclerosis and animal model of Alzheimer's disease. We postulated that the immunomodulatory effects associated to FTY720 treatments might also be beneficial to Parkinson's disease pathogenesis. Therefore, the present study investigates the effects of an oral FTY720 treatment (1 mg/kg/day, 14 days) on the behavioral and molecular alterations induced by the administration of MPTP (30 mg/kg/day, i.p., 5 days) in mice. We first established that FTY720 has the capacity to prevent the motor deficits observed in the Pole test after MPTP treatments. In the striatum of MPTP mice, Western blot analysis revealed diminutions of ~50% in the levels of tyrosine hydroxylase and dopamine transporter proteins following MPTP injections, which were both prevented by FTY720 treatments. In parallel, while striatal levels of phosphorylated extracellular signal-regulated kinases and S1PR subtype 1 were unaffected, tumor necrosis factor-alpha and glial fibrillary acidic protein levels were robustly increased in MPTP-treated mice, an outcome that was totally prevented by FTY720 treatments. Notably, FTY720 treatments was also able to prevent the reduction of brain-derived neurotrophic factor levels observed in the striatum of mice treated with MPTP. Altogether, our findings propose that oral FTY720 treatments halt the detrimental effects of MPTP on striatal dopamine terminals and motor behaviors. The mechanism of action may involve inhibition of inflammatory pathways and the modulation of brain-derived neurotrophic factor production. This study is providing novel evidence for the clinical utility of targeting S1PR in Parkinson's disease therapy.

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MTU13-10

A novel approach to detect the presence of levodopa (l-DOPA) in the polypeptide chains of proteins

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Background: Deposition of protein aggregates in neurons is a hallmark of neurodegenerative diseases. Genetic mutations in proteins can result in the synthesis of non-native proteins that accumulate in cystosolic aggregates. Certain non-protein amino acids (NPAAs) can be mischarged onto tRNA and mistakenly inserted into the polypeptide chain of proteins resulting in the sporadic generation of non-native misfolded proteins [1,2]. We utilise a novel approach to investigate the ability of the therapeutic agent levodopa (L-DOPA) to replace L-tyrosine in proteins by applying mass spectrometry (MS) whereby we can sequence peptides to reveal the presence of DOPA.

Methods: We examine the ability of high resolution accurate mass mass-spectrometry (HRAM-MS) to detect DOPA in neuronal cell proteins avoiding the need for traditional protein hydrolysis and to allow individual proteins to be identified.

Results: We successfully tested a novel approach for the detection of DOPA in peptides generated from digestion of DOPA-containing proteins and validated this method using synthetic peptides. We showed that L-DOPA can be incorporated into cell proteins. The presence of L-DOPA in proteins resulted in a decrease in solubility. Specific structural and long-lived proteins were most affected. We are now applying this approach to biological samples.

1. Rodgers, K.J., et al., *Toxic Nonprotein Amino Acids*, in Plant Toxins, 2015, Springer Netherlands: Dordrecht. p. 1-20. 2. Cox, P.A., et al.,. Proceedings of the Royal Society B: Biological Sciences, 2016. 283.

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