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Poster sessions WTH Wednesday/Thursday

WTH01 Myelination and Demyelination

WTH01-01

Autoantibody mediated CNS myelin morphology in the acute phase of experimental autoimmune encephalomyelitis

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Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating disease of the CNS. Demyelination and axonal damage are responsible for neurological deficits in MS. However, the mechanisms of demyelination and axonal damage have not been fully understood. To clarify the mechanism of demyelination in experimental autoimmune encephalomyelitis (EAE), we examined myelin morphology during the course of MOG35-55-induced EAE in the C57BL/6 mice.

Osmium-maceration scanning electron microscopic (SEM) analysis displayed ultrastructural abnormalities of myelin structure in the white matter of the EAE spinal cord. In addition, abnormal morphology of myelin was observed at early stages of EAE. While infiltrating immune cells into the CNS were not observed in the spinal cord, anti-MOG autoantibody was observed in the CNS at this point. These observations suggest that anti-MOG antibody plays an important role in the pathogenesis at the acute stages of EAE.

WTH01-02

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

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Recombinant human IgM22 (rHIgM22) binds to myelin and oligodendrocytes (OLs), and promotes remyelination in mouse models of multiple sclerosis. rHIgM22 preferentially reacts with sulfatide-positive ($O4^+$) OLs, and binding of rHIgM22 is abolished in CNS tissue slices from Cst (−/−) mice, suggesting that its binding requires the presence of a product of cerebroside sulfotransferase, possibly sulfatide, highly expressed in OLs and myelin. However the identity of the antigen recognized by this antibody remains to be elucidated. We tested the binding of rHIgM22 to purified lipids and lipid extracts from mouse brain, CNS myelin, mixed glial cells, and $O4^+$ OLs using TLC immunostaining and SPR with lipid monolayers. Our preliminary results show that rHIgM22 binds to sulfatide *in vitro*, while it does not bind to other myelin sphingolipids suggesting that sulfatide at the OLs surface might be important for the binding of rHIgM22 to these cells and to myelin. However, rHIgM22 does not bind structures expressing sulfatide outside the nervous system, so additional factors are likely relevant for the immunoreactivity of rHIgM22 in CNS. Indeed, in lipid extracts from different sources we found another lipid antigen selectively recognized by rHIgM22, whose identity is under investigation. This lipid is also present in the extracts from mixed glial cultures, which do not contain mature $O4^+$ OLs, suggesting that other glial cells in addition to OLs might be important in the response to rHIgM22.

WTH01-03

Essential role of endogenous fatty acid synthesis in CNS remyelination

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Remyelination requires adult oligodendrocyte progenitor cells (OPCs) to proliferate and subsequently differentiate into myelinating cells, hence calling for tremendous increase in lipid availability. Fatty acids are the primary constituents of cellular membrane lipids, and thus myelin itself. Furthermore, fatty acids are critical to a variety of fundamental cellular functions, including membrane targeting of proteins, energy storage, cell signalling and transcriptional regulation. While most cells are thought to mainly rely upon uptake to maintain their fatty acids pool, highly metabolically active and proliferative, i.e. cancer and precursor-/stem-cells are strongly functionally dependent on *de novo* synthesis, mediated by fatty acid synthase (FASN). The multifunctional enzyme FASN is strictly required for the synthesis of saturated fatty acids, mostly palmitate, from substrates acetyl-CoA and malonyl-CoA in the presence of NADPH as a cofactor. The relevance of this fundamental metabolic pathway during CNS remyelination has so far not been fully clarified. Thus, we addressed the functional role of *de novo* fatty acid synthesis in adult OPC-mediated remyelination. Using inducible Cre/lox system, we examined the specific effect of conditional depletion of FASN in adult OPCs on remyelination, following lysolecithin-induced demyelination. We show that FASN-mediated *de novo* fatty acid synthesis is critical to achieve efficient CNS remyelination and the maintenance of the remyelinating oligodendrocyte population. Our results add valuable information to the understanding of the regulation of the remyelination process in demyelinating conditions (including multiple sclerosis), a promising currently pursued drug target.

WTH01-04

VDR gene polymorphism BSMI in association with multiple sclerosis risk and progression in Slovak population

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Background and objectives: Vitamin D, that acts through the vitamin D receptor (VDR), has been found to be an important factor involved in the etiopathogenesis of Multiple Sclerosis (MS). Since single nucleotide polymorphism (SNP) BsmI in *VDR* gene can affect

the structure and function of VDR, we tried to identify whether this SNP is associated with the risk and progression of MS in Slovaks.
Methods: The group of examined individuals consisted of 270 MS patients and 303 healthy controls. The disease progression was evaluated by MSSS score. Genotyping was performed by polymerase chain reaction (PCR) and restriction analysis.
Results: We found that genotype BB (AA) of BsmI VDR gene polymorphism is decreasing the MS risk (recessive model, OR = 0.59, 95% CI = 0.39–0.90, $p_{\log} = 0.014$). We did not identify any association of BsmI VDR gene polymorphism with the disease progression.
Conclusion: Our findings suggest an association of VDR SNP BsmI with MS susceptibility in the cohort of Central European Slovak population. We propose the BsmI gene polymorphism to be one of the valuable genetic markers useful in the prediction of MS risk.

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WTH01-05

TSC function in CNS myelination independent of oligodendrocyte differentiation

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Our previous studies revealed that the mechanistic target of rapamycin (mTOR) promotes oligodendrocyte differentiation and developmental myelination in the CNS both *in vitro* and *in vivo* (Tyler et al., 2009; Wahl et al., 2014). Surprisingly though, recent studies showed that mice in which mTOR pathway is constitutively active in OPCs, due to a deletion of its negative upstream regulator tuberosclerosis complex (TSC), also display a hypomyelination phenotype instead of hypermyelination as originally predicted (Lebrun-Julien et al., 2014; Carson et al., 2015; Jiang et al., 2016). However, in all these studies, the deletion of TSC was introduced very early in development, during specification or differentiation of the oligodendrocyte cell lineage, and myelination independent of differentiation was not solely assessed.

The goal of this study is to assess how myelin production is affected when mTOR signaling is upregulated through deletion of TSC exclusively in the mature oligodendrocyte population, so that the differentiation process remains unperturbed. We hypothesized that loss of TSC at this later stage in oligodendrocyte maturation would lead to hypermyelination. To test this hypothesis we generated an inducible conditional knock-out mouse model for TSC1 using a tamoxifen-inducible cre recombinase expressed under the proteolipid protein (PLP) promoter. We induced deletion of TSC1 at postnatal day (PND) 7–10 and analyzed myelination in spinal cord at PND 17, 27 and 60. Our preliminary data suggest that loss of TSC in maturing oligodendrocytes has no effect on oligodendrocyte survival or differentiation. In addition, the expression levels of different myelin proteins were unchanged in the mice lacking TSC1, in sharp contrast to all previous studies showing a decrease in myelin proteins with early loss of TSC. In ongoing studies we are analyzing myelin thickness by electron microscopy to determine the impact of TSC loss in the process of myelination, independent of its effect on oligodendrocyte differentiation.

WTH01-06

A dual role for the PI3K-AKT-MTORC1 axis in peripheral nerve myelination

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Consistent with the intense anabolic challenge posed by myelination, the PI3K-Akt-mTORC1 axis has recently emerged as a fundamental player in the myelination of the peripheral and central nervous system. We and others have recently shown that genetic disruption of mTORC1 in SCs impaired myelination. However, it is not clear whether, conversely, hyperactivation of mTORC1 could augment myelin growth. Surprisingly, we show here that hyperactivation of mTORC1 following deletion of TSC1 and/or PTEN during early nerve development delayed SC myelination, rather than promoting it. Consistently, we found that mTORC1 negatively controls the expression of Krox20, a master transcription factor for the onset of SC myelination, via its downstream target S6K and that mTORC1 activity is physiologically high before the onset of myelination and declines as soon as SCs start myelinating. In sharp contrast to the outcome during early development, activation of the same pathway in SCs already committed to myelination resulted in radial hypermyelination. We, therefore, propose a unifying model according to which mTORC1 does not exert a univocal role in myelination, but at least two distinct functions: inhibition of the onset of myelination in not-yet myelinating SCs and promotion of myelin growth in myelinating SCs. The physiological decline in mTORC1 activity represents then a turning point allowing Krox20 expression to increase and SC myelination to begin, while supporting myelin growth through residual mTORC1 activity.

WTH01-07

TRKB agonists promote myelin repair in the brain

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Multiple Sclerosis is a neurodegenerative disease common in young adults, caused by an autoimmune attack against myelin. While current immune-directed treatments are successful in early disease, they do not directly stimulate myelin repair, and cannot fully prevent irreversible nerve damage, and subsequent disability. We have shown that brain-derived neurotrophic factor (BDNF) enhances myelination through activation of TrkB receptors on oligodendrocytes. To test if activation of TrkB receptors can promote myelin repair, we infused BDNF, and known TrkB agonists TDP-6, LM22A-4 and 7,8-dihydroxyflavone (DHF), into the brain of adult mice previously treated with 0.2% cuprizone to induce demyelination. Following 7 days of infusion, all TrkB agonists, but not BDNF, significantly enhanced remyelination above vehicle

controls, with increased MBP in the corpus callosum ($p < 0.001$). Each TrkB agonist had a distinct effect on oligodendrocyte populations, with TDP-6 and LM22A-4 treatment significantly increasing the density of post-mitotic oligodendrocytes ($p = 0.01$) and DHF infusion significantly increasing the density of oligodendrocyte progenitors ($p = 0.02$). Morphometry revealed TDP-6 and LM22A-4 increased the percentage of remyelinated axons ($p = 0.04$) and increased myelin sheath thickness ($p < 0.001$). BDNF treatment for 7 days did not alter oligodendrocyte density, or the percentage of remyelinated axons. The structural BDNF-mimetic TDP-6 had the greatest increase in myelin sheath thickness. To elucidate the divergent effects of the TrkB agonists, quantification of phosphorylated TrkB and Erk1/2 are being performed in conjunction with *in vitro* assays to examine signalling bias. To confirm that enhanced remyelination is an oligodendrocyte-driven effect, selected TrkB agonists have been infused into the brains of cuprizone-fed conditional knockout mice, where TrkB has been deleted from maturing oligodendrocytes. Importantly, the enhanced remyelination effect of TDP-6 and LM22A-4, in contrast to the poor response to BDNF, highlights that while native neurotrophins have poor therapeutic properties, selective modulation of neurotrophin signalling is a viable therapeutic strategy to promote myelin repair in the brain.

WTH01-08

Myelin-associated EPHRINB3 restricts schwann cell migration within central nervous system white matter

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Myelination, which allows rapid and saltatory nerve conduction, is provided by two different types of glial cells, oligodendrocytes in the central nervous system(CNS), and Schwann cells in the peripheral nervous system(PNS). These cells mainly differ in their remyelination capacity, Schwann cells being more efficient in the PNS than oligodendrocytes in the CNS. Since Schwann cells can occasionally invade the CNS under dys/demyelination conditions, these cells have been considered robust candidates for autologous cell therapy in some pathologies. However, Schwann cell survival and migration within the CNS is far from being optimal and the mechanisms involved in this restriction are poorly understood. The object of this work is to study the involvement of CNS myelin in this Schwann cell-CNS segregation. Particularly, we focused on the role of EphrinB3 as a myelin component, able to induce repulsion to other cell types. We demonstrate that Schwann cells present receptors for EphrinB3, and its presence on the Schwann cell surface impairs their adhesion on myelin and consequent migration capacities. We gained evidence that EphrinB3 response is mediated by EphA4 and EphB6 receptors. In addition, our *in vivo* studies showed that grafted Schwann cells are able to migrate towards focal demyelinated lesion in wild-type mice, but failed to mingle with CNS myelin. In contrast, these cells use blood vessels as scaffolds to pave their way towards the lesion, embedded in the extracellular matrix of endothelial cells. Finally, we show *in vitro* that EphrinB3 alters the adherence of Schwann cells to ECM components through integrin beta1. This suggest that Ephrin/Eph may act by a dual mode, repulsing Schwann cell from CNS myelin and enhancing their attraction to basal lamina, and therefore, directing their migration along CNS vasculature.

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WTH01-09

Role of RHIGM22, a remyelination-promoting antibody, in the regulation of acid sphingomyelinase activity in mixed glial cells

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Recombinant human IgM22 (rHIgM22) binds to myelin and oligodendrocytes (OLs), and promotes remyelination in mouse models of multiple sclerosis. The identity of its antigen is still under investigation but we have shown that different sphingolipids are potentially involved in its ability to bind at the cell surface. Literature strongly suggests that rHIgM22 biological activity is mediated by the reorganization of Lyn, integrin $\alpha v\beta 3$ and PDGFαR at the cell surface to form a signaling complex triggering Lyn activation which, in turn, promotes oligodendrocyte precursor cells (OPCs) survival and proliferation[1]. However, rHIgM22-mediated OPC proliferation is only detectable in mixed glial cultures (MGC), but not in purified OPCs[2]. Previous studies in OLs showed that the anti-apoptotic effect of Lyn activation might be due to reduced activity of acid sphingomyelinase (ASMase) and consequent reduced ceramide generation[3]. Ceramide generated by the action of ASMase represents an important pro-apoptotic signal, but also a signal for the re-arrangement of sphingolipid-rich signaling platforms[4]. We observed that, in MGC, Lyn is enriched in sphingolipid-enriched membrane fractions, which are also enriched in ASMase[5]. We assessed ASMase activity in MGC following a single dose treatment with rHIgM22, for either 24 or 48 h. Two different non-immunogenic human IgMs were used as a negative control. The data we obtained show a significant decrease of total ASMase activity in MGC treated with rHIgM22, with respect to control. rHIgM22-mediated increased Lyn expression and activation could result in a decrease in ASMase activity and in ceramide generation, thus inhibiting pro-apoptotic signaling and/or the organization of sphingolipid-dependent signaling platforms.

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WTH01-10

THE anti-large myelin protein zero (L-MPZ) antibody in serum modifies the peripheral nerve demyelination in Lewis rat

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Large myelin protein zero (L-MPZ) is a recently identified novel isoform of P0, in which extra 63 amino acids are added at the C-terminus. Antibodies against L-MPZ were often found in the sera from the patients with chronic demyelinating polyneuropathy (CIDP), although pathophysiological role of these antibodies are still uncertain. To determine the relevance of anti-L-MPZ antibodies to neuropathic conditions, we examined rodent demyelinating nerves in the presence and absence of anti-L-MPZ antibody.

Lewis rats were immunized with antigenic peptide of L-MPZ (244–282aa) emulsified in Freund's complete adjuvant (FCA). For control treatment, phosphate-buffered saline was used instead of L-MPZ peptide (FCA-immunized). Remarkable elevation of the L-MPZ-specific antibody titers and the slight reduction in grip power were observed, however, histological examination showed no apparent neuritis in the immunized rats, suggesting that anti-L-MPZ antibody did not induce pathological condition in the peripheral nerves. Next, we carried out the induction of demyelination with lysolecithin in the L-MPZ/FCA-immunized, FCA-immunized and non-immunized animals. Demyelinated areas were examined in the sciatic nerves of days 7 and 14 after lysolecithin injection. In non-immunized group, demyelination was observed to be peak on the day 7 and almost disappeared at the day 14 after lysolecithin injection. Demyelinated areas of L-MPZ/FCA-immunized group were considerably smaller than non-immunized group, but larger than those of FCA-immunized group on the days 7. The infiltrated $CD68^+/CD206^+$ M2 type macrophages of the L-MPZ/FCA-immunized group at the day 7 were about 50% but decreased to 10–30% at day 14, although those of FCA-immunized group at day 7 and day14 were about 20%.

Thus, present results indicate that anti-L-MPZ antibodies may influence macrophages and modulate pathological conditions during demyelination.

WTH01-11

Dynein/dynactin is necessary for anterograde transport of MBP MRNA in oligodendrocytes and for myelination in vivo

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Myelin is the lipid rich sheath that surrounds axons and promotes rapid action potential propagation in the vertebrate nervous system. In the central nervous system (CNS), myelin is produced by specialized glial cells called oligodendrocytes and disruption of myelin causes neurological disorders. Through a large scale forward genetic screen, I identified a mutant that exhibits axon and myelin defects. The phenotype results from a mutation in the gene actr10, encoding the protein Arp11, a component of the dynactin complex necessary for retrograde transport by dynein. Interestingly, *in situ* hybridization for myelin basic protein (mbp) mRNA showed that actr10 mutants have reduced mbp expression in the CNS as well as a punctate mbp phenotype, reminiscent of kinesin kif1b zebrafish mutants, leading us to hypothesize that retrograde transport was influencing anterograde mbp transport. Pulldowns of a reporter protein that binds to Mbp mRNA from rat oligodendrocytes demonstrate that Mbp mRNA granules co-immunoprecipitate with dynactin and dynein. Furthermore, treatment of rat oligodendrocyte cells with ciliobrevin, a dynein inhibitor, resulted in arrest of Mbp transport in anterograde and retrograde directions. In combination with *in vivo* data obtained from actr10 zebrafish mutants, our data highlight an unexpected role for the retrograde motor complex in anterograde Mbp mRNA trafficking.

WTH01-12

Persistent cytokine production induced in the cerebral meninges in a rat model of MS gives rise to chronic cortical pathology

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The progressive phase of multiple sclerosis (MS) is characterised by accumulating grey matter (GM) pathology. The presence of immune cell infiltrates in the meninges is associated with lymphoid tissue development, greater cortical demyelination, shorter disease duration and significant neuronal loss. Analysis of isolated meninges of MS cases has shown an increased gene expression for the pro-inflammatory cytokines: tumour necrosis factor (TNF) and

interferon-γ (IFNγ). We aimed to test the hypothesis that chronic production of pro-inflammatory cytokines in the meningeal compartment and diffusion into underlying GM can drive MS GM pathology. To do this we stereotactically injected HIV-1 based VSV-g pseudotyped lentiviral transfer vectors into the sagittal sulcus of DA rats to deliver continuous transgene expression (TNF + IFNγ) in the meninges for chronic periods through efficient transduction of meningeal cells. A neuropathology analysis was conducted at time points up to 2 months, together with RT-PCR to determine changes to TNFR1 signalling molecules. Injection of these vectors induced the formation of large immune cell aggregates in the meninges by 28 dpi, which remained at 2 months, containing CD4+ and CD8 + T-cells, CD79a+ B-cells and Iba1 + macrophages. These aggregates extended the length of the SS and across the surface of the cortex. Subpial demyelination underlying these aggregates was accompanied by widespread microglial activation and a decrease in neurofilament and NeuN staining indicating areas of potential neuronal loss. TNF/TNFR1 interactions can initiate cell death by activating pathways involved in necroptosis. RT-PCR on cortical RNA at 28 dpi showed an increase in expression of TNFR1 and downstream necroptotic genes, RIP3 and MLKL, compared to eGFP vector control animals. RIP3 + and MLKL+ immunopositive cells with the morphology of neurons were present in TNF vector injected animals. Our results suggest that TNF in the presence of IFNγ is a potent inducer of meningeal inflammation and can activate TNF signalling pathways in cortical cells leading to neuronal death and subpial demyelination and thus may contribute to clinical progression in MS.

WTH01-13

Alterations in oligodendrocyte progenitor cell populations with loss of MTOR

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Oligodendrocyte progenitor cells (OPCs) undergo several distinct stages of differentiation to form mature myelinating oligodendrocytes. The process of differentiation is highly regulated through multiple signaling pathways. Two major pathways that promote CNS myelination are PI3K/Akt/mTOR and Mek/Erk1/2. Disruption of either of these pathways in mice compromises developmental myelination, however, their functions appear only partially overlapping.

Deletion of mTOR in OPCs results in a delay in oligodendrocyte differentiation and initiation of myelination as well as long-term hypomyelination of the spinal cord. In contrast, these mice have normal myelination of the brain implying variations in cellular response to loss of mTOR. We have identified the stage of differentiation where spinal cord OPCs are delayed and accumulating in the absence of mTOR. Percentages of O^{4+} late-stage progenitor cells and PDGFRα+ early-stage progenitor cells were measured by flow cytometry. We have found decreased numbers of late-stage progenitors in the developing mTOR knockout spinal cord, with a corresponding increase in early-stage progenitors, suggesting an accumulation of many early progenitors that are unable to progress to the O^{4+} stage when mTOR is deleted. To further define alterations in OPC populations and differentiating oligodendrocytes with loss of mTOR, we have initiated experiments using Drop-seq, an innovative technology for single-cell RNA sequencing. In initial studies, we used magnetic bead columns to isolate O^{4+} OPCs from mTOR knockout and control spinal cords and simultaneously analyzed the mRNA transcripts of thousands of individually identifiable cells. Transcriptional variation across the single cells can be used to define distinct populations. Sequence data from our Drop-seq experiment is currently under analysis.

Our long term goal is to further understanding of heterogeneity of OPCs and how it contributes to differences in oligodendrocyte function. Future directions will also include studying changes in specific cellular functions and pathways with loss of mTOR.

WTH01-14

Exploring the factors causing remyelination arrest through studying cystatin F gene expression regulatory mechanism

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Demyelinating diseases are series of disorders that damage the myelin sheath in the central nervous system. At an early phase of demyelinating diseases, demyelination is accompanied by remyelination forming the shadow plaque. However at a late stage, remyelination become arrested. Cystatin F, a papain-like lysosomal cysteine proteinase inhibitor, and its main target, cathepsin C, have been demonstrated to be crucial factors in regulating remyelination arrest. A chronic demyelination mouse model, named heterozygous proteolipid protein (PLP) transgenic 4e ($PLP^{4e/-}$) mouse, was used to study cystatin F function. We found cystatin F was upregulated in the early phase (2–4 months of age) and then decreased in the chronic phase (4–8 months of age) in the $PLP^{4e/-}$ mouse. To explore the remyelination arrest mechanism in the chronic demyelinating disorders, we clarified cystatin F gene regulatory mechanisms in this study. We used a mouse line (CysF-STOP-tetO::Iba-tTA) in which the cystatin F gene expression is drived by the tetO promoter. We surprisingly found the cystatin F gene is forced to be expressed but its expression was later decreased in CysF-STOP-tetO::Iba-tTA mouse. Together with other results, we proved cystatin F expression was post transcriptionally regulated. Then, we found the factor Embryonic lethal, abnormal vision, drosophila like RNA binding protein 1 (ELAVL-1), which is an AU rich elements binding protein, stabilized cystatin F mRNA. Its expression was downregulated together with cystatin F decreased level in both $PLP^{4e/-}$ mice CysF-STOP-tetO::Iba-tTA mice. *In vitro* study showed decrease of ELAVL-1 downregulated cystatin F expression. All of these data revealed the important role of ELAVL-1 in regulating cystatin F expression. It may provide a new insight in the therapy of demyelinating disorders.

WTH01-15

Remyelination from demyelinating lesions induced by multiple sclerosis antibodies

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Multiple sclerosis (MS) is an inflammatory demyelinating disorder of the central nervous system (CNS). Following myelin loss, remyelination may occur. Current models of remyelination rely on toxin or detergent-induced injuries that are not relevant to pathological mechanisms in MS. To address this issue, we have developed new inflammatory models of demyelination and remyelination using *ex vivo* organotypic mouse cerebellar slice cultures and *in vivo* spinal cord micro-injection using recombinant antibodies (rAbs) cloned from cerebrospinal fluid (CSF) plasmablasts of MS patients. Using our novel models, we measured axonal demyelination and remyelination, oligodendrocyte loss and repopulation, axonal integrity, and astrocyte gliosis by immunohistochemistry during injury and recovery. In the *ex vivo* model, transient treatment with myelin-specific MS rAbs induced robust complement-dependent oligodendrocyte cytotoxicity and rapid demyelination. The morphology and survival of astrocytes, oligodendrocyte progenitors and neurons were unaffected, and axons remained intact. After the rAb treatment was removed, oligodendrocyte cell bodies and processes repopulated with clear evidence of new myelin protein deposition along axons. In the *in vivo* model, a clear lesion boundary delineated by the loss of oligodendrocytes and the presence of myelin debris was observed 3 days after focal injection (DPI) of MS rAb plus human complement. At 14 DPI there was continued loss of oligodendrocytes, and clearance of myelin debris within the lesion site. Axons and astrocytes were preserved. Oligodendrocyte progenitor infiltration was detected in the lesion, suggesting commencement of active remyelination. At 28 DPI remyelinated sheaths appeared outside of axons. Our results indicate that MS rAbs with complement produce a well-controlled demyelinating lesion that remyelinates *ex vivo* and *in vivo*. These new models will advance our understanding of MS inflammatory demyelination and remyelination and aid in the development of successful strategies to promote myelin repair and restore neuronal function in affected patients.

WTH01-16

Alterations of synaptic terminals in the cerebellum of chronic demyelinating mouse model

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Normal brain function depends on integrity of complex networks among neurons through synapses. Loss or disruption of myelin sheath impairs fast saltatory conduction and axonal integrity, and leads to neurological deficits in demyelinating diseases. Although it is well established that demyelination alters structures and functions of demyelinated segments of axons, influence of demyelination to axon terminals is still poorly understood. In this study, we investigated alterations of axon terminals and related axonal organelles in mouse cerebellum, using a progressive demyelination model caused by overexpression of proteolipid protein ($PLP^{4e/-}$) [1]. Morphological and three dimensional ultrastructural changes of axonal terminals and organelles including mitochondria were analyzed using serial block face scanning electron microscopy (SBF-SEM) and immunohistochemistry. At 5 months of age, demyelinated axons and axons with abnormally thin myelin were prominent in the cerebellar white matter of hemizygous $PLP^{4e/-}$ mice. In the cerebellar cortex, number and height of climbing fiber terminals were significantly reduced while quantitative SBF-SEM results showed mitochondrial volume in the terminals was increased in the hemizygous $PLP^{4e/-}$ mice compared with age-matched wild-type (WT) mice. By contrast, the numbers and mitochondrial volume of the climbing fiber terminals were similar in $PLP^{4e/-}$ and WT mice at 1 months of age. To investigate the synaptic alterations in more detail, we established organotypic cerebellar slice culture system. In the organotypic slice cultures, robust myelination in WT slices and gradual loss of myelin in $PLP^{4e/-}$ slices were observed during the entire culturing period. These results demonstrated chronic synaptic loss and enlargement of presynaptic mitochondria upon myelin loss in demyelinated axons. The organotypic cerebellar slice culture is a useful tool to observe synaptic changes in the demyelination disease model.

[1] Kagawa et al. Neuron 13 (1994) 427–42.

WTH01-17

Protective role of kolaviron on cuprizone-induced demyelination in rat models of multiple sclerosis

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This study explored the efficacy of kolaviron (kv)-a biflavonoid complex isolated from the seeds of *Garcinia kola* in providing protection against Cuprizone (CPZ)-induced demyelination in both prefrontal cortex and hippocampus of Wistar rats. Thirty rats were treated to receive (A) 0.5 mL PBS (Control); (B) 0.5 mL corn oil; (C) 0.2% CPZ; (D) 0.2% CPZ and 200 mg/kg of kv and (E) 200 mg/kg of kv and 0.2% CPZ for 6 weeks each. Rats were assessed in the open field for exploratory functions and in the elevated plus maze for anxiety-like behavior, and thereafter euthanized and perfused transcardially using 4% paraformaldehyde. The brains were removed in fixed in the same fixative. Prefrontal and hippocampal thin sections were then stained in H&E and cresyl violet for Nissl bodies. CPZ-induced demyelination resulted into behavioural impairment as seen by reduced exploratory activities, rearing behaviour, stretch attend posture, center square entry and anxiogenic characteristics. Furthermore, degenerative changes including pyknosis, kharyorrhesis, neuronal hypertrophy and reduced Nissl integrity were seen in response to CPZ administration compared to control. However, rats treated with kv before or after CPZ administration showed significant improvement in behavioural outcomes and comparatively normal cytoarchitectural profile in neural tissues. This study showed that kv provides protective roles against CPZ-induced neurotoxicity through prevention of ribosomal protein degradation.

WTH01-18

Effect of novel readthrough agents on myelin P0 translation *in vivo*

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Large myelin protein zero (L-MPZ) is an isoform of myelin protein zero (P0), containing additional 63 amino acids at the C-terminus by stop codon readthrough mechanism (Yamaguchi et al., 2012). Our recent study showed that the adhesion activity of L-MPZ is weaker than P0, suggesting that the ratio of P0 and L-MPZ in myelin is important for normal myelin structure. Recently, readthrough agents have been developed to suppress nonsense mutations in the genetic disorders, including Duchenne muscular dystrophy. Before clinical use, however, influence of readthrough agents on proteins naturally produced by this mechanism should be clarified. In the present study, we examined the activities of novel readthrough agents, negamycin analogues (Taguchi et al., 2014), on P0 gene to choose the appropriate agent for *in vivo* experiments. G418 was used as a positive control. *In vitro* transcription/translation study using human P0 cDNA demonstrated that readthrough activity (relative % ratio of L-MPZ in total of L-MPZ and P0) was nearly 10% without any agents, indicating that P0 mRNA itself has relatively high readthrough activity. One of the analogues showed higher readthrough activity (approximately 30%) compared to negamycin without inhibition of protein synthesis. The readthrough activities were also examined using the cells with stable expression of human P0 mRNA. Percent ratios of L-MPZ-positive cells were increased to 60% (~ 10% in control) by three negamycin derivatives. One of these agents was directly injected in mouse sciatic nerves. The treated nerves showed 1.3-fold increase of L-MPZ/P0 ratio compared to vehicle control. Immunohistological analysis showed the increased signal of L-MPZ in Schmidt-Lanterman incisures and paranode as well as compact myelin, which was different from normal P0 distribution. Thus, it is important to examined physiological influence of translational readthrough in the PNS myelin using this agent.

WTH01-19

Targeting neuronal Nogo receptor 1 signaling in EAE preserves axonal transport and limits demyelination

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We have previously shown that deletion of the *ngr1* allele limits experimental autoimmune encephalomyelitis (EAE) severity by preserving central nervous system (CNS) axons. What is unknown is whether this preservation is governed by myelin being intact thereby protecting axons, or axonal degeneration is limited, preventing demyelination. In this study we investigated how targeting neuronal ngr1-dependent signaling, may prevent axonal degeneration during EAE. Conditional deletion of ngr1 in axons was produced by intraocular injection of AAV2 encoding Cre (AAV2-iCre-eGFP) in $ngr1^{flx/flx}$ mice. Conversely, conditional re-introduction of NgR1 in axons was produced by intraocular injection of AAV2 encoding full-length mouse NgR1 (AAV2-NgR1-eGFP) in $ngr1^{-/-}$ mice. All mice were EAE-induced with the myelin oligodendrocyte glycoprotein (MOG_{35-55}) peptide and culled at the peak stage of disease. We found that axonal degeneration is limited in AAV2-iCre-eGFP injected $ngr1^{flx/flx}$ whereas, significant axonal damage is found in AAV2-NgR1-eGFP injected $ngr1^{-/-}$ optic nerves during EAE. As a corollary, the preservation of myelin integrity was a prominent feature in AAV2-iCre-eGFP injected $ngr1^{flx/flx}$, whereas significant demyelination was found in AAV2-NgR1-eGFP injected $ngr1^{-/-}$ optic nerves. Furthermore, the interaction between the axonal motor protein, kinesin-1 (KIF5) and collapsin response mediator protein 2 (CRMP-2) was reduced in AAV2-NgR1-eGFP injected $ngr1^{-/-}$ optic nerves with significant stalling of cholera toxin transport within the diseased optic nerve. Our data suggest that NgR1 governs axonal degeneration in the context of inflammatory-mediated demyelination through phosphorylation of CRMP-2, abrogating axonal vesicular transport. Moreover, the axon-specific deletion of *ngr1* preserves axons blunting the induction of demyelination during EAE, thereby suggesting that NgR1-dependent neurodegeneration maybe a primary mechanism during neuroinflammation.

WTH01-20

TRPA1 receptor deficiency substantially diminishes the cuprizone-induced demyelination

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Our recent studies have presented evidence that Transient Receptor Potential Ankyrin 1 (TRPA1) receptor is expressed on astrocytes in the mouse CNS and its deficiency significantly attenuated cuprizone-induced demyelination by reducing the apoptosis of mature oligodendrocytes (Saghy et al. 2016). The aim of the present study was to investigate the time course of behavioural alterations and morphological changes in cuprizone-treated TRPA1 knock out (KO) mice.Demyelination was induced by feeding wild-type (WT) and KO mice with 0.2% cuprizone mixed into standard rodent chow for 6 weeks. For the open field test, animals were placed into an open arena and filmed with a digital camera. Recordings were evaluated for the determination of the time, distance and velocity of locomotion, while the number of rearings was counted manually. Spatial working memory was investigated by Y-shaped maze. The time course of demyelination was followed by Magnetic Resonance Imaging (MRI). Myelin decompaction was analysed by Luxol Fast Blue (LFB) staining and electronmicroscopy (EM).Cuprizone-treated mice spent more time with locomotion, their mean velocity was significantly higher and the distance they travelled was also consequently longer than untreated mice at weeks 2 and 3 of treatment. No statistical difference was detected between WT and KO mice in these parameters. On the other hand,

significantly increased rearing behaviour was induced in WT mice compared to TRPA1 KO animals. On the basis of MRI, FFB, and EM analysis reduced damage of the myelin was detected in TRPA1 deficient animals in each examined time point. Inhibition of TRPA1 receptors might diminish the degenerative pathology in multiple sclerosis and could be a promising therapeutic target in demyelinating diseases.Supported by National Brain Research Program-A (KTIA_NAP_13-1-2013-0001).

WTH01-21

Inflammatory demyelination induces ependymal modifications concomitant to activation of adult (SVZ) stem cell proliferation

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Ependymal cells (E1/E2) and ciliated B1cells confer a unique pinwheel architecture to the ventricular surface of the subventricular zone (SVZ), and their cilia act as sensors to ventricular changes during development and aging. While several studies showed that forebrain demyelination reactivates the SVZ triggering proliferation, ectopic migration, and oligodendrogenesis for myelin repair, the potential role of ciliated cells in this process was not investigated. Using conventional and lateral wall whole mount preparation immunohistochemistry in addition to electron microscopy in a forebrain-targeted model of experimental autoimmune encephalomyelitis (tEAE), we show an early decrease in numbers of pinwheels, B1 cells, and E2 cells. These changes were transient and simultaneous to tEAE-induced SVZ stem cell proliferation. The early drop in B1/E2 cell numbers was followed by B1/E2 cell recovery. While E1 cell division and ependymal ribbon disruption were never observed, E1 cells showed important morphological modifications reflected by their enlargement, extended cytoskeleton, and reinforced cell-cell junction complexes overtime, possibly reflecting protective mechanisms against ventricular insults. Finally, tEAE disrupted motile cilia planar cell polarity and cilia orientation in ependymal cells.

Therefore, significant ventricular modifications in ciliated cells occur early in response to tEAE suggesting a role for these cells in SVZ stem cell signalling not only during development/aging but also during inflammatory demyelination. These observations may have major implications for understanding pathophysiology of and designing therapeutic approaches for inflammatory demyelinating diseases such as MS.

WTH01-22

The effects of GUT microbiota on myelination and oligodendroglia in the BACHD model of huntington disease

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Structural and molecular myelination deficits occur in the early stages of Huntington disease (HD), an autosomal dominant neurodegenerative disorder, characterised by progressive motor, cognitive and psychiatric deficits. Recent evidence from germ-free (GF) animal models lacking gut-associated microorganisms have suggested the microbiome to play a role in neurodegenerative and neuropsychiatric disorders. In addition, the gut-brain bidirectional communication was shown to modulate the blood-brain barrier, neurogenesis and neuronal activity, microglia responsiveness, and more specifically to our interest, to be involved in the regulation of oligodendrocyte differentiation and myelination. In this study we aimed to investigate the impact of gut microbiota on HD-related white matter phenotypes of the BACHD mouse model, and the extent to which the status of the microbiota could modulate myelination and the expression of myelin-related genes. Three months old specific-pathogen-free (SPF) and GF mice of mixed sex and genotype (wild type and BACHD) were used. Preliminary findings revealed changes in body weight in GF BACHD compared to SPF BACHD animals, as well as a reduction in brain weight in the GF groups, both in WT and BACHD, compared to the SPF groups. Analysis of transmission electron microscopy images of the corpus callosum, quantifying the thickness of myelin sheaths, suggest alterations in axonal diameter and myelin thickness, particularly evident in the GF BACHD group. On-going assessments include examination of changes in oligodendroglia cell populations and expression of myelin related proteins. Our results on the effects of the gut microbiota on myelin plasticity, in both the HD and the healthy brain, may shed light on extrinsic mechanisms regulating oligodendroglia and have important implications for therapeutic approaches and interventions for HD.

WTH01-23

Unconventional myosin id is involved in the remyelination process after cuprizone-induced demyelination

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Myelin is a multilamellar structure that ensheathes an axon and is crucial for normal neuronal function. In the CNS, myelin is produced by oligodendrocytes (OLs) those extend processes and wrap their plasma membrane around multiple axons. The dynamic membrane trafficking system, which relies on motor proteins, is required for myelin formation and maintenance. Previously, we reported that Myo1d is enriched in the outer and inner cytoplasm-containing loops in the CNS myelin, and the knockdown of Myo1d expression using specific siRNA induces morphological changes and apoptosis, and impairs myelin proteolipid protein (PLP) transport in cultured OLs (Yamazaki et al., 2014; 2016). Myo1d possibly contribute to membrane dynamics either in wrapping or

transporting of myelin membrane proteins during myelination. However, the function of Myo1d *in vivo* is still unclear. In this study, we investigated the role of Myo1d in brain by using cuprizone (CPZ)-treated de- and remyelination mouse model. Immunofluorescence signals of Myo1d and myelin basic protein (MBP) were reduced compared to those in non-treated control mice in the demyelinated corpus callosum after 5 weeks of CPZ treatment. These changes were fairly recovered to the pretreatment levels during remyelination processes. To examine the importance of Myo1d during remyelination, we injected Myo1d-siRNA into the demyelinated corpus callosum using stereotaxic technique. Knockdown of Myo1d expression induced inhibition of MBP and PLP expressions during remyelination. However, the number of CC1-positive mature OLs was not altered by siRNA treatment. To examine whether Myo1d knockdown affects cell death, we calculated the number of caspase3-positive cells. The percentage of caspase3-positive cells to total cells tended to increase after Myo1d-siRNA transfection. Furthermore, Myo1d knockdown induced activations of microglia and astrocytes during remyelination. Therefore, Myo1d knockdown possibly promoted apoptosis in OLs, sustained demyelination, and delayed remyelination process at the final stage of OLs differentiation. These results indicated that Myo1d has an important role of regeneration process after demyelination.

WTH01-24

Effects of chronic TYPE 2 diabetes on expression of hippocampal proteins in rats

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In our previous study, we demonstrated that type 2 diabetes affects blood-brain barrier integrity and ultrastructural morphology in Zucker diabetic fatty (ZDF) rats at 40 weeks of age. In the present study, we investigated the possible candidates for diabetes-related proteins in the hippocampus of ZDF rats and their control littermate (Zucker lean control, ZLC) rats by using two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). Approximately 2756 protein spots were detected by 2D-DIGE, and an increase or decrease of more than 1.4-fold was observed for 13 proteins in the hippocampal homogenates of ZDF rats relative to those of ZLC rats. Among these proteins, we found four proteins whose levels were significantly lower in the hippocampi of ZDF rats than in those of ZLC rats: glial fibrillary acidic protein (GFAP), apolipoprotein A-I preprotein (apoAI-P), myelin basic protein (MBP), and rCG39881, isoform CRA_a. Among these proteins, apoAI-P protein levels were decreased most prominently in ZDF rats than in ZLC rats based on Western blot analysis. In addition, immunohistochemical and Western blot studies demonstrated that MBP, not GFAP, immunoreactivity and protein levels were significantly decreased in the hippocampus of ZDF rats compared to ZLC rats. In addition, ultrastructural analysis showed that ZDF rats showed myelin degeneration and disarrangement in the hippocampal tissue. These results suggest that chronic type 2 diabetes affects hippocampal function via reduction of MBP and apoAI-P levels as well as disarrangement of myelin.

WTH01-25

Axon initial segment cytoskeleton shows a more complicated pattern during brain development

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Axon initial segments (AIS) and nodes of Ranvier are highly specialized axonal membrane domains enriched in Na^+ channels. These Na^+ channel clusters play essential roles in action potential initiation and propagation. AIS and nodal Na^+ channel complexes are linked to the actin cytoskeleton through βIV spectrin. However, neuronal βIV spectrin exists as two main splice variants: a longer βIVΣ1 variant with canonical N-terminal actin and αII spectrin-binding domains, and a shorter βIVΣ6 variant lacking these domains. Here, we show that the predominant neuronal βIV spectrin splice variant detected in the developing brain switches from βIVΣ1 to βIVΣ6, and that this switch is correlated with expression changes in ankyrinG splice variants. We show that βIVΣ1 is the predominant splice variant at nascent and developing AIS and nodes of Ranvier, but with increasing age and in adults βIVΣ6 becomes the main splice variant. Remarkably, super-resolution microscopy revealed that the spacing of spectrin tetramers between actin rings remains unchanged, but that shorter spectrin tetramers may also be present. Thus, during development βIV spectrin may undergo a switch in the splice variants found at AIS and nodes of Ranvier.

WTH02 Ischemia and Oxidative Stress

WTH02-01

VEGF theranostic agent promotes neuroregeneration after experimental stroke

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Angiogenesis is a processes that occurs after stroke to help the function recovery in the affected tissue, but its effectiveness is limited. Therefore, the vascular endothelial growth factor (VEGF) has been proposed as a putative therapy aimed at vascular regeneration at the brain lesion. However, due to the side effects of pathologically elevated VEGF levels such as enhance vessel permeability and leakage, and disrupt blood-brain barrier integrity, no treatment using this compound has proved its efficacy. In stroke, the peri-infarct region is a key target region, a no man's land between severely affected tissue (infarct core), with a spreading front of mediators of damage, and healthy tissue, with mediators of remodeling and recovery coming from both sides. In this abstract we report the use of a liposome-based theranostic vehicles targeted to the peri-infarct region, that encapsulate VEGF to promote angiogenesis in controlled manner, potentially avoiding high peaking doses. Liposome-based theranostic agents were constructed by extrusion with DSPC, Cholesterol, DSPE-PEG, Rhodamine-PE and GdDTPA-BSA, and anti-HSP72, loaded with or without VEGF (25 μg/kg). VEGF was also administered intravenously (50 μg/kg). As animal models of stroke we used the intraluminal transient (90 min) MCA occlusion in male rats (MCAo). Treatments were administered 24 h after MCAo. To assess angiogenesis, we used contrast-enhanced magnetic resonance imaging. Briefly, ADC, T2 and T2*-weigthed images were acquired before and after injection of ultra-small paramagnetic iron oxides. N, Q and R maps were calculated according to Boehm-Sturm *et al.*, revealing higher microvessel density and relative vessel size at the peri-infarct region in the group treated with the VEGF-encapsulated liposomes with respect to VEGF administered systemically or empty liposomes. We have provided an alternative approach to VEGF-induced angiogenesis after stroke that enhances the angiogenic effect at the desired area with lower doses, apparently reducing its side effects.

WTH02-02

Nattokinase improves blood flow by inhibiting platelet aggregation and thrombus formation

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Anti-thrombotic effects of nattokinase, an enzyme produced by *Bacillus subtilis* during fermentation of soybeans, were investigated in comparison with aspirin (a blood flow enhancer) and tissue-type plasminogen activator (t-PA, a thrombolytic drug). Nattokinase inhibited platelet aggregation and thromboxane B_2 formation *in vitro*. As a preventive mode on the thrombus formation, oral administration of nattokinase delayed $FeCl_3$-induced arterial occlusion, doubling the occlusion time at about 160 mg/kg. Especially, a high dose (400 mg/kg) of nattokinase fully prevented the arterial occlusion, as achieved with aspirin (30 mg/kg). As a therapeutic mode for thrombolysis, intravenous injection of nattokinase blocked $FeCl_3$-induced arterial occlusion, fully inhibiting at 75 mg/kg, which was achieved with about 8.5 mg/kg of t-PA. In terms of adverse-effects, t-PA caused petechial hemorrhage in the lungs and thymus at 10 mg/kg, leading to extensive bleeding at 20 mg/kg. By comparison, intravenous injection of nattokinase induced pulmonary hemorrhage from 300 mg/kg. The safety margins for t-PA and nattokinase were estimated to be 1.2 and 4.0, respectively. In addition, dexamethasone (2 mg/kg) enhanced the efficacy and safety of nattokinase, in comparison with the beneficial effect on the safety of t-PA. Dexamethasone decreased the therapeutic dose of nattokinase to 50 mg/kg, but increase the hemorrhagic dose to 400 mg/kg, leading to the safety margin of 8.0. Dexamethasone also increased the safety margin of t-PA to 2.4. Therefore, it is suggested that nattokinase could be a good candidate as functional food and/or thrombolytic drug with relatively-low hemorrhagic risk for the improvement of blood flow.

WTH02-03

Cannabinoid receptors and TRPA1 on neuroprotection in a model of retinal ischemia

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Retinal ischemia is a patological event present in several retinopathies such as diabetic retinopathy and galucoma, leading to partial or full blindness with no effective treatment available. Synthetic and endogenous cannabinoids have been described as modulators of ischemic events in the central nervous system (CNS). Thus, the present study aimed to investigate the involvement of cannabinoid system in the cell death induced by ischemia in an avascular (chick) retina. Chicks (2–7 days post-hatched) retinal segments were randomly adressed to control or ischemic for 50 min. We observed that chick retinal treatment with a combination of WIN 55212-2 and cannabinoid receptors antagonists (either AM251/O-2050 or AM630) decreased the release of lactate dehydrogenase (LDH) induced by retinal ischemia in an oxygen and glucose deprivation (OGD) model. Further, the increased availability of endocannabinoids together with cannabinoid receptors antagonists also had a neuroprotective effect. Surprisingly, retinal exposure to any of these drugs alone did not prevent the release of LDH stimulated by OGD. Since cannabinoids may also activate transiente recpetor potencial (TRP), we investigated the involvement of TRPA1 receptors (TRPA1) in retinal cell death induced by ischemic events. We demonstraded the presence of TRPA1 in the chick retina, and observed an increase in TRPA1 content after OGD, both by western blot and immunohistochemistry. In addition, the selective activation of TRPA1 by mustard oil (MO) did not worsen retinal LDH release induced by OGD, whereas the blockage of TRPA1 completely prevented the extravasation of cellular LDH in

ischemic condition. hence, these result show that during the sichemic event there is an augment of TRPA1, and the activation of this receptor is important to evoke cell death. The data also indicate that metabotropic canabinnoid receptors, both type 1 and 2, are not involved with the cell death found in the early stages of ischemia. Therefore, the study points to a potencial role of TRPA1 as a target for neuroprotective approaches in retinal ischemia.

WTH02-04

Effect of punicic acid in a cerebral ischemia model in rat

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Stroke is one of the leading causes of death and disability in the world. Ischemic stroke accounts for approximately 80–85% of all cases and is caused by the obstruction of blood flow to the brain, followed by the subsequent restoration of perfusion and oxygenation. The interruption of the blood flow initiates a complex series of metabolic events that progress to cell death. The inflammation and the oxidative stress are fundamental mechanism of damage implicated in focal ischemic stroke. Punicic acid (PA) is a polyunsaturated fatty acid found in the seed of pomegranate fruit, and it has a wide array of health beneficial properties like antidiabetic, hypolipidemic, anti-inflammatory, anticancer, antioxidant activities, and antinephrotoxic activity. Mice modeling for genetic prion disease were treated with a nanodroplet formulation of pomegranate seed oil, the nano droplet possess neuroprotective effects via its antioxidant properties. The anti-inflammatory effects have been evaluated in models of the inflammatory bowel diseases, necrotizing enterocolitis and age related bone complications. The purpose of this work was to examine the effect of a nanodroplet formulation of pomegranate seed oil on neurological deficit and morphological alterations induced in a model of ischemia and reperfusion (IR), evaluating some antioxidant and anti-inflammatory markers. Animals were divided in 6 groups: control group (CT) received only vegetal oil; IR group submitted to 1 h of ischemia and 4 days of reperfusion; 3 groups submitted to IR and administered with nanodroplet (50, 100, 200 mg/Kg) for 5 days, i.g.; and 1 group submitted to IR and administered with a single dose of 200 mg/Kg i.p. before the reperfusion. Four days of onset reperfusion, the brain of each animal was obtained to evaluate the histological damage. The treatment with PA decreases the neurological deficit and the morphological alterations induced by IR. This work was supported by CONACYT (Grant 241655).

WTH02-05

Adult mouse neural stem cell-derived microvesicles: proteomic characterization and effects on brain ischemia

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Ischemic stroke is a neurological condition provoked by the sudden occlusion of blood flow in the brain. In response to this a series of cellular and molecular events take place to prevent neuronal death and the expansion of injury. One such event involves the proliferation of neural stem cells (NSC) in the neurogenic niches of the adult brain. It has been suggested that NSC proliferation could contribute to replenish the neuronal population that died following stroke, but several studies have shown that neuronal replacement might not be as important for recovery. Rather, NSC may actively communicate with neurons to mediate protection after ischemia. Like most cells, NSC release extracellular vesicles carrying regulatory proteins, lipids and nucleic acids that are able to modulate several functions in their target cells, this mechanism of intercellular communication may underlie some protective effects produced by the ischemia-stimulated NSC expansion. In this study we investigated the possible communication mediated by exosome-enriched microvesicles between NSC and neurons in cerebral ischemia using an *in vitro* model produced by the transient deprivation of oxygen and glucose (OGD). We tested the neuroprotective effect of NSC-conditioned medium on the neurodegeneration of primary cortical neuronal cultures subjected to toxic stimuli relevant in ischemic stroke, namely, glutamate excitotoxicity, oxidative stress and induction of apoptosis. Under these conditions NSC-produced factors are capable to decrease neuronal death; the mechanism underlying such protection might be mediated through the action of molecules released in microvesicles. Therefore, we collected NSC-derived microvesicles from cells subjected to OGD and control conditions and characterized their protein content by mass spectrometry, some of the molecular hits might be involved in promoting neuronal survival and their individual characterization is underway. These results highlight the complexity of NSC-mediated signaling in promoting neuronal survival after stroke. Supported by PAPIIT-DGAPA IN226617 and CONACYT 219542.

WTH02-06

Cyclooxygenase inhibition ameliorates hypobaric hypoxia induced spatial memory impairment in rats

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Background: Hypobaric Hypoxia (HH) is an environmental stress that leads to multiple pathophysiological consequences. Prostaglandin E2(PGE2) is derived from Arachidonic acid by sequential actions of cyclooxygenases (COX-1 and COX-2). Studies demonstrate that elevated PGE2, produced from COX activity is causal factor for memory deficits**.**

Aim: To temporal quantitate PGE2 related molecules in hippocampus and cortex and effect of COX inhibitors in HH induced deficits in spatial memory.

Methdology: Male Sprague–Dawley rats were exposed to different duration of HH exposure (0,1,3,7) at 25000 ft. Levels of PGE2, PGE synthase, COX-1, COX-2 was measured in day dependent manner in hippocampus and cortex. CV staining in day dependent manner in hippocampus was performed. We examined the effect of selective COX-1 inhibitor, Valeryl salicyclate (5 mg/kg/i.p) and COX-2 inhibitor, Celecoxib (20 mg/kg/i.p) on spatial memory during HH.

Results: We found spatial memory deficit post 7 HH exposure as compared to control ($p < 0.001$) with changes in PGE2 levels ($p < 0.01$) and no. of pyknotic cells in hippocampus. The results

indicate that HH evoked PGE2, PGE synthase, COX-1, COX-2 levels in hippocampus as early as day1, reached maximum at day 3 and starts droping back at day 7. Treatment with COX-2 ($p < 0.001$) and COX-1 ($p < 0.01$) inhibitor significantly reduced path-length and latency to reach platform and no. of pyknotic cells in hippocampus as compared to HH exposed animals.

Conclusion: This suggests that PGE2 considerably contributes to spatial memory deficits at day 7. However, the time course of PGE2 up regulation suggests that HH induced PGE2 response at day 3 precedes cognitive deficit at day 7 probably via both COX-1 and COX-2 pathway. Pretreatment with specific cyclooxygenase inhibitor during HH might be ameliorating inducible PGE2 and downstream signaling, results in improved cognitive performance.

WTH02-07

Feeding perilla oil improves atherosclerosis and ischemic stroke by controlling lipid metabolism

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Since plant oils are believed to be better than animal fats for cerebrovascular and cardiovascular diseases, the effects of perilla oil on atherosclerosis and ischemic stroke were investigated. In order to evaluate anti-atherosclerotic activity, hypercholesterolemia was induced in rabbits by feeding a high-cholesterol diet (HCD) containing 0.5% cholesterol and 1% corn oil, and perilla oil (0.1 or 0.3%) was added to the diet containing 0.5% cholesterol for 10 weeks. HCD greatly increased blood total cholesterol and low-density lipoproteins, and caused thick atheromatous plaques, covering 74% of the aortic wall. Hypercholesterolemia also induced lipid accumulation in the liver and kidneys, leading to lipid peroxidation. Perilla oil not only attenuated hypercholesterolemia and atheroma formation, but also reduced fat accumulation and lipid peroxidation in hepatic and renal tissues. To evaluate anti-stroke activity, Sprague–Dawley rats were fed a diet containing various oils (10%) including perilla oil, and then body weights, blood lipids, and effects on brain infarction and physical dysfunction induced by middle cerebral artery occlusion (MCAO) were analyzed. Plant oils and trans-fat, except perilla oil, significantly increased body fats and body weight gain. Sesame oil and trans-fat specifically increased blood cholesterols and triglycerides, respectively, while perilla oil decreased both the cholesterols and triglycerides. Only perilla oil not only attenuated the cerebral infarction, but also restored the physical function in locomotor activity and rota-rod performances of MCAO rats. The results indicate that perilla oil prevents atherosclerosis and fatty liver as well as ischemic stroke by controlling lipid metabolism, and that it could be the first choice oil to reduce the risk of dietary metabolic syndrome and ischemic stroke.

