Dose- and time-dependent therapeutic and adverse effects of Mucuna pruriens extract in the 6-OHDA rat model of Parkinson’s disease

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To circumvent the limited spatial resolution of fluorescent protein imaging, we are developing genetically encoded tags for electron microscopy (EM). Arabidopsis phototropin, a photoreceptor containing flavin mononucleotide (FMN), can be engineered into a small (106-residue) Singlet Oxygen Generator (miniSOG), which efficiently generates singlet oxygen upon blue light illumination. Singlet oxygen polymerizes diaminobenzidine into an osmiophilic deposit, enabling correlative EM with nanometer spatial resolution. In an initial biological application, EM shows that the closely related cell-adhesion molecules SynCAM1 and SynCAM2, separately fused to miniSOG, predominantly localize respectively to the presynaptic and post synaptic sides of mammalian CNS synapses. MiniSOG may do for EM what GFP did for optical microscopy [Shu et al. (2011). PLoS Biology 9(4): e1001041. doi:10.1371/journal.pbio.1001041]. Combination of miniSOG with Time-STAMP [Lin et al. (2008). PNAS 105: 7744–7749] permits newly synthesized copies of a genetically specified protein to be distinguished from older copies by EM, so that new protein synthesis can be imaged with very high molecular and spatial resolution. MiniSOG also looks promising for chromophore-assisted light inactivation, e.g., of synaptic release within intact C. elegans.

A major unsolved problem in chemical neurobiology is optical imaging of action potentials with high sensitivity (>20% change in fluorescence per 100 mV) and high speed (<1 ms response time) without major phototoxocity or capacitative loading of the neuronal membrane. Voltage-dependent photoinduced electron transfer, governed by Marcus theory and implemented by synthetic molecular wires, achieves the above goals. For clinical applications, fluorescent peptides that light up peripheral nerves show surgeons where not to cut [Whitney et al. (2011). Nature Biotech. 29: 352–356].
The presentation will focus on aspects concerning the energy requirements of glutamatergic neurotransmission and neuronal-astrocytic interactions related to that. The role of glucose and lactate as neuronal energy substrates during resting and activation will be discussed. We have shown that glucose metabolism is up-regulated in cultured glutamatergic neurons during neurotransmission whereas that of lactate is not (1). Moreover, that utilization and oxidative metabolism of glucose, but not lactate correlates dose-dependently with N-methyl-D-aspartate (NMDA)-induced intracellular Ca\(^{2+}\) elevations and vesicular glutamate release in cultured neurons.

Our proposed hypothesis (2) explaining how lactate consumption may be limited by a Ca\(^{2+}\) dependent inhibition of the malate-aspartate shuttle activity will be presented. In addition, the role of glycogen as a dynamic player in brain energy metabolism will be presented. We suggest that glycogen is essential for the maintenance of glutamatergic neurotransmission and glutamate homeostasis. Glycogen seems to be important for neuronal glutamate release as well as for subsequent astrocytic glutamate uptake (3,4).

References:
Symposium 10
The mTOR Pathway in the CNS: From Neuronal Plasticity to Myelination

S10-01
MTORC1 SIGNALING IN MEMORY CONSOLIDATION AND RECONSOLIDATION
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Previous studies have shown that the mTORC1 signaling pathway regulates cap-dependent translation during protein synthesis-dependent forms of long-lasting synaptic plasticity and long-term memory in rodents. These findings have generated much excitement because they were the first demonstration of the complex biochemical regulation of translation during synaptic plasticity and memory. Using new small molecule inhibitors and novel genetically-modified mice, we have found that eIF4E-eIF4G interactions and p70 S6 kinase 1 (S6K1), two translational control mechanisms downstream of mTORC1, are differentially involved in memory consolidation and reconsolidation. These findings suggest that although memory consolidation and reconsolidation require de novo protein synthesis, there are disparate mTORC1-dependent translational control mechanisms required for these types of memory function.

S10-02
BIPHASIC ACTIVATION OF THE MTOR PATHWAY IN THE CORTEX
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Different forms of memories and synaptic plasticity require synthesis of new proteins at the time of acquisition or immediately after. We are interested in the role of translation regulation in the cortex – the brain structure assumed to store long-term memories. The mammalian target of rapamycin, mTOR (also known as FRAP and RAFT-1), is part of a key signal transduction mechanism known to regulate translation of specific subset of mRNA’s and to affect learning and synaptic plasticity. Novel taste learning induces two waves of mTOR activation in the gustatory cortex. Interestingly, the first wave can be identified both in synaptoneurosomal and cellular fractions, while the second wave is detected in the cellular fraction but not in the synaptic one. Inhibition of mTOR, specifically in the gustatory cortex, has two effects. First, biochemically, it modulates several known downstream proteins that control translation and reduces the expression of PSD-95 in vivo. Second, behaviorally, it attenuates long-term taste memory. The results suggest that the mTOR pathway in the cortex modulates both translation factor activity and protein expression, to enable normal taste memory consolidation.

S10-03
REGULATION OF CNS MYELINATION AND REMYELINATION BY MTOR
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The current studies were undertaken to investigate the role of Akt in myelination in vivo. Transgenic mice overexpressing constitutively active Akt (Akt-DD) driven by the myelin proteolipid protein promoter were generated (Plp-Akt-DD). They were analyzed from P10 through adulthood. Although Akt is a survival factor that often impacts tumor formation, Akt overexpression in oligodendrocytes did not increase progenitor cell survival or reduce apoptosis during development. On the other hand, electron microscopic analysis indicated that myelin was thicker in Plp-Akt-DD mice than WT. Thus, more myelin was generated per oligodendrocyte. As animals aged, they continued to myelinate, and they appeared unable to stop myelination. The major Akt substrate and pathway that was increased in oligodendrocytes in these animals was the mTOR pathway (mammalian target of rapamycin). Studies inhibiting this pathway with rapamycin indicated that this was the major pathway regulating the hypermyelination in these mice. Studies using rapamycin to inhibit mTOR during active myelination in normal mice also reduced the amount of myelin generated. Thus, Akt signaling through mTOR appears to be a major regulator of active myelination. On the other hand, rapamycin treatment during very early brain development led to an increase in the number of early differentiating oligodendrocytes, suggesting a different mechanism of regulation of oligodendrocyte differentiation by mTOR. In the adult, during cuprizone-induced demyelination, extensive repair by oligodendrocyte progenitor cells occurs. This can be dramatically blocked by treatment of mice with rapamycin, suggesting that mTOR is also involved in remyelination in the adult. Understanding the details of the pathways that Akt and mTOR regulate during oligodendrocyte differentiation, myelination and remyelination may provide exciting new targets for enhancing remyelination in MS. Studies supported by the National Multiple Sclerosis Society and the NIH.
Recent studies revealed that the mammalian target of rapamycin (mTOR) signaling pathway, a major target downstream of Akt, regulates oligodendrocyte differentiation/myelination (1, 2). The objectives of this study were i) to define the mTOR regulated proteome in differentiating oligodendrocyte progenitor cells (OPCs), and ii) to determine whether mTOR signaling is important for remyelination following a focal demyelinating injury. In order to define the mTOR regulated proteome, we applied an iTRAQ mass spectrometry-based proteomic approach. Among the 978 proteins identified in this study, 328 (34%) exhibited a greater than 20% change ($p < 0.05$) in control versus rapamycin treated OPCs following 4 days of differentiation in vitro. Interestingly, 197 (20%) proteins were elevated in rapamycin treated cultures, while 131 (13%) proteins were down-regulated by rapamycin. Inhibiting mTOR decreased expression of myelin proteins, proteins involved in cholesterol and fatty acid synthesis, as well as many cytoskeletal proteins, cell signaling components, and nuclear/transcriptional regulators. Of particular interest was the identification of several critical mediators of oligodendrocyte differentiation including the pro-differentiation factors Fyn and Quaking. To address whether mTOR signaling is required for remyelination, we analyzed remyelination following a cortical focal demyelination in adult mice treated with either vehicle or the mTOR inhibitor rapamycin. Analysis of lesions revealed delayed remyelination at 21 days in the rapamycin-treated animals supporting the hypothesis that mTOR signaling is required for both developmental myelination as well as remyelination.

References:
Symposium 11
Protein Tyrosine Phosphorylation Signal and Brain Functions

S11-01
SRC REGULATION OF NMDA RECEPTORS IN PAIN AND SCHIZOPHRENIA
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Regulation of the function of postsynaptic glutamate receptors is one of the principal mechanisms for producing alterations of synaptic efficacy in physiological and pathological processes in the CNS. At glutamatergic synapses, NMDA receptors (NMDARs) are upregulated by Src family tyrosine kinases which are opposed by the action of tyrosine phosphatases including STEP. Src kinase itself is a point through which multiple signaling cascades from converge to upregulate NMDA receptor activity. Src is anchored within the NMDA receptor complex by the adaptor protein ND2. This interaction is critical for holding Src in association with NMDARs when Src-mediated enhancement is normally required for triggering the synaptic potentiation that underlies learning and memory. We have discovered that excessive Src-mediated enhancement of NMDAR activity in the dorsal horn of the spinal cord mediates the hypersensitivity underlying chronic pain. On the other hand, we have found that Src-mediated enhancement of NMDAR function is interrupted by signaling through the receptor–ligand pair, neuregulin 1(NRG1) – ErbB4. NRG1/ErbB4-dependent synaptic potentiation in the hippocampus and prefrontal cortex. Increased NRG1 – ErbB4 signaling is genetically linked to schizophrenia, leading us to hypothesize that this excessive signaling suppresses NMDAR-dependent synaptic plasticity thereby producing positive symptoms of this disorder. Together our findings suggest that aberration of Src-mediated enhancement of NMDA receptor and pathological neuroplasticity is a unifying theme for several CNS disorders. Thus, normalizing Src enhancement of NMDARs is a novel therapeutic approach for CNS disorders, an approach without the deleterious consequences of directly blocking NMDARs. Supported by CIHR, Krembil Fdn, ONF and HHMI.

S11-02
ESSENTIAL ROLE OF TYROSINE PHOSPHORYLATION OF THE NMDA RECEPTOR IN MOUSE BEHAVIOR
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The NMDA receptor is essential for development, synaptic plasticity, excitototoxicity, and behavioral regulation. NMDA receptor activity is regulated by several post-translational modifications such as protein phosphorylation and palmitoylation. We previously found that the GluN2B/NR2B subunit of the NMDA receptor was tyrosine-phosphorylated, with Tyr-1472 its major phosphorylation site for Fyn tyrosine kinase. To address the role of Tyr-1472 phosphorylation in vivo, we generated mice with a knock-in mutation of the Tyr-1472 site to phenylalanine (Y1472F knock-in mice). The Y1472F knock-in mice showed impaired amygdala-dependent fear learning and reduced amygdaloid long-term potentiation, arguing that Tyr-1472 phosphorylation of GluN2B is a key mediator of fear learning and amygdaloid synaptic plasticity. We also found that Tyr-1472 phosphorylation regulated anxiety-like behavior and CRF expression in the amygdala. Furthermore, electron microscopic analyses revealed that the Y1472F mutant of the GluN2B subunit showed impaired localization at synapses. As with the case of GluN2B, we have generated Y1325F knock-in mice where Tyr-1325 of GluN2A/NR2A, the major Src-mediated phosphorylation site, was mutated to phenylalanine. The Y1325F knock-in mice showed significantly less immobility than wild-type mice in the tail suspension test and the forced swim test. We also found that the Tyr-1325 phosphorylation site was required for Src-induced potentiation of the NMDA receptor channel in the striatum, suggesting that Tyr-1325 phosphorylation of GluN2A modulates depression-related behaviors through regulation of the NMDA receptor channel activity. From these data, we conclude that each phosphorylation event on the NMDA receptor differentially regulates mouse behavior through different cellular mechanisms.

S11-03
STRESS-EVOKED TYROSINE PHOSPHORYLATION OF SIRPα REGULATES DEPRESSION-LIKE BEHAVIOR
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Severe stress induces changes in neuronal function that are implicated in stress-related disorders such as depression. The molecular mechanisms underlying the response of the brain to stress remain largely unknown, however. Signal regulatory protein ζ (SIRPζ, also known as SHPS-1) is an immunoglobulin (Ig)-superfamily protein that is highly expressed throughout the brain. This protein undergoes tyrosine phosphorylation at its cytoplasmic region and binds the protein tyrosine phosphatase Shp2. CD47 is a member of the Ig superfamily of proteins that possesses five transmembrane domains and functions as a ligand for the extracellular region of SIRPζ. CD47 is also expressed predominantly throughout the brain, with the regions in which it is abundant overlapping extensively with those enriched in SIRPζ. SIRPζ and CD47 thus constitute a cell–cell communication system that likely plays an important role in the brain. We found that mice expressing a mutant form of SIRPζ that lacks most of the cytoplasmic region manifested prolonged immobility (depression-like behavior) in the Porsolt forced swim (FS) test. FS stress induced marked tyrosine phosphorylation of SIRPζ and its formation of a complex with Shp2 in the brain. The FS stress-induced tyrosine phosphorylation of SIRPζ in the hippocampus was markedly reduced in mice deficient of Fyn, a Src family kinase. CD47-deficient mice also manifested prolonged immobility in the FS test. The FS-induced tyrosine
phosphorylation of SIRPα in the hippocampus was markedly impaired in CD47-deficient mice, suggesting the interaction of CD47 with SIRPα is important for the FS-induced tyrosine phosphorylation of SIRPα. Moreover, FS stress induced tyrosine phosphorylation of both the GluN2B subunit of the NMDA subtype of glutamate receptor and the K+-channel subunit Kv1.2 in the hippocampus, and such tyrosine phosphorylation of the proteins was altered in SIRPα mutant mice. Tyrosine phosphorylation of SIRPα through its interaction with CD47 thus mediates an antidepressant effect in the response of the brain to stress.