WTH02-08

VEGFR1-mediated neuroprotection in experimental cerebral stroke

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Cerebral stroke is a devastating disease that affects millions of people and it is the first cause of acquired disability in the word. Neuroinflammation is an important component of the pathophysiology of stroke and other neurodegenerative processes where microglia, the resident macrophages of the central nervous system, play a key role. Microglial activation can be modulated by a series of neurotrophic factors including vascular endothelial growth factor A (VEGF-A), which has been associated to neuroprotection in the acute phase of stroke, although the molecular mechanisms underlying such protection are not yet fully understood. The function of VEGF-A is mediated mainly by the activation of its canonical receptor 2 (VEGFR2), but also by receptor 1 (VEGFR1), which is preferably activated by other members of the VEGF family, such as VEGF-B. Both of these receptors are upregulated after stroke, but only VEGFR1 has been reported to be expressed in microglia. Here we studied the mechanisms of VEGF-A-mediated neuroprotection in the acute phase of stroke using an *in vivo* model produced by the transitory occlusion of the middle cerebral artery. Administering exogenous VEGF-A in the early phase after stroke, results in a significant reduction of infarct volume and increased neuronal survival. We found that the underlying mechanism of this protection involves the activation of VEGFR-1 and -2, but interestingly, pharmacologically inhibiting VEGFR2 in the presence of the exogenous ligand reduces infarct volume, limits edema, increases neuronal survival and improves neurological outcome to a greater extent than the simultaneous activation of both VEGFR1/2. Given the role of VEGFR1 on microglial responses to altered brain homeostasis, the underlying mechanisms of the VEGFR1-mediated protection could involve the modulation of the inflammatory response and microglial polarization to a neuroprotector phenotype. These results point towards VEGFR1 as an interesting therapeutic target for stroke worth of further investigation. Supported by PAPIIT-DGAPA IN226617 and CONACYT 219542.

WTH02-09

Probing nitrite and nitric oxide biochemistry in the brain *in vivo* by a novel sensing approach

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Communication between neurons and blood vessels in the brain is essential for cognition. This communication allows for an allocation of energy resources according to local demands imposed by neuronal activation, a process termed neurovascular coupling (NVC). During hypoxia, aging or neurodegeneration, NVC is compromised in part due to impaired bioavailability of nitric oxide (•NO). Nitrite has recently emerged as a metabolic precursor of •NO *in vivo* and may represent an alternative pathway for •NO

production, other than the nitric oxide synthases. The reduction of nitrite to •NO is favored via redox reaction of nitrite with ascorbate, which is present at high concentrations in the brain and is released to the extracellular medium upon glutamatergic stimulation. Thus, we hypothesized that modulation of nitrite concentration in the brain via diet may improve neurovascular coupling in emergency conditions by increasing •NO production. In this context, the development of tools to evaluate nitrite dynamics in the brain is of great interest. Here, we developed a novel sensing approach for nitrite monitoring in the brain in real-time by using fast-scan cyclic voltammetry (FSCV) associated with carbon fiber microelectrodes (CFMs). The local pressure-ejection of a nitrite solution resulted in the detection of transient signals when current was sampled at +1.1 V (*vs* Ag/AgCl). The signals had reproducible peak concentrations, rise times and decay rate constants. The developed method is a valuable tool for *in vivo* monitoring of nitrite dynamics in the brain. Furthermore, it will allow understanding the role of nitrite in neurovascular coupling and its modulation by diet.

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WTH02-10

Identification of synaptosomal receptor for extracellular protons

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Brain ischemia is accompanied by lowering of intra- and extracellular pH. Stroke often leads to irreversible damage of synaptic transmission by unknown mechanism. We investigated an influence of pH_i and pH_o lowering on free radical formation in synaptosomes. Three models of acidosis were used: pH_o 6.0 corresponding to pH_i decrease to 6.04; pH_o 7.0 corresponding to pH_i lowering to 6.92; 1 mM Amiloride corresponding to pH_i decrease to 6.65. We have shown that both types of extracellular acidification, but not intracellular acidification, increase DCF (2′,7′-Dichlorodihydrofluorescein) fluorescence that reflects free radical formation. These three treatments induce the rise of the dihydroethidium fluorescence that reports synthesis of superoxide anion. However, the impact of amiloride on superoxide anion synthesis was less than that induced by moderate extracellular acidification. Superoxide anion synthesis at pH_o 7.0 was almost completely eliminated by mitochondrial uncoupler CCCP (Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone). Using fluorescent dyes JC-1 (5,5′,6,6′-Tetrachloro-1,1′3,3′-tetraethylbenzimidazolo-carbocyanine iodide) and Rhodamine-123, we confirmed that pH_o lowering, but not intracellular acidification, led to depolarization of synaptosomal mitochondria. This suggests the presence of receptor for protons on the plasma membrane of presynaptic terminals. In this effect may be involved ASICs (Acid-Sensing Ion Channels), ion channels permeable to calcium and sodium, and OGR1 (Ovarian cancer G protein-coupled Receptor 1), g-protein associated H^+-receptor. Cu^{2+} and Zn^{2+} in micromolar concentrations can block histidine residues of OGR1 receptor responsible for proton binding. Depolarization of synaptosomal mitochondria at pH_o lowering to 7.0 is partially blocked by10 μM Cu^{2+} and 10 μM Zn^{2+}, and by 1 μM Thapsigargin, that indicating on participation of OGR1 in signal transduction. Synaptosomal ROS accumulation and depolarization of synaptosomal mitochondria at the pH_o lowering is not dependent on the presence of Ca^{2+} in the incubation medium. Also, pH_o decrease does not lead to an increase in Sodium Green fluorescence. It shows a lack of activation Ca- and Na-specific forms of ASICs. These results indicate that the main receptor for protons on the plasma membrane of the presynaptic terminal at moderate acidification is OGR1.

WTH02-11

Redox state in modulation of neuroprotection in hypoxia

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In present study the changes in the redox state and free radical activity of the rat brain tissue were analysed in acute and prolonged hypoxia. Hypoxia was modeled by pressure chamber under 310 mm Hg during 1, 4, 7, 14, 28 days.The concentration of glucose in brain under acute hypoxia was in 2.4 higher then in intact animals. But after the 4th day of hypoxia the glucose concentration falls down till normal and remains at the same value till the 14th day, when it is slightly increased. A study of energy metabolism showed that lactate/pyruvate ratio in experimental animals was 3-fold higher than in intact specimens.The substrate relations in redox-pairs malate/oxaloacetate, NAD/NADH were increased in the same manner.The rate of tissue respiration, ADP phosphorylation were decreased essentially, oxidative phosphorylation kinetics was modified. Hypoxia decreased ATP content in brain tissue. Thus, acute hypoxia is accompanied by accumulation of reducing equivalents and inhibition of tissue respiration.Then in 2 weeks of hypoxia the redox state of brain tissue returns back to the value corresponding to intact animals and restores the origin balance of NAD dependent glycolysis and the Krebs cycle reactions, demonstrated the rise again by 28 day. Changes in the redox state of the cells are correlated to the intensity of free radical oxidation. Activation of free radical oxidation is accompanied by destabilization of the cell membranes, which results in the release of neuron-specific enzymes from damaged cells into the blood.Neuron-specific enolase in the group of animals exposed to acute hypoxia was 65% higher than in intact specimens, which reflects the severity of structural and functional cnanges in the neuronal membranes. The lowest results of the intensity of free radical reactions were noted at early (4 day) and late (28 day) of hypoxia.The antioxidant potential was the same as in intact animals increasing at 28th day. The different mechanisms of recovering of redox state of the nervous cells in acute and prolonged hypoxia were postulated.

WTH02-12

Evaluation of the potential neurotoxicity of gold nanoparticles in the different rat brain regions

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The present study aims to investigate the potential adverse effects of gold nanoparticles (Au NPs) in the cortex, hippocampus, striatum, midbrain, cerebellum and medulla of adult male Wistar rat through the estimation of some oxidative stress parameters and

acetylcholinesterase (AChE) activity. Rats were divided into two main experimental groups. Animals of the 1st and 2nd groups were intraperitoneally injected with a single dose (100 μg/kg body wt) of ~ 20 nm Au NPs and decapitated after 24 h and 2 weeks of injection, respectively. Control animals were injected with saline solution and sacrificed simultaneously with the treated groups. The present data revealed that Au NPs induced several significant changes in the levels of GSH and NO and GST activities in the brain areas investigated. These changes were more prominent after 24 h than after 2 weeks of injection and varied according to the brain region examined. However, these alterations did not induce lipid peroxidation except for the cerebellum and medulla after 24 h only. In addition, Au NPs induced significant decreases in cortical and hippocampal AChE activities after 24 h. However, significant increases in cortical and cerebellar AChE activities were recorded after 2 weeks. In conclusion, although most of the early biochemical changes induced by Au NPs injection were ameliorated after 2 weeks, careful must be taken into consideration in utilization of gold nanoparticles in biological applications especially with the particle size that can penetrate the BBB.

WTH02-13

There is not a simple linear relationship between increasing numbers of mild traumatic brain injury and increasing damage

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Following mild traumatic brain injury (mTBI), some patients go on to experience long-term cognitive impairments and additional mild impacts can exacerbate negative outcomes. To compare chronic damage and deficits following increasing numbers of repeated mTBIs, we used a clinically relevant closed-head weight-drop model of repeated mTBI to deliver 1, 2 or 3 mTBIs to adult female rats at 24 h intervals under anaesthesia. Outcomes were assessed at 3 months following the first mTBI.

Neurologic function was assessed using a modified neurologic severity score and no gross motor, sensory or reflex deficits were identified ($p > 0.05$), consistent with current literature. However, vestibulomotor deficits were observed following 3 mTBI ($p \leq 0.05$). Cognitive function assessed using a Morris water maze paradigm revealed chronic memory deficits following 1 and 2, but not 3 mTBIs compared to shams ($p \leq 0.05$). Oxidative stress was assessed immunohistochemically in various brain regions, quantifying immunoreactivity of indicators of lipid peroxidation, DNA oxidation and glycoxidation. Acrolein-mediated lipid peroxidation was increased in the dentate gyrus of the hippocampus following 1 mTBI ($p \leq 0.05$), while DNA damage indicator 8-hydroxy-2′-deoxyguanosine was increased in the corpus callosum following 2 but not 3 mTBIs, realtive to shams ($p \leq 0.05$). Glycoxidation, indicated by carboxymethyl-lysine, was increased in ventral brainstem following 3 mTBI ($p \leq 0.05$). Integrity of myelin ultrastructure in the corpus callosum was assessed using transmission electron microscopy, revealing that G ratio was decreased following 1 and 2 but not 3 mTBIs compared to shams ($p \leq 0.05$). Differences in damage and deficits following 1, 2 and 3 mTBI suggests that the effects of increasing numbers of mTBIs is not simply additive. The complex picture that has emerged warrants further studies exploring mechanisms of damage as well as chronic neuroregenerative responses that may facilitate the development of therapeutic strategies to limit long term functional deficits following repeated mTBI.

WTH02-14

NRF2 expression during ischemia with and without reperfusion in rat brain

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Cerebrovascular disease is a chronic-degenerative disorder that is divided into two types: ischemic (stroke), due to the interruption of the blood supply to a region of the brain, and hemorrhagic, which is generated by the rupture of an artery in some area of the brain, whereas ischemic stroke being the third leading cause of death and permanent disability in adults worldwide. Reperfusion to ischemic brain is currently the best way to save life and limit the development of cerebral infarction. However, it is believed that ischemia-reperfusion injury (IR) is another important clinical problem in the treatment of brain damage. In addition, the exact pathogenesis of cerebral IR is still not fully understood. Evidence has shown that inflammation, reactive oxygen species (ROS) and apoptosis are mechanism involved. Cerebral IR disrupts the balance between ROS production and its inactivation by antioxidant systems, which ultimately leads to an excessive accumulation of ROS, which contribute to brain tissue damage causing cellular dysfunction and cell death. Therefore, it is reasonable for patients suffering from an IR event to benefit from reduced ROS levels in therapy in case of stroke. Nrf2 is considered one of the master regulators of endogenous antioxidant defense. In response to oxidative stress, Nrf2 promotes the expression of a wide variety of antioxidant genes, including antioxidant and non-enzymatic enzymes, by their translocation to the nucleus, binding to antioxidant response elements (AREs) and regulation of transcription of target genes. Nrf2 appears to play an important role in the protection of brain cells against ischemic brain injury. The aim of this work was study the Nrf2 levels during ischemia with and without reperfusion. Animals were submitted to 15, 30, 60 and 120 min of ischemia using the middle cerebral artery occlusion model, and the Nrf2 levels were measured by western blot in striatum, frontal cortex and hippocampus. This project was supported by CONACyT (Grant241655).

WTH02-15

Intravenous injection of minoxidil reduced neuronal damage caused by transient focal cerebral ischemia

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Intracellular potassium ion level is higher than extracellular space, which is regulated by basically sodium pump. Potassium ion generates a resting potential on the cell membrane by passing

through the leak channels. Openings of the other potassium channels on the cell membrane induce hyperpolarization and it would cancel the excitation. Potassium channels are also on the mitochondrial inner membranes. Recent studies suggested that drugs, which open potassium channels in cellular and/or mitochondrial membrane protect neuronal tissues against neurodegenerative situation including transient ischemia. Here, we have demonstrated whether Minoxidil, a potassium channel opener reduced damage on neuronal tissues that was caused by transient focal cerebral ischemia.

Transient focal cerebral ischemia was induced in the 6-week old male C57/BL mice by a 1-h middle cerebral artery occlusion (MCAO) of left hemisphere and subsequent reperfusion. Thereafter, the mice were randomly divided into 4 groups, and either saline, edaravone (6 mg/kg bw) or minoxidil (0.5 or 5 mg/kg bw) was intravenously injected, immediately. One day later, the brain was taken out, and 5 slices in total were prepared from each mouse with a thickness of 1000 μm from the bregma, 2 on the front side and 3 on the back side, using a brain slicer. The slices were stained with 2,3,5-Triphenyl tetrazolium chloride (TTC staining) to detect cellular respiration activity.

Transient focal cerebral ischemia decreased TTC staining in ipsilateral striatal area by 50% compared to the opposite side, suggested that the operation damaged the neuronal tissues. The injection of edaravone immediately after the operation completely prevented the decrease. A similar prevention was observed with the injection with 5 mg/kg bw minoxidil instead of 0.5 mg/kg bw. Minoxidil was once developed for the treatment of hypertension, but now it is famous as a treatment for androgenic alopecia and it is applied for external application. We might need to think about injecting this medicine again in anticipation of the protective action of central nervous systems.

WTH02-16

BBB damage is reduced by blockade of BETA 2-adrenergic receptor-mediated HIF-1 alpha upregulation during acute cerebral ischemia

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Aims: Disruption of the blood brain barrier (BBB) within the thrombolytic time window is an antecedent event to intracerebral hemorrhage in ischemic stroke, however, mechanism underlying BBB damage at this acute stage is not well known. Since hypoxia-inducible factor-1 alpha (HIF-1α) was discovered as a mater regulator in hypoxia, we sought to investigate the roles of HIF-1α in BBB damage as well as factors regulating HIF-1α expression after acute ischemia stroke.

Methods: *In vivo* rat middle cerebral artery occlusion (MCAO) and *in vitro* oxygen glucose deprivation (OGD) models were used to assess the integrity if BBB.

Results: pretreatment with HIF-1α inhibitor YC-1 significantly inhibited 2-h MCAO-induced BBB damage accompanied by inhibition of occludin degradation, matrix metalloproteinase 2 (MMP-2) activity and vascular endothelial growth factor (VEGF) mRNA upregulation. Interestingly, blocking β2-AR reduced ischemia-induced BBB damage by regulating HIF-1α expression. HIF-1α was shown to be colocalized with neurons but not astrocytes or endothelial cells. Of note, *in vitro* results showed that HIF-1α inhibition with YC-1 or siRNA significantly prevented 2-h OGD-promoted upregulation of VEGF mRNA and secretion of VEGF and MMP-2 in neurons. More important, blocking β2-adrenergic receptor (β2-AR) inhibited 2-h OGD-induced HIF-1α upregualtion and reduced occludin degradation induced by OGD-neuron media.

Conclusion: Taken together, acute cerebral ischemia disrupts BBB by upregulating HIF-1α and activating the neurons to secret VEGF and MMP-2, while blocking β2-AR inhibited such change. These findings provide new mechanisms underlying BBB damage within thrombolytic time window and may help reduce thrombolysis-related cerebral hemorrhage.

WTH02-17

Maintenance of steady state H2S levels rescues hypobaric hypoxia-induced pathological effect

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Hypobaric hypoxia (HH) occurs at high altitude and is associated with multiple pathophysiological conditions including spatial memory loss Recently, the role of H2S in hypoxic cerebral autoregulation has been established, however, how the endogenous H2S production is regulated in HH not known. The present study was undertaken to investigate how H2S production is modulated at 1,3 and 7 day post HH exposure.Interestingly, we observed a significant lowering of H_2S levels after HH exposure. We next tested if HH induced any change in the expression of Cystathionine beta synthase (CBS) a critical enzyme for H_2S production in the brain, under these conditions employing western blot. We observed HH culminated in marked increase in the expression of CBS at all time points studied (Day 1, 3 & 7), suggesting that the decrease in production of H_2S was not due to reduced expression of CBS in the brain. To further investigate this condition, we quantitated various amino acids including L-Cysteine, employing HPLC, from brain extracts of animals exposed to HH. we clearly observed significant decrease in the level of L-Cysteine at all time points after HH exposure. Notably, the concentration of Methionine decreased significantly only at day 3 while that of Arginine at day 1 & 3. Taken together, we inferred that the reduced level of substrate (L-Cysteine) required for production of H2S by CBS could be a possible reason for HH-induced reduction in H2S levels in the the brain. Our experiments clearly suggest that the maintenance of endogenous H_2S levels through the administration of specific L-Cysteine donor counteracts the loss of spatial reference memory in response to HH. Taken together the steady state levels of H_2S is likely to serve as the key node for preservation of neurophysiological functions during hypobaric hypoxia.

WTH02-18

Recurrent moderate hypoglycemia enhances brain injury induced by the hypoglycemic coma and leads to memory decline

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Most Type 1 Diabetes Mellitus (TIDM) patients who are under intensive insulin therapy suffer from repetitive episodes of moderate

hypoglycemia (RMH), which increase the risk for severe hypoglycemia (SH). SH can progress to the coma state, which induces neuronal death in vulnerable brain regions such as the cortex, the hippocampus and the striatum by a mechanism involving oxidative damage. However, the consequences of RMH on neuronal damage and cognitive function are not well understood, nor its effect on a subsequent period of hypoglycemic coma. The purpose of the present study was to investigate whether RMH can exacerbate neuronal damage and cognitive decline induced by a short (7–10 min) coma period in an *in vivo* model. Rats received an injection of insulin (6.5 insulin units, IU) during 7 consecutive days leading to moderate (40 mg/dl glucose) hypoglycemia. At day 8 animals received 32 IU to induce the hypoglycemic coma and were rescued with glucose after 7–10 min. Neuronal death and oxidative damage were assessed 24 after the coma by histological analysis and immunocytochemistry. Reduced glutathione (GSH) level glycogen were also assessed. Seven and 15 days after the coma, cognitive function was evaluated in two memory tests. Results show that previous RMH exacerbates oxidative damage and neuronal death induced by the hypoglycemic coma in the parietal cortex, the striatum but mainly in the hippocampus. These changes correlated with a severe decrease in GSH, glycogen increase and a significant spatial and contextual memory deficit. Results demonstrate that previous RMH enhances brain vulnerability to acute hypoglycemia by a mechanism involving decreased antioxidant defense and oxidative damage. They also highlight the relevance of an adequate control of moderate hypoglycemic episodes in TIDM.

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WTH02-19

Differential assessment of CB1R role in the neuroprotective effect of endocannabinoid system with different ways of its activation

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Endogenous cannabinoid system (ECS) regarded as a perspective target for ischemic brain injury correction. The aim of the investigation was to study the effect of ECS activation on cannabinoid receptors type 1 expression in normobaric hypoxia *in vitro*.

The experiments were carried out on primary hippocampal cultures obtained from CBA mice embryos (E18). Normobaric hypoxia was modeled on day 14 of culture development *in vitro* by replacing the normoxic cultural medium by a medium with a low oxygen for 10 min. N-arachydonoyl dopamine (10 mcM) (N-ADA) or antagonist of CB1 receptors – SR151716 (SR1) (1 mcM) or inhibitor of endocannabinoid degrading enzymes (monoacylglycerol lipase and fatty acid amide hydrolase) – JZL 195 (1 mcM) were applied into hypoxic cultural medium. The main parameters of spontaneous calcium activity, the viability of cells and the level of intravital CB1 mRNA expression by using SmartFlare RNA Detection Probes (Merck Millipore) were investigated.

ECS activation by N-ADA or JZL 195 prevented the cell death and reduction of spontaneous calcium activity in hippocampal cultures in the posthypoxic period. CB1 mRNA is synthesized by neurons and glial cells. Hypoxia caused an increase of CB1 mRNA expression. In cultures with N-ADA or JZL 195 application the number of CB1 mRNA positive cells doesn't differ from intact cultures.

Therefore, our studies revealed that activation of ECS has strong neuroprotective properties which implemented through CB1 receptors.

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WTH02-20

Zinc contributes to ischemia-induced blood-brain barrier disruption by activating mmps in cerebral microvessels

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Background: Zinc ions are stored in synaptic vesicles and cerebral ischemia triggers their release from the terminals of neurons. Zinc accumulation in neurons has been shown to play an important role in neuronal death following ischemia.
Objectives: in this study, we investigate whether zinc is involved in ischemia-induced blood–brain barrier (BBB) disruption.
Methods: We investigated the contribution of zinc to ischemia-induced acute BBB disruption and the possible molecular mechanisms using both cellular and animal models of cerebral ischemia.
Results: Zinc greatly increased BBB permeability and exacerbated the loss of tight junction proteins (Occludin and Claudin-5) in the endothelial monolayer under oxygen glucose deprivation conditions. In cerebral ischemic rats, a dramatically elevated level of zinc accumulation in microvessels themselves was observed in isolated microvessels and *in situ*, showing the direct interaction of zinc on ischemic microvessels. Treatment with a specific zinc chelator *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl) ethylenediamine (TPEN), even at 60-min post ischemia onset, could greatly attenuate BBB permeability in the ischemic rats as measured by Evan's Blue extravasation, edema volume and magnetic resonance imaging. Furthermore, zinc accumulation in microvessels activated the superoxide/matrix metalloproteinase-9/-2 pathway, which leads to the loss of tight junction proteins (Occludin and Claudin-5) and death of endothelial cells in microvessels themselves.
Conclusions: Our findings reveal a novel mechanism of cerebral ischemia-induced BBB damage, and implicate zinc as an effective and viable new target for reducing acute BBB damage following ischemic stroke.

WTH02-21

Short chemical ischemia induces death of neuroblastoma SH-SY5Y cells but not glioblastoma T98G cells

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Both translation arrest and proteasome stress associated with accumulation of ubiquitin-conjugated protein aggregates were

considered as a cause of delayed neuronal death after transient global brain ischemia however exact mechanisms as well as possible relationships are not fully understood.

The aim of this study was to compare effect of chemical ischemia and proteasome stress on cellular stress responses and viability of neuroblastoma SH-SY5Y and glioblastoma T98G cells. Chemical ischemia was induced by transient treatment of the cells with sodium azide in combination with 2-deoxyglucose. Proteasome stress was induced by treatment of the cells with bortezomib. Treatment of SH-SY5Y cells with sodium azide/2-deoxyglucose for 15 min was associated with cell death observed 24 h after treatment while glioblastoma T98G cells were resistant to the same treatment. Treatment of both SH-SY5Y and T98G cells with bortezomib was associated with cell death, accumulation of ubiquitin-conjugated proteins and increased expression of Hsp70. These typical cellular responses to proteasome stress, observed also after transient global brain ischemia, were not observed after chemical ischemia. Finally, chemical ischemia, but not proteasome stress, was in SH-SY5Y cells associated with increased phosphorylation of eIF2α, another typical cellular response triggered after transient global brain ischemia.

Our results showed that short chemical ischemia of SH-SY5Y cells is not sufficient to induce both proteasome stress associated with accumulation of ubiquitin-conjugated proteins and stress response at the level of heat shock proteins despite induction of cell death and eIF2α phosphorylation.

WTH02-22

Developed human stem cells derived neuronal model of cerebral ischemia revealing the anti ischemic potential of trans-resveratrol

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Cerebral ischemia is the largest cause of long-lasting disability in humans, which occurs due to deprived blood supply to the brain. To screen the anti-ischemic potential of drugs, no suitable human specific tool is available. We explore the applicability of human cord-blood stem cell derived neuronal cells (hCBSCNCs) as a reliable tool for the purpose. To create a model the optimum time points for oxygen-glucose deprivation (OGD) and re-oxygenation (R) have been identified. The OGD of 6 h followed by a re-oxygenation period of 24 h could be recorded as optimum under our experimental conditions. Glucose concentration during re-oxygenation was found to be one of the major factors involved in growth and proliferation of hCBSCNCs. Re-oxygenation with 4–6 mg/mL glucose concentration in medium was found to be first statistically significant parameter. This OGD-R model increases Ca^{2+} influx, which triggered the hypoxic homeostasis transcription factors like-hypoxia induced factor-1 alpha (HIF-1α), Cav-beta 3 (Cav β3), signal transducer and activator of transcription 3 (STAT3) and heat shock protein 27 (hsp-27) and subsequently induces the ROS mediated apoptotic damages in the cells. The cells viability was assessed by trypan-blue exclusion and MTT assays. We further investigated the anti-ischemic potential of trans-resveratrol (RV) in this OGD-R model when exposed to biologically safe doses (5, 10 and 25 μM) of RV in three different exposure groups i.e., 24 h prior to OGD (pre-exposure); 24 h post OGD (post-exposure) and from 24 h before OGD to end of re-oxygenation period (whole exposure). Our findings demonstrated that RV has significant potential of increasing the viability of OGD-R insulted hCBSCNCs by decreasing ROS. The whole exposure group of RV is most efficient in decreasing hypoxia induced cell death through its antioxidant properties.

WTH02-23

Interplay between the ubiquitin-proteasome system and calpains in brain ischemia

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Ischemic stroke is characterized by overactivation of glutamate receptors (excitotoxicity) thus inducing a massive accumulation of $[Ca^{2+}]i$ in the postsynaptic cells. These events will lead to the activation of the Ca^{2+}-dependent calpains, and to an overall hypofunction of the Ubiquitin-Proteasome System (UPS), by mechanisms not fully elucidated. Herein, we show that cerebrocortical neurons subjected to Oxygen-Glucose Deprivation (OGD), an *in vitro* model mimicking transient global ischemia, for 1.5 h, display reduced chymotrypsin-like activity of the proteasome when evaluated 4 h after the insult. Unexpectedly, total polyubiquitin conjugates, a marker for proteasome dysfunction, were diminished under the same conditions, while no differences were observed in total free ubiquitin levels. The decreased proteolytic activity is correlated with a disassembly of the 26S proteasome into its major constituents, the 19S regulatory and 20S catalytic particles, as shown by NATIVE-PAGE. Two non-related *in vitro* assays with recombinant calpain, identified the ATPase ring (Rpt1,3,5) proteins, along with the Rpn2 and β3 subunits, as calpain targets. Six other proteasome proteins (Rpn1,3,5,12 and USP14) may also be cleaved by calpains since their amino acid sequence contains putative PEST sequences. Together, these results suggest that calpains may impair the UPS by acting on multiple targets. An increase in calpain activity was observed observed in cortical neurons subjected to OGD, and the active protease cleaved Rpn10, Rpt3, along with Rpt1 and possibly Rpn3, by a NMDAR-dependent mechanism. Incubation of cultured cerebrocortical neurons with the USP14 inhibitor IU1, a proteasome activator, fully prevented the OGD-induced calpain activation and neuronal death, when evaluated 24 h after injury. Therefore, proteasome activation in cerebrocortical neurons subjected to OGD may be exploited to develop novel neuroprotective strategies for ischemic stroke. (Supported by FCT [SFRH/BD/51967/2012], COMPETE and Mais Centro Program).

WTH02-24

Glycyrrhizic acid attenuates ROS mediated neurodegeneration and cognitive deficit in chronic cerebral hypoperfusion rats

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Chronic cerebral hypoperfusion, a major threat for cognitive health is linked to various vascular ailments and comorbidities such as diabetes, obesity and hypertension. The study primarily aims at explaining the mitigating effects of *Glycyrrhizic acid (GA)* on cognitive health challenged by chronic cerebral hypoperfusion. Adult male Sprague–Dawley rats were segregated into four groups (i) Sham (ii) Lesion (Bilateral common carotid artery permanent occlusion) (iii) GA treated (Lesion+GA) (20 mg/kg body wt, i.p.) and (iv)Lithium chloride (Lesion+Li) (Li 40 mg/kg body wt, i.p.) After a period of 30 days postoperatively the rats were tested for behavioral alterations through a repertoire of tests like Novel Object recognition (NOR), spontaneous exploratory drive through Hole board test and spontaneous alternation by T-Maze. The brain samples harvested were used for histological and biochemical parameters. The viable pyramidal cell density in the various subfields of dorsal hippocampus was counted. White matter rarefaction in the corpus callosum was also examined and dendritic spine status was also assessed. Antioxidant propensity of GA curtailed the ROS generation by restoring the activity of Mitochondrial complex I and IV. The treated group exhibited 200% more consumption of H_2O_2 through catalase activity, lipid peroxidation was curbed by 50% and 250% increase was seen in reduced glutathione. However, Lithium chloride (Li) (a standard inhibitor of choice for GSK3 beta) exhibited significantly lower antioxidant status when compared to GA. This strong antioxidant defence has led to considerable restoration of pyramidal neuron density, prevented myelin rarefaction and restored twice as much as dendritic spines in GA treated than Li treated. GA treated rats showed 200% rise in exploration in holeboard, the spontaneous alternation was 150% increased and the discrimination index was twice as much as the lesion rats. The outcome of this study clearly implies that GA treatment posses a promising edge over the conventional Lithium chloride treatment and could mitigate the pathogenesis of neurodegeneration.

WTH02-25

Folic acid provides neuroprotection by modulating hippocampal oxidative imbalance and astrocyte response of hypoxic-ischemic rats

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The aim of this work was to investigate the effect of folic acid (FA) in rats submitted to hypoxia-ischemia (HI) evaluating memory in the ox-maze task, antioxidant defenses by superoxide dismutase (SOD) and catalase (CAT) activities and astrocyte response in the rats hippocampus. Groups of pup Wistar rats: (i) control treated with saline (CTS); (ii) CTFA; (iii) HIS and (iv) HIFA. On 7th postnatal day (P7) pups were submitted to HI model and treated with FA (P7-P22). At P22 rats were evaluated in the Ox-maze (10 sessions during 10 days to find a food reward in four boxes containing different symbols). Thereafter, the enzymes activity and glial scar (Optical density of GFAP) were measured. The HIS group had poor performance in the ox-maze, displaying a higher time to complete the task and number of incorrect nose pokes. These behavior deficits were reduced by FA administration. Additionally, a lower SOD activity was found in the hippocampus in the HIS group when compared to the others groups; the HIFA group showed only a partial decrease of enzymatic activity. The Catalase activity decreased in the HIS and HIFA comparing with the CTA group. The GFAP density increased only in the HIS group at P22. Concluding, neonatal HI resulted in cognitive deficits and FA attenuated these effects. Such behavioral impairment was associated to the decreased CAT and SOD activities and increased immunoreactivity for GFAP in the hippocampus. Folic acid administration appears to reverse, at least, partially these neurochemical parameters. Altogether, our findings suggest a potential role of FA as a neuroprotective agent on the neonatal HI.

WTH02-26

Blockade of $GABA_B$ receptor endocytosis enhances neuroprotection

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Metabotropic $GABA_B$ receptors ($GABA_BRs$) are heterodimeric G protein coupled receptors composed of R1 and R2 subunits that mediate slow inhibitory signalling in the brain. Consistent with their roles in mediating neuronal inhibition, deficits in $GABA_BR$ function play significant roles in both neurological and psychiatric disorders. We have previously reported that $GABA_BRs$ are intimately associated with protein phosphatase 2A and directly dephosphorylate S783 in the R2 subunit to enhance $GABA_BR$ endocytosis (Terunuma et al., *PNAS*, 2010). Thus it was considered that the endocytosis of $GABA_BRs$ mediated by dephosphorylation is of significance in synaptic plasticity and pathological conditions characterised by prolonged activation of glutamate receptors such as ischemia.

To test the role that phospho-dependent modulation of $GABA_BRs$ play in neuronal activity, we generated a knock-in mouse in which S783 was mutated to alanine (S783A) to prevent S783 dephosphorylation and degradation. Using these knock-in mice, we identified that S783A mice express stable $GABA_BRs$ on the plasma membrane by reducing receptor endocytosis (Terunuma et al., *J Neurosci*, 2014). Oxygen glucose deprivation (OGD) induced neuronal death in the wild-type neurons but not in S783A neurons suggesting a strong neuroprotective role of $GABA_BRs$. We also found that $GABA_BR$ signalling regulate caspase-3 activity which may be a key mechanism for neuroprotection.

WTH02-27

HIF 1-dependent normalization of pentose phosphate pathway in rat brain as a neuroprotective mechanism of hypoxic postconditioning

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Postconditioning (PostC) is an exposure of the damaged organism to extreme factors of the mild intensity to mobilize endogenous protective mechanisms. Method of PostC, which consists of three sequential episodes of mild hypobaric hypoxia, has recently been developed and validated in our laboratory. This model of hypoxic PostC was shown to improve the rats` rehabilitation after injurious severe hypobaric hypoxia (SH) by preventing neuronal loss, normalizing the lipid peroxidation process, the activity of the endocrine system and animal behaviour. In particular, it was demonstrated that hypoxic PostC up-regulates hypoxia-inducible factor-1 alpha subunits (HIF1a) level in the CA1 field of the hippocampus. Here, we have tested the hypothesis that hypoxic PostC induces neuroprotection through HIF1-dependent stimulation of pentose phosphate pathway activity. We have proved that SH suppresses the glucose-6-phosphate dehydrogenase activity in rat hippocampus, which leads to attenuation of reduced NADPH, total and reduced glutathione levels. This data correlate with decreased total antioxidant activity of cytosolic and mitochondrial subcellular fractions of rat hippocampus in this group. Meanwhile, hypoxic PostC normalizes the activity of glucose-6-phosphate dehydrogenase, stabilizes the NADP reduction process and causes a significant grow in total and reduced glutathione quantity and the rise of total antioxidant activity in the rat hippocampus. The excess reactive oxygen species generation is considered to provoke hypoxia-mediated neuronal death. Therefore, stabilization of processivity of antioxidant systems can play a key role in preventing the consequences of reoxygenation.

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WTH02-28

Stimulation of GLP-1 receptor alleviates ischemic stroke injury by elevating cerebral angiogenesis

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The study focuses on an innovative treatment in post-ischemic stroke and its mechanisms for alleviating ischemic brain injury and shortening recovering period. Maintaining neuronal viability after stress insults or traumatic injuries, such as oxidative stress or ischemic stroke, is crucial for better recovery and reducing mortality. In this study, we observed administration of exendin-4 (EX-4), an agonist of Glucagon-like peptide-1receptors, significantly enhances viability of brain cells after middle cerebral artery occlusion induced ischemic stroke. In the mean time, we found administration of EX-4 upregulated expression of VEGF and nitrogen oxide synthase (NOS) and triggered brain vasodilation and agiogenesis. Furthermore, the phosphorylated form of NOS, including neuronal NOS, inducible NOS, and endothelial NOS, were dramatically enhanced by EX-4 treatment. All lines of evidences indicated that stimulation of the GLP-1R has function on neuroprotection after ischemic stroke.

Activation of GLP-1Rs triggers several signaling pathways, including phosphatidylinositol 3 kinase, Akt; protein kinase C, mitogen-activated protein kinase; and adenylate cyclase, MEK, and Erk. According to the results, we suggested that activation of GLP-1Rs has a protective function against focal ischemic stroke via inducing expression of VEGF and NOS to enhance vasodilation and angiogenesis, which both protective effects are mediated by the GLP-1R downstream signaling pathways. We therefore postulated the enhancement of vasodilation and angiogenesis is a potential strategy for therapeutic intervention in ischemic stroke.

WTH02-29

Autophagic flux is impaired after ischemia-reperfusion exposure via cpkcgamma-MTOR signaling pathway in cortical neurons of mice

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Autophagy dysfunction has been indicated to play a critical role in cerebral ischemia. Although the intervention targeted autophagy is testified effective against ischemic injuries in a lot of studies, its regulatory signaling pathway is mostly obscure. In addition, the real significance of autophagy in ischemic injuries requires a deeply exploration. In the current study, it was found that the conversion ratio of LC3II/LC3I was increased while SQSTM1/p62 accumulation occurred in the cultured cortical neurons from mice after the oxygen glucose deprivation (OGD)/reperfusion exposure, suggesting that autophagic flux was impaired during this process. Further, the accumulation of p62 wasn't observed in PKCgamma KO mice, indicating PKCgamma took part in maintaining the smooth autophagic flux. Meanwhile, it was investigated that PKCgamma-dependent phospho-mTOR was increased at ser2481 site with western blot assay, and the co-localization of LC3 and LAMP1 was decreased with the confocal imaging. At the same time, the cell viability was decreased in PKCgamma KO mice compared with that of wild-type mice, indicating the blockage of autophagic flux was beneficial for neurons under ischemia/reperfusion exposure. The protein level of STX was not changed significantly, but its co-localization with autopahgosomes was decreased during this stage. To sum up, we draw the following conclusions from the current study: ①Autophagic flux may be impaired during the ischemia-reperfusion stage. ② PKCgamma-mTOR signaling pathway is identified to regulate the fusion between autophagosomes and lysosomes by influencing the STX anchoring to autophagosomes. ③ The impairment of autophagic flux suppressed the ischemic injuries. ④ The phosphorylation site at ser 2481 of mTOR is essential for the fusion of autophagosomes and lysosomes, and independent of the phospho-mTOR at the ser2448 that has been testified important for the upstream regulation of autophagy. ⑤ the binding of PKCgamma and mTOR is increased after ischemia-reperfusion. Together, our study provides a new insight of downstream regulation of autophagy in ischemic injuries, which may be a promising target for ischemic stroke.

WTH03 Synaptic Plasticity

WTH03-01

Assessing KCC2 functions using transport-deficient mutants

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Many neurological and psychiatric disorders such as epilepsy, schizophrenia, autism and Rett syndrome are associated with reduced expression of the neuronal chloride/potassium co-transporter KCC2. In mature neurons, chloride extrusion through KCC2 maintains low intracellular concentrations thereby ensuring chloride influx and membrane hyperpolarization upon $GABA_A$ receptor activation. However, recent studies revealed additional, ion transport-independent functions of KCC2 at excitatory synapses. Thus, chronic KCC2 down-regulation in hippocampal neurons reduces the efficacy and compromises long-term potentiation of glutamatergic synapses through modifications of actin dynamics in dendritic spines. These effects involve KCC2 interactions with intracellular partners, such as 4.1N and b-PIX that influence actin anchoring to the plasma membrane and polymerization, respectively. Whereas diuretics may be used to compensate for the loss of chloride export in pathological conditions associated with KCC2 down-regulation, they would fail to rescue ion-transport independent deficits.

In order to distinguish the impact of transport-dependent vs. independent functions of the transporter, we generated and compared several putative transport-deficient recombinant KCC2 bearing C568S, L675A or C287S/C302L/C322S/C331L mutations. Although reduced chloride efflux has been observed in heterologous cells expressing these mutants, whether this results from altered membrane expression or transport function remains unclear. We therefore compared membrane trafficking, protein interactions as well as chloride transport function of wild-type vs. mutant recombinant KCC2 both in Neuro2a cells and hippocampal neurons *in vitro*. Our data reveal that all mutants are correctly targeted to the plasma membrane. In addition, 4.1N or bPIX interactions with KCC2 mutants were compared using co-immunoprecipitation experiments in Neuro2a and $[Cl]_i$ was assessed using a highly sensitive chloride sensor for quantitative analysis. Altogether, our results will help us identify key residues involved in KCC2 function and characterize genuine, transport-deficient KCC2 retaining normal membrane expression and protein interactions. These mutants may then be used to specifically restore KCC2 transport-independent functions in pathological conditions.

WTH03-02

Changes in synapses induced by GLUN2A knockdown

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Synaptic plasticity refers to long lasting changes in synapses that have been related to the structural bases of memory and learning processes. For several years, NMDA receptors (NMDAR) have been involved in those process through different approaches. NMDAR are composed of two GluN1 obligatory subunits and two regulatory subunits GluN2 (A-D) or GluN3 (A-B). In hippocampus and other memory related brain structures, GluN2A and GluN2B are the most expressed regulatory subunits. Expression of these subunits is highly variable; GluN2B is expressed in immature and/or unstable synapses while GluN2A is expressed in mature, stable synapses. To better understand the role of GluN2A during memory acquisition and plasticity induction, we built an AAV vector carrying a shRNA anti GluN2A (AAV-sh2A) and the sequence of the eGFP protein; we also built an AAV carrying a shRNA scramble as control (AAV-shSc). In this work we analyzed changes in pre and postsynaptic markers in primary neuron cultures infected with AAV-sh2A or AAV-shSc. We observed a GluN2A expression decrease in GFP+ neurons transduced with the AAV-sh2A, compared with those transduced with the AAV-shSc. Surprisingly, GFP- neurons in cultures treated with the AAV-sh2A showed an increased GluN2A expression when compared with GFP+ and GFP- in cultures treated with the AAV-shSc. Then, we decided to investigate a presynaptic marker as Synapsin (Syn). We observed an increase in Syn dots that impact with GFP+ neurons in cultures infected with the AAV-sh2A. Finally, we asked if this increase was reflected at the postsynaptic side. We found that every Syn dot corresponded with a spine in the GFP+ neurons and, moreover, AAV-sh2A GFP+ neurons showed an increase in immature spines. We hypothesized that GluN2A decreased expression causes synaptogenesis, driven by 1) the maturation impossibility of preexisting synapses, 2) the lack of efficient connectivity in AAV-sh2A GFP+ neurons or a restriction in the dendritic arborization in those neurons.

WTH03-03

Cued memory reconsolidation in rats requires nitric oxide

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It is known that the reactivation of consolidated fear memory under protein synthesis blockade results in an impairment of memory, suggesting that the reactivated memory is destabilized and requires synthesis of new proteins for reconsolidation. It was shown earlier in our lab that nitric oxide blockade during reminder in *snails* prevented memory impairment induced by protein synthesis blockade (Balaban PM et al., 2014). In this work we tested the hypothesis of nitric oxide (NO) involvement in memory destabilization during the reconsolidation process in *rats*.

On the training day the rats were placed in Context A and after 2 min exposure to context received two auditory conditioned stimuli (CS) presentations (5 kHz, 75 dB, 30 s) that co-terminated with a 0.4 mA, 2 s foot-shock unconditioned stimulus (US). 48 h later memory reactivation test was performed by administering a single 30 s CS in context B. Immediately after the reactivation session the rats were twice intraperitoneally and subcutaneously injected with either DMSO (100%)+DMSO (1%) in NaCl 0.9% (control, $n = 15$) or DMSO (100%)+cycloheximide in DMSO (1%) (protein synthesis inhibitor, $n = 23$), or 3-Br 7-NI in DMSO (100%)+ DMSO (1%) (nNOS blockade, $n = 12$), or 3-Br-7-NI+CXM (protein synthesis and nNOS blockade, $n = 13$). 48 h later memory testing by administering a CS in context C was performed.

ANOVA Repeated Measures revealed that protein synthesis blockade after reactivation significantly impaired the fear memory to the CS (sound). Administration of the nNOS selective blockers 3-Br-7-NI alone or with CXM did not affect the freezing level. We concluded that NOS blockade in the conditions of reactivation of memory under a protein synthesis blockade prevented destabilisation of fear memory to the conditioned stimulus. Obtained results support the role of NO signaling pathways in the destabilisation of existing fear memory triggered by reactivation, and demonstrate that the disruption of this pathway during memory reconsolidation may prevent changes in long-term memory. Supported by RSF grant 14-25-00072.

WTH03-04

α-Mangostin improves hippocampal cholinergic enzyme activities and cognitive impairment in scopolamine-induced amnesic rats

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Hippocampal acetylcholine (ACh) plays a role in synaptic plasticity, including learning and memory. Hippocampal cholinergic enzymes are markedly depleted in Alzheimer's disease (AD) which is associated with cognitive deficits. The extract from the fruit rind of mangosteen (*Garcinia mangostana* L.) was recently reported to improve spatial memory in SCO-induced amnesic rats. α-Mangostin (α-MG) is an aprenylated xanthone derivative from the fruit rind of mangosteen. We examined whether α-mangostin (α-MG) improved activity of hippocampal cholinergic enzymes, behavioral alterations and cognitive impairment, in rats induced by administration of scopolamine (SCOP), anticholinergic agent that blocks the activity of the muscarinic acetylcholine receptor which commonly used as a model for AD. The ability of α-MG to improve the learning and memory performance in the SCOP-induced neurodegenerative rats was assessed using the Morris Water Maze (MWM) test. Rats injected with SCOP showed cognitive impairment and daily administration of α-MG improved memory function and increased learning behaviors. In hippocampal cholinergic system, the result found that α-MG increased the activity of choline acetyltransferase (ChAT) whereas decreased the activity of acetylcholine esterase (AChE). These findings suggest that α-MG has potential therapeutic value in alleviating SCO-induced cognitive deficits in rat hippocampus which its mechanism might be involved in regulating the hippocampal cholinergic enzymes and facilitating learning and memory.

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WTH03-05

The level of PKMζ MRNA in neuronal soma decreases after chemical stimulation of helix lucorum neurons

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Atypical protein kinase M zeta is considered to be one of the key regulators of memory formation in vertebrate and invertebrate animals. It was shown that PKMζ is synthesized *de novo* in synaptic spines after their activation. It's supposed that PKMζ concentration is regulated mostly on translational level. However, another possible way to regulate PKMζ concentration is to change transcription rate of its gene (Prkcz).

The aim of our study was to assess changes in PKMζ mRNA expression level in neurons after chemical stimulation. We used gastropod *Helix lucorum* as a model organism (it has giant neurons, which makes it possible to estimate mRNA expression even in a single neuron). Isolated snail nervous systems were activated *in vitro* by serotonin/caffein mixture application, then total RNA was extracted from ganglia or individual giant command neurons. Differential expression of Prkcz gene transcripts in experimental and control neurons was measured using RT-PCR or RNA-Seq.

Sequencing data indicated that Prkcz gene expression level decreased after neuronal activation. Using RT-PCR we measured levels of 2 different splice isoforms of PKMζ mRNA (which we discriminated earlier using 5′-RACE method), and quantity of both isoforms decreased in activated neurons.

These data may at first seem contradictory to the increase of PKMζ protein concentration in postsynapses demonstrated earlier by other researchers. If we consider that the decrease in PKMζ mRNA level revealed in our experiments was observed in somata only, but not in neurites that were not analyzed, then it's possible to speculate that after neuronal activation PKMζ mRNA is actively transported from soma to postsynapses where it's needed for LTP processes. So, the decrease of PKMζ mRNA level in soma doesn't necessary mean that transcription of the Prkcz gene is decreased. Additional experiments with mRNA level measurement in neurites are necessary to test this hypothesis.

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WTH03-06

A single ketamine neonatal exposure induces acute hippocampal susceptibility and cognitive and motor changes in adult rats

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The exposure to general anesthetics has been associated with neuronal apoptosis and changes in morphology of dendritic spines in the developing brain. Ketamine, a noncompetitive *N*-methyl-D-aspartate receptor (NMDAR) antagonist, is widely used in pediatric patients to induce general anesthesia, analgesia and perioperative

sedation. This study investigated, in an acute or long-term protocol, the hippocampal and frontal cortical cellular viability of rats exposed at postnatal day (PND) 7 to a single dose of ketamine (20 mg/kg by subcutaneous route) or saline 0.9%. In addition, biochemical and behavioral parameters were evaluated in adulthood (60 days-old rats). Neonatal administration of ketamine (PND 7) decreased the hippocampal but not frontal cortex cellular viability, 24 h after the treatment. None biochemical alteration (propidium iodide incorporation and L-[^{3}H] glutamate uptake) was observed in both hippocampus and frontal cortex of adult rats. The brain structures from neonatal rats displayed tolerance to glutamate excitotoxicity, while adult brain showed susceptibility, evidenced by the cellular viability reduction. Importantly, a single ketamine neonatal exposure prevented the glutamate-induced excitotoxicity in the frontal cortex of adult rats. Regarding behavioral analysis, an improvement in the motor function and short-memory deficit, evaluated respectively in the rotarod and novel object recognition task, was observed in the ketamine group in adulthood. Altogether, our data indicate that the hippocampal cellular viability decrease induced by a single neonatal ketamine exposure to rats can be linked, at least in part, with alteration in motor performance and short-term memory impairment in adulthood.

WTH03-07

Structural neuroplasticity of identified microcircuits investigated by Novel EM Probe Technology

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Our studies combine novel electron microscopy (EM)-probe technologies with transgenic mouse lines, aim to explore at high spatial resolution the structural modifications associated with neuroplasticity. We have previously shown that altering circuit activation in the brain by photoperiod changes the numbers of dopamine neurons. Specifically, after 1 week of long-day photoperiod, neurotransmitters released by hypothalamic neurons in the PVN switched from dopamine to somatostatin. The plasticity in neurotransmitter expression was coupled to dopamine receptor matching in the post-synaptic CRF-releasing cells and affected stress responses. Nothing is known about ultrastructural changes, like terminal sprouting and new synapse formation that might occur between pre- and postsynaptic elements during neurotransmitter switching. To this aim, we implemented novel genetically targetable probes designed for correlated light and EM (CLEM). MiniSOG is the first fluorescent protein genetically engineered for CLEM. More recently, we adapted a correlative nanobody against GFP to visualize any GFP-fused protein by light and EM via the engineered enzyme ascorbate peroxidase (APEX2), which can oxidize DAB into an EM contrasting agent. We crossed a CRE-driver line, which expresses CRE recombinase in CRF+ cells, with a TH-GFP reporter mouse line. The TH-GFP line was used as historical marker of TH expression since GFP can be detected in PVN neurons after neurotransmitter switching (when TH protein is no longer detected). The pre-synaptic cells were labeled using a nanobody against GFP fused to APEX2 introduced via viral infection. To label the post-synaptic cells, we introduced a floxed farnesylated MiniSOG via viral infection in the PVN. Next, we will reconstruct the pre-and post-synaptic cells using Serial Block face Scanning Electron Microscopy.

We expect to achieve a level of analysis on neuronal circuits with unprecedented details. The resulting tools could provide applications aimed at monitoring changes in microcircuit connectivity associated with neuroplasticity of any brain region.

WTH03-08

Autism-associated CASPR2 regulates synaptic AMPA receptors in the context of homeostatic synaptic plasticity

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During development and learning/memory-related events, the mammalian brain undergoes constant changes that can compromise its function. To prevent this, homeostatic synaptic plasticity mechanisms come into play, allowing experience-based adaptations to occur while maintaining neuronal network activity in-balance for proper brain function. One fundamental mechanism to achieve neuronal homeostasis is the dynamic regulation of AMPA receptors at glutamatergic synapses.

Herein, we describe a novel role for the cell-adhesion molecule Caspr2, implicated in autism and other neuropsychiatric disorders, in the regulation of synaptic AMPARs in the context of homeostatic plasticity. We demonstrate that loss of Caspr2 not only decreases the basal synaptic content of GluA1-containing AMPARs in cortical neurons, but also hinders the triggering of homeostatic synaptic scaling of AMPARs during prolonged neuronal inactivity. Accordingly, Caspr2 is further required for experience-dependent plasticity *in vivo*, since its loss in the mouse visual cortex (V1) prevents the scaling of AMPAR-mediated mEPSC amplitudes following chronic visual deprivation. Caspr2 is also a target antigen in autoimmune synaptic encephalitis. Remarkably, *in vitro* or *in vivo* incubation with patient-purified Caspr2 autoantibodies significantly decreases synaptic GluA1-AMPARs in cortical cultures and mEPSC amplitudes in V1.

Overall, we uncover a novel function for autism-associated Caspr2 in the regulation of synaptic AMPARs and homeostatic plasticity. Importantly, this evidence hints at a potential disruption of neuronal homeostasis following Caspr2 dysfunction in the context of disease, which is consistent with accumulating data implicating glutamatergic synapse dysfunction and impaired neuronal homeostasis as common underlying pathologies of several cognitive disorders, including autism.

WTH03-09

Identification of O-glcnacome/phosphoproteome interplay of synaptosome-associated proteins in sensorimotor cortex

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In human, a chronic reduction in neuromuscular activity through prolonged body immobilization alters motor task performance through a combination of peripheral and central factors. Studies performed in a rat model of sensorimotor perturbation have shown morphological and biochemical changes in sensorimotor cortex. However, the underlying mechanisms are still unclear. It is well known that phosphorylation regulates a wide field of the synaptic activity leading to neuroplasticity. Another post-translational modification that interplays with phosphorylation is O-linked-*N*-acetylglucosaminylation, termed OGlcNAcylation. This glycosylation is atypical, reversible and dynamic, and is involved in essential cellular and physiological processes such as synaptic activity, neuronal morphogenesis, learning and memory. Moreover, the interplay between phosphorylation and O-GlcNAcylation has been shown to play a critical role in neurodegenerative diseases. The objective of this study is to characterize the modulation of phosphoproteome/O-GlcNAcome interplay of synaptosomeassociated proteins in sensorimotor cortex after sensorimotor perturbation by differential proteomic analysis. Sensorimotor cortex synaptosomes were separated by sucrose gradient in order to isolate a subcellular compartment enriched in proteins involved in synaptic functions. Then, a multiplexed proteomic strategy was used to detect O-GlcNAcylated proteins, phosphoproteins, and the whole proteome within the same bidimensional gel. The O-GlcNAcome was revealed by the way of the Click chemistry and the azide–alkyne cycloaddition of a fluorophore on O-GlcNAc moieties. The phosphoproteome was stained by "Phospho-Tag phosphoprotein gel stain", while the whole proteome was visualized through Sypro Ruby staining. This method permitted, after sequential image acquisition, the direct in-gel detection of O-GlcNAcome, phosphoproteome, and whole proteome of synaptosome-associated proteins. Moreover, differential proteomic analysis of O-GlcNAcylated/phosphorylated proteins balance allowed us to identify key markers of synaptic plasticity induced by a period of sensorimotor perturbation.

WTH03-10

Prolonged changes in polysaccharide components of the brain extracellular matrix following photothrombotic stroke

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Perineuronal nets (PNNs) are brain extracellular matrix structures surrounding subset of GABAergic neurons. They stabilize neuronal connections, thus limiting synaptic plasticity. Decrease in PNNs densities was observed after stroke and it can be considered an attempt to create neuroplasticity conditions. We hypothesize the role of polysaccharide modifying enzymes in observed phenomenon, as PNNs are composed mainly of sugar moieties. Therefore, we investigated the expression of genes coding for enzymes directly involved in hyaluronic acid and chondroitin sulfate metabolism in mice subjected to photothrombotic stroke.

The expression of genes was analyzed in the perilesional area at earlier (4 h, 24 h, 7d) and later time points (1 month and 3 m) after unilateral photothrombotic ischemia. To investigate spatiotemporal mRNA expression qPCR method was employed. Immunohistochemical staining was used to analyze cellular localization of investigated enzymes.

We observed only ipsilateral changes, no changes in contralateral homotopic cortex were found. Analysis of early time points revealed increases in mRNA level of degrading enzymes that were accompanied with decrease in mRNA coding for some of the synthesizing enzymes. The increase in hyaluronidase 1 (HYAL1) expression was detected 24 h post-stroke and was still observed 1 month after photothrombosis. Prominent increase of hyaluronan synthase 2 (HAS2) mRNA level was detected 24 h after stroke. Elevated expression of HAS1 and HAS3, but not HAS2, was observed 1 month after stroke. At 1 month post-stroke, increased mRNA level of enzyme involved in chondroitin sulfate chain elongation, chondroitin sulfate synthase 3 (ChSy3), was observed. No change in mRNA level of enzymes modifying polysaccharide components of extracellular matrix in perilesional area was detected 3 months after stroke. Interestingly, elevation of protein level of investigated enzymes, which mRNA level change was observed at early time points, was still detected at 1 month post-photothrombosis.

Obtained data indicate prolonged changes in polysaccharide component of the brain extracellular matrix after stroke that may affect neuronal plasticity.

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WTH03-11

Development of a novel antiepileptic therapy for dravet syndrome by targeting the EEF2K/EEF2 pathway

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eEF2K is an ubiquitous Ca^{++}/Calmoduline-dependent kinase that regulates protein translation by catalyzing the phosphorylation of eEF2 (eukaryotic Elongation Factor 2). We have recently demonstrated that eEF2K/eEF2 pathway inhibits the synthesis of certain proteins involved in the function of brain inhibitory synapses (Heise et al., 2016). Thus, mice deleted of the eEF2K gene ($eEF2K^{-/-}$ mice) show potentiated GABAergic synapses and are less susceptible to drug-induced seizures than non-mutated mice. In order to investigate whether the inhibition of eEF2K can be a possible target for epilepsy treatment, we crossed $eEF2K^{-/-}$ mice with $Scn1a^{+/-}$ mice, a model of Dravet syndrome that is a genetic disease characterized by pharmaco-resistant epileptic seizures, cognitive impairment, elevated mortality and ataxia. Our preliminary data demonstrate that the phosphorylation of eEF2 in $Scn1a^{+/-}$ mice is higher compared to wild type mice, indicating a possible contribution of eEF2K/eEF2 pathway in altering excitatory/inhibitory balance in these mice. Electroencephalographic and electrophysiological experiments confirm that double $Scn1a^{+/-}$/$eEF2K^{-/-}$ mice are protected from epileptic seizures either under basal condition or under thermal stress by the increased of GABAergic transmission

(the frequency of spontaneous inhibitory post synaptic currents is higher in double *Scn1a*$^{+/-}$/eEF2K$^{-/-}$ mice than *Scn1a*$^{+/-}$ mice). Moreover our behavioral experiments suggest that also cognitive impairments of *Scn1a*$^{+/-}$ mice are rescued by the genetic deletion of eEF2K. Given that eEF2K inhibition is efficacious in reverting epileptic phenotype in another epilepsy model (Heise et al., 2016), our data suggest eEF2K as a possible new pharmacological target for the treatment of genetic form of epilepsy.

Heise C., Taha E., Murru L. *et al.,* 2016, *Cereb Cortex*:

WTH03-12

HCN channels in cerebellar and hippocampal neurons

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Amongst the various ion channels that contribute to initiation, coordination and modulation of neuronal signals, hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels play an essential role in the determination of biophysical properties of membranes. In contrast to typical voltage-dependent channels, these channels are activated at negative membrane potentials and their activation can additionally be modulated by direct binding of cAMP.

HCN channels affect neuronal excitability throughout the murine CNS. Here, we focus on two specific CNS regions, *i. e.* the hippocampus as the relay center for novel information during learning and memory processes and the cerebellum as the center of motor learning and motor control. Both regions are appealing targets for the study of synaptic plasticity due to their distinct cellular organization and defined pathways of signal relay.

First, we assessed the expression profiles of HCN channels in cerebellar and hippocampal neurons. We could show distinct expression patterns of the subunit isoforms HCN1, HCN2 and HCN4 on the RNA level as well as on the protein level. For in-depth analysis, we established cultivation of primary neurons, which retain the biochemical and electrophysiological properties of neurons *in vivo*. We addressed expression of HCN isoforms in primary neurons using super-resolution microscopy in order to gain insight on HCN isoform localization on the subcellular level.

Furthermore, we established recombinant adeno-associated viral (rAAV) vectors as efficient tools for the modification of neuronal cell function based on RNAi. Applying RNAi-mediating viral vectors *in vitro* and *in vivo* will allow us to investigate the functional contribution of individual HCN isoforms to hippocampal and cerebellar processes including neuronal excitability and learning behavior.

WTH03-13

Triclosan impairs hippocampal neuronal function

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Triclosan, an antibacterial and antifungal agent, is present in toys and in commonly used household products, such as toothpaste, detergents and soaps. However, the efficacy of triclosan is controversial and it has potential harmful effects; in the US the FDA has banned its use starting September, 2017. Nevertheless, further research on the effects of triclosan is required. Here, we investigated the effects of triclosan on a range of hippocampal functions. Addition of 1 uM triclosan to hippocampal neurons in primary cultures decreased the enhancement in spine density produced by the neurotrophin BDNF. Pre-incubation with the same concentration of 1 uM triclosan inhibited by 40% long-term potentiation induced by theta burst stimulation (CA3 to CA1) of rat hippocampal slices; higher concentrations of triclosan exerted a more drastic inhibitory effect. In addition, daily bilateral injections for 3 consecutive days of triclosan (1 ul, 10 uM) into the hippocampal CA3 area markedly reduced the ability of rats to perform a spatial navigation task. We propose that triclosan, at very low concentrations, has significant noxious effects on hippocampal function. Financial support: FONDECYT-1140545, FONDECYT-11140580 and BNI P-09-015F.

WTH03-14

Nitric oxide and ampa receptor trafficking

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The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) are composed of four types of subunits, designated as GluR1, GluR2, GluR3, and GluR4. GluR2 subunit blocks calcium influx into the cell, therefore the GluR2-lacking AMPARs are calcium-permeable (CP) channels and GluR2-containing AMPARs are calcium-impermeable (CI) channels. It has been demonstrated that long-term potentiation (LTP) in CA1 hippocampal pyramidal neurons causes rapid incorporation of GluR2-lacking calcium-permeable AMPARs: CP-AMPARs are present transiently, being replaced by GluR2-containing AMPARs ~ 25 min after LTP induction (Plant et al. 2006). A number of molecules is involved in this process including the nitric oxide (NO). Different hypotheses concerning the role of NO in AMPARs trafficking exist. One of them implies that NO regulates incorporation of GluR2-containing AMPARs into the cell membrane.

We tested whether it's true by blocking the NO-synthase (NOS) and GluR2-lacking CP-AMPARs. Experiments were performed using standard whole-cell patch clamp recordings from CA1 pyramidal neurons in acute hippocampal slices from 14-18 day old rats. CP-AMPARs blockade by adding PhTx-74 5-10 min after the LTP induction decreased the EPSC amplitude down to baseline. To our surprise, PhTx-74 application along with the NOS blockade by L-NAME did not lead to LTP reduction. Moreover, the nNOS inhibition by another blocker 3-brom-7-nitroindazole with

simultaneous CP-AMPA blockade did not reduce LTP either. Also, by measuring the rectification index we found that the balance of two types of AMPARs after LTP induction under NOS blockade is different in the control neurons. Obtained results can be explained by the fact that NO regulates GluR2-lacking AMPARs incorporation into the postsynaptic membrane.
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WTH03-15

Dopamine D1 receptor activation prevents the loss of long-term spatial memory in mice

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Hippocampal synaptic plasticity, in the form of long-term potentiation (LTP) and long-term depression (LTD), enables spatial memory formation. Dopamine, released from the ventral tegmental area particularly under conditions of reward, acts on the hippocampus, and may specifically influence the encoding of information into long-term memory. D1/D5 dopamine receptors are importantly involved in the regulation of synaptic plasticity thresholds in the CA1 region of the hippocampus and determine the direction of change in synaptic strength that occurs during novel spatial learning. Here, we explored whether D1/D5-receptors influence memory persistence without further stimulation. Using the Barnes maze paradigm, we found that mice would persist their spatial learning within 14 days, however, on the 21st day after training, they could not remember the spatial memory. Following the dopamine D1 agonist treatment, mice can remeber 40% further compared to the vehicle-treated control groups. This type of memory persistence would disappear upon dopamine D1 antagonist treatment. These findings suggest that the dopaminergic system, acting via D1/D5-receptors, influences spatial memory persistence and modulates the direction of change in synaptic strength that underlies information storage in the hippocampus. Memory reconsolidation is especially dependent on D1/D5-receptor activation. Thus, dopamine acting on D1/D5-receptors is likely to support specific experience-dependent encoding, and may influence the content of hippocampal representations of experience.

WTH03-16

Chronic treatment of combined chemotherapeutic agents alters neuronal architecture in the mouse hippocampus

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There is accumulating clinical evidence that chemotherapeutic agents induce long-term side effects, including cognitive impairment and mood disorders, in breast cancer survivors who have undergone chemotherapy. Although several preclinical studies have investigated the behavioral changes associated with hippocampal dysfunctions induced by anti-cancer drugs, the precise mechanism of chemotherapy-induced alterations in the anatomical structure of hippocampal neurons remains unknown. In this study, we investigated the detrimental effect of chronic treatment with doxorubicin (Adriamycin) and cyclophosphamide (AC) combination on neural architecture of the hippocampus in female mice. 4 weeks after chronic AC administration, histological changes in neuronal complexity and dendritic spine density and morphology in dentate gyrus (DG) granule and cornu ammonis (CA)1 pyramidal neurons were quantified using Golgi staining. Treatment of AC combination modified the dendritic morphology of hippocampal neurons, showing decreases in the total dendritic length and reduction of dendritic complexity in area CA1 apical and DG. However, AC treatment did not alter dendritic morphology in the CA1 basal dendrites. AC combination significantly reduced spine density and mature dendritic spines in the CA1, but did not alter dendritic spine density and morphology proportion in the DG. These findings indicate that AC treatment leads to alterations in micromorphometric parameters in the hippocampus in region specific manner. Thus, the alteration of neuronal architecture may be related with hippocampal dysfunctions due to anti-breast cancer chemotherapy.

WTH03-17

Hypobaric hypoxia dysregulates fear conditioning response by modulation of synaptic strength and dendritic morphology

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Fear learning is essential for survival but its generalization leads to disorders. This study aims to explore the effects of hypobaric hypoxia (HH) on synaptic plasticity correlates together with dendritic morphology in limbic brain regions and its consequence on fear conditioning.

Methods: Sprague–Dawley (SD) rats were divided into ten groups ($n = 7$): Normobaric Normoxia (NN) and HH [1, 3, 7, 14 and 21 days] each. Animals were trained for fear conditioning paradigm and exposed to HH under a simulated condition in an animal decompression chamber. Animals were tested for changes on cued and contextual fear conditioning. Synaptic plasticity in Medial Prefrontal cortex (mPFC), hippocampus, amygdala and dendritic morphology in amygdala were studied through immunohistochemistry and Golgi cox method respectively under HH.

Results: Results of the present study revealed significant decrement in fear memory as evident from decreased freezing time during 1 day ($p < 0.05$) and 3 day ($p < 0.0001$) of HH exposure, whereas no significant difference was found on day 14 and 21 of HH when compared to control group. Concurrently exposure to 3HH leads to reduction in synaptic strength by decreasing expression of PSD95 ($p < 0.05$), synaptophysin ($p < 0.05$) in hippocampus and mPFC, whereas amygdala showed increased synaptic strength by increasing expression ($p < 0.001$) of these markers. Additionally, synaptic morphology i.e. dendritic arborization, dendritic length, branch intersection and spine density significantly decreased ($p < 0.0001$) in basolateral amygdala on 3 day HH as compared to NN.

Conclusion: HH dysregulates fear conditioning which may be attributed to a differential role on synaptic strength in hippocampus, mPFC, and amygdala.

WTH03-18

Functional role of hyaluronan receptor CD44 palmitoylation in hippocampal neurons

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The extracellular matrix (ECM) and its modifiers function as important regulators of neuronal morphology and synaptic plasticity contributing to physiological processes such as learning and memory. One important player in ECM signalling is the hyaluronan receptor CD44 which has been proposed to regulate myelination, axonal growth, dendritic arborisation, synaptogenesis as well as neuronal excitability. Localisation and signalling properties of CD44 can be modified by its post-translational modifications. Palmitoylation is the most common post-translational lipid modification of proteins which represents the reversible attachment of the C16 saturated fatty acid palmitate to cysteine residue(s). Even though CD44 is known to be palmitoylated, the functional consequences of CD44 palmitoylation in the brain have not been studied yet.

Here we investigated the molecular mechanism of CD44 palmitoylation and its role in CD44-mediated regulation of neuronal morphology and synaptogenesis. We demonstrated that CD44 undergoes palmitoylation in different regions of the rodent brain. In rat hippocampal neurons, we found CD44 to be either non-palmitoylated or mono-palmitoylated. Using site-directed mutagenesis, we identified the cytoplasmic cysteine residue 298 as single palmitoylation site in rat CD44. Furthermore, by silencing endogenously expressed CD44 accompanied with the over-expressing a palmitoylation-deficient CD44 mutant we studied the effects of this lipid modification on CD44 function in hippocampal neurons.

WTH03-19

Regulation of the neuronal glycine transporter GLYT2 by P2X purinergic receptors

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Glycinergic inhibitory neurons of the spinal dorsal horn exert critical control over the conduction of nociceptive signals to higher brain areas. The neuronal glycine transporter 2 (GlyT2) is involved in the recycling of synaptic glycine from the inhibitory synaptic cleft and its activity modulates intra and extracellular glycine concentrations. In this report we show that the stimulation of P2X purinergic receptors with βγ-methylene adenosine 5′-triphosphate induces the rapid up-regulation of GlyT2 transport activity by increasing total and plasma membrane expression and reducing transporter ubiquitination. We identified the receptor subtypes involved by combining pharmacological approaches, siRNA-mediated protein knockdown, and dorsal root ganglion cell enrichment in brainstem and spinal cord primary cultures. Up-regulation of GlyT2 required the combined stimulation of homomeric $P2X_3$ and $P2X_2$ receptors or heteromeric $P2X_{2/3}$ receptors. By measuring spontaneous glycinergic currents in response to $P2X_3$ receptor agonists and glycine release and GlyT2 uptake in parallel, we could integrate GlyT2 modulation within the response of glycinergic neurotransmission to $P2X_3$ receptor activation. The recognized pro-nociceptive action of $P2X_3$ receptors suggests that the fine-tuning of GlyT2 activity may have consequences in nociceptive signal conduction.

WTH03-20

Effect of neurotrophin-4 in hippocampal synaptic plasticity induced by testosterone

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Background of the study: Neurotrophins are powerful molecular mediators of hippocampal synaptic plasticity and its electrical properties shape the structural organization of the synapse. Among these, Neurotrophins-4 (NT4) has emerged as having key roles in the neurobiological mechanisms related to learning and memory. Young hypo-gonad men, with low endogenous testosterone, are diagnosed with anxiety or depressive disorders and exhibit aberrant performance in some cognitive tasks. Indicating the sustenance or activation effect induced by testosterone towards enhances the spatial ability and the possible role of NT4 in adult hippocampus seems to be a prospective area to explore.

Method: Adult male Wistar rats were divided into four groups (n = 9): Sham, Orchidectomized (ODX), ODX+Testosterone (T) (5 mg/kg body weight) and sham+T. Animals were subjected to eight-arm radial maze (RAM) trial to evaluate the working and reference memory task (WME). Paraffin processed hippocampus tissues sections were stained with H&E, CFV, modified Trichrome and TUNEL staining. Dendritic arborizations of hippocampal pyramidal neurons were analyzed by Golgi-cox technique. Antioxidant level estimated biochemically and gene expression of NT4 signaling pathway via RT-PCR.

Results: In the RAM trial, ODX group showed a high number of WME and RME throughout the period. Whereas, T supplementation group showed significantly reduces WME and RME at the end of the trial sessions. Orchidectomized rat hippocampal cells showed altered cellular morphology, however, these changes were absent in ODX +T group. In the ODX rats, there was a reduced expression of NT4 and this has been restored in ODX+T supplemented rat hippocampus. These parameters were better in Sham+T rats.

Conclusion: Spatial memory impairment in ODX rats after T depletion confirms its positive effect on spatial learning and memory. Further, these results indicate that T presence reciprocally up-regulates the mRNA levels of NT-4. Thus, indicating the crucial role played by T in controlling NT4 expression and hippocampal neuronal plasticity, an essential modification for learning and memory.

WTH03-21

Expression of LTP-specific PRPS in prelimbic cortex region of the brain is necessary for the formation of long term memory

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Memory is one of the most fundamental processes of brain and we have not yet explored the complete notion of its underlying mechanism. Here we aimed to investigate the underlying process of "Behavioural Tagging" in long term memory (LTM) formation and to find the key factors playing role in consolidation of LTM. Behavioural tagging is a process which explains how short-term memory induced by a weak stimulus transforms into LTM when exposed to a novel environment in a critical time window. Here we have shown that how the process of "Behavioural Tagging" provides the necessary plasticity related proteins (PRPs) to stabilize LTM in adult Wistar rats. Therefore LTP-specific PRPs have been shown to play a significant role in both maintaining long term potentiation (LTP) and memory storage. For that intracerebroventricular (ICV) infusion of LTP-specific PRPs synthesis inhibitor in adult Wistar rats was done into the prelimbic cortex region of the brain which inhibited the activity of these PRPs and thus LTM which suggested that the inhibition of these LTP-specific PRPs in the prelimbic cortex region can disrupt consolidation of LTM. The results here indicate that memory consolidation-like events take place in the prelimbic cortex and these LTP-specific PRPs are essential components in LTM formation.

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WTH03-22

Lack of SEZ6 family proteins affects neuronal physiology and behaviour

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Excitatory synapse maturation and maintenance is a complex process that begins in early development and continues throughout life to facilitate learning. Characterisation of Seizure-related gene 6 (Sez6) knock-out (KO) mice revealed a role for Sez6 in patterning the dendritic arbor as well as in the development of excitatory synapses. Sez6 and related family members, Sez6 Like and Sez6 Like 2, are all expressed in neurons and have been found to have partially overlapping spatial and temporal patterns of expression indicating the possibility of functional compensation by these proteins. Additionally, it has recently been determined that all Sez6 family members are cleaved by the Alzheimer's protease β-APP cleaving enzyme 1 (BACE1). In order to investigate the roles played by Sez6 family proteins in the brain, a triple KO (TKO) mouse model was used in which all Sez6 family members are lacking. As previously reported, these Sez6 family TKO mice exhibited motor deficits on the accelerating Rotarod and reduced movement was seen in the elevated open field test. They also had behavioural deficits that indicate an impairment in cognitive flexibility. The TKO mice failed to extinguish their fear response in the context fear extinction paradigm and had difficulty changing their search strategy in the reversal portion of the Morris water maze. Along with the behavioural deficits of the TKO mice, alterations in the functional properties of neurons in the pre-limbic cortex were observed using electrophysiology. Additionally, pyramidal neurons in the somatosensory cortex of TKO mouse brains had altered dendritic spine structure with a shift away from the mature, mushroom shaped spines in favour of thin and stubby spines. Together these data reveal that the Sez6 family proteins are crucial for the structural and functional excitatory synapse plasticity that underlies flexible behaviour.

WTH03-23

Seizure-related gene 6 (SEZ6)family proteins and their influence on excitatory synapse function, motor co-ordination and cognition

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The Seizure-related gene 6 (Sez6) family of proteins, which inckudes Sez6, Sez6L and Sez6L2, are major β-secretase1 (BACE1) substrates. This protease is a therapeutic target in Alzheimer's disease and, while blocking BACE1 would be predicted to reduce toxic Ab production, it may also negatively influence Sez6 family protein dependent mechanisms. Sez6 is required for the normal development of excitatory neurons and constitutive Sez6 knockout (KO) mice display dendritic and synaptic abnormalities as well as motor and cognitive deficits. The persistence of Sez6 expression, particularly in the cortex and hippocampus, suggests ongoing roles for these proteins in the mature brain although it is not possible to separate these effects from those caused by abnormal development in the constitutive Sez6 KO mouse line. To investigate the functional roles of Sez6 family proteins, we have adopted a range of approaches utilizing an inducible Sez6 conditional KO model, Sez6L and Sez6L2 knockout mouse lines and a triple knockout (TKO) mouse model for Sez6 family proteins.

Using tamoxifen feed, we achieved near complete loss of Sez6 protein in calcium-calmodulin protein kinase II (CaMKII)-expressing hippocampal and cortical neurons. Behavioural tests indicate that Sez6 is required for normal expression of contextual fear memory in adult mice. Compared to controls, fear memory was enhanced in Sez6 cKO at 24 h and 7 days post-shock which is reminiscent of the persistent strong fear seen in Sez6 family TKO mice. Sez6L KO, Sez6L2 KO and Sez6 TKO mice exhibit deficits in behavioural tests of motor and memory function We conclude that Sez6 family proteins play important roles in the developing and adult brain particularly in learning and memory.

WTH03-24

TGF-β-sensitive neurons in the hypothalamus regulate food intake and body weight

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Regulation of feeding behavior is essential for survival and any deregulation can lead to metabolic pathologies such as obesity. In mammals, the hypothalamus is well known to orchestrate most metabolic processes including feeding. However, the specific neuronal populations and networks that control appetite and satiety are not fully understood. Here, we describe the role of distinct TGF-β-sensitive neurons in three hypothalamic regions: the paraventricular nucleus of the hypothalamus (PVN), the arcuate nucleus (ARC) and the lateral hypothalamic area (LHA) in the regulation of food intake and body weight. First, we find high numbers of TβR1- and Smad3-positive cells (TβR1: TGF-β receptor 1, Smad3: downstream transcription factor) in the PVN, ARC and LHA. To directly investigate the role TGF-β-sensitive neurons in the regulation of feeding and body weight, we used a combination of genetically engineered mice (TβR1 flox/flox, Smad3 flox/flox) and stereotactic viral injection (AAV-hsyn-GFP-Cre) that allows targeted deletion of TβR1 or Smad3. Loss of TβR1 and Smad3 in PVN neurons ($T\beta R1^{PVN}KO$ and $Smad3^{PVN}KO$) resulted in significant body weight gain and fat mass increase over time due to an increase in food intake. TβR1 deletion in the ARC ($T\beta R1^{ARC}KO$) resulted in a significant increase in body weight and food intake, but not to the same extent as the PVN. Further, Smad3 ablation in the LHA ($Smad3^{LHA}KO$) also resulted in an increase in body weight. We find that most TβR1- and Smad3 positive cells localized in the PVN express either oxytocin (OXT) or vasopressin (AVP). In the ARC, most TβR1 and Smad3-expressing neurons colocalize with POMC neurons. To further define the role of these neurons, we are conditionally activating TGF-β signaling using a "gain of function" model (LSL-TβR1CA) in the same regions. Taken together, these studies establish the importance of hypothalamic TGF-β-sensitive neurons in the central mechanisms of feeding behavior with implications to obesity pathogenesis.

WTH03-25

Presynaptic spike timing-dependent long-term depression in the CA1 region of the hippocampus

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Spike timing-dependent plasticity (STDP) is a model of synaptic plasticity that may underlie learning and memory. The aim of our research was to investigate the signalling pathway for the induction of spike timing-dependent long-term depression (t-LTD) in the hippocampus. Whole-cell recordings were made from individual CA1 cells in hippocampal slices prepared from P12-P18 mice. We have previously shown in the hippocampus that a post-before-pre pairing protocol (pairing postsynaptic action potentials with EPSCs at 0.2 Hz) produced robust input-specific t-LTD and that the induction of this form of LTD was completely blocked by D-AP5, by the broad spectrum mGluR antagonist MCPG, as well as by mGluR1 and mGluR5 selective antagonists, by phospholipase C (PLC) inhibitors and by the CB1 receptor antagonist AM251. The blockade of postsynaptic NMDARs by application of MK-801 (1 mM) through the patch pipette did not affect the induction of t-LTD (76 ± 8%, $n = 12$). We have also determined that this t-LTD requires astroglial signalling as is completely prevented by loading astrocytes with 20 mM BAPTA (129 ± 7%, $n = 5$ vs. interleaved slices 54 ± 6%, $n = 5$). Fluctuation, failures and paired-pulse ratios analysis all indicated a presynaptic locus of expression of this t-LTD. To further support a presynaptic locus of induction and expression of this form of LTD we recorded in the same neuron miniature responses before and after a post-pre pairing protocol. The frequency of miniature responses decreased from 0.4 ± 0.1 Hz (baseline) to 0.17 ± 0.06 Hz (after LTD) with no changes in mEPSCs amplitude. These results show that whereas t-LTP induction depends on postsynaptic NMDARs, the induction of t-LTD is independent of postsynaptic activation of NMDARs and likely requires presynaptic NMDA receptors. The results also show that the induction and expression of t-LTD at CA3-CA1 synapses is presynaptic.

WTH03-26

Role of matrix metalloproteinase-9 (MMP-9) in a chemically-induced synaptic plasticity

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Neuronal synapses are maintained by a complex network of adhesion molecules. MMPs known as extracellular proteases are able to modify synaptic function of which MMP-9 is of particular importance because of being able to change spine morphology by cleavage of postsynaptic adhesion molecules. However, it still remains largely unknown how MMPs may contribute to pre- and postsynaptic function. The aim of this study was to evaluate the effect of proteolytic activity of MMP-9 on the synaptic function. To test this theory, we pharmacologically inhibited the activity of MMP-9 in cultured hippocampal organotypic slices. Using whole-cell patch-clamp technique, AMPA receptor-mediated miniature excitatory postsynaptic currents (mEPSCs) were recorded from CA1 pyramidal cells. To induce synaptic plasticity, a cholinergic agonist named carbachol triggering rhythmic activity, which causes a lasting synaptic enhancement, was applied. One hour of carbachol treatment followed by an overnight incubation showed significant increase in frequency of mEPSCs. Interestingly, we observed that using MMP-9/13 inhibitor I along with carbachol further enhanced frequency of mEPSCs compared to carbachol and inhibitor I alone. Moreover, we observed the same result in MMP-9 knockout animal. Furthermore, evaluation of gelatinase activity by gel zymography in conditioned cultured medium indicated remarkable increase in the level of MMP-9 but not MMP-2 compared with control 24 h after carbachol treatment. In addition, preliminary result showed increase in spine density of group which we observed enhancement of mEPSCs frequency. Collectively, our results indicate that MMP-9 proteolytic activity can have a great impact on the synaptic plasticity.

WTH03-27

Protein methyltransferases 8 (PRMT8) restricts proteins associated with synaptic maturation in murine visual cortex

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The brain adapts to dynamic environmental conditions by altering its epigenetic state, thereby influencing neuronal transcriptional programs. An example of an epigenetic modification is protein methylation, catalyzed by protein arginine methyltransferases (PRMT). One member, *Prmt8,* is selectively expressed in the central nervous system during a crucial phase of early development, but little else is known regarding its function. We hypothesize *Prmt8* plays a role in synaptic maturation during development. To evaluate this, we used a proteome-wide approach to characterize the synaptic proteome of *Prmt8* knockout versus wildtype mice. Through comparative network-based analyses, proteins and functional clusters related to neurite development were identified to be differentially regulated between the two genotypes. One interesting protein that was differentially regulated was Tenascin-R (TNR). Chromatin immunoprecipitation demonstrated binding of PRMT8 to the *tenascin-r* (*Tnr*) promoter. TNR, a component of perineuronal nets (PNNs), preserves structural integrity of synaptic connections within neuronal networks during the development of visual-somatosensory cortices. On closer inspection, *Prmt8* removal increased net formation and decreased inhibitory parvalbumin positive (PV+) puncta on pyramidal neurons, thereby hindering the maturation of circuits. Consequently, visual acuity of the knockout mice was reduced. Our results demonstrated *Prmt8*'s involvement in synaptic maturation and its prospect as an epigenetic modulator of developmental neuroplasticity by regulating structural elements such as the PNNs.

WTH03-28

PER1-dependent molecular mechanisms behind daytime-dependent plasticity in mouse hippocampus

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The ability to convert transient stimuli into long-term changes of brain function is central to the capacity of an animal to adapt to a dynamic environment by learning. Coping with periodically recurring harmful or rewarding stimuli requires their efficient prioritization and a molecular machinery that is capable of associating, retaining, and recalling timing information. Hippocampus integrity ensures proper memory acquisition, consolidation, and retrieval. Notably, hippocampus-specific cellular and molecular dynamics that are associated with long-term memory (LTM) formation are clearly molded by time-of-day and depend on proper output from the master circadian (circa: about; dies: day) clock in the suprachiasmatic nucleus. We show that time-of-day-dependent LTM formation is tightly coupled to post-translational modifications and/or *de novo* gene expression of plasticity-related proteins, relies on intact cAMP/PKA/PKC/CREB signaling and requires chromatin remodeling. In addition, compelling evidence suggests that hippocampus-dependent LTM formation is mirrored in the plasticity of long-term potentiation (LTP): LTP efficiency, structural synaptic plasticity, synaptic excitability and the responsiveness to synaptic input follow a similarly clear circadian rhythm, depending on a dynamic expression of the clock gene PER1 in mouse hippocampus. These observations argue for an intricate interplay between the circadian system and memory, the mechanisms of which are not yet well understood. We here reveal in addition a PER1-dependent modulation of cytoplasmic-to-nuclear signaling in the murine hippocampus, providing a molecular explanation for how the circadian system potentially shapes a temporal framework for memory performance dependent on time-of-day, and adds a novel facet to the versatility of the clock gene protein PER1.

WTH03-29

PSD lattice and scaffold-adaptor protein model for PSD structure

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Postsynaptic density (PSD) is a dynamic structure, which is localized immediately underneath the postsynaptic membrane and works an essential devise for synaptic transmission and synaptic plasticity. A well-known model for architecture of PSD of type I excitatory synapse comprises of several scaffolding proteins including shank, PSD-95, GKAP and homer, to which various molecules involved in postsynaptic signaling are associated (scaffold/adaptor protein model). On the contrary, "PSD lattice" has been identified in the preparation obtained after treatment of synaptosome, SPM or Triton X-100-PSD with deoxycholate, a relatively strong detergent, and has been considered to be a basic backbone of type I PSDs before the proposal of scaffold/adaptor protein model. However, major constituents of the PSD lattice and the relationship between the PSD lattice and the scaffold/adaptor protein model have not been known. It is essential to know the details of molecular architecture of PSD for full understanding the mechanisms, at the molecular level, of dynamic nature of PSD, one of basis of synaptic plasticity. We purified a fraction that contained PSD lattice-like structures. The structure was recovered in the fraction slightly lighter than the pellet that contained PSDs. The lattice-like structure was planar, of which diameter was similar to PSD, sparser than PSD, and contained mesh-like woven fibers when observed in thin-section electron micrograph. Components of the structure were examined by Western blotting, immuno-dot blotting and immunogold negative staining electron microscopy. This study will give a new insight on the molecular architecture of type I excitatory PSD and new architecture model will be discussed.

WTH03-30

LMTK1 is a novel membrane bound kinase involved in anxiety and depression

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Lemur kinase 1 (LMTK1) is a novel Ser/Thr kinase, which is highly expressed in mammalian brain. There are two isoforms,

LMTK1A and LMTK1B; while LMTK1A binds to recycling endosome via myristoylation of the N-terminal cysteines, LMTK1B has transmembrane sequences at the N-terminal region. We report that LMTK1 regulates axon and dendrite elongation negatively through the transport of Rab11-positive recycling endosomes. Knockdown or knockout of LMTK1 enhances axonal outgrowth and dendrite arborization. We think that LMTK1 prevents overgrowth of axon and dendrites by controlling the supply of membrane components to the tip of neurites. The overgrowth of neurites caused by dysfunction of LMTK1 would result in developmental retardation or psychiatric diseases. Therefore, it is important to understand the mechanism regulating the neurite outgrowth by LMTK1. In this study, we analyzed expression of LMTK1 in mouse brains and behavior of LMTK1 knockout mice.

LMTK1 was expressed in neurons of cerebral cortex, hippocampus, cerebellum and olfactory bulb at postnatal day 5 and its expression increased gradually with aging. Quantitative PCR indicated almost equal expression of LMTK1A and LMTK1B in developing and adult brains. In adult brains, LMTK1 appeared to be also expressed in glial cells. Brain structures looked normal in LMTK1 knockout mouse when they were observed at the light microscopic level but when examined by an electron microscopy the synaptic vesicles in presynaptic terminus was more abandant in LMTK1 knockout mouse than wild-type mouse. Further, when dendritic arborization was examined in cultured cerebellum Purkinje cells, Purkinje cells of knockout mouse displayed larger dendrite expansion. LMTK1 knockout mouse exhibited abnormal behaviors, such as hyperactivity, reduced anxiety behavior and depression-like behavior. These results indicate that LMTK1 plays an important role in dendrite arborization and synaptic activity, and is involved in anxiety and depression.

WTH03-31

Remember or forget – dopaminergic modulation of auditory memory persistence in the presence or absence of alpha-synuclein

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Frequency-modulated tone discrimination (FMTD) learning of rodents induces elevated dopamine responses in auditory and prefrontal cortices during initial conditioning. We hypothesized that during early stages of FMTD learning cortical dopamine determines the efficiency of subsequent learning and memory formation. For the auditory cortex, we previously showed that local activation of D1/D5 dopamine receptors induces distinct proteome changes (including the nerve terminal-enriched protein alpha-synuclein), facilitates the stabilisation of newly acquired memory, and supports anterograde memory formation. To address the role of prefrontal dopamine, we now have utilised local infusion of D1/D5 receptor agonists into the murine medial prefrontal cortex shortly after the initial FMTD conditioning; treatment effects were monitored on subsequent 15 training days and after a training intermission of 4 weeks. Compared to vehicle-controls, prefrontal agonist treatments did not cause differences in the ascending section of the learning curve. However, at the asymptotic curve region and, in particular, during re-learning after the 4-week conditioning-free period of spontaneous forgetting, FMTD performance in D1/D5 agonist-treated mice was significantly affected. Interestingly, in mice of the C57BL/6JOlaHsd substrain, displaying a spontaneous deletion of the alpha-synuclein encoding *snca* gene, and in C57BL/6J mice with no such deletion, local post-acquisition activation of prefrontal D1/D5 receptors affected late memory performance in opposite directions, causing impairments in C57BL/6JOlaHsd mice and improvements in C57BL/6J mice. This is in line with our recent findings of substrain-dependent learning curve differences after systemic dopaminergic interference. Together, the results on the discrimination of complex sounds suggest that dopamine acting at D1/D5 receptors in different regions of the cerebral cortex during initial encoding may control both the temporary storage/retrieval of recent memories and a predisposition of neural networks essential for remote memory storage/retrieval. Opposite actions of pharmacological treatments in alpha-synuclein deficient and non-deficient mice imply a role of this protein in the dopaminergic modulation of memory consolidation.

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WTH03-32

Mechanisms of CDC42 palmitoylation in respect to neuronal functions

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Dynamic S-palmitoylation arose as an important regulator of signalling-protein functions critically involved in synaptic plasticity. The reversible attachment of the C-16 saturated fatty acid (palmitoylation) can modulate membrane insertion and sub-compartmentalisation of proteins. Palmitoylation emerged as pivotal modification of synaptic proteins, affecting key player of neuronal morphology and synaptogenesis. Here we investigated molecular details of the brain-specific isoform of small the GTPase Cdc42-palm. As a key modulator of cellular morphology, it plays an important role in regulating spine structural plasticity. We addressed the palmitoylation in more detail and explored functional consequences of neuronal signalling pathways. After identifying palmitoyltransferase DHHC5 as a protein responsible for Cdc42 palmitoylation, we could show that the enzyme favours a single cysteine residue at position 188 within Cdc42-palm as a site of palmitoylation. We also found that Cdc42-palm directly interact with the C-terminus of DHHC5. Functionally, DHHC5-mediated mono-palmitoylation of Cdc42 is necessary for the ability of Cdc42-palm to modulate dendritic morphology of hippocampal neurons and to regulate gene activation.

WTH04 Neuronal Polarity

WTH04-01

P2 receptors control the migration of medial ganglionic eminence-derived interneurons

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Cortical interneurons migration is a fundamental event for the development of the cerebral cortex. Defects in this process may underlie neurological and psychiatric conditions (Nat. Rev. Neurosci. 13(2):107-20). Thus, it is mandatory the identification of the mechanisms governing interneurons migration. It was recently shown that adenosine A_{2A} receptors contribute to interneurons migration (Sci. Trans. Med. 5:197ra104). We now found that P2Y1Rs are also expressed at mid-late stages of embryogenesis, coincident with the onset of interneurons migration. They are predominantly present in proliferative regions of mice developing telencephalon (E13), including the medial ganglionic eminence (MGE), and particularly in MGE-derived interneurons assessed by immunoreactivity and functionally identified by Ca^{2+} transients induced by the pharmacological activation of P2Y1R (MRS2365, 100 nM). In MGE explants cultures, the selective blockade of P2Y1R (MRS2179, 10 μM) significantly decreased the migration of interneurons from the MGE explants. This effect was mimicked by apyrase, which catabolizes ATP and ADP into AMP, being the migration restored upon the pharmacological activation of P2Y1R (MRS2365). This was arrested by the presence of a PKC inhibitor (BIM-1, 500 nM). These data shows that P2Y1Rs are expressed and promote MGE-derived interneurons migration through PKC activation. In contrast, the P2X-preferring agonist BzATP (1–100 μM) inhibited MGE-derived interneurons migration in a concentration-dependent manner, an effect prevented by the P2Rs antagonist PPADS (10 μM), by the selective antagonist o P2X1R, NF279 (1 μM), but not by the P2X7R antagonist, A438079 (10 μM). Moreover, we could detect immunoreactivity for P2X1R, but mostly in βIII-tubulin positive migratory neurons opposed to the predominant expression of P2Y1Rs in proliferative zones. Altogether, these evidences raise purinergic signalling in interneurons migration, supporting a scenario in which P2Y1R promotes interneurons migration as a motogenic factor in the MGE, while the P2X1R should be contributing for their guidance by transducing ATP as a repulsive cue.

WTH04-02

Novel mechanisms involving redox biology are essential to support axonal growth

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Physiological levels of ROS are important for several process in the nervous system, ranging from neuronal precursors proliferation, to axonal guidance and neurotransmission. ROS also support neurite outgrowth and axonal specification, but the mechanisms by which ROS are able to shape neurons remain unknown. We recently showed that NADPH oxidase activity is essential to sustain axon growth. Now, we report that Ca^{2+} release from the endoplasmic reticulum (ER) is coupled to ROS signaling dependent on NOX2. In this work, we explore the contribution of the link between NOX and RyR-mediated Ca^{2+} release towards axonal specification of rat hippocampal neurons. Using genetic approaches, we find that NOX activation promotes both axonal development and Rac1 activation through a RyR-mediated mechanism, which in turn activates NOX through Rac1, one of the NOX subunits. Collectively, these data suggest a feed-forward mechanism that integrates both NOX activity and RyR-mediated Ca^{2+} release to support cellular mechanisms involved in axon development. Finally, we explore the contribution of calcium entry from the extracellular milieu as a triggering factor to promote the concerted functions of NOX and RyR2 during axon specification.

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WTH04-03

Cortical principal neurons migration entails A2A receptor-driven neuronal polarization and axon formation

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Neuronal migration is a fundamental process in brain development. Indeed, impairment in neuronal migration is one of the major causes of cortical malformation (Neuron 60:273-84). Hence, it is of upmost importance to unravel the mechanisms driving neuronal migration. It was recently demonstrated that adenosine A_{2A} receptor ($A_{2A}R$) controls interneurons migration (Sci. Trans. Med. 5:197ra104). We now aimed to evaluate if $A_{2A}R$ is also involved in the migration of cortical principal neurons. We found that embryos lacking the $A_{2A}R$ ($A_{2A}R$-KO mice) showed a delayed migration of cortical principal neurons at embryonic day 17 (E17), in comparison to their wild-type littermates. Similarly, embryos exposed to the $A_{2A}R$ antagonist SCH58261 (daily 0.1 mg/kg i.p. injection in pregnant females E13-E16) presented delayed migration when compared with embryos exposed to vehicle. These effects should be due to $A_{2A}Rs$ expressed by migratory neurons, as *in utero* electroporation of plasmid encoding shRNA specific for $A_{2A}R$ at the same developmental stage (E14-E17) also delays migration. This delay in neuronal migration occurs mostly at the intermediate zone (IZ), where it is required a transition from a multipolar to a bipolar shape and the establishment of an axon-like leading process in order for neurons to proceed their migration towards the cortical plate (Nat. Neurosci. 12:1693-700). Accordingly, we found that mice

primary cortical neurons cultured in the presence of the $A_{2A}R$ antagonist SCH58261 (50 nM) leads to a reduction in the number of axons and in their length (DIV0-3). Altogether, these results demonstrate that $A_{2A}R$ is required for proper cortical principal neuronal migration, in particular for the transition from the intermediate zone into the cortical plate by controlling the establishment of neuronal polarity and axon formation.

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WTH05 Animal Model of Neuropsychiatric Disorders

WTH05-01

Changes in adult neurogenesis in chronic unpredictable mild stress and early-life inflammatory stress models in rats

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Chronic stress is a widespread condition involved in development of multiple brain disorders including depression and post-traumatic disorder. The exact way of long-term action of stressful impacts remains not completely understood. Here we provide the data concerning possible involvement of altered adult neurogenesis in the development of stress-associated brain pathology. We applied two paradigms of the chronic stress: chronic unpredictable mild stress (CUS) and early-life inflammatory stress (ELIS). In CUS paradigm, the rats were subjected to a series of stressful events including food and/or water restriction, cage tilt, crowded housing, isolation, and inversion of the light-dark schedule. Stressors were changed twice a day and presented randomly during 2 months. In the middle of CUS protocol, rats were injected with BrdU to assess the long-term effects of stress on differentiation of cells in the hippocampus. After completion of CUS protocol, behavior was analyzed, and animals were sacrificed for analysis of neurogenesis. In ELIS paradigm, bacterial lipopolysaccharide was administered intraperitoneally to rat pups on postnatal days 3 and 5 followed by BrdU injections, and behavior and neurogenesis were assessed later in adulthood, at the age of 3 months.

Two models of stress were accompanied by different changes in neurogenesis. The proliferation of precursor cells after completion of stress, assessed by PCNA staining, was unaffected in both paradigms. However, the neuronal differentiation assessed by doublecortin staining was suppressed by ELIS and enhanced by CUS. On the other hand, the number of new neurons and astrocytes generated from the cells which were born during early-life inflammation was increased in the dentate gyrus of rats subjected to ELIS. Our results suggest the difference between the ways in which these two stress paradigms influence the process of postnatal neurogenesis in the hippocampus of rats.

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WTH05-02

Optogenetic activation of striatonigral pathway is sufficient to induce obsessive-compulsive disorder-like behaviors

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fMRI studies in Human with obsessive compulsive disorder (OCD) evidenced an over-activity of the orbitofrontal cortex (OFC) to the ventral striatum (VS) projections. A recent study succeeded the modeling of OCD-like behavior in mice on the basis of clinical evidence; selective and repetitive activation of VS projecting OFC neurons induced a chronic, but not acute, over-grooming, which is relevant to human OCD phenotype. The next challenge of OCD-related circuit genetics is to clarify which cell type is involved in the pathogenesis of OCD-like behaviors. Indeed, VS projecting OFC neurons terminate on two distinct populations, called striatonigral and striatopallidal projection neurons, but which cell type mediates the pathogenesis is unknown. In this study, we hypothesized that the overactivation of striatonigral neurons caused the chronic over-grooming seen in study presented above. To selectively activate striatonigral neurons, we first generated transgenic mice in which step function channelrhodopsin2 (ChR2 (C128S)) was expressed by both striatal projection neurons (i.e. D1 receptors expressing medium spiny neurons and D2 receptors expressing medium spiny neurons). We then inserted an optic fiber onto the left ventral mesencephalon, enabling the selective illumination of striatonigral neurons axon terminals. Blue light illumination induced a contralateral rotation, suggesting the successful activation of striatonigral neurons. To further prove the opto-activation of striatonigral neurons, we examined the firing of putative connected neurons in the ventral mesencephalon. Fifty millisecond blue light illumination blocked the firing for over 15 seconds, indicating that optogenetically activated striatonigral neurons released GABA. Five second blue light illumination was given every minute, 5 times per day, for 5 consecutive days. Similarly to an OFC-VS projecting neurons overactivation, repeated activation of striatonigral neurons was sufficient to induce chronic over-grooming behaviors.

WTH05-03

Investigating mitochondrial biomarkers and function using MRS at 14.1 Tesla in a mouse model of mood disorders

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In vivo magnetic resonance imaging(MRI) and spectroscopy (MRS) are non-invasive techniques of choice for investigating and monitoring brain metabolic changes related to mitochondrial function and health. Mitochondria have been associated with many brain disorders and, among them, mood disorders. Defining and understanding mitochondrial MRI/MRS biomarkers related to mood disorders can help better characterizing endophenotypes of these psychiatric illnesses.

In this study we have investigated the MRI/MRS profile of a mouse model of mood disorders lacking an important brain

plasticity gene, *Crtc1* (CREB-regulated transcriptional coactivator 1).

Metabolic and volumetric profile alterations were determined with T_2-weighted MRI together with ^{1}H-MRS of prefrontal cortex (PFC) and dorsal hippocampus (HDors). Results indicated age-dependent alterations of glutamate and GABA levels in *Crtc1* KO mice PFC together with a constant reduction in phosphocreatine (PCr) energy metabolites in the dorsal hippocampus (PFC: Glu ($-12 \pm 3\%$), GABA($-26 \pm 11\%$); HDors: PCr($-20 \pm 8\%$)). qPCR experiments revealed no changes in electron transport chain (ETC.) gene expression but increased creatine kinase(CKMt and CKB) levels in the dentate gyrus of KO mice, confirming neuroenergetic deficiency in HDors. MtDNA copy number quantification revealed a reduction of mitochondrial mass in the dentate gyrus, which could explain the observed energetic dysfunction. Finally, preliminary ^{1}H[^{13}C]-MRS results upon infusion of [U-^{13}C] glucose suggested metabolic differences in the HDors with reduced glucose uptake or an increased glycolytic rate in KO animals. Together, these results suggest that CRTC1 might be an essential regulator of brain energy metabolism in the mouse HDors. Further investigations will aim at clarifying the mitochondrial failure of these mice and monitor its evolution with its associated MRI/MRS profile.

WTH05-04

Fluvoxamine maleate effects on dopamine signaling in the prefrontal cortex of stressed parkinsonian rats: a cognition implication

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Parkinson's disease (PD) also affects extra-striatal midbrain cells resulting in reduced extrinsic supply of dopamine (DA) to the prefrontal cortex (PFC). In the present study, we investigated the effects of reduced DA presence in the PFC on cognitive function and whether treatment with Fluvoxamine maleate (FM) attenuated these effects. Maternal separation was used to develop an animal model for early life stress that has chronic effects on brain and behavior. Sprague–Dawley rats were treated with the antidepressant FM prior to 6-hydroxydopamine (6-OHDA) lesion to model motor deficits in rats. The Morris water maze (MWM) and the forelimb use asymmetry (cylinder) tests were used to assess learning and memory impairment and motor deficits respectively. Blood plasma was used to measure corticosterone concentration and prefrontal tissue was collected for lipid peroxidation, DA, and serotonin (5-HT) analysis. Our results show that animals exposed to early life stress displayed learning and memory impairment as well as elevated basal plasma corticosterone concentration which were attenuated by treatment with FM. A 6-OHDA lesion effect was evidenced by impairment in the cylinder test as well as decreased DA and 5-HT concentration in the PFC. These effects were attenuated by FM treatment resulting in higher DA concentration in the PFC of treated animals than in non-treated animals. This study suggests that FM may ameliorate cognitive impairment in PD by preserving DA and 5-HT transmission in the PFC.

WTH05-05

Zebrafish larvae model: a novel approach to study autism

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Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder of early onset, highly variable in its clinical presentation. It includes the development of abnormal cortical circuitry that underlies autistic cognitive processes, social impairment and other behaviors. Although the animal models for autism do exist but have several disadvantages, which motivate us to design a new model for high throughput screening on autism. The aim of our study was to develop a cost and time effective model with a robust parameters to understand autism using zebrafish larvae, to overcome the shortcomings of rodent model on autism. Zebrafish embryos were treated with valproic acid and a battery of behavioral tests (markers of anxiety, fear, social impairment and irritability) was performed on larvae at seventh day post fertilization. This model shows a significant behavioural impairment in valproic acid treated larvae in comparison to control which was again supported by alteration in few marker genes and proteins involved in autism. This model was further validated using positive control drugs available for autism which reverts the phenotypic abnormalities. Thus we postulate that our 7 days larval model for autism can help in high throughput screening of new molecules on autism.

WTH05-06

Maternal and offspring MTHFR genotype contribute to autistic-like behavior in mice

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Mthylenetetrahydrofolate-reductase (MTHFR) has high prevalence of polymorphism (MTHFR677C > T) in autistic patients. We previously reported that in mouse model, maternal MTHFR-deficiency delayed morphogenesis and reflex development.

We hypothesized that maternal Mthfr$^{+/-}$ genotype increase the risk for autistic-like behavior in the adult mouse. That was tested by analyzing behaviors associated with core symptoms of autism. Susceptibility to PTZ-seizure was also tested due to high co-morbidity with epilepsy.

Three groups of 90-days old Mthfr$^{+/+}$ (Wt) and Mthfr$^{+/-}$ (Het) mice, representing maternal and offspring genotype, were tested; Wt-Wt, Het-Wt, Het-Het.

In the *open field* test, the frequency mice entered the center/center+margin was used to estimate their anxiety. Het-Het male exhibited lower anxiety compare to Wt-Wt and Het-Wt 0.33 ± 0.06; 0.23 ± 0.08; 0.18 ± 0.04, respectively ($p < 0.01$). Conversely, Het-Het female showed higher anxiety (0.14 ± 0.06; 0.22 ± 0.08; 0.26 ± 0.06; respectively, $p < 0.05$).

Mthfr$^{+/-}$ genotype interfered with recognition memory as tested in the object recognition test; offspring to Het dams spent shorter time exploring the new object versus Wt-Wt group (female-Het-Het-$25 \pm 6\%$ vs. $56 \pm 1\%$, $p < 0.03$; and male-Het-Wt $6 \pm 3\%$ vs. $29 \pm 9\%$ $p < 0.02$). In female, maternal-genotype had an additive

effect to the offspring-genotype, whereas in male it canceled the impact of offspring-genotype.

Sociability. Het-Wt male spent longer time exploring the empty versus mouse chamber whereas Wt-Wt and Het-Wt preferred the mouse chamber ($p < 0.01$). Preference to familiar versus novel mouse in Het-Het versus Wt-Wt female was indicated by longer delay to enter the unfamiliar mouse chamber (71 ± 21sec vs. 25.5 ± 8sec, $p < 0.04$). In the *resident-intruder test*, intruder Het-Wt male performed more aggressive behaviors, compared to Wt-Wt and Het-Het ($p < 0.05$).

Higher susceptibility to *PTZ-induced seizure* was obtained in Het-Wt male ($p < 0.01$ vs. Wt-Wt and Het-Het), as indicated by higher convulsions score and higher number of events ranging between head- and forelimbs-myoclonus to generalized tonic-clonic seizure.

Maternal- and offspring-Mthfr$^{+/-}$ genotype contribute to autistic-like behavior, however, results suggests the presence of some compensatory mechanism in Mthfr$^{+/-}$ offspring due to in-utero exposure to the deficiency.

WTH05-07

Ondansetron reverses depressive phenotype in diabetic mice by normalizing hippocampal neuronal atrophy and reduced bdnf levels

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It is well established that persistent diabetes may lead to neuronal atrophy, characterized by loss of synaptic connections in key limbic brain regions, implicated in depression. This is thought to occur, in part, via decreased expression and function of growth factors, such as brain-derived neurotrophic factor (BDNF), in hippocampus. We previously found that ondansetron, a selective 5-HT3 receptor antagonist ameliorated depressive phenotype evoked in streptozotocin (STZ)-induced diabetic mice. However, the plausible mechanism of its action remains unknown. Therefore, this study aimed to determine whether ondansetron was able to reverse diabetes-induced neuronal atrophy and low BDNF levels in hippocampus, along with depression-like behavior in mice. Ondansetron (0.5–1 mg/kg/day, intraperitoneally) was given to 8-week (STZ-induced) diabetic mice for 28 days followed by tail suspension test (for depression-like behavior) and open field test (for anxiety-like behavior). 24 hrs after behavioral assays, brains were collected and hippocampi were isolated, which were then subjected to Golgi-Cox stain procedure for dendritic morphological changes quantified by Sholl method and enzyme-linked immunosorbant assay for determination of BDNF levels. The results showed that STZ-induced diabetic mice exhibited a significant reduction in dendritic length and number of intersections in pyramidal neurons of CA1 region and BDNF levels of hippocampus along with pronounced depression and anxiety-like behavior. Chronic ondansetron treatment significantly reversed these behavioral, neurochemical and morphological perturbations in diabetic mice. Our results extend the previous findings demonstrating neuronal atrophy and reduced neurotrophic factors signaling associated with depression in diabetes and evidence that these processes correlate to antidepressant-like effects of ondansetron.