S11-04
A ROLE FOR THE SHP-2 PROTEIN TYROSINE PHOSPHATASE IN DEFINING THE SPACING EFFECT OF LONG-TERM MEMORY FORMATION
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This study examines roles of the PTPN11 gene and its point mutations identified in Noonan Syndrome (NS) in the spacing effects of long-term memory (LTM) formation in Drosophila. NS is an autosomal dominant genetic disorder showing learning difficulties and mental retardation. Gain-of-function (GOF) PTPN11 mutations cause about 50% of NS cases. The PTPN11 gene and its ortholog in Drosophila, corkscrew (csw), encode a protein tyrosine phosphatase SHP-2, which is recruited to many receptor tyrosine kinases upon activation and is generally a positive regulator of Ras/MAPK signaling. The spacing effect refers to a phenomenon that better memory is produced from multiple training trials spaced over time than massed together. Although such effect has been studied in psychology for over hundred years, there is almost no clue how such effect is achieved at the molecular level.

In Drosophila, long-term memory (LTM) for odor-leg electric shocking conditioning can only be induced with multiple training sessions spaced over time, which includes 10 repetitive training trials with a 15 min resting interval between trials. We show that duration of the resting interval can be regulated by manipulation of CSW expression. Overexpression of wild-type CSW in neurons of the mushroom body, a brain region critical for memory formation, shortens the resting interval required for LTM induction from a minimum requirement of 15 min to 2.5 min, whereas overexpression of NS-mutant CSW prolongs these resting intervals from 15 min to 30 or 40 min. Biochemical analysis reveals that LTM-inducing training regimens generate repetitive waves of CSW-dependent MAPK activation and the time course of MAPK activation and inactivation defines the duration of the resting interval.
Recent experimental evidence has revealed that intrinsic genetic programs endow GABAergic interneurons with an early subtype identity. It is also known that during development interneurons participate in correlated network activity. However, the possible role of electrical activity in shaping the migration and integration of specific interneuron subtypes into the cortex has not been addressed. We assessed the role of activity in the development of caudal ganglionic eminence (CGE)-derived interneuron subtypes in particular because they are born relatively late and integrate into an already active cortical network. We examined this question by specifically attenuating neuronal activity in calretinin (Cr+) reelin (Re+) and vasoactive intestinal peptide (VIP+) interneurons, three CGE-derived interneuron subtypes. We demonstrate that activity is essential for Re+ and Cr+ (but not VIP+) interneuron migration before postnatal day 3 (P3), whereas after P3 glutamate-mediated activity in these same populations controls the development of their axons and dendrites. Furthermore, we show that the engulfment and cell motility 1 gene (Elmo1), a target of the transcription factor distal-less homeobox 1 (Dlx1), is selectively expressed in Re+ and Cr+ interneurons. We observed that activity-dependent expression of this gene is both necessary and sufficient for proper interneuron migration. Our findings reveal a heretofore unknown and selective requirement for in vivo neuronal activity in shaping the cortical integration of specific neuronal subtypes.

Cortical interneurons can be divided into a large number of subpopulations according to morphological, molecular and electrophysiological criteria. It has been proposed that the combinatorial expression of transcription factors, as well as the time of birth and the specific site of origin of interneurons in the ventral forebrain, determines their subtype identity and their classification into specific neurochemical and electrophysiological subgroups. Despite considerable progress over the last several years, the transcriptional network that controls the specification and differentiation of cortical interneuron are poorly understood. The LIM-homeodomain factor Lhx6 is a key regulator of the migration and differentiation of Parvalbumin (PVA) and Somatostatin (SST)-expressing interneurons. To identify the molecular cascades that control cortical interneuron differentiation and migration we have undertaken a genome-wide gene profiling approach, in which we have compared gene expression in the brain of wild-type or Lhx6-deficient embryos. Our expression analysis identified several genes which were down-regulated in mutant brains. Some of these genes were already known to be expressed in cortical interneurons in an Lhx6-dependent manner (i.e. Kcnq1, Npas1, Npy, Som, Sox6) (Liodis et al. 2007; Zhao et al. 2008; Batista-Brito et al. 2009). Here, we have focused on a different set of genes which have not been implicated previously in cortical interneuron development but were expected, from other studies to regulate neuronal differentiation, migration and synapse formation. Using in situ hybridization, immunostaining and quantitative PCR, we initially verified that their expression is altered in Lhx6-deficient mice. We have also carried out a series of gain- and loss-of-function experiments to address their role on interneuron development. Our studies provide insight into the molecular cascades that are controlled by Lhx6 and regulate the migration, differentiation and maturation of specific subsets of cortical interneurons.

The proper function of the central nervous system requires the appropriate connections between glutamatergic neurons and GABAergic interneurons. Cortical GABAergic interneurons are characterized by extraordinary neurochemical and functional diversity. Impaired development and/or function of GABAergic interneurons can lead to severe neurodevelopmental disorders such as schizophrenia, epilepsy and autism. Recent studies have uncovered some of the molecular components underlying interneuron development, including the cellular and molecular mechanisms guiding their migration to the cortex, whereas the intracellular components involved are still unknown. Rac1, a member of the Rho subfamily of Rho GTPases, has been implicated in various cellular processes such as cell cycle dynamics, axonogenesis and migration. To address the specific role of Rac1 in interneuron progenitors originating in the medial ganglionic eminence, we have used Cre/loxP technology. The ablation of Rac1 from mitotic progenitors, results in a delayed cell cycle exit, which in turn leads to a later onset of migration towards the cortex. As a consequence, only half of GABAergic interneurons are found in the postnatal cortex. Ablation of Rac1 from postmitotic progenitors does not result in similar defects, thus underlying a novel, cell autonomous and stage-specific requirement for Rac1 activity, within proliferating progenitors of cortical interneurons. Rac1 is necessary for their transition from G1 to S phase, at least in part by regulating Cdc25 levels and Retinoblastoma protein phosphorylation.
Connectivity in the developing hippocampus displays a functional organization particularly effective in supporting network synchronization, as it includes superconnected hub neurons. We have previously shown that hub network function is carried out by a subpopulation of GABAergic interneurons that display dense and widespread axonal arborisations (Bonifazi et al. 2009). However, the fate of hub neurons remains unknown. Specifically it is unclear whether these hub cells are only transiently present or later develop into distinctive subclasses of interneurons. These questions are difficult to assess given the complexity of the GABAergic neurons and the poor expression of interneuron markers at early developmental stages. To circumvent this conundrum we used ‘genetic fate mapping’ that allows for the selective labelling of interneurons based on their place and time of origin. Following theoretical predictions, we tested the hypothesis that pioneer cells could develop into hub neurons.

Reference:
Symposium 13
Neurotransmission and Drugs of Abuse: Novel Effects on Developing and Mature Neuronal Circuits

S13-01
DEVELOPMENTAL EFFECTS OF ALCOHOL ON NEUROTRANSMISSION: IS LIGHT DRINKING SAFE IN PREGNANCY?
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Drinking alcohol during pregnancy is a significant public health problem, resulting in fetal alcohol-spectrum disorder (FASD), a condition with an estimated prevalence of 2–5%. At the more severe end of the spectrum is fetal alcohol syndrome, characterized by growth retardation, facial dysmorphology, and neuropsychiatric disorders. At the other end of the spectrum are individuals with more limited in utero alcohol exposure, who display learning disabilities or attention deficits without dysmorphology. Studies with animal models of FASD have provided convincing evidence indicating that low doses of ethanol can produce significant neurodevelopmental alterations. However, the mechanisms responsible for these effects of ethanol are unknown; as a result, few therapeutic interventions are currently available to treat FASD patients and these have limited efficacy. We recently reported that in vivo ethanol exposure during the equivalent to the human third trimester of pregnancy (i.e. neonatal period in rats) blocks brain-derived neurotrophic factor (BDNF)-dependent long-term potentiation (GABAergic transmission (GABA-LTP) in rat CA3 hippocampal pyramidal neurons (Zucca et al. 2010 J Neurosci. 30:6776-81, 2010). This effect is observed at serum ethanol concentrations as low as 0.02 g/dL (legal intoxication level in the USA = 0.08 g/dL) and is long-lasting. GABA-LTP is likely caused by degradation of L-type voltage-gated Ca2+ channels involved in retrograde BDNF release; expression of the CaV1.3 subunit was reduced to 51 ± 9% of control by in vivo ethanol exposure; n = 4, p < 0.05 by one-sample t-test versus theoretical mean of 100. The mechanisms and consequences of the reduction in L-type voltage-gated Ca2+ channel subunit expression is currently under investigation. We conclude that low doses of alcohol during late pregnancy can affect plasticity mechanisms that are critical for synapse formation, stabilization and/or pruning. These findings support the recommendation that pregnant women should not drink even low amounts alcohol at any stage of pregnancy. Supported by NIH grant AA15614.

Reference:

S13-02
RECREATIONAL DRUGS, PRESCRIPTION DRUGS AND THE ADOLESCENT BRAIN: INSIGHTS FROM ANIMAL MODELS
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Adolescence is a complex and unique developmental epoch during which the brain undergoes rapid maturation and where risky, impulsive and highly emotional behaviours are prevalent. The adolescent brain has resilience, arising from its inherent neuroplasticity, but also vulnerability, arising from the ability of stress and toxins to perturb normal developmental programs leading to adult psychopathology. Our work has used adolescent rats (aged 28–55 days) to model the impact of a variety of drugs on adolescent behavior, and also the impact of these drugs on the developing brain. With alcohol, we find that adolescent rats are particularly prone to binge drinking, and will present with impressively high blood alcohol levels after short periods of ad libitum access to beer. Hippocampal proteomic analysis shows a greater long-term impact of alcohol on the adolescent compared to the adult rat brain. With cannabinoids such as THC, we find that adolescent rats show less aversion to these drugs than adult rats. Chronic exposure to cannabinoids has disproportionate effects on adolescent, compared to adult, brains with a greater number of hippocampal protein alterations observed in adolescents after THC. Human clinical data suggest that antidepressants can be problematic in human adolescent populations with an increased risk of suicidal ideation and self-harming behaviors in teenagers given these drugs. Our work shows that the SSRI antidepressant paroxetine causes great anxiogenic effects in adolescent than adult rats. Hippocampal proteomic changes indicate neurotrophic and neuroprotective effects of paroxetine in adults, with significant downregulation of apoptotic proteins and upregulation of neurotrophic and antioxidant proteins. However, adolescent rats fail to show these neurotrophic and neuroprotective effects of paroxetine, instead displaying upregulation of proapoptotic proteins. Regional dopamine and serotonin transporter changes were also of an opposite nature in adult and adolescent rats. Overall, our results show the vulnerability of adolescent rats to self-administration of toxic doses of recreational drugs and also the differential impact of these drugs, and prescription drugs such as paroxetine, on the developing adolescent brain.

S13-03
ABUSED DRUGS DIFFERENTIALLY ALTER AMPA RECEPTOR FUNCTION IN A PATHWAY-SPECIFIC MANNER IN MIDBRAIN DOPAMINE (DA) NEURONS
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Ventral tegmental area (VTA) DA neurons play an important role in processing reward-related information and are involved in drug addiction and mental illness in humans. Information is conveyed to the VTA in large part by glutamatergic afferents that arise in a multitude of cortical and subcortical brain nuclei. One projection to VTA DA neurons arises from the subcortical
pedunculopontine nucleus (PPN) and is thought to be involved in regulating arousal and drug reward and abuse. In rat parasagittal brain slices, electrical stimulation of PPN glutamatergic afferents targeted GluR2 (GluA2)-containing AMPA receptors (AMPAR) on VTA DA neurons, and this pathway did not exhibit long-term depression (LTD). Conversely, activation of glutamatergic afferents onto the same DA neurons via electrical stimulation in the VTA evoked EPSCs mediated by either GluR2-lacking, or GluR2-containing AMPARs. Furthermore, robust LTD was observed in intra-VTA activated GluR2-lacking synapses, whereas those intra-VTA evoked EPSCs that were mediated by GluR2-containing AMPA receptors did not express LTD. Thus, greater heterogeneity in AMPAR subunit composition was observed in the intra-VTA activated glutamate pathway, as compared to the PPN-activated pathway. The effects of abused drugs on each of these glutamatergic afferents were also assessed. Twenty-four hours after single cocaine injections to rats, GluR2-lacking AMPARs were increased at both PPN- and intra-VTA-activated projections, and this permitted LTD expression in both pathways. Conversely, a single injection with the psychoactive constituent of marijuana, delta-9-tetrahydrocannabinol (D9-THC), increased GluR2-lacking AMPA receptors and permitted LTD only in the PPN-activated pathway. Our findings demonstrate that cocaine has a more global effect upon AMPAR subunit composition in VTA DA neurons, whereas D9-THC more selectively increases GluR2-lacking AMPA receptors at subcortical PPN synapses. These data further imply that distinct abused drugs may exert influence over different glutamatergic afferents to alter AMPAR subunit composition in VTA DA neurons.

S13-04
IMPLICATION OF THE HABENULA MICROCIRCUIT IN MEDIATING DRUG-RELATED MEMORIES
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Reward is a major incentive for learning. Midbrain dopamine neurons activity and therefore dopamine release are enhanced by external cues predicting a reward and at the same time are a principal target of addictive substances. A clear understanding of the neural circuits implicated in drug-mediated memories remains elusive. We combined electrophysiology, 2-photon laser photolysis and retrograde tracing to probe drug-evoked synaptic adaptation in key regions of the reward circuit. We have evidences that cocaine drastically alters the synaptic properties of excitatory inputs onto these neurons and their ability to undergo long-term plasticity providing a cellular mechanisms that mediates drug-seeking and drug-context association.

S13-05
IN UTERO EXPOSURE TO COCAINE IMPAIRS POSTNATAL SYNAPTIC MATURATION OF GLUTAMATERGIC TRANSMISSION IN THE VTA
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Maternal exposure to cocaine may perturb fetal development and affect synaptic maturation in the offspring. However, the molecular mechanism underlying such changes remains elusive. We focus on the postnatal maturation of glutamatergic transmission onto mouse ventral tegmental area (VTA) dopamine neurons. We find that during the first postnatal week, transmission is dominated by calcium-permeable (CP)-AMPAR and GluN2B-containing NMDA receptors. Subsequently we identify mGluR1 receptors as the key player in the synaptic insertion of calcium-impermeable (CI)-AMPARs and GluN2A, a process that does not occur in mGluR1 KO mice. When pregnant mice are exposed to cocaine, this glutamate receptor switch is impaired in offspring by a direct effect of cocaine on the fetal dopamine transporter. Finally, positive modulation of mGluR1 in vivo is sufficient to rescue maturation. Taken together, we identify the molecular target through which cocaine in utero impairs postnatal synaptic maturation, reveal the expression mechanism of this impairment and propose a potential rescue strategy.
Axons do not regenerate after spinal cord injury because the axons are growth incompetent, and inhibitory factors in the CNS myelin and the scar prevent the axons from regrowing. Microtubule dynamics regulate key processes during scarring, including cell proliferation, migration and differentiation. Moderate microtubule stabilization using the cancer drug Taxol prevents axonal retraction and swelling of the axon tip after CNS injury, and stimulates axon growth of cultured neurons enabling them to overcome the growth inhibitory effect of CNS myelin. Moreover, we found that moderate microtubule stabilization decreased scar formation after spinal cord injury in rodents via various cellular mechanisms, including dampening of TGF-β signalling. It prevented the accumulation of chondroitin sulfate proteoglycans (CSPGs) and rendered the lesion site permissive for axon regeneration of growth competent sensory neurons. Additionally, microtubule stabilization promoted growth of CNS axons of the Raphe-spinal tract and led to functional improvement. Thus, microtubule stabilization reduces fibrotic scarring and enhances the capacity of axons to grow. Manipulation of microtubules may offer the basis for a multi-targeted therapy after spinal cord injury.

A diverse range of cellular processes including cell division, directed cell motility, neuritogenesis and growth cone pathfinding depend on the regulated interaction between dynamic microtubules and actin filaments. The molecular mechanisms mediating this interaction, the proteins involved and how they are regulated, are now being discovered. In growth cones, dynamic microtubules interact with the actin filaments within filopodia and this interaction depends on the direct binding of the +TIP protein EB3, located on the plus-end of microtubules, and the F-actin-binding protein drebrin, bound to the proximal ends of filopodial actin filaments. Disruption of this interaction impairs growth cone formation and the extension of neurites and its role in growth cone pathfinding is now being determined. Domain mapping analysis of drebrin has revealed the presence of two actin filament-binding domains whose properties explain the specific location of drebrin to parallel actin filament bundles. In the adult nervous system drebrin regulates F-actin dynamics in dendritic spines and loss of drebrin from spines is causal to the loss of spines that underlies cognitive impairment in diseases such as Alzheimer’s. Drebrin is a phosphoprotein and current work is focused on the regulation of drebrin through phosphorylation in these different cellular contexts.
Acquisition of neuronal polarity is a complex process involving several cellular and molecular changes, including vectorial cytoplasmic flux, differential molecular sorting, local protein degradation and cytoskeleton dynamics. Most of these processes are finely regulated by signaling molecules, such as kinases and phosphatases, phosphoinositides, small GTPases and second messengers. Amongst second messengers, cAMP is important for the outgrowth and elongation of the axon. cAMP-dependent signaling had been most of the times related with changes in the activity of the protein kinase A. However, there is an alternate cAMP-dependent mechanism which involves the participation of the exchange proteins directed activated by cAMP (EPAC). EPAC proteins are guanine exchanging factor (GEF) for the Ras family members, Rap1 and Rap2. In this talk we will analyze the role of EPAC1 and 2 proteins in the development of neuronal polarity. Both proteins are differentially expressed during the transition between stages 2 and 3 of cultured hippocampal cells. A pharmacological agonist for EPAC induces multiaxonal neurons. This phenotype is verified in the presence of a specific PKA inhibitor. Additionally, a gain-of-function experiment using a constitutively active form of EPAC also induces the formation of multiaxonal neurons. The cellular and molecular mechanisms underlying EPAC function and regulation will be discussed. (Supported by Fondecyt 1095089 and ICM P05-001-F)
D-Serine in the Brain: From Neurotransmission to Neurodegeneration

S15-01
LONG-TERM POTENTIATION RELIES ON D-SERINE RELEASED FROM A NEIGHBOURING ASTROCYTE
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A common form of synaptic memory, hippocampal long-term potentiation (LTP), depends on Ca2+ influx through postsynaptic NMDA receptors. Astroglia can regulate activation of these receptors by releasing the NMDA receptor co-agonist D-serine, in a Ca2+-dependent manner. However, Ca2+ signals in astrocytes have also been associated with release of other signalling molecules such as glutamate, ATP and TNF-alpha. In addition, neurons themselves represent an important source of D-serine. The importance of astroglia for LTP induction remains intensely debated. We suppressed endogenous Ca2+ signalling in individual CA1 astrocytes by clamping their internal Ca2+ concentration and found that this procedure blocks LTP induction at nearby CA3-CA1 excitatory synapses. This LTP blockade can be reversed by exogenous D-serine or glycine whereas depletion of D-serine or inhibition of exocytosis in an individual astrocyte blocks LTP at local synapses. The underlying mechanism involves the reduced occupancy of the NMDAR co-agonist site, which depends on Ca2+-dependent activity of astrocytes. Activity in neighbouring astrocytes can have distinct effects on nearby synaptic connections, but each astrocyte can extend its influence beyond its morphological boundaries. Activity-dependent local supply of D-serine by astrocytes could thus give rise to a Hebbian mechanism regulating NMDAR-dependent plasticity in thousands of nearby synapses.

S15-02
FUNCTIONAL COUPLING BETWEEN D-SERINE SYNTHESIS AND VESICULAR TRANSPORT IN ASTROCYTES
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The concept of tripartite synapse suggests that astrocytes make up a functional synapse with pre- and postsynaptic neuronal elements to modulate synaptic transmission through the regulated release of neuromodulators called gliotransmitters. Release of gliotransmitters such as glutamate or D-serine has been shown to depend on Ca2+-dependent exocytosis. However, the origin (cytosolic vs. vesicular) of the released gliotransmitter is still a matter of debate. The existence of Ca2+-regulated exocytosis in astrocytes has been questioned mostly because the nature of secretory organelles which are loaded with gliotransmitters is unknown. Here we show the existence of a population of vesicles that uptakes and stores glutamate and D-serine in astrocytes. Immunolabeled glial organelles expressing synaptobrevin 2 (Sb2) display morphological and biochemical features very similar to synaptic vesicles. We demonstrate that these organelles not only uptake glutamate (Km ~ 1 mM) but also display a glia-specific transport activity for D-serine (Km ~ 7 mM). Furthermore, we report that serine racemase (SR), the synthesizing enzyme for D-serine, is anchored to the membrane of glial organelles allowing a local and efficient concentration of the gliotransmitter to be transported. We conclude that vesicles in astrocytes do exist with the goal to store and release D-serine, glutamate and most likely other neuromodulators.

S15-03
REDUCED EXCITOTOXICITY IN SERINE RACEMASE KNOCKOUT MICE
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D-Serine is a coagonist at the ‘glycine-site’ of the N-methyl D-aspartate (NMDA)-type glutamate receptor. About 90% of endogenous brain D-serine is directly produced from L-serine by serine racemase (SR). We have recently reported the production of the SR knockout (KO) mice with pure C57BL/6 genetic background, and the predominant neuronal localization of SR in the mouse brain. We found that SR KO mice shows attenuated in tonic-clonic seizure, c-Fos expression in the cortex, and astrogliosis in the dentate gyrus of the hippocampus are attenuated in SR KO mice. These results suggest that D-serine may be involved in controlling the extent of NMDA receptor-mediated excitotoxic insults. The control of SR activity and D-serine level in SR KO mice, we found that serine racemase (SR), the synthesizing enzyme for D-serine, is anchored to the membrane of glial organelles allowing a local and efficient concentration of the gliotransmitter to be transported. We conclude that vesicles in astrocytes do exist with the goal to store and release D-serine, glutamate and most likely other neuromodulators.

S15-04
THE SERINE SHUTTLE: A NEURON-GLIA CROSSTALK THAT PLAYS A ROLE IN NEURODEGENERATION AND SYNAPTIC PLASTICITY
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D-Serine is a transmitter-like molecule that physiologically activates N-methyl D-aspartate (NMDARs) in the brain.
Although D-serine was initially thought to be exclusively made and released by astrocytes, recent data indicate that neurons are the main source for D-serine synthesis in most brain areas. Synthesis of D-serine is carried out by serine racemase (SR), an enzyme that is predominantly expressed in neurons and converts L- into D-serine. We found that D-serine is released by neurons through depolarization and hetero-exchange catalyzed by the neuronal D-serine transporter Asc-1 both in vitro and in vivo. Functional studies indicate that neuronal D-serine is also involved in synaptic plasticity and NMDAR neurotoxicity. The data can be conceptualized by the ‘serine shuttle’ model, whereby D-serine synthesized and released by neurons can be further taken up by astrocytes for storage and activity-dependent release. On the other hand, astrocytes express little SR and are likely to export L-serine required for D-serine synthesis by the predominantly neuronal SR. The serine shuttle constitutes a new type of neuron-glia crosstalk that plays a role in NMDAR transmission and may be a therapeutic target in neurodegenerative diseases in which NMDAR dysfunction plays pathological roles.
Molecular Mechanism of Exocytosis–Endocytosis Coupling in Neurosecretory Cells

**S16-01**
**REGULATING SYNAPTIC STRENGTH ACROSS THE CLEFT BY N-CADHERINS**
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N-cadherin is a Ca2+-dependent homophilic adhesion protein that plays an important developmental role in guiding and forming synaptic connections, although it remains expressed at mature excitatory synapses. We have investigated the transsynaptic activity of N-cadherin in regulating synaptic efficacy using FM dyes to monitor vesicle turnover in cultured hippocampal neurons. Interfering with N-cadherin expression in isolated postsynaptic neurons reduces basal release probability at synaptic inputs received by the neuron. Surprisingly, this transsynaptic impairment of neurotransmitter release is accompanied by a significant slowing of vesicle endocytosis. In contrast, in neurons postsynaptically impaired for N-cadherin activity, synapses remain capable of homeostatically upregulating release probability. Our findings reveal that regulation of presynaptic efficacy is molecularly dissociable into two components by the requirement for N-cadherin: one for controlling the level of basal presynaptic strength and the other for adjusting the gain.

**S16-02**
**MULTIPLE MODES OF EXOCYTOSIS AND ENDOCYTOSIS AT CENTRAL SYNAPSES**
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Synaptic vesicle exocytosis and its subsequent endocytosis are essential signaling process for the function of the nervous system. How exocytosis and endocytosis are mediated at nerve terminals are not well understood. Here, I will describe our recent findings at a large nerve terminal, the calyx of Held using a variety of techniques, including advanced electrophysiology, electron microscopy, genetics and computer simulation. We found that there are three forms of fusion and retrieval. First, the vesicle may fuse and fully collapse with the plasma membrane, called full collapse fusion, followed by slow endocytosis. Second, vesicles may fuse with the plasma membrane without full collapse, and the fusion pore closes rapidly after the opening, called 'kiss-and-run' fusion and retrieval. Kiss-and-run with a small fusion pore size may produce a small quantal size and may provide a rapid route of vesicle recycling. Third, vesicles may fuse with each other, called compound fusion, which forms a giant vesicle, the fusion of which with the plasma membrane produces giant quantal responses. Compound fusion is also mediated by calcium and synaptotagmin, and contributes to a widely observed form of synaptic plasticity, post-tetanic potentiation. In summary, we have demonstrated three forms of fusion, full collapse, kiss-and-run, and compound fusion. Regulation of these fusion modes may provide a mechanism to control synaptic strength.

**S16-03**
**BULK ENDOCYTOSIS: FROM CHROMAFFIN CELLS TO THE NEUROMUSCULAR JUNCTION**
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Sustained nerve stimulation depletes synaptic vesicles through fusion with the presynaptic membrane. How nerve terminals adjust membrane retrieval via bulk endocytosis to precisely refill active pools of synaptic vesicle is unknown.

We have used time-lapse imaging confocal microscopy to explore the coupling between exocytosis and bulk endocytosis at the frog neuromuscular junction and in neurosecretory cells, more amenable to genetic manipulations.

Time-lapse imaging of stimulated FM1-43-labeled motor nerve terminals revealed a two step bulging and collapsing of the presynaptic membrane just preceding the formation of large endosomes surrounded by a halo of recycling vesicles. The loss of plasmalemmal surface incurred during the collapse correlated with the membrane surface generated in large endosomes and associated recycling vesicles. Disrupting actin functions blocked the bulging phase, bulk endocytosis and the recovery of neurotransmitter release following synaptic depletion. Actin therefore act together to support the bulging phase - a preparatory step that may sense the amount of membrane to be retrieved by bulk endocytosis.

To further our understanding of the role of actin during bulk endocytosis in neurosecretory cells, we have used Lifeact-GFP transfected chromaffin cells to visualise actin and have imaged the cortical actin network. Nicotine-stimulation promoted the partial disappearance of the cortical actin network closely followed by the appearance of small actin rings surrounding large endocytic vesicles. The contractile nature of these rings is suggestive of a role of actin in constricting the neck of these large invaginations of the plasma membrane to set-up the fission process. Actin therefore appears to be key player in bulk endocytosis in both systems.