WTH05-08

The oligodendroglial abnormalities in the postmortem brain from human patients and monkey depression model

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Recent postmortem brain studies of patients with psychiatric disorders have revealed several abnormalities in neurons and glia. We have developed a FACS method to count numbers of neuronal (NeuN+), oligodendroglial (Olig2 +), and astroglial/microglial (NeuN−/Olig2−) cells, which enables us to find that the number of oligodendroglia is decreased in the frontopolar cortex of postmortem brain from patients with major depressive disorder (Hayashi et al., Mol Psychiatry, 2011). The oligodendroglial progenitor cells exist in the adult mammalian brain, which are proliferating at least in rodents, and therefore, it would be reasonable to consider that the oligodendroglial abnormalities play critical roles in the pathogenesis of psychiatric diseases. In this study, in order to clarity the oligodendroglial abnormalities in major depressive disorder, we first analyzed the oligodendrocyte progenitor cells in the adult primate brain and found that Ki67 + /Olig2 + proliferating oligodendrocyte lineage cells in both gray and white matter in the cortex of macaque monkey (*macaca fascicularis*) and human. Second, we have tried to establish a non-human primate model of major depressive disorder by chronic administration of interferon-alpha, which often causes depression in human patients with hepatitis and cancer. Some behavioral changes were observed in the depression model monkeys such as the time of food intake and body shaking and preference of position in a cage after chronic administration. The oligodendroglial DNA was extracted from the monkey brain by FACS sorting and was subjected to the methylation analysis of Sox10 promoter region, which is associated with oligodendroglial differentiation. We observed a trend of hypomethylation in the Sox10 promoter of oligodendroglial DNA in the depression model monkeys as compared with that of controls.

WTH05-09

5-HT2A receptor deficiency alters the metabolic and transcriptional, but not the behavioral, consequences of CUS

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Chronic stress enhances risk for psychiatric disorders, and in animal models is known to evoke depression-like behavior accompanied by perturbed neurohormonal, metabolic, neuroarchitectural and transcriptional changes. Serotonergic neurotransmission, including serotonin$_{2A}$ (5-HT$_{2A}$) receptors, have been implicated in mediating specific aspects of stress-induced responses. Here we investigated the influence of chronic unpredictable stress (CUS) on

depression-like behavior, serum metabolic measures, and gene expression in stress-associated neurocircuitry of the prefrontal cortex (PFC) and hippocampus in 5-HT_{2A} receptor knockout (5-$HT_{2A}^{-/-}$) and wild-type mice of both genders. While 5-$HT_{2A}^{-/-}$ male and female mice exhibited a baseline anxiolytic state, this did not alter the onset or severity of behavioral despair during and at the cessation of CUS, indicating that these mice can develop stress-evoked depressive behavior. Analysis of metabolic parameters in serum revealed a CUS-evoked dyslipidemia, which was abrogated in 5-$HT_{2A}^{-/-}$ female mice with a hyperlipidemic baseline phenotype. 5-$HT_{2A}^{-/-}$ male mice in contrast did not exhibit such a baseline shift in their serum lipid profile. CUS evoked gene expression changes in specific stress-responsive genes (*Crh*, *Crhr1*, *Nr3c1, and Nr3c2*), trophic factors (*Bdnf*, *Igf1*) and immediate early genes (IEGs) (*Arc*, *Fos*, *FosB*, *Egr1–4*) in the PFC and hippocampus, with the pattern altered in 5-$HT_{2A}^{-/-}$ mice both under baseline and CUS conditions. Our results support a role for the 5-HT_{2A} receptor in specific metabolic and transcriptional, but not the behavioral, consequences of CUS, and highlight that the contribution of the 5-HT_{2A} receptor to stress-evoked changes is sexually dimorphic.

WTH05-10

Reduced interneuron density in the hippocampus and anti-despair-like behaviors in RALBP1-mutant mice

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Inhibitory interneurons in the hippocampus play an important role in the control of the network stability and the hippocampal output. Mice deficient of RalBP1, a downstream effector of the small GTPase RalA and RalB, display reduced synaptic inhibition in the hippocampus. However, the cellular mechanisms and behavioral role of reduced synaptic inhibition in the hippocampus of RalBP1-mutant mice are unknown. Here we show that RalBP1 deficiency induces reduction of interneurons in the hippocampus and anti-despair-like behaviors in mice. RalBP1-mutant mice exhibit reduced density of GABAergic interneurons in the hippocampus and decreased immobility during both the tail-suspension and forced swim tests. However, deficiency of RalBP1 does not induce anxiety and anhedonia. $GABA_A$ agonist muscimol reverted anti-despair-like behaviors in RalBP1-mutant mice. In addition, anti-despair-like behaviors were induced by suppressing GABAergic transmission in CA1 neurons of WT mice. These results suggest that inhibitory synaptic transmission in the hippocampus may regulate behavioral despair.

WTH05-11

Gestational vitamin d treatment blocks behavioural phenotypes relevant to schizophrenia induced by maternal immune activation

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Maternal infection and developmental vitamin D (DVD) deficiency are well-validated risk factors for developmental neuropsychiatric disorders such as schizophrenia. Growing evidence from animal models of maternal immune activation (MIA) using poly (I:C), a viral mimic double-stranded RNA, and DVD-deficiency suggests a shared pathophysiological pathway, altered dopamine neurodevelopment, persistently leading to behavioural phenotypes relevant to schizophrenia in offspring. To test this hypothesis, we administrated the active form of vitamin D, $1,25(OH)_2D_3$, subcutaneously to C57BL/6 mouse dams at gestation day (GD) 9 that simultaneously received intravenous injection of poly (I:C). Vitamin D treatment abolished the MIA-induced schizophrenic behavioral abnormalities in offspring, including positive symptoms (amphetamine induced hyperlocomotion and prepulse inhibition), negative symptoms (social interaction deficit) and cognitive symptoms (fear conditioning). To investigate vitamin D's protective mechanism, we considered its well-known immune regulatory functions. However, vitamin D had no effects on MIA-induced elevations of pro-inflammatory cytokines (interlukin-6, interlukin-1 beta or tumor necrosis factor alpha) in maternal plasma or fetal brain. Secondly, we assessed vitamin D's actions on the ontogeny of dopamine neurons at GD11, the earliest time point of dopamine neuronal differentiation in mice. We established an automated image analysis method using the CellProfiler software and state-of-the-art spinning disk confocal microscopy. Quantitatively immunochemistry data revealed that MIA altered the ratio of mature to immature dopamine neurons (DAs) in fetal midbrain (two-way ANOVA, $F(1,22)=14.923$, $p < 0.01$), which was restored by vitamin D (two-way ANOVA, $F(1,22)=6.536$, $p < 0.05$). In addition, we found that vitamin D increased the expression of a key dopamine differentiation factor, the nuclear receptor related 1 protein (Nurr1), and the dopamine synthesis enzyme, tyrosine hydroxylase in individual post-mitotic DAs in both control and poly (I:C)-treated fetal midbrain (two-way ANOVA, $F(1,22)=15.478$, $p < 0.01$). Taken together, these findings suggest vitamin D promotes the development of midbrain DAs and that such actions may be neuroprotective for DAs when subjected to a maternal stressor. This may account for vitamin D's ability to restore normal behaviors regulated by dopamine, and raises the possibility for future prophylactic strategies for schizophrenia using dietary vitamin D.

WTH05-12

Neuroadaptations in the dorsal striatum and escalation of methamphetamine self-administration across adolescence

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Methamphetamine (meth) is a powerful psychostimulant and the second most common abused illicit drug worldwide. In Australia, age of Meth use has significantly dropped, where two percent of Australians aged 12–14 have experimented with it recently. As adolescence is a period known for its vulnerability to develop addiction, we used the intravenous self-administration paradigm to compare meth abuse-related behaviours in adolescent and adult rats. We first observed a consistent escalation of meth intake in adolescents with dose increase following acquisition (ps < 0.01). Therefore, we hypothesised that meth during adolescence causes more drastic neuroadaptations compared to meth during adulthood.

To test this hypothesis, we performed genome wide transcriptome analysis following acquisition of meth self-administration in the dorsal striatum, which is involved in the transition from goal directed to habitual behaviour. A list of 30 differentially expressed genes (ps < 0.01, fold change > 2), in the adolescent meth compared to saline, was generated. No gene expressions were significantly changed in adult rats. Of particular interest were the downregulation of SLC18A1 (that codes vesicular mono-amine transporter 1 (VMAT1)), and upregulation of GFRA1 (that codes GDNF family receptor alpha 1). Polymorphisms in SLC18A1 are linked to schizophrenia, bipolar disorder and anxiety in humans. GFRA1 is expressed in dopamine neurons and involved in injury response in the substantia nigra. Western blotting replicated the downregulation of VMAT1 at the protein level in the dorsal striatum in meth administering adolescent rats ($p < 0.05$). GDNF protein levels are yet to be determined. Taken together, my research has developed a novel behavioural and genetic model of adolescent vulnerability to meth addiction and has found potential new targets for treatment.

WTH05-13

Neuroplastin ablation causes retrograde amnesia and circuit-dependent deficits correlated to loss of neuroplastin-PMCA complexes

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We investigate the role of Neuroplastin in memory acquisition, consolidation, storage and retrieval, which are critical processes affected in psychopathological disorders, but mechanistically not sufficiently understood. Neuroplastin cell recognition molecules are implicated in activity-dependent synaptic plasticity and intellectual abilities and essential for associative learning in conditioning paradigms e. g. two-way active avoidance and fear conditioning. In addition, neuroplastin-deficient mice reveal profound physiological and behavioral deficits, some related to depression and schizophrenia, illustrating neuroplastins' essential functions. By inducible ablation of *neuroplastin* gene expression specifically in neurons of adult mice ($Nptn^{lox/loxPrp1CreERT}$ mice), we elicit retrograde amnesia of learned associative memories and show that neuroplastins are indispensable for access and retrieval of previously acquired associative memories. In contrast, Np ablation selectively in glutamatergic neurons ($Nptn^{lox/loxEmx1Cre}$ mice) causes particular behavioral deficits indicating hippocampal, striatal, and sensorimotor dysfunctions, but intact associative learning. These results reveal that neuroplastin expression in distinct neuronal sub-types and circuits commands particular behaviors. Furthermore, neuroplastin expression by GABAergic interneurons appears to be essential for associative learning in conditioning paradigms. Potentially, neuroplastin participates in disinhibition of GABAergic cortical interneurons that is required for associative fear conditioned learning. Neuroplastin-deficient mice display reduced levels of Plasma Membrane Ca^{2+} ATPases (PMCAs), an essential regulator of the intracellular Ca^{2+} concentration ($[iCa^{2+}]$) and neuronal activity. Altered hippocampal and cortical activities correlate with reduction of distinct PMCA paralogs in $Nptn^{lox/loxEmx1Cre}$ mice and increased $[iCa^{2+}]$ in cultured mutant neurons. Human and rodent Neuroplastin enhance the post-transcriptional expression of and co-localized with PMCA paralogs in the plasma membrane of transfected cells. Our results show that Neuroplastin is essential for PMCA expression in neurons allowing proper $[iCa^{2+}]$ regulation and normal circuit activity. Neuron-type-specific Neuroplastin ablation empowers the investigation of circuit-coded learning and memory and identification of causal mechanisms leading to cognitive deterioration.

WTH05-14

Repeated ascorbic acid treatment produces antidepressant-like effect and modulates cell survival signaling pathways in swiss mice

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This study aimed to investigate the ability of a 21-day ascorbic acid administration to produce an antidepressant-like effect in the mouse tail suspension test (TST). Additionally, we examined the effect of this vitamin on hippocampal and cerebrocortical brain-derived neurotrophic factor (BDNF) immunocontent, phosphorylation of protein kinase B (AKT), extracellular signal-regulated kinase (ERK), $p38^{MAPK}$ and c-Jun N-terminal kinase (JNK) by Western Blotting. Female Swiss mice received a daily oral (by gavage) administration of ascorbic acid (0.1 and 1 mg/kg) or fluoxetine (10 mg/kg, positive control) for 21 days. The TST was performed 24 h after the last drug administration. Five minutes after the TST, the same group of animals was evaluated in the open field test as a control of general locomotor activity. Immediately after behavioral observations, hippocampi and cerebral cortices were dissected for neurochemical evaluation. Ascorbic acid (0.1 and 1 mg/kg) or fluoxetine (10 mg/kg) administration elicited an antidepressant phenotype in the TST, with no change in locomotor activity in the open fiel test. Ascorbic acid at 1 mg/kg caused an increase in AKT phosphorylation in the cerebral cortex of mice. Ascorbic acid treatment (1 mg/kg), similar to fluoxetine, decreased hippocampal $p38^{MAPK}$, but did not alter ERK or JNK phosphorylation. This study explored, for the first time, the intracellular pathways involved in the antidepressant properties of repeated ascorbic acid administration. We demonstrated that ascorbic acid anti-immobility effect in TST is accompanied by a modulation of AKT and $p38^{MAPK}$, but not BDNF, ERK and JNK, suggesting that these targets play a significant role for its behavioral response in the TST.

WTH05-15

Serotoninergic depletion generates aggressive behaviour in male Sprague–Dawley rats

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The serotoninergic system modulates appetitive, motivational and aggressive behaviour. The 5-HT synthesis reduction by drug increase aggressive behaviour in species and strains prone to it.

In this study we attempted 1- to generate aggressive behaviour with para-chlorophenylalanine (pCPA) by inhibiting tryptophan hydroxylase, and 2- to examine dose/day response related to 5-HT depletion.

Male Sprague–Dawley 60 days old rats were used. A resident/intruder paradigm was applied. Animals were divided into 5 groups: Naïve, pCPA treated rats (300 mg/kg, i.p) evaluated on the 3rd and 6th day after the administration, and the respective saline control.

Offensive behaviour (OB) was measured as attempted mounts, lateral threats and footsteps. Persecution latency time (PLT) was examined as a different parameter of OB. Bites, clinch and clinch attacks were considered as aggressive behaviour (AB). We also measured non-social interaction (freezing, lying, sitting and grooming), social interaction (sniffing and heterogrooming) and locomotor activity. The test was recorded and the videos were analysed with Kinovea 0.8.15 software. 5-HT levels were measured in plasma, olfactory bulb and raphe nucleus with HPLC fluorescence. All data were analysed by ANOVA I and Tukey *post Hoc* test.

We observed a significant decrease in PLT ($p \leq 0.001$) and a significant increase ($p \leq 0.001$) in OB between the treated, control and naïve groups. There was a significant increase ($p \leq 0.05$) of AB between the treated group tested on the 3rd and 6thday. There were no significant differences in social interaction, non-social interaction and locomotor activity. The treated groups showed a significant decrease of 5-HT levels in plasma, olfactory bulb and raphe nucleus versus control and naïve groups depending on the day of evaluation. On the 3rd day the difference was $p \leq 0.01$ and on the 6th day $p \leq 0.05$.

The depletion of 5-HT affects OB, PLT and AB. This is related to dose/day response, suggesting that a certain 5-HT level is necessary to generate aggressive behaviour.

WTH05-16

Administration of allopregnanolone and S-norfluoxetine upregulate reelin expression and improve depressive/anxiety-like behaviors

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Neuroactive steroids, including the GABA-A receptor active, allopregnanolone (ALLO) and its isomer, pregnanolone are downregulated in major depression and post-traumatic stress disorder (PTSD). SSRIs normalize their levels in treatment responders. Likewise, animal models of depression and anxiety, such as the social isolation and the forced swimming test (FST), suggest that ALLO is involved in emotional and cognitive behavioral dysfunction and its levels increase improves these deficits. Social isolation induces several other neurochemical alterations, including a corticolimbic downregulation of reelin expression. The amygdaloid nuclei are part of a crucial brain circuitry involved in emotional regulation and experimental intervention that increase ALLO in this region show antidepressant and anxiolytic effects. The aim of the present study was to compare the antidepressive and anxiolytic effects of ALLO administration with those of the neurosteroidogenic drug, S-norfluoxetine (2 weeks, twice daily) after social isolation in mice, and to analyze the corticolimbic expression of reelin mRNA after those treatments. We further investigated the behavioral effects of the bilateral intra-amygdala infusion of recombinant reelin. Social isolation in mice induced depressive-like effects (FST), aggression, and anxiety-like behavior that were improved by S-norfluoxetine and ALLO. ALLO or S-norfluoxetine also increased the expression of reelin mRNA in the hippocampus, amygdala and frontal cortex of socially isolated mice. Infusion of reelin in the amygdala induced a long-term (2 weeks) improvement of anxiety and aggression. These results suggest that the long-term improvement of emotional behavior of ALLO and S-norfluoxetine may be mediated by upregulation of reelin expression, which suggest new neural target for treatment of depression and PTSD.

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WTH05-17

Proteomic analysis of rat saliva proteins for stress biomarkers after mental and physical stress loading

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Saliva is a useful sample non-invasively collected from body fluid. Our objective in the present study is to search for saliva biomarkers for the differentiation between physical and mental stress for qality of life. Quite recently, we examined rat saliva marker proteins for mental stress by proteome using a rat mental stress model. The increased proteins by mental stress were subjected to liquid chromatography-mass spectrometry/mass spectrometry. We detected the known enzymes and secretary proteins with MW of 20–70 KDa in rat saliva proteins. In the present study, we analyzed the biomarkers for physical stress by proteome after treadmill running loading to rats. After the separation by SDS-PAGE, the increased proteins by physical stress were used for LC-MS/MS and we further analyzed the saliva proteins using a comprehensive proteomic analysis (isobaric Tags for Relative and Absolute Quantitation, iTRAQ). Finally, we might find biomarkers for mental and/or physical stress. This study (No.25350824) is supported by Grant-in-Aid for Scientific Research (KAKENHI); Grant-in-Aid for Scientific Research(C) from 2013 to 2015.

WTH05-18

Transgenerational impact of paternal exercise: rhoa gtpase family proteins and regulatory micrornas in offspring hippocampus

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There is growing evidence that perinatal paternal environments and lifestyle factors influence offspring metabolic and behavioural phenotypes. Our lab previously reported that perinatal paternal exercise is associated with impeded reinstatement of fear memory in juvenile male offspring (Short et al., Transl Psychiatry 2017). Paternal exercise has also been separately reported to enhance hippocampal-dependent learning and memory abilities to male offspring. Our lab has reported that specific microRNAs (miR-19b

and miR-133a) are differentially expressed in the sperm of C57Bl/6 mice after 4 weeks of voluntary wheel-running (Short et al., Transl Psychiatry 2017). Functional annotation analysis of validated gene targets of the microRNAs revealed significant over-representation of KEGG pathways related to axon guidance (FDR $p = 0.035$), chemokine signalling (FDR $p = 0.05$), focal adhesion (FDR $p = 0.07$) and regulation of the actin cytoskeleton (FDR $p = 0.1$). We identified two members of the small GTPase family proteins, RhoA and cdc42, that are common to those pathways. We assessed the expression of these GTPases and cytoskeletal proteins in the hippocampus of PND15 male F1 offspring using Western blot. However, we found no significant changes to RhoA, cdc42 and Rac1 protein levels in the hippocampus. We also found no significant differences in the cytoskeletal protein b-actin and the post-synaptic scaffold protein PSD-95. Thus, we conclude that the male offspring phenotype is not due to changes in GTPase family expression, and that microRNA changes in sperm are not necessarily predictive of changes to downstream gene targets in the offspring brain.

WTH05-19

Evidences of hypoconnectivity in the valproic acid rat model of autism spectrum disorder

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Autism spectrum disorders (ASD) are classified as synaptopathies and characterized by impairment in social interaction, verbal and nonverbal communication and repetitive and stereotyped behaviors. Hypoconnectivity has been suggested in ASD patients, particularly in the corpus callosum (CC). In the valproic acid (VPA) animal model of ASD, we have previously postulated local hipocamppal hypoconnectivity based on the decrease in synaptic protein synaptophysin (SYN) seen in these animals. The aim of this work was to characterize the CC structure of VPA animals and evaluate neuronal differentiation and synaptic formation of hippocampal neurons from VPA animals. Valproic acid (500 mg/kg) or saline were prenatally administered on E 10.5 (control and VPA animals, respectively). At DIV 3–5, primary hippocampal neurons from VPA animals exhibited increased complexity in dendritic and filopodia development along with increased SYN immunostaining. As differentiation proceeded (DIV 14), SYN puncta area and number as well as PSA-NCAM immunoreactivity in the VPA group were lower than in controls. Labeling of presynaptic boutons with FM4-64 dye revealed a diminution of functional synapses in the VPA group at this stage. The anterior region of CC from VPA animals showed a disorganized cellular arrangement in the absence of changes in GFAP (astrocytes) or Iba-1 (microglia) immunostainings. Immunoreativities for CC1 (mature oligodendrocytes) and myelin basic protein were reduced. Myelin of axonal tracts from VPA animals exhibited an unorganized disposition. Our results suggest that neuronal changes and myelin defects in the hippocampus and CC of VPA animals, respectively, could underlie altered connectivity postulated for these brain areas in ASD.

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WTH05-20

GLUD1 deficient mouse as a model animal of depression-like behavior

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The delta family consisting of glutamate receptor GluD1 and GluD2 has been classified as an ionotropic receptor subunit, but recent studies have revealed that GluD2 contributes to synapse formation occurring between parallel fibers and Purkinje cells in cerebellum. GluD1 is widespread in adult mouse brains, with abundant expression in the cerebral cortex, striatum, limbic regions, and cerebellar cortex. Like GluD2, GluD1 binds to neurexins via the Cbln family and their interaction induces synaptogenesis *in vitro*. Although the functional significance of GluD1 is inferred from human genetic studies reporting that the*GRID1* is a strong candidate gene for schizophrenia, bipolar disorder, major depressive disorder, and autism spectrum disorder, the relationship between molecular function of GluD1 and the onset mechanism of these diseases has been still unknown. To approach the issue, we generated GluD1 knockout mice from C57BL/6N strain RENKA ES cells with pure genetic background for behavioral analysis. GluD1 knockout mice showed increased locomotor activity in the open field test and decreased social interaction in the three-chamber test, but no significant change in anxiety-like behaviors in the light and dark test and elevated plus maze test. Under the baseline conditions, the immobility of GluD1-KO mice in the forced swim test was significantly longer than that of wild-type mice. To examine the depression-like behavior of GluD1 deficient mice, several antidepressants were administered and a forced swimming test was conducted. Interestingly, by intraperitoneal injection of saline alone, the immobility of GluD1 deficient mice lasted longer than naive GluD1 deficient mice. The decreased baseline immobility in GluD1-KO mice was remedied by pretreatment with imipramine (serotonin and norepinephrine reuptake inhibitor) and fluoxetine (selective serotonin reuptake inhibitor), whereas no difference was observed in the immobility, when treated with desipramine (selective norepinephrine reuptake inhibitor). There results indicate that GluD1-deficient mice are vulnerable to stress, and there is a possibility that the serotonin signaling system may have been involved in the depressive behavior.

WTH05-21

Synaptic proteome alterations in chronic toxoplasma GONDII-infected mice suggest interference with glutamatergic neurotransmission

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Background: Chronic infection with the intracellular parasite *Toxoplasma gondii* affects approximately 30–50% of the human population and has been implicated in the risk for psychiatric disorders like schizophrenia or major depression. The mechanisms, by which *Toxoplasma gondii* can alter neural function, behavior and disease risk, are yet incompletely understood. Here we employed a proteomic approach to investigate potential influences of latent toxoplasmosis on synaptic protein composition.
Methods: Female C57BL/6 mice received either *Toxoplasma gondii* (ME49 type II strain) or sham infection at 8 weeks of age, and brains were harvested after another 8 weeks. Synaptosomal fractions of the hippocampus and neocortex were isolated via ultracentrifugation in a sucrose gradient and submitted to mass spectrometry (MS)-based protein identification. Furthermore, in a candidate-based approach, expression levels of key synaptic proteins were compared using immunoblotting and immunofluorescence.
Results: The synaptosomal protein composition as identified with MS showed infection-related alterations of the synaptic proteome in *Toxoplasma*-infected mice, with the majority of proteins being down-regulated. Candidate-based analysis further revealed a down-regulation of the excitatory amino acid transporter (EAAT2), the vesicular glutamate transporter (VGLUT1), and postsynaptic scaffolding proteins from the Shank family (ProSAP1/Shank2, ProSAP2/Shank3) in both hippocampus and somatosensory neocortex. Immunofluorescence further revealed that the astrocytic marker protein GFAP was up-regulated in both structures.
Conclusion: Our results provide evidence for profound alterations of glutamatergic synapse composition in Toxoplasma-infected mice, with a down-regulation of key proteins involved in glutamatergic neurotransmission. Future research should assess whether these alterations are a direct effect of the parasite or rather a consequence of resulting chronic neuroinflammation.

WTH05-22

Neurotoxic effects of prenatal hyperhomocysteinemia in rats

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Adverse impacts on the maternal organism during pregnancy can lead to serious consequences on embryogenesis and postnatal development of the offspring, and their nervous system is the most vulnerable to the harmful factors. Increased serum levels of homocysteine (HC) are a risk factor for neurodegenerative diseases. It has been shown that accumulation of the products of methionine metabolism including HC in the organism is accompanied by oxidative stress and impaired catecholamine metabolism. Due to the ability to pass through the placental barrier HC may have an adverse effect on developing embryos. The content of nerve growth factors in the serum and brain structures can serve as a marker of neurodevelopmental disorders. The present work was designed to analyze the changes in the content of a neurotrophic factor NRG1 and the levels of biogenic amines in the brain of rats during embryonic and postnatal periods as well as formation of different types of memory in adult female rats subjected to prenatal hyperhomocysteinemia (PHHC). Our results demonstrate that PHHC has resulted in increased HC levels in blood serum of newborn rats and in a significant increase of NRG1 in the brain of fetuses at E20. However, despite the fact that HC content in the blood and NRG1 level in the hippocampus of female rats subjected to PHHC returned to control values by 2–2.5 months after birth, the negative effects of PHHC could still be observed when they were tested using novel object recognition and elevated 8-arm maze tests which revealed disruption of different types of memory. There was also a decrease of noradrenaline and serotonin content in the hippocampus of these rats which can underlie dysregulation of functions associated with biogenic amine transmission in the brain. Supported by RFBR 14-04-00776 and 16-04-00694, Russian State Budget for 2013-2017 (01201351571).

WTH05-23

Chronic stress induces specific responses in neurotrophin systems in brain of rats with different behavior in forced swim test

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Chronic mild unpredictable stress (CUMS) induces depressive-like behavior in laboratory animals. It has been hypothesized that depressive-like features are associated with significant modifications in the system of neurotrophic factors in the brain. In the present study we examined how the system of neurotrophic factors may impact the development CUMS-induced depressive-like behavior in rats, which are initially differed in immobility duration in the forced swim test (FST). Prior to stress exposure, male Wistar rats were

tested in the FST and divided into low immobile (LI) and high immobile (HI). Then, half of animals of each group was exposed to CUMS for eight weeks. After the end of exposure, the duration of immobility significantly decreased in stressed HI rats compared to the initial level and slightly increased in stressed LI rats. Both LI and HI exhibited increased anxiety whereas anhedonia (lower sucrose preference) was observed in the LI group only. Control LI and HI rats significantly differed in the level of BDNF mRNA in the hippocampus, but not in frontal cortex with higher level in the LI rats. LI and HI rats were similar in the expression of NGF in both structures studied. Expression of Ntrk1, Ntrk2, and Ngfr mRNA were similar in the hippocampi of LI and HI rats; however, expression of Ntrk1 mRNA was higher in the frontal cortex of the HI group. Exposure to CUMS significantly decreased the BDNF mRNA content in the hippocampus of the LI group whereas expression of NGF mRNA increased in hippocampus of the HI rats. Interestingly, in the frontal cortex of LI rats, the level of Ntrk1 mRNA increased after CUMS exposure. Thus, the initially LI rats exhibited higher expression of BDNF in the hippocampus; however, the effects of CUMS exposure (anhedonia, lower BDNF mRNA in hippocampus, and higher Ntrk1 mRNA in frontal cortex) were more expressed in this group of rats as well.

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WTH05-24

Mangiferin alleviates sleep deprivation-induced anxiety- and depressive-like behaviors, and memory deficits in mice

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Sleep is an important to strengthen immunity and functioning of nervous system thus plays central role in learning and memory consolidation. Sleep influences various predisposing factors including inflammation, oxido-nitrosative stress, excitotoxicity and Amyloid-β proteins, which are involved in the pathogenesis of anxiety, depression and memory deficits. Sleep deprivation causes release of reactive oxygen and nitrogen species which induce oxidative damage especially in hippocampus activating inflammatory process which further leads to neurobehavioral and biochemical alterations. Mangiferin (MGF), a C-glucosylxanthone, has shown to possess activities including antioxidant, anti-inflammatory, anti-anxiety, antidepressant and neuroprotection. Therefore, present study evaluated the alleviating effect of MGF pre-treatment on SD-induced anxiety- and depressive-like behaviors, and memory deficits in mice. Moreover, SD-induced changes in pro-inflammatory cytokines, ROS&RNS and BDNF level in mice were also studied to confirm their role in the pathophysiology of these disorders. Mice ($n = 10$) were pre-treated with MGF (40 mg/kg, p.o) for 14 days including 5 days of SD protocol. After SD protocol animals were subjected to Elevated Zero maze (EZM), Tail suspension test (TST) and Novel object recognition test (NOR) to assess anxiety- and depressive-like behaviors, learning and memory. Following behavioral studies, mice were sacrificed to isolate hippocampus for the analysis of IL-1β, TNF-α, BDNF, MDA, GSH and nitrite level. Results showed that chronic SD significantly decreased open arms entries and duration in EZM ($p < 0.01$), increased the immobility time in TST ($p < 0.001$) and decreased recognition index in NOR ($p < 0.001$) in mice which was significantly ($p < 0.01$) attenuated by MGF pre-treatment. Hippocampal IL-1β, TNF-α, MDA & nitrite level increased significantly ($p < 0.001$) after SD in mice which were reversed by MGF pre-treatment. Furthermore, MGF pre-treatment improved SD-induced decrease in hippocampal GSH & BDNF level. In summary, results suggested that MGF provided alleviating effect against SD-induced neurobehavioral and neurochemical alterations by impeding neuroinflammation and oxido-nitrosative stress.

WTH05-25

Role of MMP-9 in schizophrenia-like behaviors in rodents

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Schizophrenia is recognized by 3 symptoms, classified as positive, negative and cognitive. Positive symptoms may be modelled in experimental animal models by hyperlocomotion, whereas in negative symptoms lack of interest in rewards and problems in social behavior can be demonstrated. Finally, poor working memory may correlate with cognitive symptoms of schizophrenia. Herein, we employed mouse models of schizophrenia for positive, cognitive and negative symptoms and investigated the role of diminished MMP-9 in pathogenesis of schizophrenia in these animals. Mice with genetically lowered MMP-9 levels in heterozygotes (+/−, MMP-9 HET) were employed, along their wild type (WT, +/+) littermates. Since early-life stress is regarded as a factor promoting schizophrenia, we subjected the mice, in some experiments, to daily (for 21 days) encounter with an aggressive conspecific. The results indicate that alterations in the level of active MMP-9 in the brain result in increased sensitivity to locomotor hyperactivity induced by MK-801. On the other hand chronic stress, potentiates negative symptoms of schizophrenia in MMP-9 Het mice such as depressive behaviors and social behaviors impairment. Cognitive symptoms such as poor working memory can be seen in MMP-9 HET control mice. These results support the notion that MMP-9 alterations in brain may play a role in schizophrenia.

WTH06 Molecular Mechanism of Alzheimer's Disease

WTH06-01

The Alzheimer's disease transcriptome mimics the neuroprotective signature of IGF-1 receptor-deficient neurons

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Diminishing insulin-like growth factor 1 (IGF-1) signaling delays aging and alleviates neurodegeneration in several species including mammals. We previously demonstrated in a mouse model of Alzheimer-like pathology that neuroprotection can be significantly sustained by long-term suppression of IGF-1 receptor (IGF-1R). Here, we aim to decipher molecular pathways underlying the specific role of neuronal IGF-1R in neuroprotection. In the present study, we showed that suppression of IGF-1R in neurons of the aging brain efficiently protects from neuroinflammation, anxiety and memory impairments induced by intracerebroventricular injection of amyloid β oligomers. The suppression of IGF-1R signaling also invariably led to small neuronal soma size, indicative of profound changes in cellular homeodynamics. To gain insight into transcriptional signatures leading to Alzheimer's disease-relevant neuronal defense, we performed genome-wide microarray analysis on laser-dissected hippocampal CA1 after neuronal IGF-1R knockout, in the presence or absence of APP/PS1 transgenes. Functional analysis comparing neurons in early-stage Alzheimer's disease with IGF-1R knockout neurons revealed strongly convergent transcriptomic signatures, notably involving neurite growth, cytoskeleton organization, cellular stress response and neurotransmission. Moreover, in Alzheimer's disease neurons, a high proportion of genes responding to amyloid pathology showed a reversed differential expression when IGF-1R was deleted. Interestingly, the neurofilament medium polypeptide *Nefm* stood out consistently in genome wide comparisons. We found that NEFM accumulated in hippocampus with amyloid pathology, and decreased to control levels under IGF-1R deletion, suggesting that reorganized cytoskeleton likely plays a role in neuroprotection. These findings demonstrated that significant resistance of the brain to amyloid β can be achieved lifelong by suppressing neuronal IGF-1R and identified IGF-dependent molecular pathways that coordinate an intrinsic program for neuroprotection against proteotoxicity. Our data also indicate that neuronal defenses against Alzheimer's disease rely on an endogenous gene expression profile similar to the neuroprotective response activated by genetic disruption of IGF-1R signaling. This study highlights neuronal IGF-1R signaling as a relevant target for developing Alzheimer's disease prevention strategies.

WTH06-02

Investigating the role of vitamin d receptor signaling in a cell-based model of neurodegeneration

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Objectives: Sporadic Alzheimer's disease (AD) has a multifactorial etiology with interplay of genetic, environmental, metabolic and endocrine factors, although the exact pathologic mechanisms still remain obscure. Epidemiological studies from different labs including ours have reported lower serum 25OHvitamin D levels in AD patients. Further, we observed a significant association of Apa1 polymorphism on vitamin D receptor (VDR) in AD patients. Thus, it is intuitive to postulate that VDR confers genetic susceptibility to AD by modulating neuronal survival and APP processing. This study attempts to investigate the involvement of VDR/Vitamin D signaling on amyloid beta metabolism and neuronal survival in a cell based model of sporadic AD and to determine whether VDR knockdown can alter the amyloid homeostasis.

Methods: SHSY5Y human neuroblastoma cell-line was treated with oxidative/transition metal stress(ferric ammonium citrate, 20–200 μM) with or without 10–100 nM 1,25(OH)2 Vitamin D for 24–48 h. Commercially available SiRNA was used to knock down the vitamin D receptor (VDR) expression in SHSY5Y cells. Cell death, oxidative stress markers, mitochondrial function(Membrane potential, O_2 consumption rate, ATP production) VDR expression(realtime RT PCR) and changes in amyloid beta metabolism (APP, BACE expression and amyloid beta accumulation) were examined.

Results: Vitamin D was found to improve mitochondrial functions and protect the cells from iron induced death of SHSY5Y cells as measured by trypan blue exclusion, LDH release assay and propidium iodide-Hoechst staining, inhibition of NFkB and ROS production. Fe induced increase in APP and BACE expression was inhibited by vitamin D treatment. The effects of VDR knock-down on control and iron- treated cells with respect to cell viability, mitochondrial dysfunction and APP expression are currently under study.

Conclusion: Our results indicate that impaired vitamin D receptor signaling may have a role in neural cell death, and vitamin D is a putative neuroprotective agent and therapeutic candidate for AD.

WTH06-03

Profile of sumo conjugation and neuronal death in Alzheimer's disease

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Alzheimer's disease (AD) is the most common cause of chronic dementia among the elderly, with an estimated ~ 40 million patients diagnosed worldwide, a number predicted to almost double every 20 years. Therefore, the mechanisms underlying neuronal death in AD are the focus of intense research. Mitochondrial dysfunction has

been identified as a central element in the pathology of AD. The dynamic attachment of small ubiquitin-like modifier (SUMO) to target proteins is essential in all eukaryotes because it acts as a biochemical switch that alters target protein localization, stability and/or function. SUMOylation is a post-translational modification that regulates both pre- and postsynaptic function and plasticity. Recent studies suggest that protein SUMOylation can interfere with mitochondrial dynamics, which is essential for neuronal function, and may play a pivotal role in AD pathogenesis. Here we investigated global changes in protein SUMOylation, and changes in relevant proteins to mitochondrial dysfunction and neuronal death in an *in vitro* model of AD. Our data indicate that perturbations in global SUMOylation occur alongside mitochondrial impairment. In addition, the increase in SUMO2/3 conjugation (by decreasing the deSUMOylating enzyme SENP3) is an exciting potential strategy to reduce and/or prevent the neuronal death that may be initiated by mitochondrial fragmentation. In conclusion, global SUMOylation may play an important role in the mechanisms underlying AD. The identification of a SUMO substrate and the elucidation of its function and regulation by SUMOylation will lead to important insights into the pathophysiology of AD and therapeutic intervention.

WTH06-04

Phytochemical allylguaiacol exerts neuroprotective effect in hippocampal cells and ameliorates memory impairment in a mouse model

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Allylguaiacol is a phytochemical occurring in various plants such as cloves, cinnamon, basil, and nutmeg. Pharmacological activities of allylguaiacol have been reported on anti-microbe, anti-inflammation, anti-cancer, antioxidant, and neuroprotection. Although allylguaiacol has been known to have neuroprotective effects, there is no report on its regulatory mechanisms at the molecular level. In our present study, we investigated the mechanisms of allylguaiacol as an antioxidant and neuroprotective agent using hydroperoxide (H_2O_2)-treated HT22 hippocampal cells. Allylguaiacol increased the scavenging activities of free radicals 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH), and enhanced expression of antioxidant enzymes manganese superoxide dismutase (MnSOD) and catalase. In addition, allylguaiacol inhibited H_2O_2-induced damage of HT22 with increasing production of brain-derived neurotrophic factor (BDNF) and phosphorylation of phosphoinositide 3-kinase (PI3K) and cAMP response element-binding protein (CREB). In a memory impairment mouse model, allylguaiacol (2.5 or 5 mg/kg) significantly ameliorated scopolamine-mediated cognitive impairment in the passive avoidance task. In addition, allyguaiacol significantly increased the expression of TrkA and B in the hippocampus from scopolamine-treated mice. Taken together, our findings suggest that allyguaiacol exerts the neuroprotective effect through the antioxidant activation and phosphorylation of PI3K and CREB. Furthermore, the ameliorating effect of allyguaiacol may be useful for treatment of memory impairment in Alzheimer's and its related diseases.

WTH06-05

Role of circulating irisin and adiponectin in probable Alzheimer's disease

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Objectives: The hippocampus is considered as one of the principal regions affected by Alzheimer's disease (AD) and that exercise causes neurogenesis in humans reducing the risk of AD. Irisin, a novel exercise-induced myokine, has been suggested to regulate energy homeostasis and insulin sensitivity and may have a plausible role in the pathogenesis of the disease. On the other hand, adiponectin, an adipocytokine known to regulate energy and glucose metabolism, insulin sensitivity etc. has altered levels in AD which is still unclear. None of the studies has been done on irisin and thus our study attempts to explore the role of circulating irisin and adiponectin, their association with cognitive decline and their inter-relationships in AD pathogenesis.
Methods: The preliminary case-control study included 52 persons with AD who were matched for age, sex and body mass index (BMI) and 38 healthy control subjects. Non fasting serum levels of adiponectin, and irisin were measured by commercially available immune assay kits, and routine biochemical parameters were analyzed in both the study groups.
Results: The results show statistically significant lower levels of serum Irisin and higher serum adiponectin levels in AD subjects with respect to controls. The changes in the serum adiponectin were found to be positively correlated while serum irisin was inversely correlated in AD subjects with the cognitive decline. Moreover, an inverse correlation was also observed between serum adiponectin and serum Irisin in AD subjects.
Conclusion: Our results indicate the association of these hormones might act as a significant predictor in the progression of AD. Moreover, the role of serum Irisin is promising and might potentially act as a meaningful drug target in the pathogenesis of AD.

WTH06-06

The role of stress-inducible protein 1 (STI1) in cellular resilience and Alzheimer's disease

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Stress-inducible protein 1 (STI1) is a cochaperone for Hsp70/Hsp90 and secreted STI1 can signal via the Prion protein (PrP^C).

Deletion of STI1 in mice is lethal and STI1 haplo-sufficient neurons are less resistant to insults by oxidative stress and β-amyloid oligomers (AβO). AβO, a major toxin in Alzheimer's disease (AD), bind to PrP^C triggering neuronal toxicity, which can be attenuated by extracellular STI1 treatment, or overexpression of STI1. We generated several mouse lines targeting STI1, including an overexpressing mouse line (TgA) and a line with hypomorphic alleles (dTPR1), lacking exons 1 and 2. We crossed these lines with the 5XFAD (FAD) mouse model of familial AD that overexpresses mutant Amyloid precursor protein. We used biochemical and cell biology assays to characterize these lines and investigated hippocampal amyloidosis and neurodegeneration. Surprisingly, mice overexpressing STI1 presented faster and increased amyloidosis. Immunostaining suggests accumulation of extracellular STI1 and Hsp90 around plaques of TgAFAD mice. dTPR1 mice have 75% less mutated STI1 protein and show significant reduction in levels of several STI1-Hsp90 clients, including glucocorticoid receptor and tau. Cells derived from these mice are also less resistant to stress. Our preliminary results indicate a complex role for STI1 in cellular resilience and reveals a novel role for Hsp90/STI1 chaperone machinery in amyloidosis. The mouse lines we generated will be critical to understand physiological and pathological roles of STI1 in several models of protein misfolding diseases.

WTH06-07

Crosstalk between endocannabinoid and cholinergic systems in a rat model of basal forebrain cholinergic lesion

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The cholinergic hypothesis of Alzheimer's disease (AD) is based on the vulnerability of basal forebrain cholinergic pathways as responsible of learning and memory impairment. We have observed altered endocannabinoid (eCB) signaling and changes in brain lipid profile in AD patients. The objectives were to explore the crosstalk between eCB and cholinergic systems in a rat model of AD, inducing a basal forebrain cholinergic dysfunction by 192IgG-saporin. Acetylcholinesterase staining demonstrated extensive cholinergic denervation. CB_1 receptor autoradiography showed no changes in cortical [^{3}H]CP55,940 binding and up-regulation in basal forebrain (180 ± 13 and 364 ± 63 fmol/mg; $p < 0.05$) of 192IgG-saporin-treated rats. [^{35}S]GTPγS autoradiography revealed enhanced eCB signaling in several cortical regions (i.e. entorhinal: 156 ± 17% and 277 ± 30%, $p < 0.01$; somatosensory: 131 ± 29% and 218 ± 11%, $p < 0.05$) and in basal forebrain (103 ± 18% vs. 142 ± 9%, $p < 0.05$) of lesioned rats. The use of the novel and powerful MALDI-Imaging mass spectrometry (IMS) technique, allowed us to detect specific alterations of phospholipid species such as phosphatidylcholines (PC) and phosphatidylethanolamines (PE) including: [PC(36:1), 14.62 ± 0,30% and 21.64 ± 1.53%, $p < 0.01$; PC(36:4), 10.32 ± 1.01% and 18.61 ± 2.71%, $p < 0.05$; PC(40:6), 0.88 ± 0.17% and 2.25 ± 0.40%, $p < 0.01$; PE(14:1/20:4), 15.21 ± 0.70% and 11.87 ± 1.35%, $p < 0.05$]. Furthermore, the passive avoidance and Barnes Maze tests were used to evaluate learning and memory in this model. The behavioral data were compared with the specific regulation of CB_1 receptors, the degree of cholinergic damage and the relative abundance of phospholipid precursors of choline in the lesioned neurotransmission pathway.

In conclusion, CB_1 receptor-mediated signaling is potentiated in brain areas where cholinergic neurotransmission is deregulated and PE(14:1/20:4) and PC(36:4) may serve as lipid precursors for the synthesis of eCB, as well as for *de novo* synthesis of choline. A link between eCB and cholinergic systems in the CNS under neurodegenerative conditions is proposed.

WTH06-08

P2Y1 receptor is required for Aβ-induced synaptic loss, plasticity deficits and memory impairment

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Early Alzheimer's disease (eAD) is characterized by memory impairment associated to a synaptic loss in cortical and hippocampal regions (Science 298:789). These early morphological traits, already evident in mild cognitive impairment, correlate with the increased levels of Aβ oligomers (Science 297, 353–56). Moreover, there is growing evidence that extracellular levels of ATP are increased in different brain insults/pathologies and several reports have shown the involvement of P2 receptors (P2Rs) in neurodegeneration in different pathological conditions (*Front. Neurosci. 9:148*). We now found that the pharmacological blockade of P2Y1R (MRS2179, 10 μM) prevented the neuronal death of rodent hippocampal neurons exposed to $A\beta_{1–42}$ (0.5 μM, 48 h) and the initial synaptic loss observed at 24 h gauged by a decrease in the immunoreactivity of synaptic markers (e.g. synaptophysin), reflecting a reduction in synaptic density gauged by morphological analysis and by a decrease in the frequency of mEPSCs. Moreover, we found an increased density of P2Y1R in hippocampal terminals of both rats and mice 2 weeks after administration of $A\beta_{1–42}$ (2 nmol, *icv*) at a time where they displayed a mnemonic deficit (Y maze) and synaptotoxicity (reduced levels of synaptic markers) but no neuronal death (Fluoro-Jade C staining). Indeed, P2Y1R-KO mice did not display mnemonic deficit or hippocampal synaptic markers loss 2 weeks after administration of $A\beta_{1–42}$, as displayed by wild-type mice. Also, the micro-infusion of the P2Y1R antagonist MRS2500 (≈ 1 nmol/day *icv*) prevented $A\beta_{1–42}$-induced memory loss (modified Y-maze; Object displacement and Object recognition), LTP impairment (hippocampal CA1) and hippocampal synaptic loss. These data show that P2Y1Rs are responsible or contributing to synaptic dysfunction/loss underlying the cognitive deficits associated to eAD.

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WTH06-09

Oxidative stress and inflammation induced by acute and subacute ultrafine particles exposures: contribution to Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative illness affecting the elderly population, characterized by plaques of $A\beta_{42}$ aggregates, neurofibrillary tangles and neuronal loss (Allsop, 2000). Several epidemiologic and experimental studies suggest that air pollution, mainly ultrafine particles (UFPs), may exert adverse effects on central nervous system (CNS) functions (Block and Calderón-Garcidueñas, 2009). Inflammation and oxidative stress have been suggested as primary mechanisms by which UFPs exert their harmful action on CNS (Genc et al., 2012). Therefore, the aim of this work was to evaluate the activation of oxidative stress and inflammation in mice exposed to UFPs and to elucidate putative physiopathological correlations with neurodegeneration.

Male BALB/c mice were selected as in-vivo model to study oxidative stress and inflammation induction in the brain after acute and subacute intratracheal administration of UFPs from two anthropogenic sources: BC (biomass combustion generated emissions) and DEP (EURO 4 diesel engine emission) (50 μg). In parallel, control mice were always considered (sham). After treatments, RoB (rest of brain), cerebellum and hippocampus were screened for oxidative stress (HO-1, Hsp70, and Cyp1b1), inflammation (iNOS) and AD-related markers (P-APP Thr668, APP and BACE1). Moreover, sham and treated mice were submitted to fluorescence molecular tomography and brain histopathological evaluations.

BC and DEP acute peripheral exposure was able to induce oxidative stress and inflammation in mouse brain, while sub-acute exposure sustained these mechanisms and additionally it induced increase of PAHs metabolism and alteration of APP processing. Interestingly, BC resulted generally less effective than DEP in inducing the above described alterations.

In conclusion, our results suggest that both acute and subacute UFPs peripheral administration are able to induce the response of the CNS.

WTH06-10

Amyloid precursor protein level and acetylcholine esterase activity changes in neuronal cell cultures and rat brain after hypoxia

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The amyloid precursor protein (APP) and acetylcholinesterase (AChE) are multi-faceted proteins with a wide range of important functions. They are also crucially linked with the pathogenesis of Alzheimer's disease (AD). APP is the precursor of the Aβ peptide, which is the causative pathological agent in AD. AChE is linked to AD pathogenesis either by increasing cholinergic deficit or by exacerbating Aβ fibril formation and toxicity. As such, both proteins are the main targets in AD therapeutics. In our studies we have demonstrated an important interrelation in functioning of these proteins. Both can be released from the cell membrane. As we have shown AChE shedding involves a metalloproteinase-mediated mechanism which, like the α-secretase dependent cleavage of APP, is stimulated by cholinergic agonists or inhibited by batimastat, a metalloproteinase inhibitor. Overexpression of the neuronal specific isoform APP695 in neuronal cells substantially decreased levels of AChE mRNA (Hicks et al., JBC, 2013, 288:26039). In human neuroblastoma cells SH-SY5Y and NB7, AChE activity negatively correlates with the levels of their endogenous APP expression. Cultivation of NB7 cells under hypoxic conditions resulted in APP up-regulation and reduction in AChE activity. Similarly, acute hypoxia in adult rats (7% O_2, 3 h) resulted in increased APP protein levels in their cortex and reduction in AChE activity. Hence, APP influences AChE physiology under normal and pathological conditions but precise mechanisms still require elucidation. Supported by ARUK, RFBR 16-04-00694 and Russian state budget (01201351571).

WTH06-11

Aβ-induced inhibition of protein prenylation causes autophagic flux blockade

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Alzheimer's disease (AD) represents one of the most serious health problems for which treatments are urgently needed. To develop therapies we need a better understanding of the events that lead to AD. We discovered that amyloid beta peptide ($oAbeta_{42}$) inhibits the mevalonate pathway that synthesizes cholesterol and isoprenoids farnesylpyrophosphate(FPP) and geranylgeranylpyrophosphate (GGPP). FPP and GGPP are used for protein prenylation. As a consequence, protein prenylation is impaired in neurons exposed to Abeta. Moreover, protein prenylation is reduced in brains of the AD mouse model TgCRND8.

Among the several cellular processes that may be affected by hypoprenylation, autophagy is particularly susceptible because it relies heavily on prenylated proteins, particularly Rabs. Autophagy is dysfunctional in AD and reversing autophagy dysfunction in TgCRND8 mice improves the pathophysiology and rescues memory performance. Yet, the nature and cause(s) of autophagy dysfunction in AD are unclear, which prevents the development of autophagy-targeted strategies with disease-modifying value.

We determined the nature of autophagy dysfunction by examining autophagic flux using mCherry-GFP-LC3 in neurons treated with Abeta and *in vivo* in TgCRND8 mouse. We found that autophagic flux is blocked. To demonstrate that autophagy dysfunction is caused by inhibition of prenylation we tested the ability of GGPP to normalize autophagic flux and the function of Rab7. GGPP prevented Abeta-induced autophagy dysfunction. Rab7 is required for autophagy progression and is altered in AD. In Abeta-treated neurons Rab7 is hypoprenylated and its localization

to autophagosomes is reduced. GGPP corrects Rab7 prenylation and subcellular localization. Our data indicate that autophagic defects in AD are due, at least in part, to inhibition of protein prenylation, which occurs as consequence of decreased isoprenoids synthesis. Restoration of protein prenylation in cultured cells normalizes autophagy and *in vivo* could improve the pathophysiology and behavior in AD.

WTH06-12

Methylation of Aβ derived in exosomes is the diagnosis marker in Alzheimer disease

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It is a major global concern for the high and increasing incidence Alzheimer Disease (AD) worldwide. There is still limited effective treatment for AD patients, thus the early and accurate diagnosis would be the goal for retarding AD. The accumulation of amyloid-β (Aβ) levels is the symbol for AD progression, and DNA methylation of Aβ is a key epigenetic mechanism in AD. Exosomes are nanovesicles that detectable in human plasma, and they including methylated Aβ could be considered as a diagnostic marker for the development of AD. In this study, we purified the plasma exosomes of 56 AD patients by high-speed centrifuge and analyzed the contents of methylated Aβ by PCR and Western blotting. The data showed that the methylated Aβ level in exosomes of AD patients were higher than control group (health adult) ($p < 0.01$), and the methylation of Aβ increased with the development of AD ($p < 0.01$). The similar result was showed in exosomes of AD mice model that the methylated Aβ level in exosomes of AD mice were higher than control mice ($p < 0.01$). Furthermore, the treatment of methylation inhibitor 5-Aza-Cd decreased the methylation level in exosomes of Aβ in AD mice model compared with that in non-treated AD mice ($p < 0.01$). Interestingly, the survival time of the AD mice with the treatment of 5-Aza-Cd was longer (131 ± 35 days) than non-treated AD mice (101 ± 12 days). Thus, exosomes could be the diagnosis maker for AD, and inhibition of methylation of Aβ would be the target for treatment of AD also prevent the occurrence of AD development. This study could be beneficial to clinical surgical treatment for AD diagnosis as early as possible, and it would be important theoretical foundation for clinical treatment and treatment guidance.

WTH06-13

The effects of abeta oligomers on regulators of protein synthesis

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Synapse loss is a key pathophysiological feature of Alzheimer's disease (AD) and the best correlate of cognitive decline in AD patients. Nevertheless, the specific mechanisms that mediate reduction of synaptic proteins levels and, ultimately, synapse elimination in AD, remain to be fully understood. Decreased protein synthesis is a well-known feature of AD that could explain the reduction on synaptic protein levels. However, the interplay between the many regulators of protein translation are not yet well established on the course of the disease. There is also a controversy in the literature on the levels of major regulators of protein translation in AD and their role in the disease. A systematical analysis on translational regulators in different time-points is yet lacking. Here, we have investigated the levels of major regulators of protein synthesis using RT-PCR and Western Blotting in experimental models of AD such as hippocampal neuronal culture treated with Aβ oligomers (AβOs) and hippocampi extracted from mice that received intracerebroventricular (i.c.v.) injection of AβOs. We found that AβOs, increasingly recognized as proximal synaptotoxins in AD, trigger decrease in the levels of p eIF4E, p 4E-BP1, p S6K, p S6, p ERK, p mTOR and an increase on the levels of ATF4 in the hippocampi of mice 7 days after receiving an i.c.v. injection of AβOs, but not after 24 h. We also report an increase on FMRP expression and levels in neuronal cultures treated for 24 h with AβOs and in synaptosomes isolated from the hippocampi of mice 7 days after receiving i.c.v. injection of AβOs. We will further expand these analyses on APP/PS1 mice and in AD patients, but so far we show that many positive regulators of protein synthesis are inhibited, while translational repressors are enhanced in our AD models. It may be very interesting to further correlate these findings with protein translation inhibition observed in AD and further investigate the specific role of each of these proteins on the outcome protein synthesis modulation in AD.