**S16-04**
**MEMBRANE LIPID ORGANIZATION REGULATES SELECTIVE RECYCLING OF SECRETORY GRANULES IN NEUROENDOCRINE CHROMAFFIN CELLS**
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In neurons and neuroendocrine cells, release of neuropeptides and hormones occurs through calcium-regulated exocytosis. To allow secretory vesicle recycling and maintain a constant cell surface area, exocytosis must be followed by compensatory membrane uptake. How these cells, specialized for hormone release, initiate and regulate compensatory endocytosis remains poorly understood. By following the internalization of antibodies
against dopamine-ß-hydroxylase in cultured chromaffin cells, we show here that after full fusion at the plasma membrane, the granule membrane remains as a physically separate entity and is selectively recaptured through a clathrin- and actin-dependent pathway. Moreover, physical properties of individual lipid play fundamental roles in membrane trafficking by acting as scaffolding system to maintain specific machinery at restricted site of the plasma membrane. We show here that the secretagogue-induced outward transport of Phosphatidylserine occurs specifically at the exocytotic site and most likely constitutes a signal necessary for the selective recapture of the secretory granule membrane.
Symposium 17
PUFA and its Derivatives - Brain-, Neuro-Protective Agents for Senescence

S17-01
LONG TERM SUPPLEMENTED ARACHIDONIC ACID PRESERVES HIPPOCAMPAL COGNITIVE ACTIVITY IN SENESCENT RODENT
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Senescent rodents, feeding with arachidonic acid ethyl ester (AA) for longer than one month, as old arachidonic acid supplemented animals (OA) were evaluated from the viewpoint of behavior, electrophysiology, opto-physiology, biophysics, biochemistry and cell-proliferation compared with those of young animals (YC) and/or age-matched control animals with control diet (OC). Spatial cognition ability was assessed by Morris water maze: OA had tendency to show better performance than OC. Long-Term-Potentiation (LTP) was the one of evaluation measure of electrophysiology: the degree of potentiation after 1 h in OA was comparable as that of YC. Mobility of functional protein on the pyramidal neuronal membrane was evaluated with FRAP. Comparison was made on three parameters obtained from the dynamic recovery curve of fluorescence: mobile fraction (Mf), diffusion constant (D), and time constant (τ). Each of these parameters was significantly different between YC and OC suggesting the membrane fluidity is lower in OC than in YC. D and τ were comparable in OA and YC, indicating that hippocampal neuronal membranes supplemented with AA were more fluid than those in OC, whereas the fraction of diffusible protein (Mf) in aged animals remained smaller than in YC. Long-term administration of AA to senescent rats might help to preserve membrane fluidity and maintain hippocampal plasticity. Calcium mobilization in hippocampal slices was estimated following membrane depolarization and selective activation of NMDA receptors using the calcium indicator dye. The maximum increase in [Ca\(^{2+}\)] and the calcium buffering ability were significantly greater in YC than in the aged rats. Selective activation of NMDA receptors induced regional differences in Ca\(^{2+}\) elevation. In the dentate gyrus (DG), Ca\(^{2+}\) elevation in OA was comparable to that in YC, and significantly higher than that in OC. The decay in the depolarization and NMDA-induced increase in [Ca\(^{2+}\)] was more prolonged in aged CA1 and DG. Immunohistochemistry revealed that all the cell proliferated in DG was neuron and the number of neurons was twice more in OA compared to OC.

S17-02
THE POLYUNSATURATED FATTY ACID, DOCOSAPENTAENOIC ACID EXERTS A NEUROPROTECTIVE EFFECT IN AGED AND AMYLOID-β-TREATED RATS
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Age-related deficits in neuronal function and synaptic plasticity have been reported by several groups and, in animals, these deficits are characterized by impairments in performance in learning and memory tasks and by an impairment in the ability of aged animals to sustain long-term potentiation (LTP) in the hippocampus. Some of the effects of age are replicated by treatment of animals with amyloid-β (Aβ). One of the factors which contributes to the age-related deficit in LTP is a decrease in the concentration of polyunsaturated fatty acids, particularly docosahexaenoic acid, in the hippocampus. In this study, the effect of docosapentaenoic acid (DPA), a precursor of eicosapentaenoic acid, was assessed for its ability to modulate age-related and Aβ-induced changes in the rat. The evidence indicates that the age-related increase in microglial activation was attenuated by chronic treatment of rats with DPA and that this was associated with a restoration of function in a spatial learning task and in LTP. DPA also attenuated the Aβ-induced microglial activation and blocked the accumulation of Aβ that was observed in these animals following infusion for 28 days. The possible mechanisms by which these changes occurred will be discussed.

S17-03
REDUCTION OF BETA-AMYLOID LEVELS BY NOVEL PKC EPSILON ACTIVATORS
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Protein kinase C (PKC) has been shown to be a central component of memory storage in molluscs, rodents, and humans. The PKC epsilon isoform may be particularly important for memory because it is relatively brain-specific and plays an important role in synapse maturation. Therefore, isoform-specific PKC activators may be useful as therapeutic agents for the treatment of Alzheimer’s disease. We have developed a series of epsilon-specific PKC activators, made by cyclopropanation of polyunsaturated fatty acids. These activators, AA-CP4, EPA-CP5, and DHA-CP6, activate PKC epsilon in a dose-dependent manner. Unlike PKC activators such as bryostatin and phorbol esters, which produce brief activation followed by prolonged downregulation, the new activators produced sustained activation of PKC and no indication of downregulation. One possible explanation for this difference is that the new activators bind to PKC’s phosphatidylserine binding site instead of the 1,2-diacylglycerol binding site. When applied to cells expressing human APPswe/PS1Delta, which produce large quantities of Abeta, DCPLA and DHA-CP6 reduced the intracellular and secreted levels of Abeta by 60–70%. They also protected against Abeta in primary neurons, and restored the levels of synaptic markers synaptophysin and PSD-95 in a PKC-dependent manner. In contrast to the marked activation of alpha-secretase produced by PKC activators in fibroblasts, the PKC activators produced only a moderate and transient activation of alpha-secretase in neuronal cells. However, they activated endothelin-converting enzyme (ECE) to 180% of control levels, suggesting that the Abeta-lowering ability of these PKC epsilon activators is caused by increasing the rate of Abeta degradation by ECE, and not by activating nonamyloidogenic
APP metabolism. These novel PKC activators have shown no evidence of toxicity or tumorigenicity and may be useful candidates for the treatment of Alzheimer’s disease and other neurological disorders.

S17-04
ARACHIDONIC ACID DIET PREVENTS MEMORY IMPAIRMENT AND BRAIN ABETA DEPOSITION IN TG2576 MICE
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The amyloid β-protein (Aβ) plays a causative role in the development of Alzheimer’s disease (AD). The amyloid precursor protein (APP), a substrate of Aβ, is cleaved by two secretases, namely β-secretase and γ-secretase, to generate Aβ. Because APP, β-secretase, and γ-secretase are all membrane proteins, it is possible to assume that alterations in brain lipid metabolism modulate APP and/or Aβ metabolism. However, the role of arachidonic acid (ARA) one of the polyunsaturated fatty acid in Aβ metabolism remains unknown, although docosahexaenoic acid (DHA) has been shown to be involved in Alzheimer-like pathology. We report here that 4 months of treatment of Tg2576 mice with an ARA or DHA containing (ARA+ or DHA+) diet prevented memory impairment at 13 months of age. APP processing to generate soluble APP and Aβ synthesis were enhanced, but Aβ1-42/Aβ1-40 ratio was decreased, and the level of Aβ oligomers remained unchanged in 14-month-old Tg2576 mice fed with the ARA+ or DHA+ diet. The ARA+ or DHA+ diet did not alter the expression levels of APP processing and Aβ-degrading enzymes. In cortical primary neuron cultures, ARA or DHA treatment also increased soluble APP and Aβ1-40 levels. We also found that 8 months of treatment with the ARA+ diet attenuated Aβ deposition at 17 months of age compared with the control diet. These findings suggest that not only the DHA+ diet but also the ARA+ diet could prevent cognitive dysfunction and alter APP processing and Aβ1-42/Aβ1-40 ratio in Tg2576 mice through APP processing.
COMBINATORIAL ACTIONS OF A PRONEURAL BHLH TRANSCRIPTION FACTOR WITH A ZINC FINGER REPRESSOR DURING CEREBRAL CORTICOBENESIS

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During brain development, proneural bHLH transcription factors such as Neurogenin2 act as master regulatory switches to drive cerebral cortical neuron production and maturation. Within the developing cerebral cortex, neuroprogenitor cells express the proneural bHLH factor Neurogenin2 (Neurog2) in order to initiate their neurodifferentiation and then undergo active cell migration to their final destination before terminally differentiating into glutamatergic cortical projection neurons. While Neurog2 has been shown to drive neurogenesis as well as the subtype specification of cortical projection neurons, very little is known as to whether it also plays an active role in the control of neuroprogenitor cell cycle exit, or if the Neurog2-signalling cascade is tempered by negative regulators of gene expression. To address these issues, we have identified the zinc finger transcriptional repressor Znf238 to be a downstream target of Neurog2: preliminary experiments suggest that Znf238 controls the timing of migration and differentiation of newborn cortical neurons. Our RNAi experiments suggest that knockdown of Znf238 in cortical progenitors results in defects in cell cycle exit as well as a perturbation of cell migration through dysregulation of the migration promoting gene known as Rnd2. Remarkably, luciferase reporter assays for the transcriptional activity of Znf238 revealed its antagonism for signalling through Neurog2-type E-box binding sites on the Rnd2 regulatory enhancer. Our data indicates that Neurog2 coordinates the temporal progression for neurodevelopment through stimulation of a Znf238-dependent negative feedback loop for the consolidation of cell cycle exit, as well as for controlling the migration of newborn cortical neurons.

THE RB/E2F PATHWAY MODULATES NEUROGENESIS THROUGH DIRECT REGULATION OF THE DLX TRANSCRIPTION FACTORS

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Recent evidence suggests that cell cycle proteins may have novel functions beyond the control of cell division. We investigated the role of Rb/E2F pathway in the regulation of neuronal differentiation and migration during late embryonic development. We show that loss of Rb leads to terminal differentiation and radial migration defects with loss of specific interneuron subtypes in the developing brain and the olfactory bulb. This phenotype is linked to a dramatic reduction in the levels of Dlx homeodomain genes that regulate ventral telencephalic development, most significantly Dlx2. To ask if Rb plays a direct role in controlling the induction of Dlx2, we examined the regulatory regions of the Dlx1/Dlx2 locus. Using chromatin immunoprecipitation experiments, we show that Rb modulates Dlx gene expression through interaction with the Dlx forebrain-specific enhancer, 112b, the Dlx2 proximal promoter and 3'UTR region in vivo. This interaction is mediated by E2F functional sites located in 112b and acting as repressor sites. We demonstrate that in the absence of Rb, E2F7, an Rb-independent repressor, is upregulated and ectopically repress Dlx2 transcription as shown using in utero electroporation in the brain. Our data provides the first evidence for an essential role of the Rb/E2F pathway in coordinating the transition from proliferation to differentiation and maintaining terminal differentiation during neurogenesis. This work was supported by a CHRF grant and a long-term faculty development grant to N. Ghanem from AUB.

SPINAL CHOLINERGIC INTERNEURONS AND MOTOR CONTROL

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The executive component of movement – the task of determining which muscles to activate, how intensely and for how long – depends on neural circuits located in the spinal cord. At the core of these circuits are local interneurons that regulate the pattern and frequency of motor neuron firing through a combination of direct excitation and inhibition, as well as neuromodulation. Using genetic methods we have identified the source of a spinal pre-motor modular circuit mediated by muscarinic cholinergic signaling. V0c interneurons, which express the paired-like homeodomain transcription factor Pitx2, represent the sole source of C bouton cholinergic input to motor neurons. Motor neurons are not the only targets of V0c interneurons since inhibitory Ia interneurons also receive V0c derived synapses of a distinct morphology. V0c interneurons exhibit high frequency firing patterns that are phase-locked with segmental motor neuron bursting during locomotor activity, suggesting that they have a role in the modulation of motor neuron firing. In support of this idea, genetic inactivation of V0c output impairs a locomotor task-dependent increase in motor neuron firing and muscle activation. These studies have therefore uncovered a spinal modulatory interneuronal system designed to fine-tune motor output according to the demands of particular locomotor tasks.
Development of visual system circuitry requires the formation of precise synaptic connections between neurons in the retina and brain. One such connection forms between retinal ganglion cells (RGCs) and neurons within subnuclei of the lateral geniculate nucleus (LGN) – the dorsal LGN (dLGN), ventral LGN (vLGN) and intergeniculate leaflet (IGL). Functionally distinct classes of RGCs project to these subnuclei. Image-forming RGCs project to dLGN, while non-image forming RGCs project to vLGN and IGL. To identify mechanisms regulating such class-specificity of LGN targeting we screened for differentially expressed targeting molecules in developing LGN subnuclei. Reelin, an extracellular matrix protein capable of directing the growth and targeting of CNS axons, was not only enriched in vLGN and IGL, but its developmentally regulated expression coincided with the arrival and arborization of RGC axons. To assess whether reelin was necessary for retinogeniculate targeting, RGC axons were anterogradely labeled with cholera toxin β (CTB) in mice lacking functional reelin (relnrl/rl). Not only were reduced patterns of vLGN and IGL innervation observed in relnrl/rl mutants, but RGC axons were misrouted into adjacent non-retinorecipient thalamic nuclei. Using genetic reporter mice, we further demonstrated that mistargeted axons belong to a class of non-image-forming, intrinsically-photosensitive RGCs (ipRGCs). In contrast to mistargeted ipRGC axons, axons from image-forming RGCs correctly target the dLGN in relnrl/rl mutants. Collectively, these data suggest reelin is essential for the targeting of LGN subnuclei by functionally distinct classes of RGCs.
Young Investigator Colloquium 2
Degeneration and Regeneration in the CNS

YIC02-01
ABNORMAL EXOSOMAL SECRETION OF ALS-LINKED PROTEINS AND ENDOCYTIC TRANSPORT IN MOTOR NEURON DISEASE

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ALS is characterised by accumulation of misfolded proteins in vulnerable motor neurons by an unclear mechanism. Key evidence suggests a contribution of secretory pathway defects in ALS pathogenesis and leading ALS-related proteins such as SOD1, TDP-43 and FUS are themselves secreted. However, the predominant pathway underlying secretion of ALS-related proteins remains undefined. To therefore address classical secretion, we engineered N-linked glycosylation sites into SOD1, TDP-43 and FUS which were not utilised in motor neuronal cells, arguing against ER-Golgi transit as confirmed by co-localisation microscopy. To test non-classical secretion, we purified exosomes from cell conditioned medium and rodent CSF, validated by size, morphology, density and protease resistance, demonstrating that SOD1, TDP-43 and FUS were principally secreted by exosomes. ALS-linked mutant forms of these proteins were depleted in exosomes, preceding inclusion formation, ER stress, and cell death activation. Exosome deficits correlated with induction of autophagic p62, LC3 and LAMP2, impaired endosomal Rab-mediated transport and defective protein monoubiquitination, consistent with abnormal endocytic transport and lysosomal accumulation of mutant ALS-linked proteins. Spinal cord induction of endocytic Rabs was shown in presymptomatic transgenic mutant SOD1 mice, implying early contributions to pathology. We also confirmed endosomal Rab induction in spinal cords of sporadic ALS patients compared to non-neurological controls, suggesting that defects in endosome transport are common to all etiologies of ALS. Based on these multiple lines of evidence, we propose endosomal transport defects leading to impaired exosomal secretion and increased lysosomal protein burden may be an early determinant of motor neuron loss and common denominator of key pathological ALS-linked proteins. Abnormal endocytic transport is likely to point to more fundamental mechanisms of vesicle trafficking defects implicated in ALS and innovative potential therapeutic approaches.

YIC02-02
TAU OLIGOMERS-MEDIATED NEURODEGENERATION

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Pathological aggregation of the microtubule-associated protein tau and accumulation of neurofibrillary tangles (NFT) or other inclusions containing tau are defining histopathological features of Alzheimer’s disease (AD) and many other neurodegenerative diseases. The correlation between neurofibrillary tangles (NFT) and disease progression has been studied extensively with conflicting results, and the mechanisms linking the pathological aggregation of tau with neurodegeneration are poorly understood. An emerging view is that NFT themselves are not the true toxic entity in tauopathies; rather, aggregates of a size intermediate between monomers and NFT – so-called tau oligomers – are pathogenic. Investigating such oligomers requires new methods and tools. Recently, innovative work demonstrating a link between tau oligomers and AD-related phenotypes in animal models has been reported.

We developed methods to prepare homogenous populations of tau oligomers, this tau intermediate aggregate (tau oligomers) caused memory impairment and induced neurodegeneration in mice, after single ICV injection. Moreover, we developed a novel antibody specifically recognizing tau oligomers and used it to analyze tau oligomers in human brain samples and animal models for AD and tauopathies.

These studies provided the first direct evidence linking tau oligomers to neurodegeneration, and valuable information about tau oligomers, which ultimately may be useful for the design and selection of therapeutic agents and strategies that interfere with tau oligomer formation and thereby prevent their deposition and/or promote their clearance in AD and related disorders.

YIC02-03
REGULATION OF TAU PATHOLOGY BY THE MICROGLIAL FRACHTALKINE RECEPTOR

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Aggregates of the hyperphosphorylated microtubule associated protein tau (MAPT) are an invariant neuropathological feature of tauopathies. While recent studies have observed glial activation as a common feature of these tauopathies, the functional role microglia play in disease onset and progression remains unclear. Here we show that microglial neuroinflammation promotes MAPT phosphorylation and aggregation. First, lipopolysaccharide-induced microglial activation promotes hyperphosphorylation of endogenous mouse MAPT in non-transgenic mice that is further enhanced in mice lacking the microglial-specific fractalkine receptor (CX3CR1) and is dependent upon functional toll-like receptor 4 and interleukin 1 (IL1) receptors. Second, humanized MAPT transgenic mice (hTau) lacking CX3CR1 exhibited enhanced MAPT phosphorylation and aggregation as well as behavioral impairments that correlated with increased levels of active p38 MAPK. Third, in vitro experiments demonstrate that microglial activation elevates the level of active p38 MAPK and enhances MAPT hyperphosphorylation within neurons that can be blocked by administration of an interleukin 1 receptor antagonist and a specific
p38 MAPK inhibitor. Taken together, our results suggest that CX3CR1 and IL1/p38 MAPK may serve as novel therapeutic targets for human tauopathies.

YIC02-04
WNTS IMPROVES CELL REPLACEMENT THERAPY IN PARKINSON’S DISEASE
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Proof of principle for cell replacement therapy (CRT) in Parkinson’s disease (PD) has been achieved using dopamine enriched human fetal ventral midbrain (VM) tissue. Unfortunately the functional benefits of these transplants have been highly variable due to poor cell survival and integration into the host brain. Improving donor tissue and promoting axonal connectivity will enhance CRT. We have revealed important roles for Wnt proteins in DA development and shown that Wnts are capable of improving transplants in rodent models of PD.

We developed a method to selectively expand fetal tissue, increasing the yield of dopamine neurons by 10-fold. This approach was based on proliferating and differentiating VM neural progenitors, or embryonic stem cells, in the presence of key signals necessary for VM DA neuron development, including Wnt5a. These cells exhibit the transcriptional, biochemical and electrophysiological properties of VM DA cells. Furthermore, upon transplantation these cells significantly enhanced cellular and functional recovery in PD mice.

Improving graft integration into the host brain will depend on understanding and replicating the developmental events of dopamine axon guidance. We have shown select Wnts have a temporal-spatial that overlaps with the development of the dopamine pathways, suggestive of a role in DA axon growth and guidance. Our findings reveal that Wnt5a promotes neurite growth and regulates directional growth of DA axons, findings that have been verified in Wnt5a(-/-) mice. Furthermore, we show that grafts exposed to Wnt5a signaling have enhanced integration in the host brain. In summary, we have shown the ability of Wnt-5a to improve the quantity and quality of donor tissue for transplantation, and promote graft integration, thereby resulting in improved functional recovery in PD rodents.
Young Investigator Colloquium 3
Role of Neuronal Transmembrane Proteins in Neuromodulatory Mechanisms and Cell Function Dynamics

YIC03-01
TOCOTRIENOLS SUPPRESS NEUROINFLAMMATORY SIGNALING CASCADE IN ATTENUATING MPTP-INDUCED NEUROTOXICITY IN MICE
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Neuroinflammation, oxidative stress and mitochondrial dysfunction play a crucial role in the pathophysiology of Parkinson’s disease (PD). In experimental conditions, MPTP induces selective neuronal toxicity by generating reactive oxygen species, activation of proinflammatory and apoptotic pathways. The present study investigated the modulatory effect of tocotrienol (an isomer of Vitamin E) against MPTP-induced behavioral, biochemical and cellular alterations in mice. MPTP 40 mg/kg (four injections of 10 mg/kg, i.p. at an interval of 1 h) challenge significantly induced Parkinson-like symptoms (impaired locomotor activity and catatonia like behavior), oxidative damage (elevated levels of lipid peroxidation and nitrite, decreased levels of non-protein thiols) and mitochondrial enzyme complex dysfunction (decreased complex-I activity and cell viability), and increased levels of proinflammatory markers (caspase-3, NF-kB/p65, PGE2 and PGF2α levels) as compared to vehicle treated animals. Tocotrienol (25, 50 and 100 mg/kg, p.o.) pretreatment significantly attenuated the behavioral deficits, oxidative and cellular damage as well as molecular alterations in mice treated with MPTP. Present study suggests a strong correlation between oxidative stress and up regulation of neuroinflammatory cascade in MPTP-induced PD like symptoms in mice. Study further demonstrates the effectiveness of tocotrienols in the management of PD.

YIC03-02
MONITORING OF CL\(^{-}\) DISTRIBUTION IN NEURONS USING GENETIC PROBE CL-SENSOR
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Chloride (Cl\(^{-}\)) is the most abundant physiological anion. It participates in a variety of physiological functions. Possibility of monitoring chloride dynamics without perturbation of cell functioning is of a big importance and requires sensitive Cl probes allowing the quantitative estimation of intracellular Cl concentration ([Cl\(^{-}\)]) in diverse cell types. Among different tools proposed for the monitoring of (Cl\(^{-}\)), the genetically encoded Cl-sensitive indicators are most promising. Recently, a ratiometric CFP-YFP based construct, termed ‘Cl-Sensor’, with a relatively high sensitivity to chloride has been designed (Markova et al., 2008). In present study, we have developed conditions for the efficient expression of Cl-Sensor in neurons that allows its utilization for estimating the [Cl\(^{-}\)] distribution in small neuronal compartments such as dendritic spines. We also propose a new approach for the calibration of intracellularly expressed probe. The coding sequence of Cl-Sensor was inserted into a mammalian expression vector - GW1 (British Biotechnology) and thereafter successfully expressed in the cultured hippocampal and spinal neurons, CHO cells and in vivo electroporated rat retina cells. To calibrate the dependence of Cl-Sensor fluorescence on [Cl\(^{-}\)], a natural triterpenoid saponin, beta-escin, has been used. In CHO cells expressing Cl-sensor with GW1 vector, EC50 for Cl was about 30 mM. Cl-Sensor expressed in spinal cord neurons revealed an estimated EC50 for Cl of 48 mM. Using Cl-Sensor we mapped non-invasively the distribution of [Cl\(^{-}\)] in different compartments of cultured spinal and hippocampal neurons (7-8 DIV). For both neuron types, the highest [Cl\(^{-}\)] values were observed in the soma and in the dendritic branches located in a close proximity to the soma. Towards distal dendrites, a tendency of lowering of [Cl\(^{-}\)] was observed. These results demonstrate that Cl-Sensor enables monitoring non-invasively the [Cl\(^{-}\)] distribution in different types of neurons with variable morphology. This probe can be easily detected in miniature parts of neuronal branches that proves it as an effective tool for the quantitative estimation of [Cl\(^{-}\)] in various cellular compartments.

YIC03-03
THE CONTROL OF PROTEIN SYNTHESIS IN PRION AND ALZHEIMER DISEASES
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In neurons, regulated protein synthesis occurs during development, differentiation and in response to neuronal activity or neurotrophic factors. This regulated translation allows for the formation of a specific set of proteins in subcellular compartments such as the growth cones or the synapses, which can vary with time and cell necessity. The cellular prion, PrPC, is a membrane-anchored protein known for its involvement with transmissible spongiform encephalopathies, or prion diseases. In physiological conditions, PrPC mediates important neurotrophic functions through the binding of several partners. In this section, we will show that protein synthesis in neurons is enhanced by PrPC.
interaction with one of its ligands, Stress-inducible protein 1 (STI1). We will also show that neuroprotection and neuritogenesis mediated by PrPC-STI1 engagement are dependent upon this increased protein synthesis through the PI3K-mTOR signalling. Strikingly, the translational stimulation mediated by PrPC-STI1 binding is corrupted in prion infected neurons, correlating with increased levels of eukaryotic translation initiation factor 2α (eIF2α) phosphorylation.

Recently, PrPC has also been implicated in Alzheimer’s disease, as a receptor for amyloid-beta (Aβ) oligomers (ADDls). ADDls are known to decrease synaptic efficiency, impairing the formation of LTP and reducing the levels of synaptic markers. Impressively, STI1 is able to increase these synaptic markers and prevent their reduction by ADDls. All these effects are short-term and depend on new protein synthesis and on the activity of mTOR.

These data indicate that modulation of protein synthesis is critical for PrPC-STI1 neurotrophic functions, and point to the impairment of this process during protein conformational diseases, as a possible contributor to neurodegeneration.

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YIC03-04

UNIQUE GENE REGULATION OF CLUSTERED PROTOCADHERINS TO CONFER NEURONAL DIVERSITY

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The brain contains a huge number of neurons that have diverse characteristics participating in discrimination between individual neurons. It has been speculated that clustered protocadherins (Pcdhs), which encode cadherin-related transmembrane proteins, could provide this kinds of neuronal diversity. The murine clustered Pcdhs are further classified into three subfamilies: Pcdh-α (14 genes), Pcdh-β (22 genes), and Pcdh-γ (22 genes). Their loss of function in mice revealed that the clustered Pcdhs play important roles in neuronal survival, axonal projection, synaptic connectivity, and several brain functions including learning and memory. For gene expression, the clustered Pcdh genes are regulated monoallelically, resulting in the combinatorial expression of distinct clustered Pcdhs at the single-cell level. These results suggest that the unique gene regulation of Pcdhs contributes to the neuronal diversity. In order to determine the molecular basis of generating the neuronal diversity, we investigated the mechanisms of transcriptional regulation in Pcdh-α cluster. At first, bisulfite sequencing analysis and luciferase reporter assay demonstrated that high DNA methylation in Pcdh-α promoter regions is sufficient to repress transcription. Next, targeted deletion of in vitro-identified cis-regulatory element revealed its enhancer function in vivo. Finally, the links between genomic organization, DNA methylation and the enhancer element were investigated. To address this question, we genetically engineered the genomic organization of Pcdh-α in mouse, in which a 218 kb region containing first exons of 13 Pcdh-α genes with their promoters was duplicated in the original locus. The mice homozygous for mutant allele were viable and fertile with no apparent gross phenotype. The expression analysis at the whole cerebellum and at the single cell levels showed differential and reduced expressions of duplicated Pcdh-α genes, consistent with an enhancer-sharing model. For DNA methylation states, the promoters of 5′-located Pcdh-α12 and αc2 were hypermethylated than the promoters of 3′-located counterparts in mutant allele, suggesting that the promoter DNA methylation in the Pcdh-α cluster is regulated genomic position-dependent manner. In conclusion, the unusual genomic organization of the Pcdh-α cluster governs its unique expression leading to a neuronal diversity.
YIC04-01
METAL HOMEOSTASIS WITHIN THE BRAIN; HOW TO STOP AN IRON OVERLOAD
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Metals are essential cofactors for a vast number of biological processes within the cell. Although essential for life, failure of metal regulation can have lethal effects on a cell by promoting side reactions that damage macromolecules. Within the brain regulation of metals is critical as aberrant metal accumulation has been linked to the neuropathology of Parkinson’s and Alzheimer’s diseases. The divalent metal transporter 1 (DMT1) plays a central role in the regulation of iron and other metals within neurons, hence failure of DMT1 regulation is linked to human brain pathology. Recently we have discovered that DMT1 is regulated by Ndfip1, an adaptor protein that recruits E3 ligases to ubiquitinate target proteins (Howitt et al 2009). Using human primary neurons we show that Ndfip1 is upregulated and binds to DMT1 in response to metal exposure. This interaction results in the ubiquitylation and degradation of DMT1, and prevents the entry of metals into the cell. Induction of Ndfip1 expression protects neurons from metal toxicity and removal of Ndfip1 by shRNAi results in hypersensitivity to metals. We identify Ned4-2 as the E3 ligase recruited by Ndfip1 for the ubiquitylation of DMT1 within neurons. Comparison of brains from Ndfip1-/- with Ndfip1+/+ mice exposed to iron reveals that Ndfip1-/- brains accumulate iron within neurons. Together, this evidence suggests a critical role for Ndfip1 in regulating metal transport in human neurons. Significantly, analysis of post mortem brains from Parkinson’s disease (PD) patients reveals that there is an accumulation of iron within the substantia nigra, the primary region for the ubiquitylation and degradation of DMT1, and prevents the entry of metals into the cell. Induction of Ndfip1 expression protects neurons from metal toxicity and removal of Ndfip1 by shRNAi results in hypersensitivity to metals. We identify Ned4-2 as the E3 ligase recruited by Ndfip1 for the ubiquitylation of DMT1 within neurons. Comparison of brains from Ndfip1-/- with Ndfip1+/+ mice exposed to iron reveals that Ndfip1-/- brains accumulate iron within neurons. Together, this evidence suggests a critical role for Ndfip1 in regulating metal transport in human neurons. Significantly, analysis of post mortem brains from Parkinson’s disease (PD) patients reveals that there is an accumulation of iron within the substantia nigra, the primary region of neuronal degeneration in PD. Within this region we have found that Ndfip1 is upregulated in PD patients when compared to age matched control brains, suggesting a protective role for the protein in metal toxicity within the human brain.