WTH06-14

Potential crosstalk between autophagy and apoptosis in amyloid-β-induced neuronal death

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Amyloid-β (Aβ) induced neuronal death plays important role in the pathogenesis of Alzheimer's disease (AD). Among several death modalities, autophagy and apoptosis play important roles in Aβ-induced neuron death suggesting that there may be regulatory mechanisms that initiate both cell death pathways. In our study, we find that tribbles pseudokinase 3 (Trib3, an ortholog of Drosophila *Tribbles*), a novel ER stress inducible gene, is upregulated in neurons, both *in vivo* and *in vitro* upon Aβ treatment. Increased Trib3 levels inhibit the activity of Akt by interacting with it. As a result, forkhead box O1 (FoxO1), a transcription factor that is negatively regulated by Akt, is activated, translocates to the nucleus, and induces the pro-apoptotic gene *BCL2 like 11* (Bim). This leads to apoptotic death of cells via activation of caspases. We also observe that overexpression of Trib3 leads to increased accumulation of p62 and autophagic vacuoles indicating abnormal autophagy flux. Thus suggesting a role of Trib3 in autophagy. We further find that Beclin1, an autophagy induced protein is cleaved upon Aβ treatment. Studies reveal that autophagy induced Beclin1 is cleaved by active caspases, which may thwart further autophagy and induce apoptosis. Interestingly, we also find a physical interaction between Bim and Beclin1, this interaction reduces upon Aβ treatment. Thus our study reveals that induction of Trib3 leads to increased expression of Bim via the transcription factor FoxO1, which leads to apoptotic death of cells. On the other hand there is release of Beclin1 from physical interaction with Bim upon Aβ treatment. The free Beclin1 leads to enhanced autophagy, but is subsequently cleaved by caspases turning autophagy to apoptosis. We find

inhibition of both autophagy and apoptosis leads to better survival upon Aβ treatment. Most importantly, silencing endogenous Trib3 strongly protects neurons from Aβ insult. Thus, Trib3 may serve as potential therapeutic target for AD.

WTH06-15

Role of zinc in regulating calcium dependent signaling pathways during aluminium induced neurodegeneration

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Alteration of metal homeostasis has been perceived as major risk factors in the progression of neurodegeneration. Aluminium (Al) has been regarded as the third abundant element in the earth's crust (comprises nearly 8%) and has been linked to several neurodegenerative disorders including Alzheimer's disease. Despite of its abundance in nature, the molecular basis of its interaction with the physiological system are rather sparse. On the other hand, zinc (Zn) is an essential trace element that regulates a number of biological activities in our body. The objective of the present study was to explore the role of zinc, if any, in regulating calcium dependent signal transduction pathways during aluminium induced neurodegeneration in rat. Male Sprague–Dawley rats weighing 140–160 g were divided into four different groups viz: Normal control, Aluminium treated (100 mg/kg b.wt./day via oral gavage), Zinc treated (227 mg/l in drinking water) and combined aluminium and zinc treated. All the treatments were carried out for a total duration of 8 weeks. Al treatment caused impairment in the cognitive behaviour of rats, whereas zinc supplementation caused an improvement in the learning and memory of animals. Al exposure increased the levels of cAMP, intracellular calcium and calcium content in both the cerebrum and cerebellum, which however were modulated upon Zn supplementation. Further, Al treatment also decreased the Ca^{2+}ATPase activity in different regions of brain, which was found to be increased on zinc supplementation. Western blot of proteins phospholipase C (PLC), inositol triphosphate (IP3) and protein kinase A (PKA) were also found to be significantly elevated after Al treatment, which however were reversed following Zn treatment The light and electron-microscopic analysis of brain revealed alterations in neuro-histoarchitecture in the form of calcium deposits, chromatin condensation as well as mitochondrial swelling, which were appreciably improved upon zinc supplementation. Therefore, the current study suggests that zinc plays a vital role in regulating calcium dependent signal transduction pathways during aluminium induced neurodegeneration.

WTH06-16

Phosphorylation and isoform expression of tau are regulated independently during mouse brain development

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The microtubule-associated protein tau is a principal component of NFTs found in brains of Alzheimer's disease (AD). Tau in NFTs is hyperphosphorylated, but it is not known why and how those tau are hyperphosphorylated. There are 6 isoforms in tau, which are produced by alternative splicing. Interestingly, both phosphorylation and isoforms of tau are changed during development. Because phosphorylation levels of fetal tau are similar to the major hyperphosphorylated tau species in AD, it is important to understand the mechanism of high phosphorylation of fetal tau. However, it is not addressed how the isoform and phosphorylation changes are regulated during neuronal development and how it contributes mechanistically to development of AD or tauopathies. Here, we investigated regulation of the isoform and phosphorylation changes during early postnatal development in mouse.

At first, we confirmed that 3R tau was replaced by 4R tau gradually from postnatal 9 (P9) to P18 and in the similar time course as the high phosphorylation was changed to the low phosphorylation. It is known that hypothyroid delays brain development. We examined whether changes of isoforms and phosphorylation are separated by hypothyroid, which was induced by a thyroid hormone synthesis inhibitor 2-mercapto-1-methylimidazole (MMI). MMI delayed the day of dephosphorylation but did not affect the conversion of tau isoforms, indicating that the changes of isoforms and phosphorylation are not necessarily linked. Further, we examined phosphorylation of the single isoform of human tau, 3R or 4R, knocked in mouse brain. Human tau, either 3R or 4R, reduced phosphorylation levels during development even though the isoform did not change. These results show for first time that the phosphorylation and isoform changes are differently regulated during development. Our results would contribute to the understanding of the roles of tau during development but also the pathogenesis of tauopathy.

WTH06-17

Degeneration of septal cholinergic neurons caused by immunotoxin 192IGG-saporin alters gene expression in the hippocampus

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It is well known that Alzheimer disease is associated with degeneration of septal cholinergic neurons. To investigate the role of cholinergic innervation in the regulation of gene expression in the hippocampal cells, we induced degeneration of cholinergic septal neurons by intracerebroventricular injection of toxin 192 IgG-saporin. To validate effect of the toxin, we performed behavioral testing, immunohistochemical staining of septal slices and quantitative RT-PCR in the hippocampus.

Administration of the immunotoxin led to a significant increase in the total swam distance and higher velocity in the Morris Water Maze. Furthermore, during probe trial when the platform was removed from the maze, saporin-treated rats spent significantly less time in the target quadrant and swam shorter distance in it compared to the control.

Staining of brain slices from the animals that were treated with toxin showed that intracerebroventricular administration of saporin resulted in a significant decrease in the number of cholinergic neurons in the medial septum and diagonal band of Broca ($p < 0.05$). We analyzed Ig-saporin-induced changes in the mRNA expression of ribosomal genes (*mrpl27, rps23, rpl28*), microglia-

specific *slc2a5*, and *fgf1* in the hippocampus. In Ig-treated rats, *rps23* expression increased whereas expression of *mrpl27 and rpl28* was not altered. The expression of *slc2a5* significantly increased whereas the expression of fgf1 decreased. Our results suggest that Ig-saporin-induced degeneration causes not only alterations in behavior but also strongly alters expression of genes.

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WTH06-18

Caspase-3 activity in early ontogenesis is essential for regulation of neprilysin and transthyretin expression in rat brain

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Pathogenesis of the late-onset Alzheimer's disease is to a great extent linked to impaired amyloid-β peptide (Aβ) clearance from the brain caused by various pathological factors including brain ischemia and hypoxia. Epigenetic changes caused by prenatal stress are known to increase the risk of neurodegeneration in later life. Our studies in rats subjected to a single episode of prenatal hypoxia (PH) in the critical period of brain development (E14, 7% O_2, 3 h) demonstrated alterations in Aβ metabolism, impaired synaptic plasticity and cognitive deficit during postnatal development. Along with increased amyloid precursor protein (APP) expression in rat brain caused by PH we also observed activation of caspase-3 and reduced levels of a major amyloid-degrading enzyme, neprilysin (NEP), correlating with decreased levels of a transcriptional regulator AICD (C-terminal fragment of APP produced alongside Aβ) which is readily cleaved by caspases. Manipulating caspase-3 activity in PH rat brain by intraventricular injection of an inhibitor, Ac-DEVD-CHO, on P20 resulted in restoration of AICD levels, NEP activity, the number of synaptic spines in the cortex and hippocampus, and improved cognitive functions. PH hypoxia also resulted in significant changes in the levels of a transport protein transthyretin (TTR) in the choroid plexus and other brain regions whose levels also increased after injections of the caspase inhibitor. Since both NEP and TTR play an important role in Aβ clearance, alterations of their expression might lead to disruption of Aβ homeostasis leading to impaired brain functions. Understanding amyloid clearance mechanisms in the brain is of particular importance for designing strategies for prevention of neurodegeneration caused by Aβ accumulation. Supported by RFBR (16-04-00694), Alzheimer's Research (UK).

WTH07 Neurodegenerative Disease

WTH07-01

Validation of metabolic neuroimaging biomarkers in huntington disease

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Background: Evidence suggests that energy deficit plays a critical role in the pathophysiology of Huntington disease (HD). There is however lack of robust biomarkers for testing therapeutic strategies targeting brain energy metabolism. This study therefore aims at measuring dynamic parameters of brain energy metabolism in HD.

Methods: Phase one of the study involved recruiting 10 healthy individuals for method validation. The second phase – consisting in scanning 10 presymptomatic individuals, 10 patients at the early stage of HD and 10 controls – has been initiated and will be completed by May. Following our observation of abnormal energy profile in the occipital cortex, we wish to assess the rate of brain creatine kinase using 31P magnetization transfer (31P MT). Diffusion weighted spectroscopy (DWS) is also used to evaluate the diffusion properties of metabolites that reflect distinct metabolic compartments, i.e. neuronal versus glial. Furthermore, resting state functional MRI (rsfMRI) intends to capture the impact of functional connectivity on neurometabolism and vice versa.

Preliminary Results: We have successfully finished the validation phase in 10 healthy volunteers. 31P MT data showed that we fully saturated gamma-ATP without directly saturating the PCr signal. Our DWS analyses displayed similar findings to other studies performed in healthy individuals. Furthermore, the rsfMRI protocol was well tolerated with no induction of peripheral nerve stimulation and the data was of high quality. We are now actively scanning HD carriers and controls. Data analyses and interpretation will be available in August.

WTH07-02

Regulation of COPI vesicle transport via SCYL1 methylation in the pathogenesis of ER-stress induced neurodegenerative diseases

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Cumulative evidences have shown the importance of ER-stress in pathology of neurodegenerative diseases, such as Alzheimer's disease, Amyotrophic lateral sclerosis, etc. Previous studies have indicated that accumulation of unfolded protein response (UPR) by ER-stress is related to the pathology of neurodegenerative diseases. To elucidate the pathogenesis of neurodegenerative diseases from the point of view of ER-stress, we investigated the altered gene expression in SK-N-SH cells under the condition of tunicamycin-induced ER-stress by the gene fishing method. As the result, we found that Protein arginine N-methyltransferase 1 (PRMT1) is up-regulated in SK-N-SH cells under ER-stress. Based on this result, we examined the role of PRMT1 in the ER-stress pathway. PRMT1-knockdown cells showed the abnormal Golgi formation and increased UPR. To elucidate the mechanism of these alterations, we screened the methylated proteins under ER-stress condition by immunoprecipitation-mass spectroscopy, and identified Scy1-like protein 1 (Scyl1). Scyl1, a member of the Scy1-like family of catalytically inactive protein kinases, was recently reported to function in retrograde COPI-mediated intracellular transport. Interestingly, Scyl1 has also been identified as a gene product that is lost in an animal model of motor neuron disease, the muscle-deficient mouse. In the motor neuron of the above model animal, the protein circulation system between ER and Golgi apparatus was abnormal due to dysfunction of COPI transport. In consequence, UPR may be accumulated. Thus, we present the effect of Scyl1 arginine methylation on the COPI vesicle transport. This study provides novel insights into the pathogenesis of neurodegenerative diseases by UPR accumulation.

WTH07-03

Hypothalamic dysfunction and metabolic dysregulation in AD animal models

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Clinical and epidemiological studies have shown that Alzheimer`s Disease (AD) is related to diabetes. AD patients have a high risk of developing Type 2 Diabetes (T2D) and/or impaired glucose metabolism. Amyloid Beta Oligomers (ABOs), toxins that build up in AD patients brains, are known to induce ER stress and impair insulin signaling in the hippocampus of mice. In the current study we aim to investigate whether ABOs can also impact the hypothalamus and trigger peripheral metabolic dysregulation. WT mice and macaques intracerebroventricular (icv) injected with ABOs were evaluated. We further used transgenic mice models of AD (APPPS1 and CRND8) that overexpress mutant human APP and/or PSEN1 and generate high levels of human Amyloid Beta 42 (AB42) in this study. Glucose Tolerance Test (GTT) and Insulin Tolerance Test (ITT) were performed to access glucose homeostasis. Plasma insulin levels were assessed by Elisa. Levels of hypothalamic and plasma AB42 were measured in the transgenic AD mice models. Hypothalamic levels of proteins related to insulin pathway were quantified by Western Blotting and markers of inflammation were analyzed by Immunohistochemistry. Our results show that icv-infused AβOs trigger hypothalamic inflammation and impaired insulin signaling leading to glucose intolerance and insulin resistance. In the transgenic mice model we also identified glucose intolerance but not peripheral insulin resistance. These results provide evidences of metabolic dysregulation in AD models and suggest impaired hypothalamic insulin signaling as a shared molecular mechanism between AD and T2D.

WTH07-04

Ubiquitin proteasome system dysfunction in ALS patient IPSC-derived motor neurons

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Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative condition that results from the progressive death of upper and lower motor neurons. Dysfunction in the ubiquitin-proteasome system, the primary selective degradation system of the cell, is a common feature of ALS aetiology. Mutations in cyclin F, a protein central to the ubiquitin proteasome system, have recently been identified to cause ALS. Very little is known regarding the function of cyclin F in either healthy or ALS-affected motor neurons. Due to the difficulties related to culturing primary neurons, we have used patient-iPSC derived motor neurons to investigate the effects of endogenous mutant cyclin F. We aimed to utilise these motor neurons to investigate ubiquitin proteasome system dysfunction in cells derived from ALS patients with mutations including $CCNF^{S621G}$ and $SOD1^{E101G}$, compared to healthy controls. Analysis of the ubiquitin proteasome system by degron assay, a fluorescent proteasome reporter assay, indicated a significant deficit in protein degradation in ALS patient-derived motor neurons. Western blot analysis confirmed an increase in endogenous Triton X-insoluble proteins including phosphorylated TDP-43 as well as cyclin F. Identification of this disease phenotype in our motor neuron models provides a viable target for the development of future therapeutics.

WTH07-05

Regulation of neurofilament proteins by ALS-linked MIRNAS

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Neurofilaments are the most abundant cytoskeletal component in neurons. Neurofilaments determine axonal caliber and promote axonal growth, but also organize the cytoplasm to form a stable 3-dimensional lattice that supports the organization of organelles. Neurofilament protein assembly requires the correct stoichiometry among the three subunits: NFL (light), NFM (medium) and NFH (heavy). Dysregulation of neurofilament heteropolymers has been established as a cytopathological hallmark of amyotrophic lateral sclerosis (ALS), a neurodegenerative disease of motor neurons. Alterations of neurofilaments in ALS include selective suppression of *NEFL* mRNA in the human spinal cord, neurofilament proteins sequestration in inclusions and axonal swelling, and expression of variants of the *NEFH* gene. MiRNAs are evolutionary conserved non-coding RNAs that post-transcriptionally regulate the expression of most mammalian genes and play a critical role in degeneration. We have studied the expression profile of miRNAs in the spinal cord of ALS patients and the role of ALS-associated miRNAs in the regulation of NFL, NFM and NFH. We observed a massive down-regulation of miRNAs in ALS patients that was subsequently shown

to be specific to motor neurons. We developed a list of conserved miRNAs with miRNA recognition elements within human NEFL, NEFM and NEFH 3′UTRs. MiRNA *in vitro* studies showed that a total of 8 miRNAs dysregulated in spinal cord of ALS patients regulate the expression of NFL, NFM and NFH and are expressed in human spinal motor neurons. This observation is highly relevant because it implies that the alteration of a small group of miRNAs in ALS could potentially change neurofilament levels, impair its organization and induce the formation of pathological inclusions. Our results are relevant for future studies to identify novel therapeutics for ALS.

WTH07-06

Characterization of a presymptomatic stage in a drosophila Parkinson's disease model: unveiling compensatory mechanisms

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Parkinson disease (PD) is a degenerative disorder characterized by several motor symptoms including shaking, rigidity, slow movement and difficult walking, which has been associated to the death of nigro-striatal dopaminergic neurons. More than 90% of PD patients also present olfactory dysfunction. Although the molecular mechanisms responsible for this disease are not clear, hereditary PD is linked to mutations in specific genes, including the PTEN-induced putative kinase 1 (PINK1).

In this work we provide for the first time a thorough temporal description of the behavioral effects induced by a mutation in the PINK1 gene in adult *Drosophila*, a previously described animal model for PD. Our data suggests that the motor deficits associated to PD are fully revealed only by the third week of age. However, olfactory dysfunction is detected as early as the first week of age. We also provide immunofluorescence and neurochemical data that let us propose for the first time the idea that compensatory changes occur in this *Drosophila* model for PD. These compensatory changes are associated to two specific components of the dopaminergic system: Dopa decarboxylase, the enzyme responsible for the last step in dopamine biosynthesis, and the Dopamine transporter, a plasmatic membrane protein involved in maintaining dopamine extracellular levels at physiologically relevant levels.

Thus, our behavioral, immunofluorescence and neurochemical data help define for the first time presymptomatic and symptomatic phases in this PD animal model, and that compensatory changes occur in dopaminergic neurons in the presymptomatic stage.

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WTH07-07

Neuromyelitis optica immunoglobulin G targets AQP4 expressed in retinal Müller cells affecting cell volume homeostasis

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The current study evaluates if the water channel AQP4, highly expressed in Müller cells in the retina, is a pathogenic ocular target of specific serum immunoglobulin G autoantibody (NMO-IgG) produced in patients with Neuromyelitis Optica. Particularly we investigated the consequences of NMO-IgG binding to AQP4 on plasma membrane water permeability and cell volume homeostasis. Studies were performed in a human retinal Müller cell line (MIO-M1), a good model that maintains important functional characteristics of Müller cells. To avoid or to facilitate AQP4 down-regulation, cells were exposed to inactivated control or positive NMO-IgG sera in two different situations (1 hr at 4°C or 12 hr at 37°C). AQP4 expression was detected by immunofluorescence studies using a polyclonal anti-AQP4 antibody and the water permeability coefficient and cell volume regulation capacity were evaluated by fluorescence videomicroscopy. Our results showed that immediate NMO-IgG binding to AQP4 is not enough to affect water channel′s activity. However, long-term exposure to NMO patient sera clearly induced a loss of AQP4 signal from plasma membrane, along with a significant reduction of water permeability and the capacity to regulate cell volume after an osmotic swelling (RVD), a key function of Müller cells. These data demonstrate that NMO-IgG targets Müller cells AQP4, affecting its expression and its function, and subsequently cell homeostasis. Therefore, we propose that water permeability reduction after NMO-IgG binding to AQP4 in Müller cells contributes to retinal cell damage and tissue edema observed in NMO patients.

WTH07-08

The role of the autophagic pathway in muscle cell models of spinal and bulbar muscular atrophy

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Spinal and bulbar muscular atrophy is a motor neuron disease caused by an aberrant CAG expansion in the exon1 of the androgen receptor (AR) gene. This stretch is translated into a polyglutammine tract (polyQ) in the coded AR protein (ARpolyQ) that eventually prevent AR from folding correctly after binding to testosterone. Misfolded ARpolyQ may become toxic to cells and form aggregates. The protein quality control (PQC) system is in charge of protein homeostasis. It is composed by a chaperone network that tries to refold proteins; if this system fails proteins are brought to the two degradative systems: ubiquitin-proteasome system (UPS) and macroautophagy. Recently, some studies unrevealed a key role for muscle cells in SBMA. In this work we investigated ARpolyQ behaviour in muscle cells. We used muscle C2C12 stably

transfected with ARQ24 and ARQ100. We performed a filter retardation assay (FRA) (a technique that allow the detection of aggregates) on PBS extracts of both cell lines cultured in presence of testosterone. We observed that ARQ100 insoluble species retained on the membrane were higher than that of ARQ24. Interestingly, we found that ARpolyQ expression does not modify expression of PQC system-related genes measured by rtqPCR. We then performed FRA on samples previously treated with proteasome or autophagy inhibitors. We noted that only ARQ100 accumulates after autophagy inhibition. We then overexpress HspB8 a small heat shock protein that in complex with Hsp70 and Bag3 led misfolded protein/aggregates to autophagy. HspB8 overexpression counteracted ARQ100 aggregation in presence of testosterone. We then tested trehalose, an mTOR independent autophagic activator; we observed that trehalose increased HspB8 overexpression and reverted the testosterone dependent aggregation of ARpolyQ. Based on these results we hypothesized that muscle cells are a site for ARpolyQ aggregation, and that modulation of autophagy could reduce ARpolyQ aggregation in these cells.

WTH07-09

Loss of glutamine synthetase initiates a sequence of neuropathological events that culminate in epilepsy and neurodegeneration

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The enzyme glutamine synthetase (a.k.a. glutamate ammonia ligase, Glul) is enriched in astrocytes and serves as the primary pathway for synaptic glutamate clearance and brain ammonia detoxification. Loss of astrocytic Glul has been implicated in several CNS disorders, such as epilepsy; however, the mechanism by which Glul deficiency might cause disease is not understood. Thus, we selectively deleted Glul in the hippocampus and neocortex of mice to study the consequences of Glul deficiency. At 2 weeks of age, the brain cytoarchitecture and behavior of Glul deficient mice were largely unremarkable; however, the brain chemistry, microglial cells and blood vessels were altered. At 4 weeks of age, other changes became apparent, such as slowed brain growth, altered functional connectivity, reduced cerebrovascular reactivity, behavioral abnormalities, epileptic seizures and progressive neuron loss that resembled hippocampal sclerosis. Thus, loss of astroglial Glul initiates a series of molecular and cellular events that culminate in neurodegeneration and epilepsy.

WTH07-10

Ageing and oxidative stress contribute to neurodegenerative diseases-related proteins in human red blood cells

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Ageing represents the strongest predictor for developing of Neurodegenerative diseases (NDs), which are characterized by selective dysfunction and loss of neurons, associated with protein aggregates in human brain and peripheral tissues[1,2]. In particular, NDs commonly present misfolding and aggregation of one or more proteins, including primarily β-Amyloid (Aβ), α-synuclein (a-syn) and tau[1].

ND etiology remains to be fully elucidated, however increased oxidative stress seems one of the potential common factor. Oxidative stress, accumulating in ageing and NDs, has been related to impaired mitochondrial activity, lipid peroxidation, protein modification, DNA damage and apoptosis[3].

Herein, red blood cells were used as peripheral cellular model to investigate the correlation between ND-related proteins and the extent of the antioxidant capability (AOC), a key marker of oxidative stress in ageing-related pathologies. In particular, the content of a-syn, Aβ and tau and of their oligomeric/phosphorylated forms were determined by immunoenzymatic assays in a cohort of 110 human subjects.

Both plasma AOC toward hydroxyl radicals and total α-syn content were reduced in older subjects; in contrast, tau and Aβ accumulated in elderly subjects and showed an inverse correlation with hydroxyl AOC.

The positive correlation between antioxidant capability and reduced protein accumulation was confirmed by these data, and suggest that peripheral content of ND-related proteins should be further investigated as potential markers of neurodegeneration. In this respect, preliminary data on patients affected by Parkinson's disease will be shown.

[1]Biochem Pharmacol. 2017; pii:S0006-2952(17)30047-3.
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WTH07-11

Altered glycogen metabolism in the brain of insulin-resistant Goto-Kakizaki rats: a 13C magnetic resonance spectroscopy study in V

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Impaired insulin signalling affects brain structure and function leading to behavioural and cognitive alterations. The role of glycogen in the diabetic brain remains to be elucidated. In the present study we investigated insulin resistance-induced alterations of brain glycogen metabolism in the living brain by means of magnetic resonance spectroscopy (MRS). MRS experiments *in vivo* were performed on a 14.1 T spectrometer using a home-built surface coil. [1-^{13}C]glucose was infused into adult Wistar and insulin-resistant Goto-Kakizaki (GK) rats under isoflurane anaesthesia.

Localised ^{13}C MRS was performed in a volume of 600 μL within the brain with a modified SIRENE pulse sequence. The ^{13}C MRS experiment measured brain glucose and glycogen signals over at least 8 h. Then, rats were sacrificed with a focused microwave fixation device, and the brain was stored for extraction of glycogen and water-soluble metabolites. Fractional enrichment (FE) and content of glucose and glycogen were determined by MRS *in vitro*. Time courses of glycogen ^{13}C labelling measured *in vivo* were modelled together with FE and concentration determined in brain extracts to estimate glycogen turnover. The glucose infusion rate was adjusted to reach similar glucose levels and FE in the plasma of both GK and Wistar rats (~17 mM). Under such conditions, brain glycogen concentration was similar (5.5 ± 0.9 and 5.0 ± 0.4 μmol/g in Wistar and GK rats, respectively), the rate labelling incorporation from [1-^{13}C]glucose into glycogen was 0.24 ± 0.05 and 0.48 ± 0.09 μmol/g/h in GK and Wistar rats ($p < 0.05$), respectively. Taking in account brain glycogen concentration, glycogen turnover time τ was 26.4 ± 4.9 h in GK rats and 14.5 ± 1.7 h in Wistar rats ($p < 0.05$). In sum, we demonstrate that brain glycogen mobilisation is slower in insulin resistance despite normal brain glycogen content, which may have implications for the adequate support of neuronal function.

WTH07-12

Co-localization of RGNEF with TDP-43 into micronuclei-like structures induced by cellular metabolic stress

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Amyotrophic lateral sclerosis (ALS) is an adult-onset progressive disorder characterized by degeneration of motor neurons. Although the cause of the disease remains elusive, a common neuropathological hallmark is the formation of neuronal cytoplasmic inclusions (NCIs) in motor neurons, which include RNA-binding proteins such as TDP-43 and Rho Guanine Nucleotide Exchange Factor (RGNEF). Cellular stress seems to be highly relevant to the pathogenesis of ALS. Oxidative and osmotic stress have been extensively studied in regards to the role of stress granules in the formation of NCIs in ALS. However, the role of cellular metabolic stress in this pathology has yet to be explored. Previously, we determined that the Leucine-rich domain in the amino terminal region of RGNEF is critical for proper regulation of its RNA-destabilizing activity. Considering the fact that the Leucine-rich domains are commonly involved in protein-protein interactions, its presence could be critical in the regulation of protein complexes where RGNEF is involved.

We hypothesized that under cellular metabolic stress there is formation of cytoplasmic inclusions containing ALS-related proteins and the Leucine-rich domain of RGNEF is critical for the recruitment of RGNEF into those inclusions.

Interestingly, under cellular metabolic stress we observed the formation of cytoplasmic inclusions containing TDP-43 which resemble micronuclei structures. The nature of this micronuclei-like structures was confirmed using the nuclear markers SIRT-1, Histone H1 and NPCP (Nuclear Pore Complex Proteins). Flag-RGNEF-L-rich protein forms intracellular inclusions in stressed HEK293T cells which co-localized with endogenous TDP-43 into micronuclei-like structures. Endogenous RGNEF was also observed to co-localize with endogenous TDP-43 in micronuclei-like structures.

This is the first cellular model of recruitment of endogenous TDP43 into cytoplasmic inclusions *in vitro* induced by cellular metabolic stress. Notably, these inclusions were micronuclei-like structures. Also, our results indicate a critical role for the Leucine-rich domain of RGNEF in the formation of RGNEF inclusions under pathological conditions. This study suggests that the metabolic stress could have an important role on the pathogenesis of ALS.

WTH07-13

Modulation of tau isoforms by RNA reprogramming: analysis of phenotypic rescue in a mouse model of tauopathy

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Tauopathies are major neurodegenerative diseases characterized by the presence of intraneuronal aggregates of the Tau protein in insoluble neurofibrillary tangles. Tau is a microtubule-associated protein expressed in neurons, involved in cellular functions such as microtubule stabilization and axonal transport, is encoded by the MAPT gene. The Exon 10 (E10) can be alternatively spliced, giving rise isoforms with three (3R) or four (4R) repeats of microtubule binding domains, both isoforms are expressed in equal amounts in the normal adult human brain. Several tauopathies are associated with mutations in the MAPT gene which modify E10 alternative splicing, leading to an imbalance between the 3R and 4R Tau isoforms. Correction of that imbalance might represent a therapeutical approach for those tauopathies. We evaluate the phenotypes of mice carrying a human Tau transgene with an abnormal ratio of Tau isoforms (hTau mice), proposed as a model. Htau mice have an excess of 3R Tau in several brain areas. We sought to correct that Tau isoforms imbalance and analyze if such restoration produces a phenotypic rescue. We used RNA pre-trans-splicing molecules (PTM) to promote the inclusion of E10 in the endogenous Tau transcript. PTMs were delivered into specific areas of the mouse brain by lentiviral vectors. Cognitive performance was tested in the novel object recognition task. The content of 3R and 4R Tau isoforms was determined by qPCR and Western blot. The presence of hyperphosphorilated Tau was detected by immunohistochemistry, the content of insoluble forms of Tau was measure by western blot and the neuronal firing was recorded. Htau mice rescued by trans-splicing restored some cognitive and neurochemical phenotypes, indicating that RNA reprogramming is a suitable tool to achieve a phenotypic recovery. Our results raise perspectives about using this technique to treat tauopathies.

WTH07-14

Reduction of mutant huntingtin in oligodendroglia rescues myelination and behavioural deficits in a model of Huntington disease

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Clear evidence from human and animal studies indicates that white matter structures are profoundly affected in Huntington disease (HD). Although its etiology is not fully understood, white matter atrophy appears very early in the disease course suggesting that it may be a primary event preceding neuronal loss. We hypothesize that abnormalities in white matter reflect dysfunction that involves the direct effects of mutant huntingtin (mHTT) on oligodendrocytes, the myelinating cells of the central nervous system. Using the BACHD mouse model of HD, which expresses full-length human mHTT and mimics many of the behavioural and neuropathological features of the human condition, we genetically reduced mHTT expression in oligodendroglial cells by crossing BACHD mice to NG2-Cre mice. Using electron microscopy analysis of myelinated fibers of the corpus callosum at 1 and 12 months of age, we show that myelin sheaths are thinner and less compact in BACHD mice. Reduction of mHTT expression in oligodendroglial cells rescues the deficits in thickness and compactness of myelin sheaths, supporting cell intrinsic effects of mHTT on oligodendrocytes. We further show that silencing mHTT in oligodendroglia improves aspects of behavioural dysfunction in the HD mice, including motor and psychiatric-like phenotypes. Our findings suggest that the expression of mHTT in oligodendrocytes contributes to myelin abnormalities and certain behavioural manifestations in HD. Our study provides novel insights into the etiology of white matter pathology in HD.

WTH07-15

Evaluation of selective PKR inhibition in mouse models of memory deficits and neurodegeneration

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The pro-apoptotic Protein Kinase RNA-activated (PKR) is activated by auto-phosphorylation, which in turn phosphorylates translation initiation factor eIF2α, in response to Alzheimer's disease pathogenic mechanisms, e.g. high levels of beta-amyloid species, neuro-inflammation, or presence of the ApoE4 risk-promoting allele. The activity of a potent and selective small molecule PKR inhibitor (SAR489883) was evaluated for target engagement *in vivo* in two animal models: transgenic mouse model expressing human APOE4 (APOE4 knock-in mice), and mice with intracerebral ventricular (ICV) injection of toxic Ab oligomers (AbOs). After oral treatment in ApoE4 KI mice and in C57Bl/6 mice in which AbOs were administered ICV, PKR target engagement was evaluated from brain samples by measuring both PKR occupancy, using KiNativ™ technology, and brain levels of PKR substrate (phospho-eIF2α/eIF2a) using semi-automated Simple-Western). In AbOs-injected mice, effects of SAR489883 on synapse loss were assessed by measuring brain levels of synaptic biomarkers SNAP25, PSD95 and synaptophysin, a hallmark of neuroinflammation, brain IL1β was investigated using ELISA. The effects of SAR489883 were characterized on cognitive functions using the Barnes maze test in ApoE4 KI mice and the Morris water maze test in AβO-injected mice. In ApoE4 KI mice, 1-week oral BID administration of SAR489883 dose-dependently reduced learning and memory deficits. In AβOs-injected mice, a 2-week administration of SAR489883 in the diet also dose-dependently reduced cognitive impairment, rescued deficits of synaptic proteins and reduced IL-1b. In both mouse models, the effects of SAR489883 were associated with a dose-dependent PKR occupancy and reduction of brain levels of peIF2a. An innovative combination of recent technologies (ActivX/KiNativ, Simple-Western), provides evidence of specific and robust PKR engagement by SAR489883 in brain which can be applied in blood cells and may be a promising approach to monitor these biomarkers in human.

WTH07-16

Shrinkage of peripheral nerve fibers in KCC3-T991A mutant mice

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KCC3 is an electroneutral transporter mediating the efflux of K^+, Cl^-, and obligatory water molecules to maintain cell volume. Loss-of-function mutations in KCC3 result in Agenesis of the Corpus Callosum with Peripheral Neuropathy (ACCPN). ACCPN is prevalent in a region of Quebec affecting approximately 1 in 2000 live births. Individuals suffering from ACCPN are homozygous for a mutation that truncates KCC3, rendering it non-functional. Patients exhibit severe sensorimotor neuropathy. Analysis of peripheral nerves in patients and a mouse model of the disease demonstrated swelling of axons. The relationship between axonal volume and the neuropathy remains unknown. The activity of KCC3 is regulated by phosphorylation/dephosphorylation of two key residues, Thr991 and Thr1048, located in the cytosolic C-terminus of the protein. Phosphorylation of these residues results in loss of KCC3 activity, whereas dephosphorylation by phosphatase results in its activation. We recently documented the case of a young boy exhibiting a *de novo* mutation in the KCC3 gene substituting Thr991 into alanine (T991A). The patient exhibits a greater motor neuropathy than sensory and displays none of the ACCPN brain deficits. We created a mouse model recapitulating the mutation of this patient and observed that only the homozygous mice display severe locomotor deficits. To determine the size of the nerve fibers, we isolated the sciatic nerve of wild-type, heterozygote, and homozygote mice, separating the proximal (close to spinal cord) and distal portions (between knee and foot) of the nerves. We fixed these samples for transmission electron microscopy analysis and observed that nerve fibers are significantly shrunken in both heterozygous and homozygous mice compared to wild-type mice ($p < 0.001$), with greater shrinkage in homozygote versus

heterozygote animals ($p < 0.036$). These data are consistent with a gain-of-function mutation rendering KCC3 constitutively active, resulting in shrinkage of the nerve fibers, in contrast to loss-of-function mutations in KCC3 that lead to swelling of the fibers. Our data highlight the critical role of KCC3 in cell volume homeostasis for the integrity of peripheral nerve fibers.

WTH07-17

Evaluation of pridopidine in the transgenic YAC128 mouse model of Huntington disease

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Pridopidine is currently in clinical development for the treatment of Huntington disease (HD) and investigations to increase the understanding of its therapeutic benefit and mode of action are ongoing. Here we aim to investigate the efficacy and mechanism of action of pridopidine using the transgenic YAC128 mouse model of HD. Pridopidine was administered to animals starting at early (1.5 months of age) or late stages of disease (8 months of age). In the early treatment cohort, animals were divided into three groups receiving 0, 10, or 30 mg/kg of pridopidine for a period of 10.5 months. In the late cohort, animals were divided into two groups receiving either 0 mg/kg or an escalating dose of pridopidine (10 mg/kg in week 1, 20 mg/kg in week 2, and 30 mg/kg in weeks 3–8). Pridopidine-treated animals were evaluated using a battery of behavioural tests. Our analysis reveals that chronic treatment with pridopidine improves behavioural measures including motor learning, motor performance and affective phenotypes in the YAC128 HD mice. Specifically, pridopidine improved motor learning in the rotarod test, and motor performance in the accelerating rotarod and climbing tests, reduced immobility in the forced swim test of depression, and decreased anxiety-like behaviour in the open field test and elevated plus maze. Assessment of neuropathology revealed no effect of pridopidine on striatal and corpus callosum volumes, or forebrain weights. Finally, RNA-Seq analysis revealed that pridopidine treatment results in significant reversal of transcriptional deficits in YAC128 HD mice and highlighted potential mechanisms of pridopidine-mediated functional improvements. Overall, our study supports continued clinical development of pridopidine for HD.

WTH07-18

RAGE inhibition in substantia nigra of rats prevents 6-OHDA-induced parkinsonism

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The receptor for advanced glycation end products (RAGE) is a pattern-recognition receptor associated with inflammation in most cell types. RAGE up-regulates the expression of proinflammatory mediators and its own expression via activation of NF-kB. Recent works have proposed a role for RAGE in Parkinson's disease (PD). In this study, we used the multimodal blocker of RAGE FPS-ZM1, which has become available recently, to selectively inhibit RAGE in the substantia nigra (SN) of rats intracranially injected with 6-hydroxydopamine (6-OHDA). FPS-ZM1 (40 mg per rat), injected concomitantly with 6-OHDA (10 mg per rat) into the SN, inhibited the increase in RAGE, activation of ERK1/2, Src and nuclear translocation of NF-kB p65 subunit in the SN. RAGE inhibition blocked glial fibrillary acidic protein and Iba-1 upregulation as well as associated astrocyte and microglia activation. Circulating cytokines in serum and CSF were also decreased by FPS-ZM1 injection. The loss of tyrosine hydroxylase and NeuN-positive neurons was significantly inhibited by RAGE blocking. Finally, FPS-ZM1 attenuated locomotory and exploratory deficits induced by 6-OHDA. Our results demonstrate that RAGE is an essential component in the neuroinflammation and neurodegeneration induced by the parkinsonian agent 6-OHDA in the SN. Selective inhibition of RAGE may offer perspectives for therapeutic approaches.

WTH07-19

Characterisation of EIF2B bodies in vanishing white matter disease

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Leukoencephalopathy with Vanishing white matter (VWM) is a fatal neurological disorder. It arises through autosomal recessive mutations within eukaryotic initiation factor 2B (eIF2B); a guanine nucleotide exchange factor (GEF) for the protein eukaryotic initiation factor 2 (eIF2). eIF2B provides a critical control point in the regulation of protein synthesis. Although eIF2B is a global regulator of protein synthesis, the phenotypic effect of VWM mutations is predominately observed in oligodendrocytes and astrocytes. Structurally, eIF2B is composed of five subunits: α, β, δ, γ and ε. Over 150 mutations, within all subunits of eIF2B, have been identified as causative of VWM. Although VWM predominately affects infants, disease onset, course, and severity, is highly variable amongst patients, and currently no genotype-phenotype link has been established. Using cell biology techniques we are developing a model to assess the functional effects of VWM mutations *in vivo*, offering scope for more accurate patient prognosis. Localisation studies in Yeast have shown that eIF2B localises to specific cytoplasmic foci, termed eIF2B bodies. eIF2 shuttles through these bodies, suggesting they are sites of GEF

activity. Using a GFP tagged eIF2Bε subunit, we have identified eIF2B bodies in mammalian glial cells through live cell imaging studies. The distribution of eIF2B bodies in these cells revealed heterogeneous populations, differing in size and abundance. Co-localisation studies of all subunits of eIF2B, via immunocytological techniques, identified these different bodies as subcomplexes of eIF2B; correlating with those recently identified via MS. Furthermore, utilising fluorescent recovery after photobleaching (FRAP) technology, we have evidence to suggest that populations of eIF2B bodies possess different levels of GEF activity, complementing previous biochemical assays. Additionally, the various eIF2B body populations display diverse responses to cellular stress, demonstrating differing levels of regulation within the body populations. Investigation into eIF2B body populations in various cells types, demonstrates the importance of this regulation to individual cell function. These data suggest that different compositions of eIF2B bodies allow cells to have varying characteristic responses to stress.

WTH07-20

Phenotypic differences between brain- and liver-specific glutamine synthetase knockout mice

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Hepatic encephalopathy (HE) is a common manifestation of hyperammonemia in patients with severe liver disease. In the brain, the astroglial enzyme glutamine synthetase (a.k.a. glutamate ammonia ligase, Glul) is vital for ammonia detoxification and neurotransmitter inactivation. Hyperammonemia is associated with inactivation of brain Glul via protein tyrosine nitration, and it has been suggested that such inactivation worsens the symptoms of HE (Gorg et al., 2010). Further, lack of Glul in the liver can also lead to chronic hyperammonia (Qvartskhava et al., 2015). Here we created conditional knockout mice lacking GS in the liver (L-Glul cKO) and cerebral cortex (C-Glul cKO), respectively, and compared the phenotypes of the two lines. The L-Glul cKO exhibited normal locomotor activity and innate rodent behaviors such as digging and sheltering by the open-field, nest-building and marble-bury tests. In contrast, the C-Glul cKO exhibited altered locomotor activity and impairments in digging and sheltering behaviors. Furthermore, the L-Glul cKO mice had unremarkable gross brain histology, whereas C-Glul cKO had altered microglia, astrogliosis and neurodegeneration. Thus, the knockout phenotypes are markedly different, with a greater complexity of alterations caused by the loss of Glul in the brain.

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WTH07-21

ALS mutant SOD1 affects pathological modifications of TDP-43

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Amyotrophic lateral sclerosis (ALS) is a fatal, adult-onset, and progressive neurodegenerative disorder with no cure. Cu/Zn-superoxide dismutase (SOD1) was the first identified protein associated with familial ALS; mutant SOD1 form abnormal aggregates. Recently, phosphorylated and truncated TAR DNA-binding protein 43 (TDP-43) is a principal component of ubiquitinated cytoplasmic inclusions in neuronal and glial cells in ALS. However, it remains unclear whether these ALS-linked proteins partly have a shared pathogenesis. The main purpose of this study was to determine the association between mutant SOD1 and TDP-43 in SOD1 G93A transgenic mice model, ALS cell line model, and spinal cord tissues and induced pluripotent stem cells (iPSCs) derived motor neurons from familial ALS patient. We examined the pathological TDP-43 modifications in SOD1 G93A transgenic mice model, ALS cell line model, and spinal cord tissues and iPSCs-derived motor neurons from familial ALS patient. In the present study, we demonstrated an age-dependent increase in TDP-43 C-terminal fragments and phosphorylation at serine 409/410 in spinal cord motor neurons and glial cells of ALS transgenic mice and a similar increase in TDP-43 modifications in spinal cord glial cells of patients with ALS. The cytoplasmic mislocalization of TDP-43 was also observed in iPSCs-derived motor neurons from familial ALS patient. Moreover, we observe that mutant SOD1 interacts with TDP-43 by co-immunoprecipitation assays using WT-hSOD1 and mutant (G93A) hSOD1-transfected motor neuronal cell lines. Mutant SOD1 overexpression led to an increased amount of mutant SOD1 and its interacting proteins including TDP-43 C-terminal fragments and phosphorylation in the detergent-insoluble fraction in the spinal cord of SOD1 G93A transgenic mice and familial ALS patient. These findings suggest that mutant SOD1 could affect the solubility/insolubility of its interacting TDP-43 through physical interactions and pathological modifications of TDP-43 in glial cells may be involved in motor neuron death in the spinal cord of SOD1 G93A transgenic mice and familial ALS patient.

WTH07-22

Oxidative aggregation of AMPK underlying age-related impairment of selective autophagic clearance in the hippocampus

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AMP-activated protein kinase (AMPK) regulates energy states and autophagy in response to various stresses. Aging-associated disturbance of autophagy is closely related to impaired neuronal function and survival. Although AMPK is thought to be critical for maintaining autophagy and the clearance of damaged organelles in the aged brain, little is known about AMPK-associated changes in the brain during aging. Therefore, the aim of this study was to elucidate the impact of aging on AMPK physiology and signaling in

the hippocampus of aged mice. Young (8–10 weeks) and old (18 months) C57BL6 mice were used and acute restraint stress was given to them for 6 h. The changes in AMPK signaling and selective autophagic clearance were investigated through western blot analysis, immunofluorescent staining, co-immunoprecipitation and electron microscopic observation. We found that AMPK formed oxidized aggregates in the hippocampus of old mice, which occurred alongside a decline of thioredoxin 1 (Trx1). Moreover, old mice showed abnormal perinuclear mitochondrial clustering that colocalized with oxidized AMPK aggregates in hippocampal pyramidal neurons and impaired AMPK-mediated selective autophagic capacity. Interestingly, the oxidative aggregation of AMPK and perinuclear mitochondrial clustering were ameliorated by overexpression of Trx1, suggesting that age-related changes in AMPK physiology and consequent accumulation of dysfunctional mitochondria are reversible. These findings could be used to develop new therapeutic strategies for overcoming age-related hippocampal dysfunction and dementia.

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WTH07-23

MIR-18B dysregulation triggers apoptosis by inhibition of MCTP1 and RARB in fALS linked SOD1 mutation

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MicroRNAs (miRNAs) are endogenous noncoding RNAs that regulate gene expression at the post-transcriptional level and key modulators of neurodegenerative disease. Overexpressed miRNAs have an important role in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). However, the pathogenic mechanisms of dysregulated miRNAs are still unclear. Here, we aimed to determine dysfunction of RNA metabolism including miRNAs in fALS. We compared transcriptional profiling of NSC-34 and NSC-34 (G93A) mutant cell lines and indentified upregulation of hypoxia inducible factor 1 alpha (Hif1α) and myocyte specific enhance factor 2c (Mef2c) and downregulation of multiple C2 and transmembrane domain containing protein 1 (Mctp1) and retinoic acid receptor beta (Rarb) in NSC-34 (G93A) mutant cell lines. We demonstrated that Hif1α which is increased by miR-18b dysregulation is associated with Mef2c expression. Decreased both Mctp1 and Rarb were directly regulated by miR-206 which also is increased by Mef2c. Thus, the simultaneously down regulation of Mctp1 and Rarb accelerates Bax which is apoptotic regulatory proteins in NSC-34 (G93A) mutant cells. The inhibition of Mctp1 and Rarb induce intracellular Ca^{2+} level and reduce cell differentiation, respectively. This finding suggested that miR-18b dysregulation is involved in apoptosis cell death in SOD1 mutation. Furthermore, miR-18b signaling pathway was precisely discovered in G93A TG mice, and SOD1 ALS patients as NSC-34 cells. Taken together, our data indicate that SOD1 (G93A) mutation decreases miR-18b which is sequentially regulates several genes (Hif1α, Mef2c, Rarb and Mctp1) and miR-206. These results strongly suggest new insights into the dysregulation of miRNAs dependent pathogenic mechanism in ALS and FTLD.

WTH07-24

Vitamin A (retinol) protects against neurodegeneration in a 6-hydroxydopamine rat model

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Introduction: Vitamin A (retinol) exerts fundamental role in cellular processes regulation, such as growing, cell division and apoptosis. In recent years, several studies have been proposing an anti-inflamatory and antioxidant effect of retinol, however, series of pro-oxidant actions have been shown in different *in vivo* and *in vitro* conditions, demonstrating a dualistic redox activity of this molecule. The role of vitamins as antioxidants in neurodegenerative diseases has also been extensively debated, and some results showed a positive correlation between serum levels of retinol and reduced cognitive decline. However, the effect of dietary intake of vitamin A on the risk of Parkinson's disease (or its prevention) is uncertain as available epidemiological data are limited.

Objective: To investigate the preventive role of vitamin A supplementation against 6-hydroxydopamine (6-OHDA) neurotoxicity in a Parkinson's disease rat model.

Results: Rats received oral supplementation of retinol as retinyl palmitate (3000 IU/Kg/Day^{-1}) for 30 days prior to 6-OHDA injection into substantia nigra (SN). Rotatrod test, imunohistochemistry and western blot analyses were performed 15 days after 6-OHDA injection. Retinol supplementation significantly protected against 6-OHDA-induced locomotory deficit in rotarod test. The decreases in TH^+ cells as well as TH protein levels in SN were prevented by retinol supplementation. Serum analysis revealed that retinol was able to reduce the amount of IL-1β and TNF-α pro-inflammatory cytokines, and also was able to reduce the levels of carboxymethyl-lysine, an advanced glycation end product, in cerebrospinal fluid, suggesting a possible involvement of receptor for advanced glycation endproducts (RAGE) in oxidative/inflammatory process.

Conclusions: Vitamin A supplementation had a preventive property in 6-OHDA-induced motor deficit, dopaminergic degeneration and induction of pro-inflammatory cytokines.

WTH07-25

SC79, a AKT activator, rescues Alzheimer's disease-associated memory impairments and aberrant synaptic plasticity

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Aβ is as a key mediator for synaptic dysfunction and cognitive impairment observed in Alzheimer's disease (AD). However, the

precise mechanism of the effect of Aβ is still not complete. Akt is known to be aberrantly regulated in AD brain. However, its possibility in therapeutic target for AD-associated memory impairment is not studied. Here we examined the effects of direct Akt activator, SC79, in hippocampal-dependent memory using Aβ-treated and 5XFAD mice AD models. We found that pharmacological activation of Akt rescued memory impairments and aberrant synaptic plasticity in both of Aβ-treated and 5XFAD mice. These results suggest that Akt might be a therapeutic target for memory impairment observed in AD.

WTH07-26

PTUPB, dual inhibitor of soluble epoxide hydrolase and cyclooxygenase-2, mitigates rotenone induced neurotoxicity

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Epoxyeicosatrienoic acids (EETs), are the metabolites of arachidonic acid cascade, plays a crucial role in cytoprotection by attenuating oxidative stress, inflammation and apoptosis. EETs are rapidly metabolised *in vivo* by soluble epoxide hydrolase (sEH). Elevating the half life of EETs by inhibiting sEH is a novel strategy for neuroprotection and the simultaneous inhibition of COX-2 will have an added advantage in neuroprotection. In the present study, PTUPB was evaluated for its anti-Parkinson activity against rotenone induced mitochondrial dysfunction, oxidative stress and neuroinflammation in N27 dopaminergic cell lines and *Drosophila melanogaster* model of Parkinson disease (PD). The *in vitro* neuroprotective efficiency was evaluated by measuring cell survival assays, oxidative stress parameters (intracellular ROS, protein oxidation, lipid peroxidation, and mitochondrial membrane potential), inflammatory markers (IL-6, COX-1 and COX-2), and apoptotic markers (c-jun, P-c-jun, JNK, P-JNK, pro and active caspase-3). Further, *in vivo* neuroprotective efficiency was confirmed by measuring Survival rate, negative geotaxis, dopamine and its metabolites (LCMS) and oxidative stress parameters. PTUPB pre-treatment significantly improved cell viability, through amelioration of ROS production, proteins oxidation and lipids peroxidation. It also attenuated the mitochondrial damage by improving mitochondrial membrane potential and complex I activity. PTUPB normalizes the altered mRNA expression levels of inflammatory markers and antioxidant enzymes as assessed by RT-PCR. PTUPB also decreased the phosphorylation of apoptotic markers like JNK and c-jun leading to alleviated levels of cleaved caspase-3. These results were in corroboration with *in vivo* results with improved survival rate, negative geotaxis, dopamine levels, antioxidants and anti-inflammatory status in *Drosophila* model of PD. These results substantiate the neuroprotective efficiency of PTUPB indicating its potential therapeutic benefits in the treatment of PD.

WTH07-27

Surveillance of human prion diseases in Brazil data from 2005 to 2017

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Global surveillance of CJD and its subtypes was recommended by WHO for investigate the presence of the variant of Creutzfeldt Jakob Disease, for a better understanding of the iatrogenic causes as well as the distribution of hereditary CJD forms. The compulsory notification of diseases caused by prion began in Brazil in 2005 as an initiative of the Ministry of Health. So far, we have received 620 blood samples from notified cases of suspected CJD. They were analyzed by genomic sequencing to identify mutations and polymorphisms in the *PRNP* gene. The average age of our patients was 60.6 years (10-94y). PRNP polymorphisms analysis at codon 129 showed that 50% of cases were homozygous for methionine, 31% were heterozygous and 19% were homozygous for valine. Regarding genetic diseases, we found fifteen patients with CJD, in which the mutation E200K (nine cases), D178N (two cases), T183A (one case), V180I (one case) and octarepeat insertion (two cases) were detected. We also diagnosed two patients with GSS syndrome (P102L) and three patients with fatal familial insomnia (129M+178N). After clinical and exams evaluation, the notified cases were classified according to the WHO criteria. Among of them, 8% were classified as sporadic CJD, 42% as probable CJD, 20% as possible CJD, 4,5% as genetic prion disease, 18.5% as suspected CJD and 7% were non-CJD. This study provides the first epidemiologic data about human prion diseases in Brazil. Similar to any other country the availability of brain tissue from these patients is a limiting factor to confirm the diagnosis of prion diseases. In this way, the present work represents an important tool for prion-prevention policies and shows great importance for future implementation of clinical trials.

WTH07-28

Trimethyltin-induced hippocampal neurodegeneration

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Trimethyltin (TMT), a toxic organotin compound, induces neurodegeneration selectively involving the limbic system and especially prominent in the hippocampus. Neurodegeneration-associated behavioral abnormalities, such as hyperactivity, aggression, cognitive deficits, and epileptic seizures, occur in both exposed humans and experimental animal models. Previously, TMT had been used generally in industry and agriculture, but the use of TMT

has been limited because of its dangers to people. TMT has also been used to make a promising *in vivo* rodent model of neurodegeneration because of its region-specific characteristics. Several studies have demonstrated that TMT-treated animal models of epileptic seizures can be used as tools for researching hippocampus-specific neurotoxicity as well as the molecular mechanisms leading to hippocampal neurodegeneration. This review summarizes the *in vivo* and *in vitro* underlying mechanisms of TMT-induced hippocampal neurodegeneration (oxidative stress, inflammatory responses, and neuronal death/survival). Thus, the present review may be helpful to provide general insights into TMT-induced neurodegeneration and approaches to therapeutic interventions for neurodegenerative diseases, including temporal lobe epilepsy.