Reference:
1. Howitt et al. (2009). PNAS

YIC04-02
PRE-CONDITIONING TRIGGERED BY CARBON MONOXIDE: NEW STRATEGIES TO PREVENT BRAIN DAMAGE DUE TO HYPOXIA-ISCHEMIA AND REPERFUSION
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Low doses of the endogenously produced molecule carbon monoxide (CO) has beneficial effects on several tissues, acting, as an anti-inflammatory, anti-proliferative or anti-apoptotic. Recently, it has been demonstrated that reactive oxygen species (ROS) are important signalling molecules for the cytoprotective role of CO, suggesting a pre-conditioning (PC) mode of action. Hypoxia-ischemia and reperfusion (HIR) is the main cause of brain damage leading to mortality and morbidity. In adults HIR is mainly due to stroke whether, in newborns is caused by perinatal complications. Cerebral tolerance induced by PC reveals to be an efficient strategy to protect brain against HIR. Cerebral HIR in newborns can be partially predicted by detection of suffering signals during intrauterine infant’s life or during the birth. Additionally, preterm newborns represent a high risk population for brain injury.

In neurons, CO exposure provides PC and increases resistance against apoptosis induced by excitotoxicity. PC was mediated by ROS generation, NO production and guanylyl cyclase activation. In astrocytes, CO anti-apoptotic role was clearly attributed to inhibition of mitochondrial membrane permeabilisation. In isolated non-synaptic mitochondria CO inhibits loss of potential, inner membrane permeabilisation, swelling and cytochrome c release. Yet, CO presents ROS as signalling molecules and modulates adenine nucleotide translocase function by glutathionylation. CO also inhibits astrocytic apoptosis by reinforcing oxidative phosphorylation. This gaseous transmitter enhances ATP production, cytochrome c oxidase (COX) activity, mitochondrial biosynthesis and decreases glycolysis. Furthermore, physical interaction between COX and Bcl-2 is higher in the presence of CO.

Finally, in the in vivo perinatal model of cerebral HIR (Vannucci model), rat pups were exposed to CO and followed by ischemia insult. CO-treated pups present low levels of apoptosis in the hippocampus. Indeed, CO prevents cytochrome c release from mitochondria and caspase-3 activation and increases Bcl-2 expression, which were assessed in hippocampal extracts.

YIC04-03
UNRAVELING NEW PATHOBIOLGICAL MECHANISM OF DEVELOPMENTAL BRAIN DAMAGE BY ENVIRONMENTAL TOXICANTS
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Exposure to heavy metals have a detrimental effect on developing brain. One major phenomenon by which this adverse impact is brought about is by influencing astrocytes. GFAP levels are altered by metals causing astrocyte damage.

GFAP exists in the form of GFAPα, GFAPβ, GFAPγ, GFAPγ, GFAPα, GFAP partial and GFAPc. We hypothesized that metal toxicity altered the physiological stoichiometry of GFAP isoforms.

Because the ontogenic profiling of the different isoforms has never been done, we performed that first. Further, the pregnant, lactating and post-natal rats were exposed to the metals, and mRNA levels of GFAP isoforms were determined at critical time points of neuro- and gliogenesis.

Ontogenic profiling revealed the decrease in level of GFAP α (27% ± 2), GFAP β (18% ± 2), GFAP γ (33% ± 2) and GFAP delta (30% ± 2) in brain of control as well as metal mixture (MM)-treated rats. Upon MM-treatment, there was (27% ± 2) fall in GFAPα, and increase in GFAP β (22% ± 2). The level of GFAP partial decreased (50% ± 3) from pd0 to pd 60 in control rats while in case of treated
an increase (42% ± 3) was observed. The level of GFAP 7e was constant from pd0 to pd 60 in control, but reduced significantly (44% ± 2) upon MM-treatment. The level of gfap e was constant from pd0 to pd 60 in control while in treated rats an increase was observed.

In control, there was no change in GFAP α /total GFAP from pd0 to pd60 while in case of treated, a significant (25% ± 2) fall in the ratio was observed. There was no change in the GFAP β /total GFAP ratio for both sets. GFAP α /total GFAP decreased (47% ± 3) in control, but remained constant in treated. GFAP partial /total GFAP (65% ± 3) reduced while GFAP e /total GFAP increased in treated, but remained constant in control. GFAP 7e /total GFAP increased (33% ± 2) in control, but remained constant in treated rats.

Conclusion: GFAPα appears to be the major splice variant among total GFAP in control that undergoes consistent suppression in developing brain.

YIC04-04
THE ROLE OF THE EXTRACELLULAR MATRIX OF THE BRAIN IN REGULATION OF NEURONAL ACTIVITY
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During late neuronal development after the establishment of neuronal networks, a condensed, brain specific form of the extracellular matrix, the so-called perineuronal net (PNN) is formed. It is made of a meshwork of glycoproteins and proteoglycans of both neuronal and glial origin. The PNN is thought to function in synapse stabilization and it has been found that mutants in several components of the ECM exhibit impairments in synaptic long-term potentiation. We have recently found that the mature form of the brains ECM hinders lateral diffusion of AMPA receptors and thereby modulates short-term plasticity. In this study we focused on excitatory neurons, which have a well established but less pronounced form of the ECM than found on inhibitory neurons. Therefore we now analyse lateral diffusion of AMPA receptors selectively on GABA-ergic neurons and compare diffusion rates to those found on excitatory neurons. Further we test for the impact of the PNN on lateral diffusion by enzymatic removal of this specialized form of the ECM.
Ischemic injuries and neurodegenerative disorders lead to death or impairment of neurons in the central nervous system. Application of stem cell based therapies, namely stimulation of endogenous neurogenesis or cell transplantation, are promising strategies and currently under investigation. The human embryonic teratocarcinoma stem cell line, NT2, is reported as model of neuronal differentiation. NT2 cells are pluripotent, characterized by high proliferation yields in vitro and neurally committed progenitor cells. Carbon monoxide (CO) is an endogenous product of heme degradation by heme-oxygenase. Administration of CO at low concentrations produces several beneficial effects in distinct tissues, such as anti-inflammation, anti-proliferation, anti-apoptotic or neuroprotection. Although there is no data reporting CO as a factor involved in stem cell differentiation, several evidences support this hypothesis. This gasotransmitter is antiproliferative in smooth muscle cells, induces mitochondrial biogenesis in cardiomyocytes and generates ROS as signaling molecules. These cellular processes are broadly described to be involved in cell differentiation. In order to assess the effect of CO in neuronal differentiation, NT2 progenitor cells were treated in the presence or absence of CO. In the presence of CO, post-mitotic neurons were obtained by treatment of NT2 with retinoic acid (RA) supplemented with CO. While, as control, neurons were generated by treatment with RA only. After differentiation process and isolation of post-mitotic neurons, neuronal quantification was performed by microscopic counting and neuronal characterization by immunofluorescent microscopy, western blot and RT-PCR assays. CO does increase proliferation yields of post-mitotic neurons, presenting similar features as standard RA-treated NT2 neurons. Thus, one can speculate that CO improves the final yield of neuronal differentiation by increasing mitochondrial biogenesis, modulating proliferation and/or ROS signaling. In conclusion, CO appears as a promising therapeutic molecule for stimulating endogenous neurogenesis or for improving neuronal production for potential cell transplantation.

**TU01-02**

**CYTOSKELETON-DEPENDENT CANNABINOID RECEPTOR 1 SIGNALING IN NEURAL CELLS**

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GPCRs may mediate their effects on neuronal growth and differentiation through activation of ERK1/2. In analyzing the proximal signaling of the GPCR cannabinoid receptor 1 (CB1R) in primary cortical neurons, we have shown that Methanandamide (R(+)-MA) induces a biphasic ERK1/2 activation at 5 and 15 min, mediated by sequential activation of Gq/11/PLC/PKCζ/Src-Fyn, and Gi/o/Src/Fyn/FGFR, respectively. Recruitment of molecules increases with time of exposure to R(+)-MA, suggesting that it also serves receptor trafficking. Concurrently to these intermolecular signaling interactions, F-actin cytoskeleton associated proteins MARCKS and p120catenin were drastically modified by phosphorylation of PKCζ and Src, respectively. We therefore investigated the role of actin filaments and microtubules in the CB1R-dependent signaling, using the specific disruptors cytochalasin D or nocodazole in primary neurons. We found that both inhibited the second activation peak of ERK at 15 min, but not the first, indicating that cytoskeleton integrity is a pre-requisite for CB1R recycling to membrane lipid rafts. These receptor-proximal signaling events led to induction of neuritic outgrowth in the long term. Specifically, by 48 h, the average length of the major neuritic process in R(+)-MA-treated neurons was increased by a statistical significant 37.5% over the vehicle-treated. The significance of actin cytoskeleton as an integrator of CB1R signaling was further confirmed with studies in glioma cell lines where the R(+)-MA-induced phenotypic changes were reversed by cytochalasin D. Taken together these results present evidence that both the cortical and microtubule cytoskeleton play important roles in the regulation of CB1R signaling in developing neurons (support: PENED 03EAD778/GGET/EU to DM).

**TU01-03**

**ALTERATIONS IN RAT BRAIN NEUROGENESIS FOLLOWING EXPOSURE TO FRACTIONATED DOSES OF IONIZING RADIATION**

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Ionizing radiation commonly used in the radiotherapy of brain tumours can cause adverse side effects to surrounding normal brain tissue. The adult mammalian subventricular zone (SVZ) of the brain lateral ventricles (LV) and their subsequent lateral ventricular extension, the rostral migratory stream (RMS), is one of the few areas, which retains the ability to generate new neurons and glial cells throughout life. The aim of the present study was to investigate the occurrence of radiation-induced alterations of forebrain’s neurogenesis. Adult male Wistar rats were investigated 30, 60 or 90 days after whole-body irradiation with fractionated doses of gamma rays (the total dose of 4 Gy). For the study of alterations of the numbers of proliferating cells and precise identification of cell specific phenotype through the migratory pathway, the immunohistochemistry
TU01-04
NEUROPEPTIDE Y PROMOTES NEUROGENESIS AND PROTECTION AGAINST METHAMPHETAMINE-INDUCED TOXICITY IN DENTATE GYRUS CELL CULTURES
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Methamphetamine (METH) is a toxic drug of abuse that can damage the hippocampus leading to cognitive deficits. Moreover, hippocampal neurogenesis plays an important role in cognition and recent studies suggest that METH alters neurogenesis. Therefore, our aim was to investigate the effect of METH on dentate gyrus (DG) neurogenesis, focusing on cell survival, proliferation and differentiation, and to elucidate the protective role of NPY under METH-induced toxicity. DG cells were obtained from mice, developed as neurospheres. Cultures were exposed to METH (1–1000 nM) for 24 h, 48 h and 7 days to evaluate cell death, proliferation and neuronal differentiation, respectively. Then, cells were pre-incubated with 1 μM NPY and co-exposed with 10 nM METH for cell death studies. Cultures were pre-incubated with 1 μM NPY and co-exposed with 1 μM METH to assess neuronal differentiation. So, METH increases cell death and did not affect cell proliferation, but decreases the number of NeuN-stained cells at 1μM METH. Also, NPY increases the number of BrdU-labeled cells and of mature neurons via Y1 receptor. Moreover, NPY prevents cell death induced by 10 nM METH through the activation of Y1 and Y2 receptor. Regarding neuronal differentiation, NPY completely blocked the effect of METH via Y1 receptor activation. In conclusion, METH is toxic to DG cells affecting both cell viability and neuronal differentiation. Besides, NPY is pro-proliferative, pro-neurogenic and protective to DG cells under METH-induced toxicity.