WTH07-29

Aberrant expression and possible pathogenic role of S100B-RAGE in ALS

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Neuroinflammation is one of the major players in amyotrophic lateral sclerosis (ALS) pathogenesis, and astrocytes are significantly involved in this process. S100B, a calcium-binding protein mainly expressed by astrocytes in the CNS, can be released in pathological states, activating the receptor for advanced glycation end-products (RAGE). In ALS, different indications point to an aberrant expression of S100B and RAGE; this work provides a comprehensive picture of their localization, expression timing and possible roles in SOD1G93A models of ALS. We observed that S100B and RAGE are progressively upregulated selectively in astrocytes of diseased rats with a timing pattern that may be linked to the level of neurodegeneration. Also the expression of the full length and soluble RAGE isoforms, which display antagonistic functions, is likely correlated to the features of tissue damage. Moreover, we showed that the mere presence of mutant SOD1 is able to increase the intracellular levels and release of S100B from astrocytes, suggesting that increased astrocytic expression of S100B might be an early event during the progression of the disease. Finally, our findings indicate that the protein may exert a pro-inflammatory role in the disease, since its inhibition in astrocytes derived from SOD1G93A mice dowmregulates the expression of reactive/ pro-inflammatory genes (GFAP, TNFalpha, CCL6, CXCL10), indicating that the protein migkt promote a pro-inflammatory phenotype in astrocytes.. Thus, our findings candidate the S100B-RAGE axis as an effective contributor to the pathogenesis of the disease, so that its blockade may be regarded as a rational target for therapeutic intervention in ALS.

WTH07-30

Role of G quadruplex rna structure in ALS/FTD

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are multisystem disorders with overlapping functional and genetic causes. Errors in the production of the DNA/RNA binding proteins Tar-DNA binding protein 43 (TDP-43) and fused in sarcoma/translocated in liposarcoma protein (FUS/TLS) are causative of ALS and FTD, as these proteins are the major protein components in over 90% of ALS and over 50% of FTD inclusions. In 2011 it was discovered that a hexanucleotide GGGGCC expansion in the *C9ORF72* gene is a common genetic cause for ALS and FTD. The mechanisms by which the mutations in FUS and the GGGGCC expansion lead to ALS/FTD are not known, hindering the development of therapeutic agents against these diseases. In this study we advance the hypothesis that the G-quadruplex RNA structure plays an essential role in the pathogenic mechanisms of both FUS and C9ORF72 hexanucleotide expansion in ALS and FTD. We show by biophysical methods that the GGGGCC expansion adopts preferentially the G quadruplex over an extended hairpin structure. Moreover, wild type FUS and ALS causing FUS mutants bind G quadruplex forming mRNAs, including the GGGGCC expansion using the C-terminal arginine-glycine-glycine motif.

WTH07-31

Postnatal exposure augments neurotoxicity of ZINC/ paraquat exposed adult rats on oxidative stress, monoamine transporters, apoptosis

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Pesticides and heavy metals are established as the major environmental risk factors for Parkinson's disease (PD). Developmental exposure to pesticides enhances the susceptibility for dopaminergic neurodegeneration in re-challenged adult rats. However, the effect of postnatal pesticide exposure on the heavy metal-induced neurotoxicity or vice versa is not yet clearly explored. The present study aimed to investigate the effect of postnatal exposure of zinc (Zn) or paraquat (PQ) on the nigrostriatal dopaminergic neurodegeneration in re-challenged adult rats. Male Wistar rats were treated with Zn/PQ during post natal (5-19) days followed by re-exposure upon adulthood (twice weekly) for 12 weeks. Striatal dopamine content, oxidative stress indicators and expression of tyrosine hydroxylase (TH), dopamine transporter (DAT) and vesicular monoamine transporter-2 (VMAT-2) were measured in the control and treated groups. Besides, the mitochondrial cytochrome-c release and caspase3/9 activation were also analyzed. A marked reduction was obtained in the striatal dopamine and glutathione content with concomitant increase in lipid peroxidation and protein carbonyls in adulthood exposed and postnatal + adulthood-exposed groups. While significant reduction in the expression of VMAT-2 and TH was observed, the mitochondrial cytochrome-c release and caspase-3/9 activation along with DAT expression were found to be elevated in adulthood exposed groups.

The changes were more pronounced in postnatal + adulthood exposed groups as compared with adulthood alone. Postnatal exposure per se did not show any noticeable change in any of the aforementioned parameters. Results of the study demonstrate that postnatal pre- exposure of Zn/PQ augments oxidative stress, alters DAT/VMAT-2 expression and induces intrinsic apoptosis leading to dopaminergic neurodegeneration in re-challenged adult rats.

WTH07-32

Functional impact of phosphorylation of mutant HTT at serine-421 in human neurons

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Huntington disease (HD) is a neurodegenerative disease caused by an autosomal dominant mutation in a single gene, Huntingtin (*HTT*), which results in an elongated polyglutamine tract in the HTT protein. The phosphorylation at serine-421 (pS421) of mutant HTT (mHTT) downstream of IGF-1/Akt signaling axis was previously reported to be neuroprotective. Though certain molecular mechanisms have been described, the effects of pS421 on mHTT toxicity and the exact cellular processes involved remain incompletely understood. Here we demonstrate that pS421 of mHTT ameliorates certain mitochondrial alterations seen in HD human neural cells. Using genome editing, we generated isogenic HD human pluripotent stem cell (hiPSC) lines in which the S421 site in mHTT has been mutated into a phosphomimetic glutamic acid (S421D) or phospho-resistant aspartic acid (S421A). We observed significant differences in mitochondrial function and structure for hiPSC-derived neural cells with the S421D compared with S421S and S421A forms of mHTT. We further observed amelioration of transcriptional changes in mitochondrial-related genes in neural cells with S421D but not the S421A form of mHTT. Our results show that post-translational modification at S421 may modulate the toxicity of the full-length mHTT protein at least in part by affecting HD-associated mitochondrial alterations. Our study highlights a facet of the relationship between mHTT and mitochondrial dysfunction in the context of human physiology, with potential relevance to the pathogenesis of HD.

WTH07-33

Ankyring malfunction and neurodegenerative process in HuC KO mice

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Hu proteins (the neuronal Elav-like: nElavl) are the mammalian homologue of the Drosophila Elav, an RNA-binding protein expressed in the nervous system. Hu proteins bind to target RNAs and regulate alternative splicing and translational process.

Almost all of the brain regions express HuC together with HuB and/or HuD. However, cerebellar Purkinje cells (PCs) express only HuC. Then if HuC gene is knocked out, PCs specifically become all of the neuronal Hu null. Dysfunction of PCs in HuC KO mice leads the intentional tremor, gain abnormality and ataxia. Prior to the onset, the axons of PCs underwent morphological changes; swollen and retracted at the cerebellar nuclei. To reveal the mechanisms of the axonal degeneration, components of the spheroids were investigated with histological analysis. Most spheroids were accumulated with mitochondria and endoplasmic reticulums. Surprisingly, cytosolic organelle such as nuclei and ribosomes were also observed in the spheroids. These abnormal distributions of cytosolic organelle were thought to be caused by dysfunction of selective filter between soma and axon. Based on these data, AnkyrinG was suspected to be a causing factor. In neurons, AnkyrinG is located in the axon initial segment (AIS) and forms the selective filter between soma and axon. This system is required to define the delicate protein distribution in neuron. Our studies showed that HuC regulates the alternative splicing of AnkyrinG, and the splicing process was disrupted in HuC KO cerebellum. Moreover, particular splicing variant, which increased in HuC KO cerebellum, was identified as embryo-specific variant of AnkyrinG. Furthermore, the embryo-specific variant exhibited differential binding affinity to Spectrin compared to adult variant. These data indicate that HuC maintains the homeostasis of axons probably through controlling alternative splicing of AnkyrinG.

WTH07-34

A novel contrast agent to detect apoptotic cells in stroke and Alzheimer's disease

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Apoptosis-related neurodegeneration is directly linked with the loss of brain function in stroke and Alzheimer's disease (AD). So far there are no clinical means to detect apoptotic cells *in vivo*. Here we present a new contrast agent (CA), bearing a fluorescent and a radioactive labels, that is designed to accumulate in apoptotic cells due to increased cleaved caspase-3 (CC3) activity. In neuronal culture models of neurodegeneration, including oxygen-glucose deprivation, camptothecin and beta-amyloid oligomer toxicity, CA accumulated predominantly in apoptotic cells. In live animals, positron emission tomography (PET) showed CA accumulated on the injury side of stroke mouse brains (transient middle cerebral artery occlusion, MCAO). Similarly, PET showed CA accumulation in forebrain of AD mice (5xFAD), but not in wild-type controls. Confocal microscopy on excised post-injection brains confirmed the spatial distribution of CA in both cases. In MCAO brains CA fluorescence correlated with the increase in CC3-positive cells on the operated side. In optically cleared MCAO brains CA and CC3

were co-localized on the injury side. In 5xFAD brains, CA fluorescence was elevated in hippocampus and cortex, compared to wild-type controls. Co-staining with thioflavinS revealed that CA accumulated in single cells and around amyloid plaques. Our studies in brain disease models show that this CA targeting caspase-3 activity could become an important tool for *in vivo* detection of apoptosis-affected regions in neurodegenerative diseases or other conditions or therapies associated with apoptosis, providing a proof of principle for its potential utility in humans.

WTH07-35

Neuroactive steroid levels in sciatic nerve: effects of blunted *de novo* fatty acid synthesis

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Neuroactive steroids are cholesterol-derived molecules that function as protective agents in central and peripheral nervous system. We recently described that a genetic model of reduced fatty acid synthesis, the sterol regulatory binding factor-1c knock-out mice (Srebp-1cKO), developed peripheral neuropathy over time. At 10 months of age, we found that Srebp-1KO sciatic nerves showed an apparent hypermyelination of small-caliber fibers due to changes in myelin periodicity resulting in myelin instability and Remak bundle degeneration. We decide to evaluate the levels of neuroactive steroids in plasma and in sciatic nerve of Srebp-1cKO male mice at 2 and 10 months of age using LC-MS/MS. At 2 months of age, we found an increase of pregnenolone, associated with decrease of progesterone and further metabolites. The levels of testosterone were also increased. Interestingly, changes in pregnenolone, progesterone and testosterone were not observed in plasma but were restricted to sciatic nerve. These results were further corroborated by gene expression analysis. The expression of P450scc, the enzyme involved in the first step of steroidogenesis, was increased. Moreover, both 5a-reductase (5a-R) and 3b-hydroxysteroid oxidoreductase (3a-HSOR) mRNA levels were also induced.

At 10 months of age, the neuroactive steroid profile showed further differences. Indeed, the levels of pregnenolone were decreased while those of dihydroprogesterone, tetrahydroprogesterone and isopregnanolone were increased. Furthermore, testosterone and its metabolites were decreased. Moreover, plasma levels of neuroactive steroids were unaffected confirming that the observed changes occurred in the sciatic nerve. At this age, we also found a significant decrease of P450scc gene expression associated with an increase of 5a-R and 3a-HSOR mRNA levels.

Altogether, our data support the concept that the cross-talk between fatty acid synthesis and neuroactive steroids, may represent a possible therapeutic strategy for peripheral neuropathy.

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WTH07-36

Comparative analysis of cerebral 64CU and F-18 FDG uptake in APPSWE TRANSGENIC MOUSE MODELS OF ALZHEIMER'S DISEASES WITH PET/CT

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Objectives: Copper is required for brain development and function. Copper deficiency causes malfunction and loss of neurons in Menkes disease and excess copper accumulated in brain tissue causes neuronal damage in Wilson's disease. To explore cerebral ^{64}Cu uptake as a biomarker in pathophysiology of AD, the objective of this study was to compare ^{64}Cu and F-18 FDG uptake in the brains of APPSWE transgenic mice measured with sequential $^{64}CuCl_2$-PET/CT and F-18 FDG PET/CT.

Methods: APPSWE Tg2576 transgenic mice (n = 5) were subjected to sequential PET/CT after intravenous injection of $^{64}CuCl_2$ and F-18 FDG as a tracer, respectively. A quantitative PET analysis was conducted to compare ^{64}Cu and F-18 FDG uptake in the brains of APPSWE transgenic mice, in comparison to ^{64}Cu and F-18 FDG uptake in the brains of control C57BL/6 mice (n = 5) after intravenous injection of $^{64}CuCl_2$ as a tracer.

Results: Different biodistribution of $^{64}CuCl_2$ and F-18 FDG in APPSWE Tg2576 transgenic mice and wild type C57BL/6 mice was visualized on PET/CT images, showing high F-18 FDG and low ^{64}Cu uptake in the brains of the mice. There was increased ^{64}Cu uptake in the brains of young adult APPSWE Tg2576 transgenic mice (0.71 $\pm$ 0.13%ID/g) compared to ^{64}Cu uptake in the brains of young adult C57BL/6 mice. In contrast, there was no difference of F-18 FDG uptake in the brains of APPSWE mice (5.0 $\pm$ 0.72%ID/g) and C57BL/6 mice (5.6 $\pm$ 1.30%ID/g).

Conclusion: Increased ^{64}Cu uptake was detected in the brains of young adult APPSWE Tg2576 transgenic mice compared with the ^{64}Cu uptake in the brains of C57BL/6, supporting further investigation of age-dependent changes of ^{64}Cu and F-18 FDG uptake in the brains of APPSWE Tg2576 transgenic mice, in comparison to control C57BL/6 mice.

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WTH07-37

Cytosolic CU(II) is modulated by glutathione

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Copper is an essential micronutrient that regulates several cellular processes, including mitochondrial oxidative phosphorylation, iron metabolism, free radical detoxification and neurotransmission. Copper is a redox-active transition metal that readily oxidizes from Cu(I) to Cu(II) in aerobic solutions. Several studies indicate that under physiological conditions, the main copper redox state in the cytosol is Cu(I), which is maintained by reducing agents,

hypothesized to predominantly glutathione. Brain glutathione depletion as well as copper dyshomeostasis, are features of neurodegeneration. We hypothesized that intracellular Cu(II) is elevated by the loss of glutathione in these diseases. There is no previous direct evidence for the normal presence of Cu(II) in the cellular cytoplasm. In this study, using XANES and a Cu(II)-sensor, we demonstrate for the first time that Cu(II) is present in the cytosol. Moreover, our results show that a decrease of intracellular glutathione by *N*-ethylmaleimide or L-buthionine sulfoximine, increases the intracellular levels of Cu(II), indicating that the copper redox-state is indeed regulated by glutathione. Furthermore, we show that under oxidative stress conditions, such as H_2O_2 or glutamate treatments, Cu(II) intracellular levels are also increased. Together, our results indicate that Cu(II) can emerge in the cytosol under oxidative stress that is typical of neurodegenerative diseases. Loss of Cu(I) and the emergence of redox-catalytic Cu(II) may exacerbate the metabolic injuries in these disorders.

WTH07-38

3-Hydroxykynurenine and 3-hydroxyanthranilic acid enhance the toxicity induced by copper in rat astrocytes culture

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Copper is a heavy metal and an integral component of various enzymes and biological functions;however, excess copper is neurotoxic and has been implicated with neurodegenerative diseases as Alzheimer. This metal is able to modify the cellular redox environment. In this context, kynurenine pathway (KP) is modulated by the redox environment and produces some metabolites with redox properties as 3-hydroxykynurenine (3-HK) and 3-hydroxyantrhanilic acid (3-HANA). The imbalance in the production of these kynurenines is related with some neuropathologies, in which the common factors are oxidative stress, inflammation and cell death. The aim of this study was to evaluate the effect of these kynurenines on the cupper toxicity in astrocytes cultures. First, we evaluated the $CuSO_4$ (0–500 μM) effect on MTT reduction, ROS production, mitochondrial membrane potential (MMP) and cell viability on primary cultured astrocytes. Then was evaluated the effect of the co-incubation of $CuSO_4$ (350 μM) whit metabolites (100 μM) in the same parameters that were previously tested, also GHS levels and the chelating copper effect of 3-HK and 3-HANA. Our results showed that $CuSO_4$ decreased MTT reduction and MMP, while it increased ROS production and cell death in a concentration-dependent manner. The co-incubation with metabolites enhances the toxic effect of copper in MTT reduction, MMP and cell death. Copper also decreased GSH levels around 50% and co-incubation with the kynurenines decreased 70% GSH levels. However, the increase in ROS production by copper was abolished by metabolites. Both metabolites are able to chelate cupper in a concentration dependent manner. These data suggest that 3-HK and 3-HANA increased copper toxicity in an independent manner to ROS production; however, their effect on GSH levels could play an important role in the potentiation of cell damage induced by copper.

WTH07-39

Lysosomal impairment causes the onset of neurodegeneration in mouse granule neurons: the side effect of sphingolipid metabolism

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Several lines of evidence implicate lysosomal dysfunction in the neuropathology associated with Lysosomal Storage Diseases. Nevertheless, the mechanistic link between the altered lysosomal homeostasis and the neuronal damage is still unknown. In this context, promising results obtained in a fibroblast-model of lysosomal impairment suggest a possible role played by the alteration of sphingolipid metabolism. With the aim to investigate the effects of lysosomal impairment in neurons, I developed a new *in vitro* model of lysosomal engulfment represented by differentiated mouse cerebellar granule neurons loaded with sucrose. Interestingly, in sucrose loaded neurons I found an increased lysosomal biogenesis due to the nuclear translocation of the Transcription Factor EB, master regulator of lysosomal genes. Furthermore, sucrose loading induces the activation of autophagy and a decrease in cell viability accompanied by the onset of neurodegeneration. Remarkably, after sucrose loading I found an alteration of sphingolipid composition characterized by the reduction of polysialoganglioside and sphingomyelin contents followed by the increase of ceramide level. These findings suggest that sucrose loading induces an activation of the sphingolipid catabolism as confirmed by the increased activities of the main sphingolipid hydrolytic enzymes, both intracellularly and at the plasma membrane level. These results let to speculate that sucrose loading causes an augmented fusion between lysosomes and the cell surface resulting in the increase of sphingolipids hydrolases *in-situ*. The relationship between these events and the production of ceramide at the plasma membrane level unveils a new role of sphingolipid metabolism in the activation of downstream pathways responsible for the onset of cell damage and neurodegeneration.

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WTH07-40

Locus coeruleus lesion by 6-hydroxydopamine induces recognition memory deficits in rats: involvement of prefrontal cortex

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Locus coeruleus (LC) degeneration, the main source of cerebral noradrenaline (NA), has been related with neurodegenerative disorders such as Alzheimer's (AD) and Parkinson's diseases (PD). Growing evidence support earlier NA deficiency in several brain areas resulting from selective degeneration of LC neurons. Additionally, LC projections to the prefrontal cortex (PFC) have a critical role on the cognitive functions. Therefore, here we evaluated the learning and memory of rats after a 6-hydroxydopamine (6-OHDA)-induced selective noradrenergic lesion in LC and the involvement of the likely resulting NA deficit in the PFC. For this,

adult male Wistar rats received stereotaxic bilateral injections of 6-OHDA (5 μg/side) into the LC and two stainless-steel guide cannulas were implanted aimed at the PFC. This 6-OHDA dose did not cause any motor alterations. SHAM group received just vehicle (0.2% ascorbic acid in saline). Selective noradrenergic lesion was reached by nomifensine administration (10 mg/kg/ml, i.p.) 1 h before of 6-OHDA infusion. LC lesion caused short- and long-term recognition memory impairments addressed on object recognition test 14 days after 6-OHDA administration. Moreover, LC slices from 6-OHDA-injected rats showed an elevation in the mitochondrial membrane potential. The propidium iodide (PI) incorporation and the nitroxidative stress production were not altered in the LC slices from lesioned rats. Importantly, PFC slices from 6-OHDA group exhibited cell damage evaluated by PI incorporation, nitroxidative stress production increase and mitochondrial membrane potential disruption when compared to the SHAM group. These outcomes suggest that the irregular action of LC neurons caused neurochemical changes in the PFC. Corroborating with the hypothesis of PFC noradrenergic deficit, bilateral NA infusion (1 μg/side) into this region immediately before of the training session restored the 6-OHDA-induced recognition memory dysfunctions. Thus, our results suggest that the LC lesion and the consequent dysregulation network with the PFC could be involved in the recognition memory impairments observed in both AD and PD patients.

WTH07-41

Participation of MAPK signaling pathway in a model of neuronal degeneration in striatal of rat

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Quinolinic acid (QUIN), an endogenous metabolite from kynurenine pathway, acts as a competitive agonist on NMDAr and its intrastriatal administration to rats has been used to reproduce some alterations similar to those observed in some chronic-degenerative disorders. Oxidative stress-depended signaling pathways, such as MAPK, are related with the physiopathology of some brain diseases, promoting cell death (JNK and p38 activation) or declining survival cell (ERK1/2 inhibition). Besides, MAPK activation has been related to changes on some systems antioxidants such as Nrf2 and CREB and to suitable levels of neurotrophins. In this study, we evaluate the activation of MAPK in the cell death in the striatum induced with QUIN, and its participation on transcription factors related to antioxidant response. Animals were intrastriatally infused with QUIN (30, 60, 120 and 240 nmol/ml). Right striatum was dissected at 2 h, 24 h and 7 days after QUIN injection. MAPK levels were detected by western blot and IHC. Histological analysis was done by H&E and FJ-B at 7 days after QUIN injection. Motor evaluation was done 6 days after operation. We found that QUIN (120 and 240 nmol/ml) significantly increased the activation of pathways related to death cell (p-JNK y p-p38) and decreased the activation of pathways that promote survival cell (p-ERK1/2) at 7 days. The sustained activation of p-JNK with QUIN 120 nmol from 2 h to 7 days could be involved in the onset of morphological alterations observed up to 7 days. Moreover, cell death in the striatum could also be due to decreased levels of Nrf2, CREB and BDNF levels. The decrease of preserved cells may be the cause of the deficit in motor evaluation with higher doses with QUIN. These data suggest that activation of p-JNK could be participating in the mechanism of damage of QUIN in the striatal cells of rats. CONACYT (Grant 241655).

WTH07-42

Validation of histocultures from adult human brain as a tool to study age-associated neurodegenerative diseases

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Histocultures from adult human brain are a powerful model to study cellular and molecular aspects of neurologic diseases. The advantages of this approach over 2-D cultures include the preservation of brain cytoarchitecture and neuronal connectivity. Few studies so far have assessed the morphofunctional state of adult human brain-derived tissue along the days in culture. Here, we have evaluated cell survival and function of cortical slices from adult human brains (median age 39 ± 11) along several days in culture. Tissues were obtained from patients submitted to partial lobectomy for the treatment of pharmaco-resistant temporal lobe epilepsy (Ethics Committee HCRP17578/15). Only cortical tissue resected to get access to the hippocampus was used. Fragments (ca. 1 cm^3) were collected at the surgical room, immersed in ice-cold, oxygenated buffered saline, sliced using a vibratome, and cultured. Tissue integrity was not affected by processing, as revealed by HE staining. MTT assay indicated no significant reduction in cell viability up to day 4. Immunohistochemistry revealed neuronal and astrocyte number stability along the days *in vitro*. Importantly, neurons remained synaptically active throughout the period in culture, as probed by ERK phosphorylation and neurotransmitter release after KCl-induced depolarization. The attack of Alzheimer's disease-associated Abeta oligomers to cultured slices was assessed by both IHC and ELISA. A massive binding of oligomers was evident, suggesting that this histoculture is amenable for modeling neurodegenerative diseases. This protocol may facilitate the application of histocultures from adult human brains in studies on new therapeutics for neurodegenerative disorders.

WTH07-43

Chronic stress triggers tau pathology through autophagy inhibition and induction of stress granules

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Imbalance of neuronal proteostasis associated with misfolding and aggregation of Tau protein is a common neurodegenerative feature in Alzheimer's disease (AD) and other Tauopathies. Consistent with suggestions that lifetime stress maybe an important precipitating factor of AD, we previously reported that environmental stress and high glucocorticoid (GC) levels evoke accumulation of aggregated Tau; however, the underlying molecular mechanisms remain unclear. We now demonstrate that chronic stress and GC trigger an mTOR-dependent inhibition of autophagic process, the cardinal clearance pathway for aggregated proteins, leading to accumulation of Tau aggregates and cell death in mice and cells stably expressing P301L-Tau. Considering the interplay of autophagy with Stress granules (SGs) dynamics, we also show that environmental stress/GC stimulate the induction of SGs, recently shown to promote Tau misfolding, aggregation and neurotoxicity. Notably, pharmacological intervention that stimulates autophagic process (Temsirolimus) attenuates the GC-driven elevation of Tau, SGs and cell death. This work provides novel insights into the mechanisms through which neuronal cells convey the detrimental impact of prolong environmental (HPA-related) stress to intracellular "stress" signaling, causing Tau-driven brain pathology.

WTH07-44

Activation of NRF2 in striatum is oxidative stress independent and dependent of P62 and DPP3 proteins

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Nrf2 is the most important protein that regulates the expression of genes involved in the celular redox homeostasis. Its activation in a non-canonical pathway, involves the disruption of Keap1-Nrf2 complex by direct interaction of some proteins with Keap1, like p62 and DPP3. It has been reported that quinolinic acid (QUIN), a selective agonist of NMDAr, it is capable to induce an oxidative/nitrosative state in the cell, so its intrastriatal administration has been used as an excitotoxic/pro-oxidant model. Unexpectedly, we found that QUIN administration increase the Nrf2 activation 30 min after its injection in the striatum, without increase of ROS production. In this work we propose to evaluate the effect of different doses of QUIN on Nrf2/Keap1 interaction in an *in vivo* model in the rat striatum. We administrated 1 μL of isotonic saline or QUIN (15, 30, 60, 120 and 240 nmol) in the striatum of male Wistar rats (260–300 g) and at 30 min after injection, striatum was collected. The Nrf2, Keap1, p62 and DPP3 levels was measured by western blot, the oxidative stress was evaluated by dihydroethidium oxidation and Xantine Oxidase (XO) and NAD(P)H oxidase (NOX) activity. Finally, the localization of protein expression (Nrf2 and p62) was identified by Immunofluorescence and the evaluation of protein interaction between Keap1 and p62 or DPP3 by Immunoprecipitation. Total amount of p62 and DPP3 proteins at 30 min, shows no change with all doses of QUIN, whereas total Keap1 are increased. However, an increase in p62, p-p62 and Nrf2 nuclear levels was observed. Dihydroethidium oxidation, XO and NOX activity showed not changes. The interaction between Keap1 and DPP3 or p62 increases and we found that this process is carried out in striatal neurons. These results suggest that at 30 min, the activation of Nrf2 is associated with the Keap1-Nrf2 disruption by DPP3 and p62 in neuronal cells, and this effect is oxidative stress independent. CONACyT (Grant): 241655.

WTH07-45

Ankyrin-R is required for cerebellar purkinje cell survival

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Ankyrin (Ank) proteins, are found throughout the body and act as the primary link between the spectrin-based cytoskeleton and the cytoplasmic domain of many membrane-associated proteins. Although AnkG and AnkB are well recognized as important domain organizers within the nervous system, few studies have investigated AnkR's role. Our lab recently showed AnkR can compensate for a loss of AnkG and cluster Na^+ channels at nodes of Ranvier. Additionally, multiple studies have indicated various neurological disturbances have disruptions in AnkR, including cerebellar dysfunction. However, the role of AnkR in the nervous system remains poorly understood. Our expression analyses show, unlike the other ankyrin proteins found widely throughout the brain, AnkR is only present in a subset of neurons, including cerebellar Purkinje cells. To elucidate the role of AnkR in these cells, I have examined AnkR knockout mice (AnkRpale/pale). Data reveals AnkRpale/pale mice have progressive Purkinje cell degeneration marked by increasing amounts of abnormal protein accumulation and dystrophic axons, resulting in cell loss in aged mice. Additionally, gait analyses show ataxia in null animals. Interestingly, mutations of bIII spectrin underlie spinocerebellar ataxia type 5 (SCA5), characterized by disrupted gait and progressive Purkinje cell degeneration, phenotypically similar to AnkRpale/pale mice. Although the precise molecular mechanisms underlying these changes remain unknown, our data confirms AnkR and bIII spectrin interact in the brain. Taken together, these data suggest AnkR plays an important role in stabilizing the spectrin cytoskeleton in Purkinje cells. Future studies using our novel AnkR cKO will further explore the role of AnkR in Purkinje cells, and other neuronal populations of interest.

WTH07-46

A novel model: optical stimulation causes axonal degeneration mediated by axoplasmic calcium

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Axonal degeneration contributes to neurological deficits in nervous system disorders, and is characterized by axonal swelling. Since energy failure and Na^+/Ca^{2+} overload play central roles in the axonal degeneration, abnormal functions of mitochondria are implicated in the axonal degeneration. However spatio-temporal changes of mitochondrial dynamics in relation to degenerative alterations of axons are still poorly understood. In this study, we investigated morphological changes and underlying mechanisms in the acute degeneration of sensory nerve axons observed with optogenetic stimulations, which enables spatio-temporal regulation of stimulations causing degenerative changes. Mixed dorsal root ganglion (DRG) cultures were obtained from rat embryo, and codon-optimized ChIEF (oChIEF), a channel rhodopsin variant, conjugated with red fluorescent mCherry was introduced into the DRG neurons with lentiviral vectors. Simultaneously, green Ca^{2+}-indicator, GCaMP3 or mitochondria-targeted green fluorescent Dendra2 (mitoDendra2) was introduced with other lentiviral vectors. Dominant negative mutant of Drp1, a mitochondrial fission protein (Drp1K38A), was also introduced in some cultures. Optogenetic stimulation of oChIEF caused axonal swelling following by axonal fragmentation in a manner dependent on duration of stimulation. GCaMP3 imaging demonstrated that axoplasmic Ca^{2+} increase precedes the axonal swellings, and treatments with Ca^{2+} chelators and Ca^{2+} channel blockers ameliorated the axonal swellings. Inhibition of mitochondrial fission by overexpression of Drp1K38A elongated stationary mitochondria, inhibited mitochondrial fragmentation upon optogenetic stimulation and decreased axonal swelling. During photo-stimulation, some axons exhibited rapid amelioration of axonal swelling, and the amelioration accompanied simultaneous decrease of GCaMP3 fluorescence. These results suggest that optical stimulation of channel rhodopsin variants causes axonal degeneration mediated by axoplasmic Ca^{2+} increase in sensory axons, and mitochondrial fission mediated by Drp1 exacerbates the initiation of axonal degeneration. Furthermore, intrinsic mechanisms reversing axoplasmic Ca^{2+} increase may be beneficial for axonal survival during Ca^{2+} induced axonal degeneration.

WTH07-47

Identification and characterization of novel dystonia musculorum mutant mice

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We identified a novel spontaneous mutant mouse showing motor symptoms that are similar to those of *dystonia musculorum (dt)* mouse. The observations suggested that the mutant mice inherited the mild *dt* phenotype as an autosomal recessive trait. Linkage analysis showed that the causative gene was located on chromosome 1, which are close to the dystonin (*Dst*) gene locus. To investigate whether *Dst* is the causative gene of the novel mutant, we crossed the mutant with *Dst* gene trap (Dst^{Gt}) mice. Compound heterozygotes showed a typical *dt* phenotype. Mutation analysis indicates a nonsense mutation in the spectrin repeat of plakin domain. The novel mutant mouse was named $Dst^{dt-23Rbrc}$. Histological analyses showed abnormal neurofilament (NF) accumulation in the nervous system of $Dst^{dt-23Rbrc}$ mice, which is characteristic of the *dt* phenotype. We mapped the distribution of abnormal NF-accumulated neurons in the brain and found that they were located specifically in the brainstem, spinal cord, and in regions such as the vestibular nucleus, reticular nucleus, and red nucleus, which are implicated in posture and motor coordination pathways. Therefore, we have identified a novel mutant allele of *dt*, which causes histological abnormalities in the central nervous system that may account for the abnormal motor phenotype. This novel spontaneously occurring mutant may become a good model of hereditary sensory and autonomic neuropathy 6, which is caused by mutations in human *DST* gene.

WTH07-48

Inhibition of HDAC4 with sodium butyrate does not prevent AMPA-induced excitotoxic degeneration of spinal motoneurons *in vivo*

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Selective motoneuron (MN) loss is the pathological hallmark of motoneuron diseases (MND). Chronic excitotoxicity is a fundamental mechanism proposed to explain this selective MN loss, which causes muscle denervation and atrophy. Muscle-nerve communication at neuromuscular junctions (NMJ) requires the synthesis and secretion of muscle-derived growth factors, and histone deacetylase 4 (HDAC4) activity in muscle has been

established as a critical regulator of NMJ maintenance (*Science* 326:1549, 2007). Furthermore, HDAC4 is overexpressed in skeletal muscle under conditions that result in NMJ disruption, such as nerve injury (*J Biol Chem* 282:33752, 2007) or MN disease (*Brain* 136:2359, 2013). These data suggest that HDAC4 inhibition might have protective effects against NMJ degeneration. Therefore, we studied the effects of the pan-HDAC inhibitor sodium butyrate (BA) in a model of chronic spinal MN death and paralysis induced by the chronic infusion of AMPA into the spinal cord of healthy rats using osmotic minipumps (*J Neuropathol Exp Neurol* 66:913, 2007). BA was administered intraperitoneally once daily for 6 days, beginning 1 day after osmotic minipump implantation. We observed that BA did not prevent paralysis, as assessed by two motor behavioral tasks, and did not reduce MN loss. In spite of this lack of protection, BA treatment induced an increase in histone H3 K9-14 acetylation in hindlimb muscles and spinal cord tissue, as determined by Western blots, suggesting HDAC inhibition by BA. These results indicate that either HDAC4 has only a minor role in excitotoxic MN death, or that BA inhibits other potential beneficial HDACs. In fact, recent reports have pointed out the relevant role of other HDAC members, such as HDAC7, in NMJ maintenance (*Muscle Nerve* 52:109, 2015). This work was supported by DGAPA, UNAM (Project IN204516) and CONACYT, México (project 240817). MP-G is recipient of a scholarship from CONACYT.

WTH07-49

Novel UBQLN2 mutations linked to amyotrophic lateral sclerosis and spastic paraplegia through defective proteolysis

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Amyotrophic lateral sclerosis (ALS), characterized by the degeneration of upper and lower motor neurons in the cortex, the brainstem and the spinal cord, is fatal, usually within 5 years. Frontotemporal dementia (FTD), due to the loss of frontal and temporal neurons, occurs in 15% of ALS patients. Most ALS cases are sporadic (SALS) whereas ~10% are familial (FALS). Mutations in *UBQLN2* have been associated with X-linked juvenile and adult forms of ALS and ALS/FTD. Ubiquilin-2 is a component of the ubiquitin inclusions detected in ALS spinal cord. We performed genetic analysis of 400 FALS and 770 SALS and identified three novel mutations in the PXX repeat domain of UBQLN2, a hot spot domain for ALS/FTD mutations. One of these mutations was also identified in patients with spastic paraplegia, affecting only the upper motor neurons of the limbs. These mutations, predicted to be deleterious, were absent from control databases. Experiments performed on patient lymphoblasts carrying these mutations showed that proteolysis pathways were improperly regulated. Our results confirm the role of PXX repeat in ALS pathogenesis, expand the clinical spectrum of *UBQLN2* mutations to spastic paraplegia phenotype and underline the pivotal role of ubiquilin-2 in proteolysis regulation pathways.

WTH07-50

Association between mitochondria and endoplasmic reticulum in dysmyelinated axons

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Myelin ensheathment maintains axonal integrity and enhances the transmission of electrical impulses along the axons. Diseases of myelin lead to axonal degeneration, but the underlying mechanisms are still unclear. Mitochondria associated membranes (MAM), the structural connections between endoplasmic reticulum and mitochondria, are important for physiological functions, such as Ca^{2+} signaling, mitochondrial lipid metabolism, and autophagy. Disruption of MAM has been implicated in mitochondrial dysfunction, which has been proposed as a major contributor of axonal degeneration in diseases of myelin. In this study, we investigated mitochondrial changes and the association with MAM in a chronic demyelination model. We used three dimensional ultrastructural analyses with serial block-face scanning electron microscopy (SBF-SEM). In mouse model of a chronic demyelination caused by extra-copies of proteolipid protein (PLP4e), most axons are myelinated at 1 month-old (mo) but chronic demyelination is observed at 5 months. Quantitative SBF-SEM analyses demonstrated that mitochondrial volume and surface areas and the total MAM areas of individual mitochondria were larger in the demyelinated axons of 5 months PLP4e compared with myelinated axons of 5 months wild-type mice. The increase of total MAM areas in demyelinated axons of PLP4e was attributable to enlargement of individual MAM, since MAM density (number of MAM / mitochondrial surface area) was similar. In the myelinated axons of 1 month PLP4e and wild-type mice, sizes of individual MAM and MAM density were similar. These results demonstrate usefulness of SBF-SEM in observing MAM and suggest that enlargement of MAM is caused by chronic loss of myelin. We propose increased MAM is beneficial for the mitochondrial functions and axonal survival in demyelinating diseases.

WTH07-51

Oligodendroglial conditional knockout of DARS2 results in white matter atrophy and neurobehavioral changes in mice

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Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL) is caused by mutations in *Dars2*, a gene encoding the mitochondrial enzyme aspartyl-tRNA synthetase. LBSL results in a rare, progressive, neurological disease that manifests as white matter signal abnormalities in the cerebral white matter and spinal cord, as well as slowly progressive dorsal column spasticity, dysarthria, and ataxia. To date, no animal model or treatment exists. Previous attempts to develop an animal model of LBSL through the complete or conditional neuronal knock-out of *Dars2* have been unsuccessful. Here, to mimic the clinical presentation in white matter, we developed a conditional knock-out of *Dars2* expression using Cre-lox recombination in *Pdgfrα*-expressing oligodendrocyte precursors. *Pdgfrα*$^{Cre+}$;*Dars2* $^{fl/fl}$ animals show a slight progressive behavioral phenotype with a reduction in both locomotor activity and rearing in open field over time, consistent with slowly progressive ataxia seen in LBSL. Preliminary data from these animals also suggests a reduction in oligodendrocyte transcription factor (OLIG2)-expressing cells per area in the corpus callosum and an overall reduction in corpus callosum area relative to age-matched control littermates, consistent with white matter abnormalities observed in the clinic. This novel mouse model has the potential to elucidate mechanisms of LBSL and may allow for translation to clinical discoveries for the treatment of LBSL.

WTH07-52

Quantification of GABA, glutamate and glutamine in a single measurement by magnetic resonance spectroscopy in human subjects

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Purpose: GABA and glutamate (Glu) are the major inhibitory and excitatory neurotransmitters in the brain and can be measured by magnetic resonance spectroscopy (MRS) *in vivo*, though GABA requires an additional measurement using the MEGA-PRESS editing sequence. Glu and glutamine (Gln) co-edit with GABA providing the possibility of measuring all three from a single MEGA-PRESS acquisition. Here we evaluate using phantom data whether Glu and Gln separation in GABA MEGA-PRESS can be achieved and use MEGA-PRESS spectra acquired *in vivo* to identify quality criteria of spectra from which Glu and Gln can be reliably estimated.

Method: Phantoms containing Glu, Gln, GABA and *N*-acetylaspartate at different concentrations were scanned using MEGA-PRESS optimized for GABA in a 3T *Philips Achieva* scanner. Spectra were also acquired *in vivo* from 5 different brain regions from 36 healthy volunteers. Quality assessment was performed on the data to determine the characteristics that were shared by spectra which returned Glu/Gln ratios in the physiological range after quantification using the QUEST routine in the jMRUI software package.

Results: Glu and Gln were estimated accurately in all phantoms with a linear relationship between measured and true concentration, R^2= 0.95 for Glu and R^2= 0.91 for Gln. The quality assessment framework was based on measurements from the spectra which, after quantification, returned physiological ratios of Glu/Gln (70% of all spectra). The signal-to-noise of the edited GABA signal, the linewidth of the *N*-acetylaspartate signal and the Cramer-Rao lower bound of the composite Glu + Gln signal were measured using AMARES in jMRUI. Data from the remaining 30% of spectra which had a Glu/Gln ratio outside the physiological range were from spectra which failed at least one of the quality criteria.

Conclusion: Glu and Gln can be reliably quantified from GABA optimized MEGA-PRESS acquisitions provided spectra meet certain quality criteria.

WTH08 Psychiatric Disorders and Drug Abuse

WTH08-01

Effects of nicotine on glia activation and dopaminergic system neurotoxicity induced by mdma in adolescent BALB/c mice

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The interaction between MDMA and Nicotine affects multiple brain centers and neurotransmitter systems (serotonin, dopamine and glutamate) involved in motor coordination and cognition. In this study, we elucidated the effect of prolonged (4 weeks) MDMA, nicotine and a combined Nicotine-MDMA treatment on motor-cognitive neural functions. In addition, we have shown the correlation between the observed behavioural change and neural structural changes induced by these treatments in BALB/c male mice. 20 Male periadolescent BALB/c mice (P.21) were treated for a period of 4 weeks with the vehicle, Nicotine, MDMA and Nicotine + MDMA. The control group received normal saline (subcutaneous, s.c). MDMA was administered subcutaneously to a set of 5 animals (2 mg/Kg body weight) at 2 days interval. The Nicotine treated group received 2 mg/kg BW of Nicotine daily while the Nicotine-MDMA group was treated with Nicotine (2 mg/Kg BW; s.c.) daily and MDMA (2 mg/ Kg; s.c.) at 2 days interval. We observed that MDMA (2 mg/Kg body weight; s.c) induced a decline in motor function, while Nicotine (2 mg/Kg body weight; s.c) improved motor function in male mice. In combined treatment, Nicotine reduced the motor function decline observed in MDMA treatment, thus no significant change in motor function for the combined treatment versus the control. Nicotine or MDMA treatment reduced memory function and altered dopaminergic and serotonergic, microglia and astrocytes activities in striatum and nucleus accumbens (core and shell). Similarly, a combined Nicotine-MDMA treatment reduced memory function when compared with the control.

WTH08-02

Proteomics and immunocytochemistry of rat neural stem cells, neurons and astrocytes exposed to alcohol: implications for FASD

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Neural stem cells obtained from rat embryos were exposed to various concentrations of ethanol (25 to 100 mM) for up to 96 h. There were no significant changes in the morphology of the cells but the numbers of neuron-like i.e. MAP- (microtubule associated protein 2-) expressing cells were reduced by ethanol in a dose-dependent manner, especially at 50 and 100 mM. The protein composition of the neural stem cells was analysed by proteomics (MALDI-TOF/Mass Spectroscopy) and, in the case of selected proteins, the changes were verified by western blotting. In the proteome analysis a total of 29 identified proteins were altered by ethanol (50 mM for 96 h) relative to ethanol-free control. Of these proteins some were related to cytoskelet (dihydropyrimidinase related proteins 2 and 3 involved in neural development and remodelling), others were involved in transcription/translation (e.g. nucleophosmin, dead end homolog protein, heterogeneous nuclear ribonucleoproteins H and C, and spliceosome RNA helicase bat1), energy metabolism (e.g. enolase-α and ADP-ribosylarginine hydrolase), signal transduction (e.g. RAB GDP dissociation inhibitors α and β, serine/threonin protein phosphatase and ras homolog gene family, member G) and oxidative stress (glutathione-S-transferase and heat shock proteins HSP 60, 70 and 90). Two of the proteins, nucleophosmin (NPM) and dead end protein homolog 1 (DND1) were further studied by immunocytochemical techniques in cultured neurons and astrocytes. NPM and DND1 displayed a similar pattern of ethanol-induced changes in both types of cells. Thus the ethanol exposure may alter and disturb a range of mechanisms needed for the normal function of neural stem cells eventually leading to the proliferation of seriously impaired neurons and glia. The processes of development, differentiation and repair using such cells in ethanol-exposed brains could result, *inter alia*, in abnormalities such as those typically encountered in the foetal alcohol spectrum disorder and/or in alcoholism later in life.

WTH08-03

Determination of neurosteroids in patients with internet addiction disorder by liquid chromatography-tandem mass spectrometry

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Neurosteroids are synthesized in the nervous system from cholesterol or steroidal precursors and regulate various functions such as development, neuronal plasticity, cognition, mood control, and social behavior in the central and peripheral nervous system. In this study, to investigate the alteration of neurosteroids in urine from patients with internet addiction disorder, an improved analytical method was developed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Urine samples were extracted by an Oasis HLB extraction cartridge after enzymatic hydrolysis with β-glucuronidase/arylsulfatase cocktail. Neurosteroids were separated using Waters ACQUITY @ BEH Phenyl column (2.1 × 100 mm, 1.7 μm) and a mobile phase consisting of eluent A (0.1% acetic acid in 95% water) and eluent B (0.1% acetic acid in 95% acetonitrile) with a gradient program at a flow rate of 0.4 mL/min and were monitored in Multiple Reaction Monitoring (MRM) mode by tandem mass spectrometry (MS/MS). The alteration of neurosteroids in human urine and excretion pattern may play important role to understanding probable internet addiction disorder, and the described methods could be used to evaluate and monitor patients with internet addiction disorder.

WTH08-04

Impairments in laterodorsal tegmentum to VTA projections underlie glucocorticoid triggered reward deficits

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Ventral tegmental area (VTA) activity is critical for motivated behaviours and reinforcement. Importantly, VTA activity is tightly modulated by afferents arising from the laterodorsal tegmentum (LDT). Disruption of this circuit can ultimately increase the risk for the development of neuropsychiatric disorders, including those associated with reward deficits, such as depression, anxiety, obsessive-compulsive disorder, obesity, addiction or antisocial behaviour. Additionally, the VTA region is particularly vulnerable to the effects of stress/glucocorticoids (GCs). Previous studies revealed that *in utero* exposure to glucocorticoids (iuGC) triggers prominent reward deficits later in life but nothing is known about the impact of this exposure in the LDT-VTA circuit.

Here, we show that iuGC animals have long-lasting changes in the expression of cholinergic markers in the LDT, and *in vivo* singe-cell electrophysiology revealed that LDT basal activity was decreased.

Interestingly, we observe a bidirectional effect in LDT-VTA inputs: upon LDT stimulation, iuGC animals present a decrease in the magnitude of excitation and an increase in the magnitude of inhibition in the VTA. While in control animals most of the inhibitory responses arise from putative GABAergic neurons, in iuGC group there is a shift in the type of cells presenting inhibitory responses, with a significant increase in the number of dopaminergic neurons.

In agreement with LDT-VTA dysfunction, we show that iuGC animals present motivational deficits that are rescued by selective optogenetic activation of this pathway. Importantly, we also show that LDTVTA optogenetic stimulation is reinforcing, and that iuGC animals are more susceptible to the reinforcing properties of LDT-VTA stimulation.

WTH08-05

Cannabidiol (CBD) treatment decreased the sensitized locomotor response to repeated methamphetamine exposure in male rats

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Background: Cannabidiol (CBD) is a non-psychoactive component of the cannabis plant and is showing potential as a promising treatment for mental disorders, such as psychosis. An animal model that is commonly used to mimic the neurochemical changes underlying psychosis is methamphetamine (METH) sensitisation, where repeat administration of the psychostimulant progressively increases locomotor activity. The ability of CBD to modulate METH-induced psychosis within a preclinical setting has not yet been examined. The aim of this study was to determine, in a preclinical psychosis model, whether CBD could attenuate locomotor activity in methamphetamine (METH)-induced sensitisation rats.
Methods: Male Sprague Dawley rats (n = 38) were subjected to daily METH (1 mg/kg days 2 and 8, 5 mg/kg days 3–7; i.p.) or saline (1 mg/kg; i.p.) injections for 7 days. After 3 weeks of withdrawal, METH-induced locomotor sensitisation was examined across 3 challenge days, whereby rats received a CBD injection (0, 40 or 80 mg/kg; i.p.) followed by a METH (1 mg/kg) or saline injection 30 min later. Locomotor activity was then measured for 60 min.
Results: Rats pretreated with METH showed a significant sensitised locomotor response on the veh + METH challenge day when compared to saline controls. Furthermore, METH-induced sensitisation was reduced following CBD treatment at both 40 and 80 mg/kg. In rats chronically treated with saline, CBD administration at 40 mg/kg significantly reduced acute METH-induced locomotor activity.
Conclusion: These results demonstrate that 40 and 80 mg/kg doses of CBD were able to reduce locomotor activity in METH-sensitised rats and may provide avenues of drug development for the reduction of behaviours associated with chronic METH abuse.

WTH08-06

Methamphetamine and modafinil elicit differential epigenetic and functional profiles in the mouse medial prefrontal cortex

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Methamphetamine (METH) addiction presents with specific behavioral alterations that suggest long-lasting changes in gene regulation within brain nuclei of the reward circuitry, including the medial prefrontal cortex (mPFC). METH negatively impacts the mPFC function, leading to decreased function and longstanding cognitive decline both in humans and animal models. Given the persistence of the addiction phenotype at both behavioral and transcriptional levels, increasing evidence implicate epigenetic mechanisms of gene regulation behind the neurobehavioral adaptions induced by psychostimulants. Also, psychostimulant drugs are known by their pro-cognitive effects, in part by its ability to increase PFC function, such as modafinil. Interestingly, modafinil has shown little abuse liability. The aim of the present study is to identify differential markers of METH and modafinil actions on epigenetic and functional targets in the mPFC, that may help identify pathways associated with addictive versus cognitive enhancing traits of these stimulants. Mice received METH (1 mg/kg) or modafinil (90 mg/kg) *single dose acute treatment* (sacrifice 1 hr later) or *subchronic daily7 days-treatment* (sacrifice withdrawal day 4). METH single dose treatment induced paired-pulse facilitation of EPSCs in D1-expressing layer V pyramidal neurons (patch clamp in BAC-Drd1a-tdTomato), suggesting reduced presynaptic probability of glutamate release, whereas modafinil had no effect. We found reduced dopamine receptors Drd1a and Drd2 mRNA expression after METH, whereas modafinil increased expression of Drd2 and c-

Fos compared to controls. Both stimulants acutely decreased H4ac and increased H3ac, HDAC2 and NMDA GluR1, compared to controls. H4ac, HDAC2 and GluR1 effects were blocked by D1 antagonist pretreatment, whereas H3ac effect was not. Subchronic METH and modafinil decreased H4ac and GluR1 expression, whereas only METH showed decreased H3ac and HDAC2. These differences could be related to METH-dependent detrimental effects on mPFC versus the pro-cognitive profile induced by modafinil in experimental and clinical settings.

WTH08-07

Differential role of CB1 receptors within accumbal subregions in stress-induced reinstatement of cocaine-conditioned preference

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Stress is considered one of the most important factors known to induce relapse in human addicts and in animal models of drug addiction. Research from our laboratory demonstrates that, using a conditioned place preference (CPP) paradigm, an acute restraint stress exposure triggers reinstatement of cocaine-CPP. With regard to the neurobiological mechanisms underlying relapse, there are numerous evidences for the participation of the endocannabinoid system (ECS), primarily through their actions at the widely distributed CB1 receptors (CB1R). Nevertheless, the role of ECS in stress-induced reinstatement has not been extensively studied. Considering that subregions of the Nucleus Accumbens (NAc) contribute significantly, but differently, to the impact of drug and stress on addiction, the present study has been designed to evaluate the involvement of CB1R within Core and Shell compartments of NAc in a restraint stress–induced reinstatement model. Male Wistar rats (220–300 g) that extinguished cocaine-CPP were microinjected into the Core or into the Shell of NAc with a CB1R agonist (ACEA; 0.001 or 0.01fmol/side) or a CB1R antagonist (AM251; 5 or 10ug/side), subsequently assigned to the different treatments of restraint stress exposure and then tested for reinstatement of cocaine-CPP. Results show that the intra-Core administration of AM251 abrogated restraint stress-induced reinstatement, and ACEA facilitated reinstatement after a non-reinstatement stress exposure. Moreover, the facilitating effect of ACEA was prevented by pretreatment with a microinjection of AM251. Interestingly, these effects were not observed after CB1R ligands microinjection into the NAc Shell compartment. Our results support the hypothesis of the preferential influence of CB1R within NAc Core, but not Shell, in the reinstatement of cocaine seeking behavior. This conclusion is in accordance with previous results of our lab that demonstrate the preferential role of glutamatergic transmission within NAc Core in the same model. Futures studies will attempt to confirm a possible glutamate dependent mechanism underpinning the effects of CB1R ligands on the restraint stress-induced reinstatement of cocaine-CPP responses.

WTH08-08

Effects of 4-phenylbutyric acid in abnormal behavior-displayed mice

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Valproic acid (VPA) is known as an anti-convulsant and a mood stabilizer. However, it has shown that exposure to VPA in pregnant causes high risk of autism and cognitive deficits in offspring mice. Previously, we reported that the offspring is under high endoplasmic reticulum stress. In addition, we found that the neuron of the offspring might suppress neurite outgrowth such as axon and dendrite in the process of embryonic development. 4-Phenylbutyric acid (4-PBA) is known as a chemical chaperone and a histone deacetylase inhibitor. In this study, we investigated the effects of 4-PBA in brain of the offspring. We injected VPA into pregnant mice at 12.5 days gestation. The offspring born from VPA-treated mothers were subjected to the experiment as abnormal behavioral mice. Prenatal exposure to 4-PBA did not improve abnormal behaviors such as locomotor activity and social communication in the VPA-treated offspring. However, 4-PBA led to improvement of decreased postsynaptic Shank3, density protein 95, neuroligin 1 and cell adhesion molecule 1 expression in cerebral cortex. These molecules have relevance to the pathogenesis of autism spectrum disorders (ASD). Therefore, prenatal exposure to 4-PBA have no influence on abnormal behaviors induced by VPA but, nevertheless, 4-PBA improved expression of the synaptogenic factors which related to ASD in cerebral cortex.

WTH08-09

Translational control by EIF2ALPHA regulates acute and persistent effects of cocaine

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Drug addiction is a major global mental health problem; however, the underlying neurobiological mechanisms remain elusive. While the effects of drugs of abuse require *de novo* protein synthesis, the translational control mechanism(s) targeted by drugs of abuse are not known. Our goal is to understand: a) how acute exposure to drugs of abuse usurp specific translational control mechanism(s), and b) how it leads to persistent changes in the reward circuits in the brain to cause maladaptive reward learning and reinforce compulsive drug-seeking behavior.

We discovered that translational control by phosphorylation of eukaryotic initiation factor 2 α-subunit (p-eIF2α) regulates the vulnerability to the effects of cocaine. We found that cocaine reduces p-eIF2α levels in the ventral tegmental area (VTA)—a key reward center in the brain—more readily in adolescent mice compared to adults. Specifically, in adolescent mice but not in adults, a sub-threshold dose of cocaine reduced p-eIF2α levels and potentiated synaptic inputs onto dopaminergic neurons in the VTA, and elicited drug-reinforced behavior (place preference).

Strikingly, in a series of gain- and loss-of-function experiments, we found that increasing or decreasing p-eIF2α levels genetically or pharmacologically render mice more resistant and more vulnerable, respectively, to the acute effects of cocaine. Consistent with these

findings, metabotropic glutamate receptor-mediated long-term depression—whose disruption is postulated to increase vulnerability to addiction—was impaired in the VTA of both adolescent mice and adult mice with reduced p-eIF2α-mediated translation.

Moreover, we also found that genetically or pharmacologically reducing p-eIF2α-mediated translation facilitates the progression of the transient effects of acute cocaine to a more persistent one. Taken together, our data suggest that: a) cocaine hijacks p-eIF2α-mediated translational program to elicit synaptic potentiation in VTA dopaminergic neurons that contributes to addiction-related behavior, and b) p-eIF2α-mediated translation could be a key mechanism gating the progression from transient to persistent effects of cocaine. Thus, modulating p-eIF2α mediated translation could be a therapeutic approach to prevent the persistent effects of drugs of abuse and may hold promise for new treatments for addiction.

WTH08-10

Investigating the role of dopamine receptor- and parvalbumin-expressing neurons in extinction and retrieval of conditioned fear

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Pavlovian fear conditioning and extinction have been studied extensively in the laboratory to understand learning and memory processes. There is significant interest in finding ways to enhance the strength of extinction learning, as it forms the basis for exposure therapy used to treat many anxiety disorders. A better understanding of the neurobiological basis of extinction is a critical step in achieving this goal. The aim of the present study was to examine the pattern o activation of neurons that express dopamine receptors 1 and 2 (D1R and D2R), and parvalbumin (PV) in mice that underwent extinction or retrieval of a fear memory. Adult male transgenic mice expressing D1R or D2R tagged with green fluorescent protein (GFP) were fear conditioned with 6 tone-shock pairings. The following day they were randomly divided into one of four experimental groups: handled, context, retrieval or extinction. Extinction groups were exposed to 45 tone presentations, retrieval groups were exposed to 5 tone presentations and the context groups were exposed to the extinction context without any tones. 90 min following their assigned treatment, mice were perfused and brain tissue processed for Fos/GFP/PV immunohistochemistry. The number of Fos, GFP and PV expressing cells were quantified in the prelimbic cortex (PrL), infralimbic cortex (IL) and basolateral amygdala (BLA). Extinction led to increased Fos expression in the IL and a decrease in the number of D2R+ cells in the IL compared to all other groups. Fear memory retrieval resulted in increased activation of D2R+ cells in the PrL compared to all other groups. These results highlight the complexity of dopamine's involvement in fear retrieval and extinction learning, and provide nuanced insights into the roles of specific dopamine receptor subtypes. This will be valuable for informing future research that aims to strengthen extinction learning via dopaminergic mechanisms.

WTH08-11

Sexually dimorphic function of dopamine in the postnatal neurodevelopment

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Many neuropsychiatric disorders share both developmental and dopaminergic hypotheses. Several studies showed that dopamine (DA) regulates neurogenesis, and we recently showed that DA is involved in GABAergic system development. However, these are early neurodevelopmental processes and it is possible that DA has different functions in postnatal brain development as well. The first five postnatal days of mouse brain development are important for synaptogenesis. Because of this, we investigated if DA imbalance at this developmental window affects prepubertal mouse behavior.

Objective: Evaluate the prepubertal behavioral consequences of dopaminergic imbalance in the first five postnatal days of mice.

Methods: Newborn Swiss mice were i.p. daily treated with saline, L-DOPA/benserazide (10/5 or 50/25 mg/kg) or quinpirole (0,5 mg/kg) from P1 to P5. Naïve and sham groups were also used as control. At 1 month of age, animal's behavior were investigated by open field test (OFT) and elevated plus maze (EPM). Another group, that were also treated with saline or L-DOPA/benserazide from P1 to P5, received an acute treatment (saline or L-DOPA/benserazide) 30 min before going through the tests. Data were analyzed using One Way ANOVA/One Way ANOVA on Ranks and Tukey test.

Results: We observed a decrease of total distance in all females treated, but not males (OFT). We also observed an increase in the number of rearings in females treated with Quinpirole and males treated with L-DOPA (OFT). In EPM test, only females treated with Quinpirole showed a decrease in the number of entries and time in open arms. Males previously treated with L-DOPA and acutely challenged with L-DOPA before the tests showed a higher decrease in the number of rearings in OFT and EPM, but not females.

Conclusion: Our data suggest a sexually dimorphic function of DA in the postnatal neurodevelopment. The DA challenge also suggest a sexually dimorphic consequence in the dopaminergic signaling when there is DA imbalance in early postnatal development. However, further studies are necessary to understand the molecular and biochemical mechanisms involved in this regulation.

WTH08-12

Ethanol withdrawal affects the mechanism of fear memory labilization in the basolateral amygdala complex: effect of D-cycloserine

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Contextual fear memory formed under withdrawal from chronic ethanol consumption is resistant to pharmacological interference with the reconsolidation process; indicating a resistance to the occurrence of the labilization phase after recall. In addition, pre-retrieval D-cycloserine (DCS) administration facilitates the reconsolidation interference of this resistant memory. The molecular mechanisms underlying the influence of ethanol withdrawal or DCS on fear memory labilization have not been established yet. Thus, here we evaluated the ubiquitin-proteasome system (UPS) activity in

the basolateral amygdala complex (BLA) after fear memory retrieval in ethanol withdrawn (ETOH) animals, and the influence of DCS on this molecular pathway. For this, we examined the polyubiquitinated proteins levels by Western Blot and proteasome chymotrypsin-like activity by enzymatic assay. Male Wistar rats were made dependent via an ethanol containing liquid diet (6% v/v) for 14 days. The respective control (CON) group was pair-fed with the same diet without ethanol. Contextual fear conditioning was performed on day 3 of withdrawal. Seven days after, rats were subjected or not to memory retrieval and were sacrificed 60 min later. In addition, separated CON and ETOH animals received DCS (5 mg/kg, i.p) or saline (SAL) injection 30 min before retrieval and were sacrificed 60 min later. Our results indicated that the retrieval only induced an increase in polyubiquitinated proteins expression and proteasome activity in the BLA from CON rats, whereas those effects were not observed in ETOH rats. These animals showed UPS activity patterns similar to those of the groups not subjected to retrieval. In the second experiment, we observed that ETOH rats treated with DCS before retrieval displayed elevated and similar UPS activity to CON rats after recall. In summary, ethanol withdrawal affects the neurobiological mechanisms involved in the fear memory labilization and DCS favors this molecular pathway in ethanol ETOH rats.

WTH08-13

Cocaine reward susceptibility is related to pubertal risk-taking behaviour in prenatally stressed offspring

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Gestational stress induces long-lasting neurochemical changes in the offspring and increases vulnerability to drug-seeking behaviour during adulthood. Since sensitivity to drug-induced reward is highly heterogeneous among individuals there is an urgent need to recognize predictive factors of drug reward vulnerability for a proper diagnosis and development of effective treatments. The aim of the present study was to identify early behavioural traits related to an adult increased vulnerability to cocaine reward. Employing a prenatal restraint stress model in rats, we evaluated novelty response, anxiety-like and risk-taking behaviours during puberty and its relationship with individual differences in cocaine-induced conditioning place preference during adulthood. Our results show that prenatal stress impacts differently in the pubertal offspring behaviour leading to two different populations: a low anxiety/high risk-taking population during puberty that will search for the rewarding properties of cocaine later in life and a high anxiety/low risk-taking population with low preference for cocaine during adulthood. This study clearly underscores the importance of early detection of behavioural traits opening the possibility of timely intervention to avoid the devastating consequences of drug addiction later in life. Moreover, studying individual differences of drug responsiveness is a key strategy to understand the underlying molecular mechanisms of vulnerability or resilience to the establishment of substance use disorders following drug exposure.

WTH08-14

Neonatal exposure to estradiol valerate increases morphine-induced locomotor activity and accumbal dopamine release in adult rats

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Neonatal exposure to sex hormones reprograms reproductive and non-reproductive tissues such as the brain in adult rats. Our lab has demonstrated that neonatal administration to Estradiol Valerate (EV) and Testosterone Propionate (TP) increases the content and release of dopamine (DA) in brain areas related to reward and locomotion in adult rats. On the other hand, it has been shown that sex hormones can modulate the expression of the mu-opioid receptor in different brain areas. Therefore, neonatal reprogramming with hormones could alter morphine response during adulthood in rats and predispose to addiction.

Our results show that locomotor activity induced by morphine is higher compared to locomotor activity induced by saline. However, neonatal reprogramming with EV significantly increases morphine-induced locomotor activity compared to control male rats. This increase in locomotor activity induced by morphine in EV rats is associated to a greater NAcc DA release induced by morphine compared to control male rats.

These results demonstrate that neonatal reprogramming with EV increases sensitivity to morphine effects possibly through increasing expression of mu-opioid receptors in GABAergic interneurons of the ventral tegmental area.

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WTH08-15

Lack of the SEZ6 protein attenuates cocaine relapse

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A significant problem of cocaine dependency is the propensity to relapse to cocaine-use even after extended periods of abstinence. A novel protein that influences cocaine relapse is Seizure-related gene 6 (Sez6). Sez6 is a neuron-specific protein, highly conserved between mice and humans, which plays an essential role in dendritic branching, dendritic spine formation and excitatory synaptic transmission. In the mature brain, Sez6 is strongly expressed in the dorsal striatum and nucleus accumbens (NAc), integral brain structures of the mesocorticolimbic dopamine pathway, which is pathologically altered by repeated exposure to cocaine. To investigate the function of Sez6 in cocaine dependence, mice with conditional deletion of Sez6 in all CaMKIIα-expressing forebrain projection neurons (Sez6 cKO) underwent investigator-administered (cocaine conditioned place preference (CPP)) and intravenously self-administered (IVSA) cocaine conditioning paradigms. Sez6 cKO and control mice

demonstrated equivalent cocaine CPP and stable self-administration of cocaine. However, following extinction of cocaine CPP and a subsequent low-dose cocaine prime, Sez6 cKO mice did not reinstate cocaine-seeking behaviour, unlike control mice. This behaviour was correlated with a reduced number of mature dendritic spines on NAc core medium spiny neurons of Sez6 cKO mice, compared to controls, immediately after cocaine-primed reinstatement. Similarly, after 1 month of abstinence from stable cocaine IVSA, Sez6 cKO mice did not relapse to cocaine-seeking upon presentation of drug-associated cues to the same extent as control mice. Together, these data indicate that Sez6 plays an important role in the synaptic changes that underpin cocaine relapse.

WTH08-16

Synthetic oxytocin-like treatment decreased the sensitized locomotor response to repeated methamphetamine exposure in male rats

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Background: Synthetic Oxytocin-Like Compound-1 (SOC-1) is a new compound that is currently being investigated for it's oxytocin-like effects in reducing drug dependence and social deficits. Repeat administration of the psychostimulant methamphetamine (METH) is associated with progressively increasing locomotor activity; a phenomenon described as behavioural sensitisation. As SOC-1 has been minimally investigated for it's modulation of METH-related behaviours, the aim of this study was to determine whether SOC-1 could reduce the heightened locomotor activity evident in METH sensitised rats.
Methods: Male Sprague–Dawley rats (n = 44) were subjected to daily METH (1 mg/kg on days 2 and 8, 5 mg/kg on days 3-7; i.p.) or saline (0.9%; i.p.) injections for 7 days. After 5 weeks of withdrawal, behavioural changes were examined over 4 challenge days where SOC-1 (0, 2.5, 5, and 10 mg/kg; i.p.) was administered 5 min prior to METH (1 mg/kg) or saline, after which locomotor activity was measured for 60 min.
Results: Rats pretreated with METH showed a significant sensitised locomotor response on the veh + METH challenge day when compared to saline controls. Additionally, SOC-1 dose-dependently reduced locomotor activity in METH sensitised rats when compared with saline. A significant difference in locomotor activity was also evident when comparing the 2.5 mg/kg and 10 mg/kg SOC-1 doses.
Conclusion: These results show that all doses of SOC-1 were able to reduce locomotor activity in METH-sensitised rats and may have specific effects to reduce behaviours associated with chronic METH abuse.

WTH08-17

Redox regulation via alpha-lipoic acid influences cocaine reinstatement behaviour

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Substance use disorders impose a heavy burden on affected individuals and society yet current treatment options are limited. There is thus a pressing need to more closely examine pathophysiological mechanisms underlying these disorders if new therapeutic options are to be developed. Elevated dopaminergic neurotransmission is the primary means by which drugs of abuse elicit a feeling of reward. However, this enhanced dopaminergic signalling is a source of reactive oxygen species and alters redox status with downstream effects on drug-induced neuroplasticity, signalling and behaviour. We therefore sought to determine whether targeting redox regulation with the antioxidant alpha-lipoic acid (ALA) may moderate cocaine extinction and/or reinstatement behaviour using a self-administration model and place preference paradigm. Male Sprague–Dawley rats were randomly assigned to receive either ALA (n = 7) or PBS (n = 6) and were trained to self-administer cocaine in operant chambers using a fixed ratio schedule. After successful acquisition of cocaine self-administration, rats underwent 3 weeks of extinction training followed by cue-primed reinstatement. The number of presses on the cocaine-paired lever was recorded across the experiment. To investigate whether ALA is intrinsically rewarding, we employed a compressed 7-day conditioned place preference paradigm with rats divided into 4 treatment groups: PBS (n = 8), ALA (n = 9), ALA and the μ opioid receptor agonist Naloxone (n = 8), or ALA and the dopamine type 1 receptor antagonist SCH23390 (n = 9). Analysis of the self-administration data indicated that ALA and PBS groups did not differ in their acquisition of cocaine self-administration; however, ALA reduced active lever pressing during reinstatement ($t(11)=4.298$, p = 0.001). Rats did not display any preference for the ALA-paired chamber in the place preference paradigm, and rats that received SCH23390 or Naoloxone did not differ to their ALA-treated counterparts. Combined these results suggest that ALA reduces the motivation to consume cocaine without eliciting reward itself. Moreover, these results provide further evidence that redox regulation of drug-induced pathophysiology is a valid target in the search for new therapies.

WTH09 Mechanism of Neuroprotection

WTH09-01

Cognitive-enhancing effects of beta-sitosterol in vanadium-induced neurotoxicity in mice

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Environmental discharge of vanadium causes physiological, cognitive and behavioural impairments in humans and animals via production of reactive oxygen species leading to lipid peroxidation and alteration in antioxidant defence system. The current study was carried out to investigate the cognitive-enhancing ability of beta-sitosterol in vanadium-induced neurotoxicity.

Forty eight mice were divided into four groups (A-D). Group A (control) received distilled water, B (standard group); α-tocopherol (500 mg/kg) every 72 hrs orally and sodium metavanadate (3 mg/kg) intraperitoneally (i/p), C; a single oral dose of β-sitosterol (100 μg) and sodium metavanadate (3 mg/kg) i/p while group D received sodium metavanadate (3 mg/kg) only i/p. All experimental groups received treatment for 7 consecutive days.

Cognitive, locomotor and antioxidant activities were evaluated by behavioural tests (Morris water maze, open field and hanging wire tests), antioxidant enzymes assay (catalase, SOD, GPx, GSH), and oxidative stress markers (MDA, NO and H_2O_2) measurements respectively. Immunohistochemical expression of Myelin Basic Protein (MBP) in the brain was also studied.

Beta-sitosterol significantly attenuated spatial learning deficits; improved motor coordination and reduced anxiety in vanadium neurotoxicity even better than the standard. Significant ($\alpha \leq 0.05$) increase in free radicals formation, decreased antioxidant enzyme activities, structural damage to myelin sheaths and decrease expression of MBP were observed in the sodium metavanadate only group, co-administration of beta-sitosterol however decreased these pathologic features and immunohistochemistry features were not significantly different from control mice.

The present study revealed that β-sitosterol possesses potent cognitive-enhancing, antioxidant and myelo-protective activities.

WTH09-02

LPS preconditioning attenuates neuroinflammation via gene reprogramming in rat model of epilepsy

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Background: Neuroinflammation seems to contribute to epileptogenesis, and reciprocally, prolonged seizures induce inflammation. Based on this, modulation of inflammation in the brain can be offered as one of the therapeutic approach to attenuate epileptic seizures. Preconditioning with sub-lethal dose of lipopolysaccharide (LPS) represented a state of neuroprotection to attenuate brain damage in rodent models. The purpose of this study was to clarify the effect of single brain LPS preconditioning on inflammatory profile induced by epileptic seizure in Pentylenetetrazol (PTZ) model of epilepsy.

Method: To determine the neuroprotective effect of brain LPS preconditioning on inflammatory profile induced by PTZ, all animals were evaluated for seizure duration. In addition, the effect of the LPS preconditioning on neuronal damage is observed in the hippocampal regions by histological assessments using nissl staining. Finally, the molecular assays were applied to analyze gene and protein expression associated with different cascades induced by LPS preconditioning such as TLR4 and inflammatory signaling pathways.

Result: Preconditioned animals performed significantly better on the behavioral tests to decrease seizure duration. Furthermore, this data were also consistent with the histological assessments. Additionally, molecular analysis showed that LPS preconditioning was accompanied by a reduction in pro-inflammatory mediators, whereas the expression of anti-inflammatory markers increased during tolerance. Additionally, expression of NFκB inhibitors such as SHIP1 and TOLLIP, which are known for their function in the reduction of pro-inflammatory reaction, was also enhanced. These findings were confirmed by western blot analysis.

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

WTH09-03

Dihydroprogesterone treatment restores myelin lipid profile in rat cerebral cortex

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Diabetes causes functional and structural changes in the nervous system leading to complications known as peripheral neuropathy, and encephalopathy. While the causes of diabetic peripheral neuropathy have been studied, the impact of diabetes on central nervous system (CNS) and its myelin compartment remains elusive. CNS myelin is a specialized membrane with high lipid to protein ratio to sustain CNS structure and function. Studies performed in an experimental model of diabetes using streptozotocin (STZ) injection revealed that the gene expression of some myelin proteins was significantly decreased by the pathology. Instead, myelin is highly enriched in lipids, such as cholesterol, glycosphingolipids and

plasmalogens contributing to myelin maintenance. Despite their important role, the effect of diabetes on myelin lipid profile of CNS has been poorly studied.

Data obtained by mass spec experiments showed that 3 months of diabetes induced an extensive impact on the levels of phosphatidylcholines (Ctrl: 376 ± 55 pg/μg of protein vs. STZ: 205 ± 41 pg/μg of protein, $p < 0.05$) and phosphatidylethanolamines (Ctrl: 602 ± 21 pg/μg of protein vs. STZ: 292 ± 46 pg/μg of protein, $p < 0.001$), plasmalogens (Ctrl: 153 ± 11 pg/μg of protein vs. STZ: 66 ± 12 pg/μg of protein, $p < 0.001$) as well as phosphatidylserines (Ctrl: 119 ± 23 pg/μg of protein vs. STZ: 45 ± 11 pg/μg of protein, $p < 0.001$) and phosphatidylinositols (Ctrl: 9.7 ± 0.2 pg/μg of protein vs. STZ: 4.03 ± 0.9 pg/μg of protein, $p < 0.01$). In addition, the levels of cholesterol (Ctrl: 6.1 ± 1.5 ng/μg of protein vs. STZ: 2.9 ± 0.55 ng/μg of protein, $p < 0.01$) and myelin basic protein were also decreased in the myelin of the same brain area. Interestingly, 1-month treatment with a neuroprotective molecule such as dihydroprogesterone, a metabolite of progesterone, restored the lipid and protein myelin profiles to the levels observed in non-diabetic animals.

Given the key functional and structural roles of lipid and protein in myelin, our data indicate, for the first time, that cerebral cortex myelin is severely compromised in diabetic status.

WTH09-04

Tetrahydroisoquinoline in ayurvedic medicine is neuroprotective in experimental Parkinson's disease

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Ancient '*Ayurvedic'* medicine from India could be an unexplored treasure for treating the incurable and debilitating neurodegenerative Parkinson's disease (PD). '*Kampavata'* the Vedic analogue of PD indicates *Ayurvedic* herbal formulation that could be neuroprotective. Tetrahydroisoquinoline (TIQ) an identified *Ayurvedic* alkaloid was assessed for its potential therapeutic effect *in vitro* and *in vivo* models for PD. *In vitro*, murine neuronal (Neuro2a), microglial (EOC20) and astrocytic (C8D30) cells exposed to MPP^+ dopaminergic neurotoxin were treated with TIQ (0.1–10 μM) for 24 h. Using indirect contact of dbcAMP-differentiated dopaminergic Neuro2a cells with astrocytes + microglia, mitochondrial superoxide radicals and survival within neurons was determined by Live Dead assay and MitoSOX flow cytometry. TIQ (10 μM) treatment significantly attenuated (37%) MPP^+-induced loss of live (Calcein AM-positive) differentiated neurons compared to MPP^+ alone. Further, MPP^+-induced mitochondrial accumulation of toxic superoxide radicals in dopamine neurons was significantly reduced (30%) by TIQ (10 μM). *In vivo,* adult C57/BL6 MPTP-PD mice (acute intraperitoneal MPTP 16 mg/kg dose, 4 times at 2 h intervals) were administered TIQ (per-oral 200 mg/kg body weight, bi-daily, 7 days post MPTP). Control mice were PBS injected or gavaged with TIQ alone. TIQ ameliorated dopaminergic neurotoxicity in mice causing a significant 16% increase in striatal dopamine level on the 7th day post-MPTP as detected by HPLC electrochemistry. Western blot for striatal expression of tyrosine hydroxylase (TH) the rate limiting enzyme of dopamine synthesis revealed 1.2-fold upregulation from its diminished expression post MPTP intoxication. This study indicates the anti-parkinsonian neuroprotective potential of TIQ in MPP^+-exposed cell co-culture and in MPTP mice. TIQ-mediated neuroprotection reflects through reduction in MPP^+-induced toxic buildup of mitochondrial superoxide radicals along with recovery in striatal dopamine levels and tyrosine hydroxylase expression. Knowing the molecular basis for the neuroprotective efficacy and safety of TIQ shall assist in translational therapeutic benefit in PD patients.

WTH09-05

Astrocyte-derived exosomes reduce infarct volume and improve neurological recovery in an *in vivo* model of focal cerebral ischemia

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Stroke is the second cause of death worldwide and induces permanent disabilities in surviving patients. Nowadays, the endogenous mechanisms that participate in neuronal rescue after stroke are not fully elucidated. Therefore, we aimed to assess how different cellular components of the brain interact to promote tissue recovery by establishing pathways of intercellular communication driven by exosomes. It has been proposed that exosomes derived from different cell types can mediate cellular responses able to reduce damage in neurological diseases, but there is limited information about their role in the protection against ischemic stroke. Here, we explored whether astrocytes contribute to protect neurons after ischemia by releasing exosomes. For this, we cultured rat primary cortical astrocytes under normoxia conditions for 48 h and collected the exosomes released to the medium. We also collected exosomes from astrocytes subjected to 6 h of hypoxia followed by 48 h of recovery. We tested whether theses vesicles induce a protective effect in the brain of rats subjected to experimental stroke, induced by a 90 min occlusion of the middle cerebral artery (tMCAO) in adult rats. Exosomes suspensions collected under normoxia and hypoxia were injected into the lateral ventricle of stroked rats 30 min after reperfusion. After 24 h of stroke, we analyzed the effect of exosomes administration on the infarct volume, neuronal survival, blood-brain barrier (BBB) integrity and neurological outcome. Notably, exosomes derived from astrocytes cultured under normoxia significantly reduced the infarct volume evaluated by triphenyl-tetrazolium chloride staining, attenuated BBB permeability alterations and improved neurological scores. Conversely, exosomes derived from astrocytes subjected to hypoxia were not as affective at reducing infarct volume, improving neurological recovery or limiting the BBB permeability alterations. These results show how the environment and molecular cues generated by support cells contribute to rescue neurons under ischemic conditions. Supported by PAPIIT-DGAPA IN226617 and CONACYT 219542.

WTH09-06

Melatonin attenuates βAPP processing VIA PIN1/NF-κB pathway in Aβ42-induced cellular model of Alzheimer's disease

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Alzheimer's disease (AD) is the most common cause of age-related dementia. AD-affected brain present extracellular deposits, which are the senile plaque composed of a set of hydrophobic peptides call amyloid β-peptide (Aβ). Soluble Aβ oligomers are the primary pathogenic factor leading to neuronal dysfunction in AD. Aβ-induced neurotoxicity exacerbates a vicious cycle of amyloidogenic processing stimulation and inflammation. Aβ is a normal product of β-amyloid precursor protein (βAPP) process resulting from the sequential cleavage of β-secretase (BACE1) and γ-secretase (PS1). Melatonin due to its incomparable functional versatility has multiple effects relevant to intervention in AD. Our previous studies demonstrated that melatonin regulated the non-amyloidogenic and amyloidogenic processing of βAPP by stimulating the α-secretases and down regulating both β- and γ-secretases. In the present study we proposed an Aβ-induced cellular model of AD to evaluate the therapeutic potential of melatonin and its underlying mechanisms of action. We confirmed this model in $A\beta_{42}$ treated SH-SY5Y neuroblastoma cell cultures by analyzing the levels of ADAM 10, BACE1 and PS1 expression. Pre-treatment with melatonin alleviated $A\beta_{42}$-induced alterations in the βAPP processing secretases. Considering neuroinflammation induced by Aβ played a critical role in the pathogenesis of AD, we observed the signaling pathway of Aβ-induced enhancement of nuclear factor-κB phosphorylation (pNF-κB) levels. The results indicated that $A\beta_{42}$ induced increased NF-κB p65 and up-regulated BACE1 possibly via Pin1/NF-κB signaling pathway. Pin1 is a key player in the pathogenesis of AD since the levels are compromised leading to increased Aβ formation. We showed that $A\beta_{42}$ led to Pin1 reduction causing destabilization of the p65/Rel subunit of NF-κB further resulting in its activation. Overall our present study demonstrated that melatonin prevented $A\beta_{42}$ induced alterations in βAPP processing secretases via Pin1/NF-κB signaling pathway.

WTH09-07

Investigating neuroprotective actions of irisin in the central nervous system

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Recent studies have pointed out that irisin, an exercise-induced myokine first identified as a regulator of adipocyte metabolism, may play important roles in brain function. Irisin was recently reported to stimulate hippocampal BDNF expression, which, in turn, promotes neuronal survival, synaptic plasticity and memory. However, it is still unclear whether peripheral irisin crosses the blood brain barrier (BBB) and what are the mechanisms underlying its effects in the brain. To address these questions, we have produced recombinant irisin and tested its ability to modulate signaling pathways relevant to brain homeostasis. First, we detected a slight increase in brain irisin levels after intravenous injection of recombinant irisin in mice. To investigate the signaling pathway underlying the neuronal actions of irisin, neuronal hippocampal cultures were treated with irisin, followed by quantification of the expression of BDNF and of genes related to the unfolded protein response (UPR), as these factors play key pathogenic roles in several neurodegenerative diseases. Irisin significantly increased BDNF and reduced UPR-related gene expression. Our results support the notion that irisin has neuromodulatory effects in the hippocampus, which could be of relevance to learning and memory processes. We are currently investigating additional effects irisin might have in the central nervous system and if peripherally delivered irisin triggers similar responses.

WTH09-08

Neuroprotective effect of whey protein concentrate (WPC) during aging

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Aging is a progressive multifactorial process exclusively marked by loss of cellular, molecular, and physiological functionality. The anatomical and physiological changes in the brain are fairly correlative with advancing of age. Moreover, aging causes substantial molecular to morphological changes in brain, the brain cells being more susceptible toward oxidative stress mediated damages due to the presence of high lipid content and higher oxygen consumption. It has been well documented that brain aging is also associated with decreased cellular uptake of L-cysteine, an amino acid essential for glutathione biosynthesis. Glutathione plays a critical role in protecting cells from oxidative stress and maintaining the thiol redox state in the central nervous system. The level of glutathione in brain is relatively lower than other organs. Whey protein concentrate (WPC) is a rich source of sulfur-containing amino acids such as methionine and cysteine and consumed as a functional food with wide range of nutritional attributes. Thus, the attempts were made to investigate the neuroprotective, anti-inflammatory and antioxidant efficacy of dietary supplementation of whey protein in brain tissue of old aged rats. Young (4 months) and old (24 months) male Wistar rats were supplemented with WPC (300 mg/kg b.w.) for 28 days. The data demonstrated that WPC augmented the decreased levels of FRAP, total thiol and acetyl cholinesterase in brain of old aged rats as compared to young control rats. Furthermore, WPC treated groups exhibited significant ($p < 0.001$) reduction in levels of lipid peroxide, protein carbonyls, reactive oxygen species and nitric oxide in aged rats. WPC supplementation also down-regulated the expression of inflammatory markers such as IL-1β and TNF-α, whereas up-regulated the expression of autophagy markers such as Atg3, LC3B and Beclin-1 in old aged rats. Taken together, the data confirmed the anti-aging and neuroprotective role of cysteine rich WPC that may also offers a better strategy to counteract age-dependent changes in brain.

WTH09-09

Quercetin attenuates the cadmium induced neurotoxicity by altering the autophagy via PI3K/AKT/MTOR signaling pathway

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Quercetin, a polyphenolic flavonoid has widely been present in varieties of foods has been regarded as nutraceutical. Having the potent antioxidant properties, protective role of quercetin in various neurodegenerative diseases like Alzheimer's has also been established. Further, Dysregulation of autophagy led to neuronal cell death in neurodegenerative diseases. Although studies carried out previously demonstrated the mechanism of cadmium induced neurotoxicity, however, the cellular and molecular mechanism underlying the role of autophagy in cadmium mediated neuronal death has not been fully understood. So, in order to gain insight into signaling cascade involved in cadmium mediated autophagy, the present study has been carried out to understand the protective role of quercetin in cadmium mediated increase in autophagy flux that led to neuronal death. We observed that exposure to cadmium altered the expression of autophagy proteins as LC3-II, Beclin1 and other Atg like proteins and GFP-LC3 puncta cells. Cadmium exposure in presence of bafilomycin A1 increased the levels of LC3-II and SQSTM1 levels in cholinergic rich areas of the brain. Further, interesting to it, combine treatment of cadmium with rapamycin (pharmacological activator of autophagy) mitigated such effects and in presence of 3MA (Pharmacological inhibitor of autophagy) cadmium induced neurotoxicity aggravated. Cadmium treatment activated the PI3K/Akt led to the down regulation in mTOR pathway. Further, cadmium also resulted in mitochondrial loss, bioenergetics deficits, and increased ROS generation leading to neuronal death. However, simultaneous treatment with quercetin ameliorated such changes. The results of the present study exhibit that cadmium-mediated neurotoxicity is associated with impaired autophagy through the PI3K/Akt/mTOR Signaling and these changes were further protected by quercetin.

WTH09-10

Neuroprotective effect of apelin against retinal ganglion cell death induced by retinal ischemia-reperfusion injury

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Glaucoma is a neurodegenerative optic neuropathy characterized by the loss of retinal ganglion cells, resulting in irreversible blindness. It is suggested that the loss of retinal ganglion cell is caused by several factors including glutamate excitotoxicity via *N*-methyl-D-aspartate (NMDA) receptors and retinal ischemia-reperfusion injury. We have recently reported that apelin, the oligopeptide ligand for the G protein-coupled receptor APJ, protects retinal ganglion cells from NMDA-induced excitotoxicity in mice. In this study, we investigated whether apelin protects against retinal ganglion cell death induced by retinal ischemia-reperfusion injury. Retinal ischemia-reperfusion injury was performed in apelin knockout mice and wild type mice using the high intraocular pressure method. Retinal ganglion cell death was assessed by counting the number of cells in the retinal ganglion cell layer in retinal sections stained with hematoxylin and eosin. TUNEL assay was conducted to detect apoptotic cells in the retinal ganglion cell layer. Electroretinography was performed to assess the electro-responses of retinal ganglion cells. Apelin deficiency in mice facilitated the decrease of cells in the retinal ganglion cell layer in retinas following retinal ischemia-reperfusion injury. Apelin deficiency also accelerated apoptosis in the retinal ganglion cell layer induced by retinal ischemia-reperfusion injury. Consistent with the retinal histopathological findings, electroretinography revealed that apelin deficiency enhanced the reduction of electro-responses in the retinas after retinal ischemia-reperfusion injury. These results suggest that endogenous apelin protects against retinal ganglion cell death induced by retinal ischemia-reperfusion injury.

WTH09-11

Protective role of aqueous extract of terminalia arjuna against cerebral ischemia induced mmp mediated blood brain barrier damage

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Compromised Blood brain barrier (BBB) following focal cerebral ischemia plays key role in infiltration of inflammatory cells which results in reperfusion injury and brain edema. Activated Matrix Metalloproteinases (MMPs) mediated degradation of tight junction (TJ) proteins is critical in disruption of BBB. To combat the multi-directional cerebral ischemic cascade events, a holistic approach is needed. *Terminalia arjuna,* a traditional medicinal plant well known cardiotonic was documented for its effective role in cardioprotection. Its antioxidant, hypolipidemic, anti-inflammatory, neuroprotective and hypertensive properties claim its effects. The present study intended to assess the ameliorative effect of *Terminalia arjuna* (*aeTA*) against transient focal cerebral ischemia. Adult male Sprague–Dawley rats divided into three groups ($n = 9$): Sham, Lesion and pretreated groups of 15 days *aeTA* were subjected to 2 hrs of Middle cerebral artery occlusion (MCAO) for 2 hrs followed by reperfusion. After 24 hrs of reperfusion the rats were assessed for behavioral deficits by Modified neurological severity scoring (mNSS), Evans blue (EB) extravasatation evaluation for BBB status, Triphenyl tetrazolium chloride (TTC) stain for Infarct measurement, Western blot analysis of MMPs (MMP2 & MMP9) and TJ proteins (Claudin5 & Occludin). The pretreatment with *aeTA* for 15 days exhibited significant improvement in neurological behavioral outcome and reduced infarct volume compared with the MCAO rats. Also the *aeTA* treatment was found to reduce BBB breach confirmed through decreased EB extravasations, significant reduction in matrix degrading MMPs (MMP2 & MMP9) and maintaining the levels of tight junction proteins (Claudin5 & Occludin). Thus pretreatment with *aeTA* treatment could reduce the BBB breach against focal cerebral ischemia and improve neurobehavioral outcome.

WTH09-12

Melatonin ameliorates cognitive deficits and synaptic dysfunction in streptozotocin-induced hyperglycemic state in rats

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Imperative evidences have indicated that deregulated glucose metabolism is a risk factor for cognitive dysfunction and developing Alzheimer's disease (AD). However, the underlying mechanisms are not fully elucidated. Melatonin is a neurohormone whose levels has been associated with the development of diabetes and AD patients. Melatonin exerts multiple complementary mechanisms of action against AD in animal models. Therefore, the present study examined whether melatonin can exert beneficial effects upon hippocampal-dependent cognitive function and synaptic plasticity in rats induced hyperglycemia by injection of streptozotocin (STZ). Adult Wistar rats were administered with one injection of STZ at (60 mg/kg; i.p.) and melatonin at (10 mg/kg/day, i.p.) for 42 consecutive days. Morris water maze (MWM) assay, western blotting and immunofluorescence staining in the hippocampus were performed to evaluate the effects of melatonin administration. The efficacious effect of melatonin was manifested in significantly ameliorated the spatial learning and memory impairment and alterations in expression of synaptic proteins (synaptophysin and PSD95) in STZ-induced rats. These results demonstrated that melatonin administration ameliorated memory deficit in hyperglycemic rats by up-regulating the plasticity-related proteins in hippocampus. However, further investigations are needed to explore the underlying mechanism of melatonin regulation in hyperglycemic rats.

WTH09-13

Melatonin improves cerebral blood flow decrease in aged mice

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Age-related degeneration of the brain vasculature may reduce the blood flow and stem cells' neurogenic potentials, which leads to reduced cognition. In our previous study, we found that the blood flow in old mice's brains is lower than that in young mice and the old mice had more curved pial arteries and fewer pial artery junctions than young mice. This decreased of cerebral blood flow and vascular alterations may reduce the arteriolar vasodilatory capacity and distensibility, thus contribute to the vascular cognition disease in seniors. Melatonin, which is a widely known and potent free radical scavenger and antioxidant. Furthermore, melatonin has been reported that vasoconstriction cerebral arterioles via activation of either MT_1 and MT_2 G-protein-linked membrane receptors. In this study, we examined the effects of melatonin treatment on cerebral blood flow and vascular structure. 12 months male Balb/c mice drank water with melatonin (10 mg/mL with ethanol; dilute in water) for 4 months and measured of cerebral blood flow every month. Using indocyanine green (ICG) fluorescence tracer, we measured and compared the normal aging mice (sham control) and melatonin-treated aging mice. The mean transit time (MTT) and T_{rising} (the time between of the first appearance of ICG fluorescence) were significantly faster in melatonin-treated aging mice than in normal aging mice. Moreover, blood flow index (BFI) was higher in the melatonin-treated aging mice than in the normal aging mice. These data suggest that melatonin may improve cerebral blood flow in aging animals.

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WTH09-14

Beneficial effects of enzyme-treated asparagus extract and buckwheat hull extract on memory functions in Sprague–Dawley rats

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Enzyme-Treated Asparagus extract (ETAS) is extracted from the residual lower parts of Asparagus (*Asparagus officinalis* L.) grown in Hokkaido, Japan. ETAS has been shown to have various benefical effects on health, such as anti-oxidative, anti-inflammatory or anti-stress activity. Buckwheat hull extract (BWHE) is a mixture of some flavonoids including rutin. We have shown that BWHE has protective effects on toxicant-induced hippocampal injury in rats. The objective of this study is to investigate the beneficial effects of ETAS, BWHE and rutin on spatial memory functions in rats. Male SD rats aged 4-weeks were fed chow containing 0.75% (w/w) ETAS, BWHE or rutin for 33 days. The rats were subjected to the Morris water maze task to examine memory acquisition and memory retrieval. Our results showed that ETAS supplementation facilitated memory acquisition in an early stage. On the other hand, BWHE supplementation seemed to facilitate memory retrieval. Rutin supplementation shown to have little effect both on memory acquisition and retrieval. In conclusion, these flavonoids have beneficial effects on different stages of memory formation processes in rats.

WTH09-15

Prevention of excitotoxicity associated changes in GLUN2B and TRKB levels by NMDA receptor inhibitors *in vivo*

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Excitotoxicity due to uncontrolled activation of NMDA receptor (NMDAR) causes neuronal death in various neurodegenerative diseases. Molecular mechanisms leading to cell death under excitotoxic conditions are still not clearly understood. Monosodiumglutamate (MSG) is known to cause excitotoxicity *in vivo*. In this

study we are observing the changes at the level of protein expression as well as their post translational modifications under excitotoxic conditions in the MSG treated rat model system. Activation of calcium signalling via NMDAR is expected upon chronic treatment with MSG. Briefly, methodology includes 15 days of MSG injection via i.p route in 100–150 gm adult male rat, which were given orally either vehicle or an NMDAR inhibitory plant extract. A group of animals were also fed with a known NMDAR inhibitory drug, dextromethorphan. Analysis by Morris water maze (MWM) test showed that the behavioural impairment caused by MSG administration could be ameliorated by simultaneous treatment with one of the NMDAR inhibitors, either the plant extract or dextromethorphan. Reduced GluN2B level was observed by immunoblotting in hippocampal and cortical tissues in MSG treated animals, which was reversed in the group fed with NMDAR inhibitory plant extract. Changes in the level of, TrkB upon MSG treatment was also found to be prevented in the group fed with NMDAR inhibitoray plant extract. Other proteins related to calcium signalling and cell death such as p-GluA1-Ser831, BDNF, p-PP1 and Bcl2 are also being analysed. Elucidation of the pathways that are altered would reveal the mechanism of action of neuroprotection by the NMDAR inhibitory plant extract.

WTH09-16

Lotus, a neural circuit formation factor, blocks PIRB-mediated axon growth inhibition

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The inability of damaged axons in the adult mammalian central nervous system (CNS) to regenerate is attributed to axonal growth inhibitors such as Nogo proteins, myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMgp). Nogo receptor-1 (NgR1) is a common receptor for these inhibitors. Recently, paired immunoglobulin-like receptor B (PirB), which originally regulates the immune system, has also been identified as a novel receptor for these inhibitors. We previously identified lateral olfactory tract usher substance (LOTUS) as a novel key molecule for axonal bundling of lateral olfactory tract by antagonizing NgR1. However, another function of LOTUS remains unknown. In this study, we found that LOTUS interacted not only with NgR1 but also with PirB. Overexpression of LOTUS with PirB in COS7 cells interfered with the binding of Nogo to PirB. Soluble form of LOTUS suppressed Nogo-induced growth cone collapse and neurite outgrowth inhibition in cultured dorsal root ganglion neurons from *ngr1*-deficient mice. These results suggest that LOTUS also exerts the antagonistic activity on PirB as well as NgR1, raising the possibility that LOTUS may enable injured CNS neurons to re-elongate their axons and thereby to overcome the limitation of axonal regeneration.

WTH09-17

The role of lysophosphatidic acid (LPA) and in the maintenance of the blood retina barrier and photoreceptor function

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Retinal degeneration may be mediated by loss of outer blood retinal barrier (BRB) integrity, leading to injury of the retinal pigment epithelium and photoreceptor death. Lysophosphatidic acid (LPA), and autotaxin (ATX), have been implicated in inflammation, angiogenesis and apoptosis. Given that ATX is a signature RPE marker, we hypothesize that it plays an important role in maintaining the microenvironment of the retina.

Human pluripotent stem cell (hPSC) lines were differentiated into RPE cells (Lidgerwood et al., 2016), and were shown to express high levels of *ATX* and *LPA* receptors (*LPARs*) by qRTPCR. Measurements of endogenous LPA and ATX were determined using LC-MS and western blot, respectively, and indicate that RPE cells secrete low basal amounts of LPA (0.1-0.4 nM), but secrete large amounts of functional ATX (apical). Addition of exogenous LPA to hPSC-RPE cultures did not affect RPE markers or cell morphology, suggesting RPE-derived LPA serves as a paracrine signal for neighbouring cells. hPSC-RPE cells were co-cultured with 661W photoreceptors, and indicate that high doses of LPA (> 10 μM) resulted in cytoskeletal changes in photoreceptors. CD4 + and CD8 + T cells treated with LPA have reduced activation, suggesting RPE-derived LPA may play a role in the maintenance of the immunoprivaleged retina.

WTH09-18

Evaluation of guanosine effects in 6-OHDA model of non-motor symptoms of Parkinson's disease

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Parkinson's disease (PD) is a progressive neurodegenerative disorder associated with dopaminergic neurodegeneration in dorsolateral striatum (DLS) and *substantia nigra pars compacta* (SNpc).

Degeneration in SNpc affects other brain areas including the prefrontal cortex (PFC), which has been associated with anhedonia and depression. 6-hydroxydopamine (6-OHDA) is a neurotoxin-model of PD and undergoes a non-enzymatic auto-oxidation, generating reactive oxygen species (ROS) and inhibition of mitochondrial complex I. A bilateral injection of 6-OHDA (10 μg/hemisphere) in the DLS induced a partial degeneration (about 60%) of dopaminergic neurons in the SNpc and non-motor impairments, mimicking an early premotor stage of PD. In this study, we investigated the effects of the neuroprotective nucleoside guanosine (GUO) treatment in a temporal evaluation of behavioral tasks after 6-OHDA-induced damage. 6-OHDA infusion induced anhedonic-like behavior in the splash test (after 8 days), but it did not alter the sucrose consumption preference (after 5-8 days). 6-OHDA also increased immobility time in the forced swimming test (FST, after 21 days) and induced short memory impairment in the object recognition test (ORT, after 14-15 days). However, no alterations in olfactory discrimination (after 3 days), motor performance in the open field (after 14 days), anxiety-like behavior in Y-maze test (after 20 days) and social interaction (after 22 days). Biochemical analyses were performed in cortical, striatal and hippocampal slices of rats 22 days after 6-OHDA. ROS levels and mitochondrial membrane potential were not changed 22 days after 6-OHDA infusion. GUO presented a partial effect on anhedonic-like behavior and prevented the development of increased immobility time associated with depressive-like behavior. GUO present no alterations in biochemical assays. In summary, these results provide for the first time the GUO effects on anhedonic-like and defense behaviors relevant to depression in 6-OHDA-lesioned rat, a model of non-motor symptoms associated with PD.

WTH09-19

Intracellular calcium homeostasis and signal transduction in brain cortex in conditions of hypokinesia

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Lack of intracellular calcium ([Ca^{2+}]in) has a great role in signal transduction disturbances implicated in the pathogenesis of the circulatory and metabolic disturbances in brain. Our objective was investigation of [Ca^{2+}]in homeostasis and phosphatidylinositol cycle (PIC) signaling pathway in brain cortex in conditions of hypokinesia (HK). Male mature white rats were used. HK was modeled in individually cramped cages. Labeled [14C]-arachidonic acid (AA) and 45CaCl2 were used for radioisotopic measurement of 45Ca^{2+} influx/translocation and membrane-dependent phospholypase (PLase)activity. Our results evidence enhancement of inward current of labeled 45Ca 2 + ions with elevation of intracellular Ca^{2+} concentration in conditions of HK, as well as activation of the Ca^{2+}-dependent enzymes, such as PLaseA1, A2, C, activation of the PIC signaling pathway which was manifested in elevation of diacylglycerol - one of the secong messenger of PIC and elevation of free AA contentin brain cortical synaptosomes. PIC stimulation leads to the Ca-induced Ca-release from the endoplasmic reticulum (ER) which contributes to the additional rise in cytosolic Ca^{2+}. This phenomenon is confirmed by our results testifying that the content of $Ca^{+}2$ ions within the ER is changed insignificantly. Moreover, Ca^{2+} ions are mainly accumulated within the mintochondrial fraction (MF). Thus, HK is involved in the pathogenesi of ischemic brain injury due to ceasing of protective influx of the elevated cytosolic Ca^{2+} into ER and facilitating their accumulation within the MF, promoting irreversible damage of synaptosome in brain cortex, which was contributed also by accumulation of the free AA thereby leading to alteration of presynaptic function of neurocytes of the brain cortex.

WTH09-20

Effects of polyamines on protein elongation and autophagy in neurons

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During aging neurons have to face multiple challenges including lesions in their nucleic acids or oxidative stress. On top of that impaired autophagy and the accumulation of misfolded, and, therefore, non functional or even toxic proteins impair the physiological functions of neuronal cells. Bearing this in mind, a rescue of autophagy and the restoring of protein homeostasis is considered to be essential in term of healthy aging.

Supplementation of polyamines results in eliminating of so called stalled ribosomes (a phenomenon occuring when protein elongation is disturbed) most likely due to a higher amount of hypusinylated factor eIF5A.

We characterized synaptoneurosomes prepared from 3, 18 and 26 month old mice using metabolic labeling of nascent proteins via BONCAT and subsequent Western Blot analysis. First results show a clear restoration of *de novo* synthesized synaptic proteins including Shank2, IRS1, and also of Homer1. Furthermore, we also use a polysome profiling approach to characterize ongoing translation in young and aged primary neuronal cell cultures without treatment or under supply of polyamines.

As polyamines also positively effect autophagy in aged neurons, visualization of autophagosomes via staining with Monodansylcadaverine in fixed cells an live staining revealed a distinct but difficult to interpret effect of the supplementation of polyamines. In a more generell sense, autophagosome formation via the LC3- or the GABARAP-system seems to be influenced in aging too, whether this process is affected by polyamines is still unclear.

Our results underline a potential therapeutic benefit of polyamines with respect to protein homeostasis and autophagy.

WTH09-21

In-vivo modulation of transferrin receptor protein-1 by a complex vitamin molecule reverses Alzheimer's-type pathology

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Metal ions are crucial for normal neurochemical signalling, while perturbations in their regulation are associated with

neurodegenerative processes in Alzheimer's disease (AD). Hypothesizing that metal chelators, antioxidants and anti-inflammatory agents could improve disease outcomes via modulation of transferrin receptor protein-1 (TfP-1)-a metal ion regulator, we investigated the efficacy of a complex vitamin supplement (CVS) formulated with several B-vitamins and ascorbic acid in reversing AD-type neurodegeneration. Forty Wistar rats (8 weeks-old) were assigned into five groups ($n = 8$), including controls and those administered CVS (400 mg/kg/day) orally for 2 weeks before or after aluminium chloride ($AlCl_3$; 100 mg/kg)-induced neurotoxicity. Rats were assessed for standard behavioural functions related to cognition, learning, memory and anxiety. The prefrontal cortex (PFC), hippocampus and amygdala were prepared for spectrophotometry, histology, histochemistry and immunohistochemistry. Our data showed that CVS significantly reversed reduction of exploratory/working memory ($p < 0.05$), frontal-dependent motor deficits ($p < 0.01$), cognitive decline ($p < 0.005$), memory dysfunction ($p < 0.05$) and anxiety ($p < 0.01$) compared to $AlCl_3$ (neurotoxic) group. These findings correlated with CVS-dependent modulation of TfP-1 expression within the PFC, hippocampus and amygdala that were accompanied by significant reversal of neural oxidative stress in expressed superoxide dismutase, nitric oxide, catalase, glutathione peroxidase and malondialdehyde. Through modulation of TfP-1, CVS inhibited neural bioenergetics dysfunction as increased labelling of glucokinase within PFC and hippocampus (but not amygdala) correlated with increased glucose-6-phosphate dehydrogenase and decreased lactate dehydrogenase expressions. These further relates to inhibition of over-expressed acetylcholinesterase and increased total protein synthesis. H&E and Nissl staining of thin sections corroborated roles of CVS in reversing $AlCl_3$-induced AD-like pathology and were accompanied by related changes in astrocytes and neurofilaments (cytoskeleton) immunohistochemical analyses. Summarily, we showed that CVS modulates neural TfP-1 overexpression, thereby normalising neurochemical signalling pathways linking concurrent progression of oxidative stress, bioenergetics deficits, synaptic dysfunction, cytoskeletal dysregulation and cellular hypertrophy in AD.

WTH09-22

Garcinol reduces streptozotocin-induced Alzheimer's like phenotypes and neuropathology in rats

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Sporadic Alzheimer's disease (sAD) is a progressive neurodegenerative disorder with multi-factorial pathophysiology. It involves cholinergic malfunction and mitochondrial dysfunction, and probably oxidative stress plays a significant role in its progressive nature. There is keen interest in identifying compounds related to nutraceuticals that can attenuate oxidative stress and amyloidosis in sAD that can improve memory. Streptozotocin (STZ) intracerebroventricular (ICV) administration has been used for developing sAD model in rats. The aim of the current study is to illustrate the potential role of garcinol (ICV infusion) a polyisoprenylated benzophenone in this rodent sAD model in relation to mitochondrial impairment, oxidative stress and memory functions. Cognitive abilities were assessed by Morris water maze (MWM) and elevated plus maze (EPM). Reactive oxygen species (ROS) generation, caspase-3, mitochondrial complex-I and V activities were estimated by employing spectrophotometry and fluorometry respectively. Further, we investigated the protein expression of choline acetyltransferase, glial fibrillary acidic protein (GFAP), and mitochondrial dynamin regulated proteins (Drp1) by Western blot. STZ-ICV animals exhibited memory deficits, which was effectively recovered by garcinol ICV treatment (1 and 10 μg), and was comparable to Tacrine treated STZ-ICV rats. Furthermore, garcinol significantly attenuated STZ-ICV-mediated choline acetyltransferase, mitochondrial complexes I and V activities sAD animals. Garcinol prevented STZ-ICV-induced elevations in ROS production, caspase-3 over activation, and over expression of GFAP and DRP1 proteins. Garcinol 10 μg inhibited amyloidosis and cell death in STZ intoxicated animals. Our results demonstrated that garcinol could be a potent memory enhancer due to its mitochondrial rejuvenating effect and antioxidant capabilities. Our findings suggest that garcinol could be dominant nutraceutical used in AD specifically related to modify defective cholinergic functions, mitochondrial functions, caspase–3 over activation and oxidative stress.

WTH09-23

Administration of L-tyrosine with levodopa could be neuroprotective in Parkinson's disease

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Introduction: The 20 canonical amino acids (AAs) account for less than 2% of all AAs in Nature. Most non-protein amino acids (NPAAs) are made by plants and their natural toxicity is often utilised to protect against predation or inhibit the growth of competing plants. In some cases toxicity arises from the ability of a NPAA to be charged onto tRNA in place of a structurally similar protein AA and become inserted into a polypeptide chain. L-DOPA (levodopa), which is present in mucuna plants, replaces L-tyrosine in protein synthesis and effectively kills larvae. We have shown that L-DOPA and can induce protein aggregation and apoptosis in human neurons in vitro[1] and proteins containing incorporated DOPA are present in brain and plasma of L-DOPA-treated patients[2]. Here we test the ability of L-tyrosine to prevent L-DOPA incorporation into proteins *in vivo*.
Methods: Rats ($n = 22$) were administered L-DOPA (6.5 mg/kg) IP, twice daily with or without L-tyrosine (100 mg/kg). After sacrifice at 21 days, proteins were extracted from the motor cortex (MC), substantia nigra (SN) and striatum (CPu) and levels of DOPA-containing proteins measured by HPLC.
Results: Plasma tyrosine levels were 3 fold higher in the L-tyrosine-treated group 0.5 h after IP injection but returned to control levels after 2.5 h. DOPA in hydrolyzed proteins increased 5 fold in the CPu of DOPA-treated rats, and this was significantly reduced in the group of rats that received L-tyrosine with L-DOPA ($p < 0.01$).
Discussion: Mischarging of tRNAtyr with L-DOPA and incorporation into neuronal cell proteins is a mechanism of L-DOPA toxicity that has been largely overlooked. In the present study we demonstrate that after a short exposure to L-DOPA, proteins containing incorporated L-DOPA are detectable in the rat brain and incorporation can be significantly reduced with L-tyrosine, suggesting a potentially protective effect *in vivo*.

1. Rodgers, KJ., et al., J Neurochem (2006). 2. Chan, S. W., et al. Exp Neurol 238, 29-37, 9 (2012).

WTH09-24

Dibenzoylthiamine, a lipophilic thiamine precursor, protects against oxidative damage in neuroblastoma cells

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Recent evidence suggests that thiamine (vitamin B1) and some of its derivatives can exert prominent neuroprotective effects in the mammalian brain, particularly in mouse models of Alzheimer's disease and tauopathies. As orally administered thiamine crosses intestinal and blood-brain barriers only slowly, precursors with higher bioavailability e.g. sulbutiamine, benfotiamine and dibenzoylthiamine, have been developed. We investigated the protective effects of thiamine and those precursors in neuroblastoma cells cultured in a medium containing minimal amounts of thiamine (10 nM), but sufficient to sustain normal growth. We induced oxidative stress by incubating the cells (24 h) in the presence of the neurotoxic agent paraquat (0.25 mM). This treatment reduced cell viability by 40%. When thiamine or the precursors were present simultaneously, we observed protective effects by the precursors while free thiamine was ineffective. Dibenzoylthiamine was most efficient, affording complete protection of cells at 10-20 μM. It also caused the highest increase in intracellular thiamine, suggesting that the protection from oxidative damage is linked to increased levels of free thiamine (rather than thiamine disphophate) in the neuroblastoma cells. The mechanism of this protective effect is presently under investigation. These results and others from our laboratory raise the possibility that dibenzoylthiamine might useful as a neuroprotective agent in neurodegenerative disease.