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TU01-05
COMPOUND SM2 PROMOTES PROLIFERATION OF BRDU-POSITIVE CELLS IN THE SUBGRANULAR ZONE OF HIPOCAMPAL DENTATE GYRUS OF ADULT MICE
Bastos, G.N.1, Nascimento, M.V.3, Magalhães, R.C.1, Santos, I.V.1, Silva, E.O.2, Tavares, D.V.3, Carvalho, L.4, Reis, R.A.4, Crespo-Lopez, M.E.5, Da Silva, M.N.3 and Do Nascimento, J.L.1,2
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Newborn neurons emerge from neural stem cells (NSCs) from niches in the mammalian adult brain. These cells are incorporated into functional circuits and may be important to acquisition and retention of memory. Therefore, the search for new compounds that enhance proliferation and differentiation of neural stem cells in the hippocampus represent a significant scientific challenge with great promise. Here we have used SM2 (phytosterol) on the neurogenesis in the subgranular zone of hippocampal dentate gyrus of adult mice using 5'-bromo-2'-deoxyuridine (BrDU)-pulse chase method. Increased doses (0.1; 1; 5 mg/Kg) of SM2 were given to adult male BALB/c mice; or 0.9% NaCl (control). Mice were sacrificed at 24 h or 7 days after the BrdU administration, and hippocampal slices were processed for immunohistochemistry. We found that SM2 did not modify the mice behavior at any dose used, but increased the number of BrdU-positive cells in the subgranular zone of hippocampal dentate gyrus 24 h or 7 days after injection. Using an in vitro methods, SM2 treatment nestin was highly expressed in the neural progenitors when compared with control. SM2 not showed BrdU-positive cells out subgranule cell layer (ectopic neurogenesis). These results suggest that SM2 increase proliferation of nestin-positive cells and also BrdU-positive cells in differentiation process in the sub granular zone of hippocampus of adult mice. In conclusion, we have evidence that SM2 compound induce proliferation of newborn cells in hippocampal neurogenic niche

TU01-06
STI1 IS ESSENTIAL FOR EMBRYONIC DEVELOPMENT AND PLAYS AN IMPORTANT ROLE IN NEURODEGENERATIVE DISEASES
Beraldo, F.H.1, Santos, T.G.2, Soares, I.1, De Jaeger, X.1, Calder, M.3, Guimaraes, A.L.1, Hajj, G.N.2, Fan, J.1, Watson, A.1, Prado, V.1, Martins, V.2 and Prado, M.1
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Stress Inducible Protein 1 (STI1) is co-chaperone secreted by astrocytes and promotes intracellular Ca2+ increase leading to neuronal differentiation and neuroprotection in a PrPC-dependent way. To understand the roles that STI1 play in vivo we generated genetically modified mice with a disruption in the STI1 gene. Heterozygous mutant mice have 50% decrease in STI1 mRNA and protein expression and were born apparently normal. However, homozygous mutant STI1 mice were not recovered alive and during development only few embryos reach close to E7.5 days. This is remarkable because STI1 is not required for survival in yeast or C. elegans. STI1 has previously been shown to participate in cognitive functions via its interaction with PrPC. However, in heterozygous,
reduction of ST11 did not affect muscle strength or memory in the
heart force or in the novel object recognition test. Interestingly, ST11
heterozygous mutant mice were hyperactive but not due to
increased anxiety as mutants performed as well as wild-type mice
in the elevated plus maze. To investigate if ST11 could play a role in
neurodegenerative diseases, we used the AD transgenic mouse
model (APPSwe/Ps1ΔE9), which have increased Abeta levels,
develop plaques and have cognitive dysfunction at 6 months of age.
ST11 protein levels were reduced by 50% in 6-month-old APPSwe/
Ps1ΔE9 mice, but mRNA levels were identical to control mice. In
contrast, in 9-month-old APPSwe/Ps1ΔE9 mice ST11 mRNA levels
are increased fourfold and protein levels are the same found in
control mice. These results indicate that ST11 has a versatile role in
signalling mechanisms with functions in early embryogenesis and
regulation of ST11 mRNA levels appears to compensate for the
decrease in the protein levels found in the APPSwe/Ps1ΔE9 mice.

TU01-07
ROLE OF E3 UBIQUITINE LIGASE APC/C-CDH1 IN NEURONAL SURVIVAL IN VIVO
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The E3 ubiquitine ligase APC/C (Anaphase Promoting Complex/
Ciclosome) is a multiprotein complex that catalyzes, apart from
other substrates of the cell cycle, cyclin B1 polubiquitination for its
subsequent proteasomal degradation. This allows a fast degradation
of the complex Cdh1/cyclinB1 at the end of mitosis, and keeps this
complex inactive during G1 phase of the cell cycle. Recently, we
have demonstrated that Cdh1 is the main activator of APC/C in rat
cortical neurons. Moreover, APC/C-Cdh1 is essential for neuronal
survival, as it promotes the continuous degradation of cyclin B1, avoiding the nuclear accumulation of cyclin and the subsequent
activation of the cell cycle machinery and neuronal apoptosis.
Nowadays it is known that Cdh1 regulates neuronal survival, axonal
growth, synaptogenesis and glucidic metabolism in primary
cultured neurons. However, the significance of these functions in
vivo remains unknown. In this work we aimed to elucidate Cdh1
function in the in vivo brain. For achieving this objective we used
specific neuronal encephalic cortex Cdh1 knocked out mice. Our
results show that Cdh1 depletion induces a shortening of the II and
III layers of the cerebral cortex in a time dependent manner,
suggesting a selective neuronal loss. These results corroborate, the
essential function of Cdh1 in neuronal survival in vivo.

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TU01-08
SEL-1L INFLUENCES SELF-RENEWAL IN NEURAL STEM CELLS
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Murine SEL-1L (mSEL-1L) belongs to the Unfolded Protein Response (UPR) gene family, acting as a ‘gate keeper’ in the control of
newly synthesized soluble and membrane proteins. It is essential
during mouse development since homozygous mSEL-1L deficient
mice are embryonic lethal due to growth impairment with the brain
being the most affect region. In the study here presented, we explore
the role of this protein in stemness maintenance, analyzing its
contribution in Neural Stem Cells (NSCs) self-renewal.

We demonstrate that mSEL-1L expression is associated with
pluripotency and multipotency states, but is lost during NSCs
terminal differentiation into astrocytes, oligodendrocytes and neu-
rons. Interestingly, the protein silencing is partially mediated by the
refined post-transcriptional regulation of mmu-miR-183.

Our studies support the hypothesis that mSEL-1L protein is
responsible of self-renewal control, since its deprivation in NSCs
determines in vitro a significant down-modulation of the early
neural progenitor markers PAX-6 and OLIG-2 and a severe
proliferation defect. This might be due to an alteration of the Notch
pathway, as revealed by the drastic reduction of its effector HES-5.
Furthermore, these cells exhibit a premature differentiation tен-
dency, showing high levels of the pro-neural factor Neurogenin 2
and of both the astrocytic and neuronal markers GFAP and Beta-III
Tubulin, while the principal NSC stemness makers Nestin and
SOX-2 are strongly down-modulated.

In conclusion, we propose that the lack mSEL-1L is responsible
of the progressive progenitor pool depletion, which ultimately leads
to NSC death likely due to the misregulating of the Notch
signalling.

TU01-09
ADULT NEUROGENESIS IN SEVERAL MICROBAT AND MEGABAT SPECIES
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Considerable species differences have been reported on the
occurrence of adult neurogenesis in the order Chiroptera indicating
low or complete absence of cell proliferation. This report presents
findings from investigation of adult neurogenesis in two megachi-
roroptera species and six microchiroptera species, which were not
reported previously in literature. The animals were euthanized and
transcardially perfused with saline followed by 4% paraformalde-
hyde in 0.1 M phosphate buffer (PB). Brains were removed and
post fixed in the same fixative overnight. Following equilibration in 30% sucrose in PB, 50 μm frozen section were cut in sagittal planes. Ki-67 and doublecortin (DCX) immunohistochemistry was undertaken to demonstrate proliferating cell and immature neurons. A combination of Ki-67 and DCX immunostainings confirmed adult neurogenesis in the subventricular zone (SVZ), rostral migratory stream (RMS), olfactory bulb and dentate gyrus (DG) of the hippocampus in the two megabat species (Eidolon helvum, Epomophorus wahlbergi) and five microbat species (Cardioderma cor, Chaerophon pumilus, Hipposideros commersoni, Miniopterus schreibersii and Triacanthops persicus). DCX positive cells were observed only in the cerebral cortex in the sixth microbat species (Coleura afra). In addition, neurogenesis was observed in other potential sites such as the cerebral cortex in the Cardioderma cor, Coleura afra, Hipposideros commersoni, Miniopterus schreibersii, Triacanthops persicus and Eidolon helvum, and the amygdala in both megabats. In conclusion, despite the suggestion that mega- and microchiroptera could have a paraplythic origin based on the huge neuroanatomical differences between the suborders, there were no such differences in adult neurogenesis as it is evident in the brains of both mega- and microchiroptera.

TU01-10

COMPOUND A INCREASE THE NUMBER OF NON-ECTOPIC BRDU+ CELLS IN THE HIPPOCAMPAL SUBGRANULE ZONE

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The search for new compounds that enhance proliferation and differentiation of neuronal stem cells in the hippocampus represent a significant scientific challenge. Newborn neurons emerge from neuronal stem cells (NSCs) from niches in the mammalian adult brain. Neurogenesis is also observed in seizures models with hilar ectopic granule cells location. We have analyzed the compound A (CA-polyacetylenic compound) extracted from Amazon plant, which produces convulsion behavior in rats in high doses. The aim of this study was evaluated if in low doses CA produces neurogenesis in the subgranular zone of hippocampal dentate gyrus of adult mice using the 5'-bromo-2'-deoxyuridine (BrdU)-pulse chase method. Increased dose of CA (0.25, 0.5 and 1 mg/kg) or vehicle were given 5 h before BrdU injection. Mice were sacrificed at 7 days after BrdU administration, and hippocampal slices were processed for immunohistochemistry. We found that CA not promoted seizure behavior in any dose in this work. CA at 0.25 mg/kg had no effect (49. 3 ± 20.18 cells) when compared with vehicle (42 ± 8 cells). However, 0.5 and 1 mg/kg of CA induces an increase on BrdU+cells in subgranular zone of hippocampal dentate gyrus (71 ± 21.96 and 76.6 ± 6.6 cells; respectively) but not showed any hilar ectopic BrdU+cells. These results suggest that CA could be stimulating the hippocampal neurogenesis.

TU01-11

THE δ-OPIOID RECEPTOR-MEDIATED NEURITE OUTGROWTH INVOLVES G PROTEINS AND THE SIGNAL TRANSducer AND ACTIVATOR OF TRANSCRIPTION 5B

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Opioid receptors (μ, δ, κ) are prototypical Gi/o-coupled receptors and participate in mechanisms controlling neural growth, differentiation and synaptic plasticity (1). We have recently demonstrated that δ- and μ-opioid receptors (δ-OR and μ-OR) form multi-component signaling complexes, consisting of Signal Transducers and Activators of Transcription 5A/B (STAT5A/B), e-Src kinase and selective G protein subunits, leading to STAT5A/B phosphorylation (2, 3). We, therefore, wondered whether these dynamic protein complexes are implicated in a molecular mechanism through which opioid receptors may regulate transcription, differentiation and survival in neuronal cells. To examine the effect of δ-OR-induced STAT5B activation on neuronal survival and neurite outgrowth, we used Neuro-2A cells transiently transfected with the δ-OR and/or a dominant negative construct of STAT5B that cannot be phosphorylated (DN-STAT5B). The cells were treated with the δ-opioid agonist DSLET and (i) the number of live cells was visualized and counted under a microscope in the presence of trypan blue, or (ii) the length of the neurites was measured. A higher percentage of surviving cells was detected in the presence of DSLET, an effect that was reversed either by antagonist treatment or the expression of the DN-STAT5B construct prior to agonist administration. Similarly, DSLET activation of δ-OR resulted to increased neurite outgrowth and this effect was blocked by pertussis toxin pre-treatment, which inactivates Gi/o proteins, and the presence of the DN-STAT5B construct. Collectively, our results suggest that δ-OR activation leads to neuronal cell survival and neurite outgrowth via a signaling pathway involving Gαi/o proteins and STAT5B transcription factor.

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References:

TU01-12

GENERATION OF DISTINCT NEURONAL CELL TYPES FROM EMBRYONIC STEM CELLS FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

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Embryonic development of the CNS involves the coordinated generation of an extraordinary diverse array of neurons. The directed differentiation of embryonic stem cells (ESCs) into neuronal stem cells (NSCs) of specific identities and the identification of endogenous pathways that may mediate expansion of NSCs are fundamental goals for the treatment of degenerative disorders and trauma of the nervous system. We have previously shown that timely induction of a single Hox gene (Hoxb1) at the right cellular context can direct the generation of region specific neural progenitors. (Gouti et al., Stem Cells 2008). The advantage of this system is the generation of precise neural populations (NP) along
the AP axis as well as the identification of novel target genes and cellular processes using whole-genome expression profiling. Molecular analyses suggested that Hoxb1 ES-derived NSCs exhibited a preference for dorsal neural tube fates. The most dorsal population generated in the neural tube are neural crest (NC) cells and further analysis suggested that Hoxb1 participates in NC cell induction in vivo. To further examine this in vivo we used chick in ovo electroporation were we reported that anterior Hox patterning genes participate in NC specification and EMT by interacting with NC-inducing signaling pathways and regulating the expression of key genes involved in these processes (Gouti et al., Stem Cells 2011). We have extended this approach using different Hox genes and found that we can induce distinct NS cell fates corresponding to different levels of the developing spinal cord. The identity of these neural progenitors is further restricted using specific ventral signals in order to generate distinct motor neuron progenitors for hindbrain, brachial and thoracic levels. The developmental potential of these progenitors is currently analyzed by transplantations in newborn mice. This project may establish an approach that could provide us with diverse motor neuron cell types necessary for an effective cell therapy approach to treat motor neuron degenerative disorders.

TU01-13

PROFILES OF IFRD1 AS A NOVEL DIFFERENTIATION REGULATOR IN NEURAL PROGENITORS

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We have previously identified interferon-related developmental regulator-1 (Ifrd1) as the gene responsible for the predominant suppression of neuronal differentiation in neural progenitors isolated from adult mouse hippocampus. In the mouse embryonic carcinoma P19 cells endowed to differentiate into neuronal and astroglial cells, marked but transient expression was at first seen in nestin mRNA within 4 days in culture under floating conditions, followed by MAP2 mRNA expression during culture under adherent conditions after dispersion and subsequent Ifrd1 mRNA expression in line with GFAP mRNA. In P19 cells with transient overexpression of Ifrd1, a significant decrease was found in mRNA expression of MAP2, but not of GFAP, within 72 h after transfection. Prior to the decrease in MAP mRNA expression, a significant decrease was seen in mRNA expression of the proneural gene NeuroD1 in P19 cells 48 h after the transfection of Ifrd1 expression vector. By contrast, no significant changes were found in mRNA expression of different proneural genes up to 72 h after transfection. These included Hes1, Hes5, Mash1, Math1, Math3, Neurogenin2 and Neurogenin3. In P19 cells transfected with a luciferase reporter plasmid linked to NeuroD1 promoter, a drastic decrease was seen in luciferase activity in cells with a NeuroD1 promoter, but not in those with an empty vector, within 4 days in culture with retinoic acid. On analysis using mutated deletion constructs, however, a significant decrease was still seen in luciferase activity in cells transfected with a reporter construct containing – 112bp upstream of NeuroD1 promoter. These results suggest that Ifrd1 may suppress neuronal differentiation through a mechanism relevant to predominant inhibition of transactivation of NeuroD1 in undifferentiated neural progenitors.