WTH09-25

Apelin inhibits *N*-methyl-D-aspartate-induced retinal ganglion cell death by activating AKT and ERK via APJ receptor in mice

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Retinal ganglion cell death is a hallmark of retinal diseases including glaucoma and diabetic retinopathy. The loss of retinal ganglion cells in these diseases has been suggested to be caused by excessive glutamate-induced excitotoxicity as mediated via *N*-methyl-D-aspartate (NMDA) receptors. It has been reported that apelin, the bioactive peptide, has neuroprotective properties against excitotoxicity in the cultured-neuronal cells prepared from hippocampus via APJ receptors, the G protein-coupled receptors. In this study, we investigated whether apelin inhibits retinal ganglion cell death induced by NMDA in mice. The number of retinal ganglion cells was counted in retinal sections stained by hematoxylin-eosin. TUNEL assay was performed to detect apoptotic cells in retinal ganglion cell layer. APJ expression in the retina was identified by immunohistochemistry. Activations of Akt and extracellular signal-regulated kinase (ERK) were assessed by western blotting. APJ immunoreactivities were observed in retinal ganglion cells immunostained with Brn-3a antibody. Apelin injection into the vitreous body suppressed the decrease of retinal ganglion cells induced by following an intravitreal injection of NMDA. This protective effect was blocked by co-injection of ML-221, an APJ antagonist. An intravitreal injection of apelin induced activations of Akt and ERK in retinas, and these activations by apelin were completely inhibited by ML221. Inhibitors of Akt and ERK signaling pathways blocked the protective effect of apelin on NMDA-induced apoptosis in retinal ganglion cell layer. These results suggest that apelin inhibits NMDA-induced retinal ganglion cell death by activating Akt and ERK via APJ receptors.

WTH09-26

Rapamycin induced activation of autophagy protects hippocampal neurons of aging rat brain through MTOR/AKT1/CREB pathway

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Autophagy is a highly conserved catabolic process involved in continuous removal of toxic protein aggregates and cellular organelles to maintain cellular homeostasis and functional integrity. The mechanistic understanding of autophagy mediated neuroprotection during aging remains elusive. Here, we investigated the potential role of rapamycin-induced activation of autophagy and mTOR/Akt1/CREB pathway(s) in the neuroprotection of hippocampal neurons of aging rat brain. In hippocampus region of aging rat brain, we found impaired redox balance, synaptic neurotransmission and cognitive functions, and suppressed pro-survival signaling as compared to normal young control rats. Rapamycin (0.5 mg/kg b.w., oral, daily for 30 days) administration caused a significant reduction of mTOR complex 1 phosphorylation at Ser2481 and a significant increase in levels of autophagy markers such as microtubule-associated protein-1 light chain-3 (LC3), Beclin-1, sequestosome-1/p62, unc-51-like kinase 1 (ULK1). In addition, rapamycin-induced the activation of autophagy that further activated p-Akt1 (Ser473) and p-CREB (Ser183) expression in aged rats. The activated autophagy markedly reversed age-associated impaired redox homeostasis by decreasing the levels of pro-oxidants such as ROS generation, intracellular Ca^{2+} flux and lipid peroxidation, and increasing the levels of antioxidants such as superoxide dismutase, catalase and reduced glutathione. The activated autophagy also provided significant neuroprotection against age-associated synaptic dysfunction by increasing the expression of synapsin-I, synaptophysin and PSD95, neurotransmission dysfunction by increasing the levels of CHRM2, DAD2 receptor, NMDA receptor and AMPA receptor, and ultimately improved cognitive ability in aged rats. Moreover, wortmannin (an autophagy inhibitor) administration significantly reduced the expression of autophagy markers, p-Akt1 and p-CREB as well as the autophagy mediated neuroprotective effect. Our study demonstrates that autophagy can be an integrated part of pro-survival (mTOR/Akt1/CREB) signaling and autophagic activation restores the oxidative defense mechanism(s) and maintains the integrity of synapse and neurotransmission in aging rat brain.

WTH09-27

Neuroprotective effect of spermidine as a caloric restriction mimetics in accelerated senescence model of rat

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Brain aging is a degenerative process that undergoes a progressive decline and opens a new window of vulnerability towards oxidative damages. Caloric restriction (CR) has been well documented to have health-promoting and lifespan extending effects. However, long-term CR would be highly problematic because of compliance challenge and other unpleasant side effects. Thus, to develop or explore the molecules, either natural or synthetic that mimic the beneficial effects of CR, is attracting the more attention and interest. Such candidate's molecules are usually known as CR mimetics (CRMs). Caloric restriction, through autophagy induction, is widely accepted as a lifespan-prolonging and health-promoting measure. Spermidine is a polyamine found in a variety of food and also known as potential CRM. The present study aimed to investigate the potential anti-aging effects of Spermidine in an accelerated senescence aging rat model. Thus, the attempts were made to investigate the anti-aging effects of spermidine (10 mg/kg b.w., oral) on brain tissues in D-galactose (500 mg/kg b.w., subcutaneous 45 days) induced aging model as well as naturally aged. We found that D-galactose significantly increased the level oxidative stress biomarkers lipid peroxidation (LPO) and protein oxidation (protein carbonyl content, PCO) along with simultaneous decrease in ferric reducing antioxidant potential (FRAP), reduced glutathione (GSH), ion channels (Na^+/K^+ ATPase and Ca^{2+}ATPase activity) and acetylcholinesterase (AChE) activity in brain. Spermidine reverses the effects of D-galactose by significantly ($p < 0.01$) decreasing the level of LPO, PCO closer to the control value, and significantly increasing FRAP, GSH, ion channels (Na^+/K^+ ATPase activity, Ca^{2+}ATPase) and AChE activity. Moreover, spermidine also restored the level of antioxidative markers. The RT-PCR and western blot data for expression of Beclin-2, LC-3, Atg-3, IL-6, TNF-α, Sirt-1, Sirt-2 and synaptophysin further confirmed a neuroprotective and anti-aging effect of spermidine in aging rats. Thus, data confirmed that spermidine is a promising compound for preventing neurodegeneration during aging brain.

WTH09-28

Biphasic responses of trans-resveratrol on proliferation of neural progenitor cells and aged rat hippocampal neurogenesis

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The plethora of literature has supported the potential benefits of Resveratrol (RV) as a life-extending as well as an anticancer compound. However, these two functional discrepancies resulted at different concentration ranges. Likewise, the role of Resveratrol on adult neurogenesis still remains controversial and less understood despite its well documented health benefits. To gather insight into the biological effects of RV on neurogenesis, we assessed the potential effects of the compound on the proliferation and survival of neural progenitor cells (NPCs) in culture, and in the hippocampus of aged rats. Resveratrol exerted biphasic effects on NPCs; low concentrations (10 μM) stimulated cell proliferation mediated by increased phosphorylation of extracellular signal-regulated kinases (ERKs) and p38 kinases, whereas high concentrations (> 20 μM) exhibited inhibitory effects. Administration of Resveratrol (20 mg/kg body weight) to adult rats significantly increased the number of newly generated cells in the hippocampus, with upregulation of p-CREB and SIRT1 proteins implicated in neuronal survival and lifespan extension respectively. We have successfully demonstrated that Resveratrol exhibits dose dependent discrepancies and at a lower concentration can have a positive impact on the proliferation, survival of NPCs and aged rat hippocampal neurogenesis implicating its potential as a candidate for restorative therapies against age related disorders.

WTH09-29

Neuroprotection of ketamine against cell death caused by oxidative stress during epileptogenesis in the mouse pilocarpine model

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Oxidative stress contribute to epileptogenesis and constitute an extremely deleterious event considered to be a cause of prolonged seizures.The mechanism contributing to hyperexcitability induced oxidative stress is not very well-defined and the mechanism understanding can lead to a novel anti-epileptogenic therapies. The objective of this study was to investigate the effect of ketamine on oxidative stress during the epileptogenesis phase in a mouse model of temporal lobe epilepsy induced by pilocarpine. In our study, animals received intraperitoneally either 0.9% saline (control group), or pilocarpine (100 mg/kg) every 20 min still the beginning of status epilepticus (epileptic group),or Ketamine 10 mg/kg (ketamine group),or a combination of pilocarpine and ketamine (epileptic/ketamine group)(ketamine was administered 15 min after pilocarpine injection), or a combination of pilocarpine and ketamine (ketamine/epileptic group) (ketamine was administered 15 min before pilocarpine injection). Mice were sacrificed 10 days after treatment. The status of oxidative stress was investigated by measuring malondialdehyde (MDA), nitric oxide (NO) content and free iron (Fe) in brains animals. Brain antioxidants enzymes activities levels were also determined. Data showed that in the epileptic group, we obtained a highly significant increase of lipid peroxidation, nitric oxide and free iron but we noted a highly significant decrease of catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) activities. Ketamine treated alone decreased significantly MDA, increased significantly NO but has no effect on free iron. Ketamine increased highly significant the 3 antioxidants enzymes activities. Administration of Ketamine before or after the epileptic induction showed that there is a highly significant decrease of the parameters MDA, NO and Fe. Regarding antioxidants enzymes activities, we noted a decrease of CAT and POD but a

highly significant increase of SOD. So ketamine administration increasing SOD activity and decreasing POD and NO content. SOD and CAT activity are involved in mechanisms responsible for eliminating oxygen free radicals during the establishment of epileptogenesis. Ketamine play an antioxidant role in the brain during epileptogenesis.

WTH09-30

Autophagy induction in brain by severe hypoglycemia and its modulation by the ketone body beta-hydroxybutyrate

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During hypoglycemia alternative substrates to glucose such as the ketone bodies (KB), acetoacetate (AcAc) and β-hydroxybutyrate (BHB) can be used as energy in brain. Furthermore diverse studies have shown that KB prevent neuronal death in different injury models. Nevertheless, the mechanisms by which KB prevent neuronal damage are still not well understood. Previous studies from our group have suggested that autophagy, a lisosomal-dependent degradation process activated during energy failure, participates in neuronal death induced by glucose deprivation in cultured cortical neurons. In these conditions, D-BHB, stimulates the autophagic flux and prevents neuronal death (J.Neurosci.Res. 41:600). In the present study we aimed to investigate whether autophagy is activated *in vivo* during insulin-induced hypoglycemia and glucose reperfusion and whether the neuroprotective effect of D-BHB, is related to autophagy. We analyzed the changes in the content of the autophagy proteins, LC3-II, used as index of autophagosome formation, and p62/SQSTM1, involved in autophagic degradation by western blot, in the cortex and hippocampus of hypoglycemic rats treated or not with BHB. Results show that autophagosome accumulation is promoted after 2 h of severe hypoglycemia in all studied cerebral regions as evidenced by the increased levels. 6 h after glucose reperfusion LC3-II content decreased to basal levels, suggesting less autophagosome formation or stimulated autophagosome degradation. However, after 24 h a second increase in LC3-II was observed in rats exposed to the hypoglycemic coma, while in those treated with D-BHB a significant decrease in LC3-II content was observed, suggesting that less autophagosomes are formed. No changes in p62/SQSTM1 were observed in the cortex, while in the hippocampus, a significant decrease in p62/SQSTM1 content was observed at 24 h in animals treated with D-BHB, suggesting the stimulation of the autophagic flux. Altogether these results suggests that D-BHB prevent autophagosome formation and stimulates the autophagic flux during *in vivo* hypoglycemia.

WTH09-31

Anti-amyloid antibody SAR228810 is neuroprotective against oligomeric ABETA-42-induced toxicity in mouse primary neuronal cultures

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Alzheimer's disease (AD) is characterized by the accumulation of extracellular deposition of beta-amyloid peptide in senile plaques and the intracellular aggregation of tau protein in neurofibrillary tangles associated with cognitive deficit. Although the initial neurotoxic event leading to onset of AD remains to be defined, it is considered to be highly linked in experimental models for AD to the presence of protofibrillar forms of amyloid-β peptide 42 (Aβ42) in the brain. Two monoclonal antibodies developed against protofibrillar forms of Aβ42 have been evaluated in an *in vitro* model of neurotoxicity. In neuronal cultures isolated from mouse cerebral cortex, we showed that oligomeric Aβ42 preparations induced a marked increase in caspase-3/7 enzymatic activity assessed by caspase 3/7 assay (+649 ± 124%) and a reduction in neurite outgrowth (-47 ± 5%) assessed by image analysis of immuno-stained MAP2-positive neurons. SAR228810, a humanized anti-amyloid antibody, and SAR255952, its murine version, significantly inhibited the oAβ42-induced neurotoxicity in a concentration-dependent manner. The concentration of 1 μM SAR228810 showed a near full reversion of the oAβ42-induced increase in caspase 3/7 activity and reduction of neurite outgrowth. Nor SAR228810 or SAR255952 did exhibit any significant effect in the absence of oAβ42, nor IgG isotype controls on neuronal cultures. Our results demonstrate that the anti-amyloid monoclonal antibodies, SAR228810 and SAR255952, have neuro-protective activity against oAβ42-induced neurotoxicity *in vitro*. These data are in support of a therapeutic use of antibodies directed against protofibrillar amyloid b peptide in AD.

WTH09-32

Melatonin attenuates learning and memory impairment in mice after methamphetamine administration

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Methamphetamine (METH) is an psychostimulant drug which is highly abuse. It has been shown that METH is directly neurotoxic and can cause changes of brain structure and function in brain. The cognitive skills including memory and working memory impairment in correlate to METH use haves been reported. The previous studies have shown that melatonin (MEL), a neurohormone secretes by pineal gland, has beneficial roles in cognitive decline patients with mild cognitive impairment. Thus, in this study, the effect of MEL on METH-induced cognitive impairment were investigated. Adult male ICR mice were received METH (1 mg/kg body weight) or saline

subcutaneously (s.c.) once daily for 14 consecutive days. One day after last METH injection, METH-treated mice were further administered with saline or MEL (5 or 10 mg/kg body weight) s.c. once daily for 30 days. After that Morris water maze (MWM) test was used to evaluate the effect of MEL on METH-induced learning and memory impairment. Stereotypey behaviors were used to observed the behavior change between groups on the day before and after each drugs treatment. During the training trial, all mice gradually learned to locate the platform. However, mice treated with METH spend longer time to reach the platform when compare with the control and other groups. The escape latency in probe trial task increased in the METH-treated animals when compare with control saline-treated group. Mice treated with both at 5 mg/kg and 10 mg/kg MEL after induced by METH spent less time of the escape latency when compare with METH-treated animals, whereas MEL alone showed no effect in the time spend to reach the platform. Taken together, these results suggested that exposure to METH causes learning and memory impairment, which can be attenuated by treated with MEL. This work was supported by the Thailand Research Fund (TRF) under the TRF Research Career Development Grant (RSA5980041) to SM.

WTH09-33

Melatonin modulates permeability transition pore by inhibition of mitochondrial KATP channels in isolated brain mitochondria

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There is increasing recognition of the importance of mitochondria in neurodegenerative disorders. Mitochondria play a key role in apoptotic and necrotic cell death. Melatonin (Mel), an indoleamine produced in several organs including the pineal gland has been known for its neuroprotective actions. In our study, we have investigated whether the mitochondrial ATP sensitive potassium (mtK_{ATP}) channel blocker 5-hydroxydecanoate (5-HD) and calcium (Ca^{2+}) affects permeability transition pore (PTP) alterations in isolated brain mitochondria treated with melatonin (Mel) and cyclosporin A (CsA). Mitochondrial swelling, mitochondrial membrane potential ($\Delta\psi m$), ROS measurement and mitochondrial respiration were evaluated in isolated brain mitochondria. In our results, mitochondrial swelling stimulated by exposing Ca^{2+} ions and 5-HD associated by mPTP opening as depicted by modulation of CsA and Mel. In addition, Ca^{2+} and 5-HD decreased $\Delta\psi m$, depleted intracellular ROS, and inhibition of mitochondrial respiration (state 3 and state 4) in isolated brain mitochondria. Addition of Mel and CsA has shown significant restoration in mitochondrial swelling, $\Delta\psi m$, intracellular ROS measurement and mitochondrial respiration in isolated brain mitochondria. Therefore, we speculate the modulatory effect of Mel and CsA in mitochondria treated with 5-HD and Ca^{2+} hinders the mPTP-mediated mitochondrial dysfunction and cellular oxidative stress. We conclude that inhibition of mPT is one likely mechanism of CsA's and its neuroprotective actions. Development of neuroprotective agents including Mel targeting the mPTP therefore bears hope for future treatment of severe neurodegenerative diseases.

WTH09-34

Kynurenic acid prevents cytoskeletal disorganization induced by quinolinic acid in mixed cultures of rat striatum

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Kynurenic acid (KYNA) is a neuroactive metabolite of tryptophan known to modulate a number of mechanisms involved in neural dysfunction.Although its activity in the brain has been widely studied, the effect of KYNA counteracting the actions of quinolinic acid (QUIN) remains unknown. The present study aims at describing the ability of 100 μM KYNA preventing cytoskeletal disruption provoked by QUIN in astrocyte/neuron/microglia mixed culture.KYNA totally preserved cytoskeletal organization, cell morphology andredox imbalance in mixed cultures exposed to QUIN. However, KYNA only partially prevented morphologic alteration in isolated primary astrocytes, and failed to protect the morphology of neurons in which redox equilibrium was not disrupted by QUIN exposure.Moreover, KYNA prevented QUIN-induced microglial activation andupregulation of Iba-1, and partially preserved TNF-α level in mixed cultures. TNF-α level was also partially preserved in astrocytes.In addition to the mechanisms dependent on redox imbalance and microglial activation, KYNA prevented downregulation of connexin-43 and the loss of functionality of gap junctions (GJs), preserving cell-cell contact,cytoskeletal organization and cell morphologyin QUIN-treated cells.Furthermore, the toxicity of QUIN targeting the cytoskeleton of mixed cultureswas not mediated by *N*-methyl-D-aspartate (NMDA) signaling. We suggestthat KYNA protects the integrity of the cytoskeleton of mixed cultures modulating microglial activation, which in turn is upstream of oxidative imbalance andmisregulated GJs leading to disrupted cytoskeleton in QUIN-treated cells.This study contributes to elucidation of the molecular basis of KYNA protection against QUIN-induced cell damage directed to the cytoskeleton of neural cells.

WTH10 Cell Death

WTH10-01

Class IV semaphorin SEMA4A from CSF of multiple sclerosis patients induces oligodendrocyte cell death

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Multiple Sclerosis (MS) is a progressive demyelinating disease of the central nervous system (CNS) whose causes are not yet well understood. It is thought to be caused by demyelination followed by a lack of or suboptimal re-myelination by oligodendrocytes in the CNS, however the extrinsic and intrinsic factors relating to oligodendrocyte death in MS are also not well understood. Identifying how alterations in oligodendrocyte biology contribute to MS pathogenesis is an essential prerequisite for developing better intervention strategies in MS treatment.

We have previously shown a dose-dependent cytotoxic effect of the Class IV Semaphorin Sema4A on primary rodent oligodendrocytes. In rodents, Sema4A binds to Tim-2, a member of the T-cell immunoglobulin domain and mucin containing domain (Tim) family, a receptor that is expressed on activated T cells as well as oligodendrocytes. We recently discovered that Tim-2 is also the primary receptor for the iron delivery protein H-ferritin (Hft) and that oligodendrocytes are unique in the brain for taking up iron via this protein. However, the receptor for binding has yet to be identified in human oligodendrocytes as the gene for Tim-2 has not been detected in humans. We currently show that both recombinant Sema4A protein and Sema4A in the CSF of MS patients have a dose-dependent cytotoxic relationship with oligodendrocytes. Our data demonstrates that cell death following exposure to Sema4A is likely mediated via apoptosis. Our data also suggest that Sema4A levels in the CSF of MS patients could be used in combination with current standards of biomarkers for disease progression. Additionally, we have data that suggests addition of H-ferritin can prevent Sema4A cytotoxicity. Together, the data strongly suggest that identifying the receptor for both H-ferritin and Sema4A will provide novel targeting for potential therapeutic treatments of demyelinating disorders.

WTH10-02

Prognostic significance and survival statistics of astrocytoma patients' in a cohort with assessment of glioblastoma with oligodendroglial component

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Astrocytoma constitutes the most noted malignancies of central nervous system with worse clinical outcomes in Grade IV astrocytoma or glioblastoma multiformae. In present studies, we have seen anatomic distribution of astrocytoma subtypes in a cohort of 479 patients and correlated it with survival outcomes. Anatomic location was confirmed by MRI (magnetic resonance imaging) images. We have also looked into overall survival particulars in astrocytoma subtypes and further prognostically assessed significance of glioblastoma with oligodendroglial component (GBMO). Pattern of MIB1 and p53 expression was evaluated by immunohistochemistry studies in respective cases. Our findings highlights that in total cases, tumor location was dominated by frontal and temporal lobes. Survival analysis in high grade (Grade III, $p = 0.03$) (Grade IV, $p = 0.01$) astrocytic tumors confirms poor outcomes with temporal, parietal and occipital location as compared to frontal lobe. Overall survival studies demonstrates glioblastoma multiformae (GBM) was associated with worse prognosis as compared to astrocytoma subtypes ($p < 0.0001$). In high grade astrocytomas, anaplastic astrocytoma was found with 19 months of median survival age while 12 months in case of glioblastoma multiformae. These was statistically significant difference among survival of glioblastoma patients'. Glioblastoma multiformae patients with oligodendroglial component was found to have median survival of 16 months (Chi square= 11.09, $p = 0.0009$, 95% CI= 1.37 to 3.4). In conclusion, we state that in our cohort, parietal, temporal and occipital lobes were found with significantly poor prognosis in grade II, grade III and grade IV astrocytoma patients. Among astrocytoma subtypes, patients with glioblastoma multiformae were associated with worse survival outcomes. Glioblastoma multiformae patients with oligodendroglial components found to have significantly longer survival than glioblastoma patients' and respond well to chemo and radiotherapy.

WTH10-03

Autophagy fails to prevent energy stress-induced neuronal death due to calpain-mediated lysosomal dysfuction

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Autophagy is triggered during nutrient and energy deprivation in a variety of cells as a homeostatic response to metabolic stress. In the CNS deficient autophagy has been implicated in neurodegenerative diseases and ischemic brain injury. However, its role in hypoglycemic damage is poorly understood and the dynamics of autophagy during the hypoglycemic and the glucose reperfusion periods has not been fully described. In the present study we analyzed the changes in the content of the autophagy proteins BECN1, LC3-II and p62/SQSTM1 by Western blot and autophagosome formation was followed through time-lapse experiments, during glucose deprivation (GD) and glucose reintroduction (GR) in cortical cultures. According to the results, autophagosome formation rapidly increased during GD, and was followed by an active autophagic flux early after glucose replenishment. However, cells progressively died during GR and autophagy inhibition reduced neuronal death. Neurons undergoing apoptosis during GR did not form autophagosomes, while those surviving up to late GR showed a second weave of autophagosome formation. Calpain activity strongly increased after GR and remained elevated during progressive neuronal death. Its activation led to lysosome permeabilization

and the loss of lysosomal cathepsin B. Calpain inhibition increased cell viability and the number of neurons containing lysosomes, autophagosomes and cathepsin B immunoreactivity. Taken together, the present results suggest that calpain-mediated lysosome dysfunction during GR turns an adaptive autophagy response to energy stress into a defective autophagy pathway which contributes to neuronal apoptosis. In these conditions, autophagy inhibition results in the improvement of cell survival.

WTH10-04

Bax activation blocks self-renewal and induces apoptosis of human glioblastoma stem cells

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Glioblastoma (GBM) is characterized by a poor response to conventional chemotherapeutic agents, attributed to the insurgence of drug resistance mechanisms and to the presence of a subpopulation of glioma stem cells (GSCs)[1]. GBM cells and GSCs present, among others, an overexpression of anti-apoptotic proteins and an inhibition of pro-apoptotic ones, which help to escape apoptosis. Among pro-apoptotic inducers, the Bcl-2 family protein Bax has been recently emerged as a promising new target in cancer therapy along with first BAX activators (BAM7, Compound 106 and SMBA1)[2]. Herein, a derivative of BAM-7, named BTC-8[3], was employed to explore the effects of Bax activation in different human GBM cells and in their stem cell subpopulation. BTC-8 inhibited GBM cell proliferation, arrested cell-cycle arrest and induced apoptotis through the induction of mitochondrial membrane permeabilization. Most importantly, BTC-8 blocked proliferation and self-renewal of GSCs, and induced their apoptosis. Noteworthy, BTC-8 was demonstrated to sensitize both GBM cells and GSCs to the alkylating agent temozolomide. Overall, our findings shed light on the effects and on the relative molecular mechanisms related to Bax activation in GBM, and suggest Bax-targeting compounds as promising therapeutic tools against the GSC reservoir.

[1]*EMBO Mol. Med. 2014;6:1359-70.*
[2]*Nature 2008;455:1076-81.*
[3]*J. Med. Chem. 2015;58:2135-48.*

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WTH11 Autonomic-Autonomic/Neuroendocrine Systems

WTH11-01

Integrative microrna-mrna expression changes in vmh is associated with neuronal maladaptive response to hypoglycemia

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The brain is highly sensitive to proper fuel availability as evidenced by the neuronal maladaptive function during ischemic attacks and recurrent episodes of hypoglycemia (RH). Studies have demonstrated that RH induces the changes in gene (or mRNA) expression involved in neuronal glucose sensing, which limits the ability of brain to sense subsequent hypoglycemia (i.e. impaired glucose sensing), thereby compromises the neuroendocrine regulation. The current study tests the hypothesis that impaired neuronal glucose sensing in RH is mediated by coordinated changes in microRNAs (negatively regulate expression of genes at posttranscriptional level) and mRNAs expression in ventromedial hypothalamus (VMH), a well characterized glucose sensing brain region. In brief, adult male Sprague–Dawley rats were administered with either subcutaneous insulin (2-1.2 U/kg) or saline injections for 3 days to induce RH (30-50 mg/dl, $n = 10$) and control (recurrent saline, RS; $n = 10$) group respectively. On 4th day, both groups were subjected to either 1) hypoglycemic (40-50 mg/dl) clamps to assess neuroendocrine response to hypoglycemia, or 2) were euthanized to obtain RNA from the punch biopsy of VMH to determine genome-wide microRNAome and transcriptome profiling changes with a high-throughput RNA-sequencing and bioinformatics approach. Results indicate that RH leads to an impaired neuronal glucose sensing as demonstrated by blunted neuroendocrine (i.e. epinephrine) response to hypoglycemia. In RNA-sequencing analysis, a total of 205 microRNAs and 1013 mRNAs were differentially expressed between groups, in which, 82 microRNAs pair to 402 mRNAs at false discovery rate (FDR) < 0.05, analyzed by microRNA target filtering using Ingenuity Pathway Analysis software. A microRNA-mRNA integrative network analysis of RH induced genomic changes, identified miR-23a-3p and miR-7a-5p based on their large predicted network and association with mRNA target changes involved in neuronal processes, specifically *Clcn3* and *Mknk2* (gene products involved in GABAergic synaptic vesicles release and neurite outgrowth, respectively). These finding suggest that miR-23a-3p and miR-7a-5p might be potential mediators or regulators of the neuronal maladaptive response occurring in repetitive hypoglycemia.

WTH11-02

Gonadotropin-releasing hormone neurons are regulated by gut peptides at the level of the neuroterminals in the median eminence

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Gonadotropin-releasing hormone (GnRH) is produced in neurons in the preoptic area which project to the median eminence (ME), where the peptide is secreted into hypophysial portal blood. The neurosecretory terminals of GnRH neurons are outside the blood brain barrier in the external zone of the ME and hypothalamic factors such as kisspeptin control secretion at this level. Given that the GnRH neurosecretory terminals are freely accessible to blood-borne factors, gut hormones could also modulate GnRH secretion. We aimed to determine the effects of $NPY_{13\text{-}36}$, ghrelin, GLP-1 (and its agonist Exendin-4) on GnRH secretion in sheep. Ovariectomised ewes ($n = 6$/group), received guide tubes directed to 2 mm above the ME. Microinjections of each peptide (0.5-1 nmol) were made into the ME during continuous blood sampling to measure pulsatile luteinising hormone secretion. $NPY_{13\text{-}36}$, has similar properties to PYY and, when injected into the ME, where the neuroterminals of GnRH are found, it suppressed LH levels (reflecting an effect on GnRH secretion). Mean levels were reduced from 1.7 ± 0.23 to 1.1 ± 0.18 ng/mL ($p < 0.05$). An i.v. injection of $NPY_{13\text{-}36}$ also reduced LH secretion. Immunostaining and *in situ* hybridisation showed that GnRH neurons do not express Y2 receptor, so the effect may be via the Y5 receptor. Microinjection of 0.5 nmol ghrelin into the ME also lowered LH levels from 3.3 ± 0.52 to 2.6 ± 0.49 ng/mL ($p < 0.05$). Ghrelin receptors were colocalised to GnRH neurosecretory terminals, by immunocytochemistry. To test effects of GLP-1 and Exendin-4, OVX ewes were treated with estrogen and progesterone to suppress GnRH/LH levels. Doses of 0.5 nmol of GLP-1 or Exendin-4 increased LH levels (from 0.5 ± 0.11 to 0.8 ± 0.17 ng/mL; $p < 0.05$ for GLP-1 and from 0.9 ± 0.22 to 2.3 ± 0.12 ng/mL; $p < 0.005$ for Exendin-4). Exendin-4 induced a more sustained elevation in LH secretion than GLP-1. We conclude that reproductive function is affected by gut peptides acting on GnRH neurosecretory terminals in the ME.

WTH11-03

A neuroanatomical evaluation of cholinergic, catecholaminergic, serotonergic and orexinergic neural systems in mammals pertaining

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One of the few remaining mysteries in mammalian phylogeny is the issue of chiropteran phylogeny. In order to further investigate the diphyletic hypothesis that states that megachiroptera evolved from primate-like gliders and that microchiroptera evolved from insectivores, the cholinergic, catecholaminergic, serotonergic and orexinergic systems were analyzed in five insectivores and three prosimian primates.

Brains were coronally sectioned and stained according to standard immunohistochemical methods and the presence or absence of 93 nuclei within the neuromodulatory systems was entered into modern cladistics software.

The three shrews lacked the cholinergic parabigeminal and Edinger-Westphal nuclei, had a mediodorsal arch of the cholinergic laterodorsal tegmental nucleus, lacked the catecholaminergic A4 and A15d nuclei and presented with an incipient ventral division of the substantia nigra which is identical to previously studied microchiroptera. All three prosimians presented with a central compact division of catecholaminergic locus coeruleus (A6c) surrounded by a shell of less densely packed (A6d) tyrosine hydroxylase immunopositive neurons. This combination of compact and diffuse divisions of the locus coeruleus complex is only found in primates and megachiropterans of all the mammalian species studied to date.

Our neuroanatomical analysis suggests a phylogenetic relationship between the Soricidae (shrews) and the microchiropterans, supports the phylogenetic grouping of primates with megachiropterans, and suggests that primates are phylogenetically closer to megachiroptera than to any members of the Euarchontoglires. The cladistic analysis confirmed the neuroanatomical analysis.

WTH11-04

Mode of action of RB150, an aminopeptidase a inhibitor, as a central-acting antihypertensive agent in DOCA-salt hypertensive rats

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In the brain, angiotensin III (AngIII) generated from angiotensin II (AngII) by aminopeptidase A (APA) exerts a tonic stimulatory action on the control of blood pressure (BP) in hypertensive rats. RB150, a prodrug of the specific and selective APA inhibitor is the prototype of a new class of centrally-acting antihypertensive agents responsible for the inhibition of brain APA activity leading to a decrease in BP in hypertensive rats. Since following brain AngIII formation blockade by RB150, no AngII accumulation was observed, we aimed to delineate if RB150 induces the activation of another metabolic pathway of brain AngII, in particular angiotensin converting enzyme type 2 (ACE2) which converts AngII into angiotensin 1-7 (Ang1-7). For this purpose, we used a model of salt-dependent hypertension, the DOCA-salt rat. RB150 and MLN4760, an ACE2 inhibitor were administered by intracerebroventricular (ICV) route and mean arterial BP (MABP) was recorded in alert hypertensive rats. Maximal MABP decrease and area under the curve (AUC) of the variations in MABP were calculated. ICV administration of RB150 (100 μg) significantly decreased MABP compared to vehicle (-30.41 ± 5.68 mmHg vs. -8.05 ± 6.14 mmHg, $p < 0.01$; AUC: 43.35 ± 10.25 vs. 3.87 ± 9.82 mmHg.min, $p < 0.01$). Administration of MLN4760 (10 μg) did not induce any significant change in MABP compared to vehicle (-14,19 ± 5.42 mmHg, $p > 0.05$; AUC: 9.39 ± 5.97 mmHg.min, $p > 0.05$). The combination of MLN4760 with RB150 co-administered by ICV route partially inhibited the RB150-induced antihypertensive effect (-16.26 ± 5.66 mmHg, $p < 0,01$; AUC: 23.84 ± 14.81 mmHg.min $p < 0,05$, compared to RB150 alone). These results suggest that inhibition of brain APA by RB150 triggers ACE2 metabolic pathway, resulting in the formation of Ang 1-7, which, by its action on the Mas receptor, could participate to the blood pressure decrease in the hypertensive rat.

WTH13 Sensory Systems

WTH13-01

Function of presynaptic TRPV1 receptors in the spinal cord dorsal horn is modulated by chemotherapeutic drug paclitaxel

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Neuropathic pain is major treatment-limiting factor accompanying cancer treatment with paclitaxel (PAC). We have reported previously (Li *et al.*, J. Neurosci., 35:13487–13500, 2015) that acute application of PAC (50 nM) modulated miniature excitatory postsynaptic currents (mEPSC) frequency and diminished tachyphylaxis of TRPV1 (transient receptor potential vanilloid 1) receptors mediated response after repeated capsaicin application via TLR4 receptors activation. Here we studied the signaling pathways involved in PAC-induced modulation. Whole-cell patch clamp recordings of mEPSC from spinal cord neurons in lamina I/II from adult male mice were used. Von Frey filament measurements were used to evaluate the presence of mechanical allodynia. PAC-neuropathy was induced by single dose application of PAC (8 mg/kg, *i.p.*). In naïve animals mEPSCs frequency evoked by second capsaicin application was reduced to 33% of the first one. After acute PAC-treatment the second response was 91% of the first one. Our data show that the second capsaicin response tachyphylaxis was diminished also 1 day (72%) and 8 days (83%) after single systemic *in-vivo* PAC-treatment. Effect of PAC treatment on tachyphylaxis was significantly reduced by PI3-Kinase antagonist wortmannin or LY294002 and by wide-spectrum kinases inhibitor staurosporine. These results suggest that PI3-Kinase and other kinases may play important role in the signaling between TLR4 and TRPV1 receptors in the spinal cord dorsal horn and may be involved in the development of painful states after PAC treatment. Targeting these molecules may represent a possible option for analgesic treatment in states of paclitaxel induced neuropathic pain. Our work was supported by grant support: GAUK 138215, LQ1604 BIOCEV-FAR, GACR 15-11138S, LH15279, GACR P304/12/G069, CZ.1.05/1.1.00/02.0109, RVO67985823.

WTH13-02

Peptidergic modulation of pain and anxiety: forebrain relaxin-3/RXFP3 networks and descending control of nociception in mice

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Persistent pain can hinder normal function and behaviour, with a negative impact on quality-of-life. In persistent pain conditions, patients develop conditions such as anxiety, which worsens pain sensation, creating a feedback loop between pain and this comorbid state. Anxiety is linked to altered function in brain areas innervated by relaxin-3 neurons. Indeed, activation of its receptor, Relaxin/Insulin Family Peptide Receptor 3 (RXFP3), can alter arousal, stress- and anxiety-related and reward-seeking behaviours in rodents. These data suggest a possible link between RXFP3 activity and control of pain sensitivity. Thus, these studies assessed the effect of RXFP3 activation/inhibition on the control of mechanical and thermal pain sensitivity in normal and persistent pain conditions in mice. Intracerebroventricular (icv) administration of RXFP3 agonist peptide reduced mechanical, but not thermal, pain sensation in C57BL/6J mouse model of inflammatory pain ($n = 5$ mice/group, $p < 0.01$). These effects were associated with decreased activity of nociceptive neurons in spinal cord ($n = 6$ mice, $p < 0.05$). In addition, RXFP3 antagonist augmented mechanical and thermal pain sensitivity ($n = 7$ mice/group, $p > 0.05$). These data suggest that relaxin-3 provides a tonic drive to maintain mechanical and thermal pain thresholds. In parallel, we sought to identify the neuronal circuits responsible for the observed effects. Using neural tract-tracing, we identified brain areas that receive relaxin-3 inputs, which in turn innervate the rostroventral medulla (RVM), a region that gates descending pain control. These regions include anterior cingulate cortex, central amygdala, bed nucleus of the stria terminalis and hypothalamus, which are functionally related to pain sensation and comorbidities. Together, these data suggest RXFP3 as a therapeutic target for pain management, and further studies of the specific circuits and mechanisms involved are warranted.

WTH13-03

Bifurcate spinal dorsal neurons projecting simultaneously to supraspinal centers by the dorsal column and the anterolateral system

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Sensory information arriving at the spinal dorsal horn (SDH) neurons can be transmitted to the supraspinal centers by two main systems: the dorsal column-medial lemniscal (DC-ML) or by the antero-lateral system (AL). These systems are well recognized and studied and the main conclusion is their independence or lack of interactions. To our knowledge no study has proposed their interactions as well as their bilateral projections. In order to test this hypothesis, we perform some neurochemical (retrograde neuronal tracing) and electrophysiological experiments in rats. We study principal SDH neurons at the L2-L5 segments having the characteristic of sending projections to the *Gracilis* nuclei (GRA) from the DC-ML or to the ventral postero lateral thalamic (VPL) nuclei from the AL. True Blue (TB) tracer was placed into the left GRA and Fluoro Gold (FG) or Fluor Rubi (FR) tracer was injected in the right VPL. After 13 days, the animals were perfused to localized the stained cells in spinal cord (SC). In order to test the functionality of the neurons founded we used concentric bipolar

electrodes to apply electric stimulation at the left GRA and at the right VPL, and recordings (using glass micropipettes) of the single unit SC cells (left side at L2-L5 SC level). The neurons were classified as SDH wide dynamic range (WDR) cells. A collision test was performed as follows: spontaneous or evoked spikes by receptive field SC stimulation triggered the GRA or VPL stimulation, evoking an antidromic spike. Our data showed that some spinal dorsal horn neurons presented a double staining with TB and FG. These double stained neurons were found in the left and right side of the dorsal horn of SC. The main projection was to the left side (3 to 1). Regarding the electrophysiological experiments, we found neurons showing C-fibers activation which also presenting collision activities for either GRA or VPL antidromic electric activation. Our data show bilateral SC cells projecting to the GRA and VPL. It is possible that this neuronal arrangement serves to assure the alarm system of nociception and generate a diffuse descending response.

WTH13-04

Non-inflammatory autoantibody-induced glia activation in a passive transfer-trauma mouse model of complex regional pain syndrome

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Complex Regional Pain Syndrome (CRPS) is a severe chronic pain condition after small injuries. Abnormal immune response against sensory nerve-derived antigens and complex neuro-immune interactions are suggested to be responsible for the symptoms, but the mechanisms are unclear and the therapy is not satisfactory. In our passive-transfer translational mouse model, we investigated the behavioural, immunological and neurochemical changes characteristic of the disease.

Plantar incision was performed in C57Bl/6 mice daily treated i.p. with purified serum-IgG of CRPS patients or healthy volunteers for 3-13 days. Paw mechanonociceptive threshold was measured by aesthesiometry, volume by plethysmometry, neutrophil/macrophage myeloperoxidase activity by luminescence *in vivo* imaging, sensory neuropeptides and cytokines by immunassays, glia markers in pain-related brain regions with immunochemistry.

CRPS IgG significantly increased and prolonged hyperalgesia and swelling of the incised paw compared to healthy IgG particularly in the second week, when swelling resolved. Myeloperoxidase activity and substance P-like immunoreactivity moderately, but significantly increased in CRPS IgG-treated mice transiently on days 2 and 7, but calcitonin gene-related peptide and inflammatory cytokines were not altered. CRPS IgG significantly increased the density of astrocyte-related glial fibrillary acidic protein (GFAP) and microglia-staining Iba1 in L4-L5 spinal dorsal horn, periaquaductal gray and somatosensory cortex. GFAP increased in the first week on both sides, Iba1 elevated only ipsilaterally mainly later.

The main symptoms of CRPS with special emphasis on hyperalgesia can be reliably transferred from patients to mice by IgG. Peripheral inflammation is not likely to play a crucial role in this pathological pain, but glia-mediated central sensitization is more likely to be involved.

WTH13-05

Microrna deregulation in the spared nerve injury mouse model for neuropathic pain

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Micro-RNAs (miRNAs) are small non-coding RNAs that regulate gene expression by interacting with the 3′-untranslated region of mRNAs. During the past few years, miRNAs emerged as potential biomarkers and an increasing number of miRNAs are found deregulated and associated with signatures of neuropathic pain, induced by experimental nerve injury. In relevant models, the neurons in the dorsal root ganglia (DRG) exhibit increased neuronal excitability, which results in augmented nociceptive signaling from the periphery to the central nervous system. Therefore, we set out to explore signatures of nociceptor hyperexcitability and correlate them with miRNA expression patterns.

Neuropathic pain was induced in mice by using the spared nerve injury model (SNI). Seven days post lesion, a combination of techniques (miRNA and RNA sequencing, *in vitro* electrophysiology, qRT-PCR, *in situ* hybridization) was employed for the identification of pain-related miRNAs, their *in silico* predicted mRNA targets and related functional alterations. Several up-regulated miRNAs, and down-regulated mRNAs, were found to be associated with the pain-phenotype displayed by SNI-treated mice. Targeting these miRNAs could potentially provide novel therapeutic approaches in the management of neuropathic pain.

WTH13-06

Oxytocin induce thermal analgesia via vasopressin-1A receptor by modulating TRPV1 and K-conductance

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Recent studies have provided several lines of evidence that peripheral administrations of oxytocin (OT) induces analgesia in human and rodents. However, the exact underlying mechanism of the analgesia still remains unclear. In the present study, we aimed to identify the receptor which mediates analgesic property of OT and its cellular mechanisms in thermal pain behavior. When we examined whether intraperitoneal (IP) injection of specific antagonist for oxytocin and vasopressin (AVP) receptors reverse the oxytocin-induced analgesia in Hargreaves' thermal pain behavioral tests in rats. We found that oxytocin-induced analgesia was reversed by AVP1a-RA, the antagonist for AVP1a receptor, but not by OTRA, oxytocin receptor antagonist. Single cell RT-PCR analysis revealed that while AVP1a receptor, compared to OT, AVP1b, and AVP2 receptors, was more profoundly expressed in DRG neurons,

expression of AVP1a receptor was predominant in TRPV1-expressing DRG neuons. The Fura-2 based Calcium imaging experiments showed that capsaicin-induced calcium transient was significantly inhibited by oxytocin, which was again reversed by AVP1a-RA, AVP1a receptor antagonist. These results suggest that AVP1a receptor in the DRG cells mediate oxytocin-induced thermal analgesia. Additionaly, the whole cell patch clamp recording demonstrated that oxytocin increasespotassium channel conductance via AVP1a receptor in the DRG cells.

Taken together, our findings suggests that the analgesic effects produced by peripheral administration of oxytocin are attributable to the activation of AVP1a receptor amongst oxytocin receptors, which results in the regulation of TRPV1 channels and increase of potassium conductance in DRG neurons.

WTH13-07

Mechanisms of pain hypersensitivity in a pharmacological mouse model of attention-deficit/hyperactivity disorder

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Aim: The 6-hydroxy-dopamine (6-OHDA) neonatal lesion induces brain deficits influencing cognitive and motor behaviors. It is accepted as a pharmacological model of ADHD with good face and construct validity. Recent clinical evidence pointed to pain hypersensitivity of ADHD patients. Here, we investigated the effects of neonatal dopamine depletion (P5) in mouse on pain thresholds at adulthood (P40).

Methods: We analyzed nociceptive behavioural responses to thermal and mechanical stimuli in 6-OHDA lesioned adult mice. Neuronal activity to mechanical stimulus was further examined *in vivo* by unit recording of spinal nociceptive neurons.

Results: 1). Neonatal dopamine (DA) depletion resulted in behavioral characteristics similar to those seen in patients with ADHD. At P24, ADHD-like mice exhibit hyperactivity. At P40, ADHD-like mice show anxiety, antisocial behavior, increased aggressiveness, mildly impaired latent inhibition and short-term memory. We also demonstrated attention deficit and increased impulsivity in ADHD-like mice. 2). Mice with neonatal dopamine depletion exhibited a marked increase in both thermal (heat and cold) and mechanical sensitivity. Dopamine depletion also increased pain sensitivity in persistent pain conditions, i.e. at 4 days after Complete Freund's Adjuvant injection in the hindpaw. Interestingly, ADHD symptoms were not modified in inflammatory conditions suggesting that ADHD influences pain sensitivity while the reverse was not true. 3). Electrophysiological recordings showed increased activity of spinal neurons in response to both innocuous and noxious stimuli only in the ADHD-like group. Moreover, our data indicated that ACC neurons are hyper-activated in ADHD-like mice. Finally, we found that the electrical stimulation of controlateral ACC (100 Hz; 10, 20, and 30 V; 10 s) increases the responses (amplitude and mean spike frequency) of WDR neurons to innocuous and noxious stimuli.

Conclusion: Our results demonstrated the validity of the neonatal 6-OHDA model to mimic ADHD syndrome. Taken together, our data demonstrate that ADHD conditions induce WDR spinal cord neurons hyperactivation and pain hypersensitivity. We also suggest that the deregulation of ACC may be the trigger for spinal neuron dysfunction

WTH13-08

Immunohistochemical and physiological analyses of histamine receptors on ganglion cells in the developing gerbil retina

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Histamine is an important neurotransmitter in the central nervous system. There are efferent nerve terminals from the brain to the retina, and histamine is reported to be released to the retina. Also, mammalian retinal neurons have been reported to express several types of histamine receptors. In order to confirm the presence of histaminergic pathway in the developing and adult retina, calcium-imaging and immunohistochemical analyses were used in the gerbil (*Meriones unguiculatus*). First, the activity of histamine receptors was examined by fura-2 based calcium-imaging technique. A bath application of 100 μM histamine or histamine agonists increased the intracellular calcium concentration in some retina ganglion cells. Next, we examined the localizations of H1, H2 and H3 receptors in the gerbil retinae from 1 to 350 postnatal days. We found that H1, H2 and H3 receptors expressed respectively on retinal ganglion cells. H1 receptor expresses through the retinal maturation. On the other hand, the expressions of H2 and H3 receptors became maximum from 14 to 21 postnatal days. Histidine decarboxylase, which produces histamine from histidine, also expressed in retinal ganglion cells, and moreover, each of histamine receptors and histidine decarboxylase were co-localized at the same retinal ganglion cells. Since the gerbil opens the eyes at 3 weeks old, it is considered that the H2 and H3 receptors play some specific roles at the formation of the early visual system. Colocalization of histidine decarboxylase and histamine receptors in retinal ganglion cells suggest that retinal ganglion cells may interact with each other via histamine. Therefore, histamine may be one of the important neurotransmitters and/or neuromodulators in the visual information processings of the mammalian retina.

WTH13-09

The role of PAR2 receptors in modulation of nociceptive transmission at spinal cord level under inflammatory conditions

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Modulation of synaptic transmission in the spinal cord dorsal horn plays a key role in the development of pathological pain states. Protease-activated receptors (PARs) are family of four G-protein-coupled receptors (PAR1-4) activated by proteases that cleave the N-terminal domain of the receptor unmasking a tethered peptide ligand sequence. The role of PAR2 in pain perception is closely related to their presence in a subpopulation of dorsal root ganglion

(DRG) neurons, where they are often co-expressed with TRPV1 receptors. Our previous work documented an important role of PAR2 receptors on the presynaptic endings of primary afferents in the spinal cord (Mrozkova et al.,PLoS ONE, 2016, 11, 10, e0163991). The present work aimed to study the role of PAR2 and the possible interaction with TRPV1 receptors at the spinal cord level under inflammatory conditions.

Whole-cell patch clamp recordings of miniature excitatory postsynaptic currents (mEPSCs), spontaneous (sEPSCs) and dorsal root stimulation evoked (eEPSCs) were made from superficial dorsal horn neurons in lumbar spinal cord slices of young (21 days) Wistar rats. Peripheral inflammation was induced by application of 3% carrageenan into the paw 24 h before the experiment. Application SLIGKV-NH2 (PAR2 AP, 100 μM) was used to activate PAR2 receptors.

Application of PAR2 agonist increased frequency of mEPSCs (136.8 ± 10.0%; $p < 0.01$), sEPSCs (127.2 ± 9.5%; $p < 0.05$) and also amplitude of the evoked EPSCs (154.4 ± 12.6%; $p < 0.01$). Administration of TRPV1 antagonist (SB366789) together with the PAR2-AP prevented the frequency (mEPSC, sEPSC) and amplitude (eEPSC) increases.

Our results suggest that presynaptic PAR2 receptors and their interaction with TRPV1 receptors may play an important role in modulation of nociceptive synaptic transmission in the spinal cord dorsal horn, particularly under the conditions of peripheral inflammation.

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WTH14 Limbic and other Systems

WTH14-01

Signaling by striatal cholinergic interneurons in mice is required for cue detection and behavioural flexibility

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While the role striatal dopamine plays in controlling goal-directed behaviour has been extensively studied, less is known about the importance of striatal cholinergic interneurons (CINs). CINs control striatal circuits by releasing two different neurotransmitters: acetylcholine (ACh) and glutamate (Glu). Initial findings suggest that both ACh and Glu released by CINs can increase and decrease the release of striatal dopamine, respectively, which could contribute to the control of goal-directed behaviour by CINs. To test this possibility, we generated three genetically modified mouse lines with a specific deletion of the vesicular acetylcholine transporter (VAChT) and/or vesicular glutamate transporter 3 (VGLUT3), necessary for the release of ACh and Glu from CINs. In VAChT-deficient mice, we found deficits in two different behavioural paradigms in which habit and goal like-behaviour were examined. Moreover, we reproduced these deficits by AAV virus-mediated deletion of VAChT limited to the dorsal striatum. Using fast cycling voltammetry we found a significant decrease of dopamine release in the dorsomedial but not in the dorsolateral striatum of VAChT-deficient mice. In contrast to these findings, the VGLUT3-deficient mice did not show a deficit in cognitive flexibility and they even performed slightly better than wildtype mice in some of the task parameters. We also found that the mice with a deletion of both VAChT and VGLUT3 from CINs performed worse in motivational tasks. However, the deficit did not seem to be caused by decreased motivation to work for a reward, but rather by an impaired ability to find salience in the reward-related stimulus. We conclude that modulation of striatal circuits by CINs is necessary for intact goal-directed behavior and that ACh released by CINs is specifically important for cognitive flexibility.

WTH14-02

Chronic relaxin-3 receptor activation in ventral hippocampus and medial amygdala reciprocally modulates anxiety-like behaviour

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The neuropeptide, relaxin-3, preferentially activates the $G_{i/o}$-protein-coupled receptor, RXFP3. Relaxin-3/GABA neurons constitute a conserved ascending neural network in mammalian brain, enriched in limbic areas involved in stress, arousal and emotion-related behaviours, such as amygdala, ventral hippocampus (vHip), medial and lateral septum, and the prefrontal cortex. We have previously observed differential effects of *acute* RXFP3 activation on conditioned fear in rats, which were associated with the site of administration (icv vs. local) and presumed differential sites and extent of action. In this study, we characterized the effects of *chronic* RXFP3 activation in two key limbic regions on 'affective' behaviours, including anxiety and social avoidance. Adeno-associated viral vectors driving local secretion of the RXFP3 agonist, R3/I5, were bilaterally injected into the vHip *or* medial amygdala (MeA) of adult male, Sprague–Dawley rats (7-10/group). Chronic vHip RXFP3 activation *decreased* time and distance travelled in the open arms of the elevated plus maze (EPM) *and* the aversive light zone of the light-dark box (LDB); and decreased social interaction with a conspecific stranger, compared to control (all $p < 0.05$). This was associated with a significant *decrease* in somatostatin (SOM) immunoreactivity in neurons in the 'virally-transduced' region. Conversely, chronic RXFP3 activation in the MeA *increased* time and distance travelled in EPM open arms *and* centre of a large open field (LOF), but did not alter behavior in the LDB or social assays. We are currently assessing effects of chronic RXFP3 activation on neuronal SOM levels in MeA; and the neurochemical identity of RXFP3-expressing neurons in the vHip and MeA. Our data suggest 'topographic' and 'disruptive' effects of persistent RXFP3 signalling on anxiety-related behaviour, related to precise site(s) and timing of the exogenous relaxin-3 actions, which likely contrast to those of endogenous peptide inputs, and receptor activation patterns. These studies provide a better understanding of the neurochemical basis of anxiety-related behaviour, with the potential for identifying novel therapeutic targets for anxiety disorders.

WTH14-03

Nucleus accumbens D2-dependent increase in motivation: impact of selective manipulation of local microcircuits

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The nucleus accumbens (NAc) is a key brain region of the reward circuit, playing an important role in reward processing, reinforcement and motivation toward such reward. Because of its biological function, NAc dysfunction has been implicated in various neurological and neuropsychiatric disorders, that include depression, obsessive-compulsive disorder, anxiety, and in addiction. Around 95% of the NAc neurons are medium spiny neurons (MSNs), mainly divided into those expressing dopamine receptor D1 (D1R, D1-MSNs) and those expressing dopamine receptor D2 (D2R, D2-MSNs). The remaining 5% are different subtypes of interneurons. Previous work from our group identified a relevant involvement of D2-MSNs in motivation and positive reinforcement; however, the intrinsic accumbal microcircuit wiring behind this positive effect is still unclear. In order to understand what type of neurons were involved in this behavioural effect, we combined optogenetic activation of NAc D2-MSNs with *in loco* pharmacological delivery

of specific neurotransmitter antagonists to show that accumbal D2-MSN-mediated enhancement in motivation requires dopamine release from VTA terminals in a cholinergic-mediated manner, that acts in both D1R and D2R.

In conclusion, this work highlights the fact that activation of D2-MSNs during cue exposure increases motivation, and that a concerted action of different neurotransmitter systems is required for this behavioural effect.