TU01-14

APP-BP1 KNOCKDOWN REDUCES NEURAL DIFFERENTIATION OF FETAL NEURAL STEM CELLS

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Amyloid precursor protein binding protein-1 (APP-BP1) was first identified as an interacting protein of the intracellular carboxyl (C-) terminus of amyloid precursor protein (APP) and is known as a cell cycle protein that mediates the NEDD8 conjugation pathway. However, its physiological function is still not fully understood. In this study, we explored whether APP-BP1 plays a role in the neural differentiation of fetal neural stem cells by altering APP intracellular domain (AICD) production and by affecting the gene transcriptional activity of APP in fetal neural stem cells. APP-BP1 knockdown by siRNA treatment was found to down-regulate neural differentiation and to up-regulate AICD generation in fetal neural stem cells. In addition, the suppression of APP-BP1 expression reduced the gene transcriptional activity of APP assessed by a Ga4 transactivation assay. Given these factors, APP-BP1 may modulate neurogenesis by regulating gene transcriptional activity of AICD in fetal neural stem cells.

TU01-15

PROX1 IS INVOLVED IN THE GENE REGULATORY NETWORKS CONTROLLING GENERIC AND SUB-TYPE SPECIFIC NEUROGENESIS

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Spinal cord neurons acquire two basic specialized identities, namely motor neurons (MNs) and interneurons. MNs are generated from a pool of Olig2 positive progenitors in the ventral spinal cord that defines the pMN domain. However, the upstream molecular mechanisms that control this neuronal specification is not well understood. We have previously shown that Prox1, a transcription repressor and downstream target of proneural genes, regulates differentiation of neural progenitors (NPs) via direct suppression of Notch1 expression (Kaltezioti et al. 2010, PLoS Biol). Active Notch1 signaling is necessary for the correct specification of MNs, raising the possibility that Prox1 may also be associated with this regulation. Accordingly, we show here that Prox1 is mainly expressed in NPs destined to generate interneurons, and only transiently expressed into pMN domain during early stages of MN specification. Most important, gain-and-loss of function studies in the chick neural tube and mouse NPs show that Prox1 is sufficient and necessary for the suppression of MN identity in the spinal cord. Mechanistically, activated Notch1 signaling cannot rescue the Prox1 effect on MNs, suggesting an alternative mode of action. In agreement, Prox1 is sufficient to directly suppress Olig2 gene expression in the pMN domain, which is a key regulator for the
initial specification of the pMN domain and MN identity. Conversely, shRNA-mediated knockdown of Prox1 in the chick neural tube indicates that Prox1 is necessary for the suppression of Olig2 outside the pMN domain. Plasmid-based luciferase assays and ChIP analysis showed that Prox1 directly suppresses the proximal promoter of the Olig2 gene locus and K23 enhancer, which specifically drives Olig2 gene expression into the pMN domain. Collectively, these observations indicate that Prox1 is essential for the suppression of MN fate in NPs via direct transcriptional repression of Olig2.

TU01-16
HUMAN INDUCED PLURIPOTENT STEM CELLS FOR MODELING HUMAN DEVELOPMENT AND DISEASE
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Pluripotent cells, such as embryonic stem cells, comprise a promising tool for unraveling the molecular mechanisms of cellular differentiation and development and may have important implications in Regenerative Medicine. Despite the incredible growth in knowledge that has occurred in stem cell research within the last two decades, the study of cellular reprogramming and pluripotency has recently begun and is essential to facilitate the ultimate use of these cells in the clinic. Human somatic cells have been reprogrammed directly to pluripotent stem cells (hiPSC) by ectopic expression of four transcription factors, Oct4, Klf4, Sox2 and Myc. Recent methodological improvements increase the efficiency and detection of the reprogramming process. We have used four bicistronic lentiviral vectors encoding the four reprogramming factors, each co-expressed with a distinct fluorescent protein, namely vexGFP (violet light excited-green fluorescent protein), mCitrine, mCherry, and mCerulean (Papapetrou et al. 2009). By co-transduction of adult human dermal fibroblasts (HDF) with these vectors several hiPSC lines were generated that expressed pluripotency markers. Human embryonic stem cells and hiPSC were subsequently differentiated to dopaminergic and motor neurons and their properties studied. Additionally, dopaminergic neurons which represent the type of cells destroyed in Parkinson’s disease were also derived from reprogrammed fibroblasts of healthy human individual and Parkinsonian patients. These hiPSC lines are being used to model mechanisms of neural development and neurodegeneration as well as to develop stem cell therapeutic approaches for human neurodegenerative diseases and neurotrauma.

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Reference:

TU01-17
NEURAL STEM/PRECURSOR CELLS SECRETING IGF-I CAN HAVE A NEUROPROTECTIVE ROLE IN AN ANIMAL MODEL OF TEMPORAL LOBE EPILEPSY
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Epilepsy is a neurodegenerative disease with a prevalence of roughly 1% of the population. Temporal lobe epilepsy (TLE) is among the most frequent types of drug-resistant epilepsy, making the need for new neuroprotective agents to alleviate hippocampal degeneration that follows TLE seizures a pressing issue. Insulin-like growth factor 1 (IGF-I) has been shown to have neuroprotective activity following a number of experimental insults to the nervous system, and in a variety of animal models of neurodegenerative diseases. In the present work we investigated the possible neuroprotective effects of IGF-I following unilateral intrahippocampal administration of kainic acid (KA), an animal model of TLE. We show that IGF-I, either administered intrahippocampally or secreted by neural stem/precursor cells (NPCs) transduced with its gene and transplanted in the hippocampus, decreased neurodegeneration as assessed by cresyl violet staining and GFAP- or CD11b-immunostaining. Additionally, we studied the differentiation potential of control or IGF-I-overexpressing NPC grafts in the KA-injured hippocampal environment, at 8, 30 or 60 days after transplantation. Transplanted NPCs transduced with the IGF-I gene differentiated earlier than non-transduced NPCs into Nestin-, Doublecortin- or NeuN-immunopositive cells. Based on the above, we can conclude that IGF-I is an important neuroprotective agent which could possibly be used to therapeutically address TLE in the future. Moreover, these results demonstrate a significant effect of IGF-1 transduction in regulation of NPC functions and provide a potential strategy of enhancing the prospective repair potential of NPCs.

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TU01-18
GEMININ COORDINATES SELF-RENEWAL AND DIFFERENTIATION DECISIONS IN NEURAL PROGENITORS OF THE DEVELOPING MOUSE CORTEX
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In the developing mouse cortex neurons are generated from three distinct types of neuronal progenitor cells, the neuroepithelial
and radial glial cells that divide in the ventricular zone of the cerebral cortex and the basal progenitors that divide in the basal surface of the ventricular zone, the subventricular zone. Progenitor cells must balance between self-renewal that allows maintenance of their population and differentiating into different cell types. A key feature in this process that should be tightly regulated in time and space is the decision of progenitor cells to remain in a proliferative state or to exit the cell cycle and begin to differentiate. It has been proposed that Geminin regulates decisions between proliferation and differentiation, through interactions with chromatin remodeling complexes and transcriptional factors. At early stages of nervous system development, Geminin has been suggested to promote neural cell fate acquisition, while at later stages it promotes neuronal differentiation. Moreover, it has been suggested that Geminin has an essential role in T cell proliferation. To gain insight into the mechanisms regulating self-renewal and differentiation of cortical progenitors, we have generated mice that lack Geminin expression in the developing nervous system. Our data show that early cortical progenitor cells, in the absence of Geminin, remained in a proliferative state rather than differentiate into neurons. This bias towards cortical progenitor self-renewal seems to involve early cortical progenitors as it was not observed in later developmental stages. Moreover, Geminin overexpression in cortical progenitor cells reduces cortical progenitor cell population and exhibit premature cell cycle exit towards neuronal differentiation. Our data suggest that Geminin regulates cortical progenitor cells decision between self-renewal and differentiation.

TU01-19
HUMAN NEURAL STEM CELLS TRANSDUCED WITH OLG2 TRANSCRIPTION FACTOR AMELIORATE EXPERIMENTAL NEONATAL PERIVENTRICULAR LEUKOMALACIA

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Oligodendrocytes have relevance for production and maintenance of the central nervous system myelin, which facilitates salutary conduction of nervous impulses along axons. Since intrauterine infection and/or perinatal asphyxia causes loss of oligodendrocyte progenitors, leading to periventricular leukomalacia (PVL), we established oligodendrocyte progenitor cells (F3.olg2) by transducing human neural stem cells (F3 NSCs) with Olig2 transcription factor. Seven-day-old male rats were subjected to hypoxia-ischemia-lipopolysaccharide (HIL), and intracerebroventricularly transplanted with F3.olg2 (1 x 10^6 cells/rat) once (on day 10) or 4 times (on days 10, 17, 27 and 37). Neurobehavioral abnormalities were evaluated on days 14, 20, 30 and 40 via cylinder test, locomotor activity and rota-rod performance, and learning/memory function was tested on days 41-44 through passive avoidance performance. F3.olg2 recovered using rate of contralateral forelimb in cylinder test, improved locomotor activity, and near-fully restored rota-rod performance of PVL animals, in addition to marked improvement of cognitive function. It was confirmed that transplanted F3.olg2 cells migrated to damaged areas; periventricular white matter, internal capsule and corpus callosum, and that the cells differentiated into mature oligodendrocytes, i.e., positive for immunostaining to myelin basic protein. The results indicate that transplanted F3.olg2 restored neurobehavioral function via myelination, and that human oligodendrocyte progenitor cells could be a candidate for cell therapy of perinatal hypoxic-ischemic and infectious brain injuries including PVL and cerebral palsy.

TU01-20
ENHANCED NEURAL PROGENITOR/STEM CELL SELF-RENEWAL VIA THE INTERACTION OF STRESS INDUCIBLE PROTEIN 1 WITH THE PRION PROTEIN


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Prion protein (PrPC), when associated with the secreted form of the stress inducible protein 1 (STI1), plays an important role in neural survival, neurogenesis, and memory formation. However, the role of the PrPC-STI1 complex in the physiology of neural progenitor/stem cells is unknown. In the current report, neurospheres cultured from fetal forebrain of wild-type (Pmp^+/+) and PrPC-null (Pmp0/0) mice were maintained for several passages without the loss of self-renewal or multipotetiality, as assessed by their continued capacity to generate neurons, astrocytes, and oligodendrocytes. The homogeneous expression and co-localization of STI1 and PrPC suggests that they may associate and function as a complex in neurosphere-derived stem cells. The formation of neurospheres from Pmp0/0 mice was reduced significantly compared to their wild-type counterparts. In addition, blockade of secreted STI1, as well as its cell surface ligand, PrPC, with specific antibodies, impaired Pmp^+/+ neurosphere formation without further impairing the formation of Pmp0/0 neuropheres. Alternatively, neurosphere formation was enhanced by recombiant STI1 application in cells expressing PrPC, but not in cells from Pmp0/0 mice. The STI1-PrPC interaction was able to stimulate cell proliferation in the neurosphere-forming assay, whereas no effect upon cell survival or the expression of neural markers was observed. These data suggest that the STI1-PrPC complex may play a critical role in neural progenitor/stem self-renewal via the modulation of cell proliferation, leading to the control of the stemness capacity of these cells during nervous system development.
TU01-21
EFFECT OF MELATONIN ON CELL PROLIFERATION IN HIPPOCAMPAL DENTATE GYRUS BY DEXAMETHASONE-INDUCED STRESS MICE

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Adult hippocampal cell proliferation has been demonstrated in several species and is regulated by both environmental and pharmacological stimuli. Hippocampal cell proliferation is decreased by exposure to stress or administration of glucocorticoid or dexamethasone (DEX). The reduction of cell proliferation associated with the brain cognitive dysfunction leads to learning and memory impairment. The aim of this study is to investigate the cell proliferative modulation under metadione pre-treatment in dexamethasone induced stress mice. The mice were treated with dexamethasone 60 mg/kg (i.p.) for 21 days. Melatonin (10 mg/kg, i.p.) was injected 30 min before the dexamethasone treatment. Cell proliferation in dentate gyrus of hippocampus was investigated by using 5-bromo-2-deoxy-uridine (BrdU) as a marker for dividing cell. Our studies demonstrate that dexamethasone treatment (60 mg/kg) significantly decreased the number of BrdU-positive cells in the hippocampal dentate gyrus. Administration of metadione before dexamethasone treatment significantly restores cell proliferation in the dentate gyrus. The results suggest that melatonin may have a protective effect of the cell proliferation impairment resulting from dexamethasone, which may be helpful for improving brain cognitive function.

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TU01-22
INTERPLAY BETWEEN NEDCIN AND BMI1 REGULATES PROLIFERATION OF EMBRYONIC NEURAL STEM CELLS

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Neural stem cells (NSCs) divide in two ways; symmetric division for self-renewal and asymmetric division for differentiation into neurons and glial cells. During early periods of brain development, NSCs proliferate rapidly through symmetric division, which contributes primarily to the determination of total neuronal number in mature brain. However, the molecular mechanisms underlying symmetric division of NSCs remain elusive. Necdin is expressed predominantly in post-mitotic neurons during the brain development. Necdin interacts with many regulatory proteins such as the cell cycle-related transcription factors E2F and p53 and the neurotrophin receptors TrkA and p75NTR. Through these interactions, necdin inhibits both proliferation and apoptosis of proliferative cells and promotes differentiation and survival of differentiated neurons. In this study, we examined whether necdin is involved in cell cycle regulation of embryonic NSCs. Necdin was expressed in cultured NSCs prepared from mouse embryos at E14.5. In necdin-deficient NSCs prepared from paternal necdin gene-mutated mice, the expression levels of cyclin-dependent kinase inhibitor p16Ink4a mRNA was significantly decreased. Because expression of p16Ink4a is negatively regulated by Bmi1, a polycomb-group transcriptional repressor that is highly expressed in NSCs and promotes their proliferation, we examined the physical and functional interactions between necdin and Bmi1 in the cell cycle control of embryonic NSCs. Double-immunostaining analysis demonstrated that necdin and Bmi1 colocalized in the nucleus of cultured NSCs prepared from mouse embryonic forebrain. Coinmunoprecipitation and in vitro binding assays revealed that necdin directly bound to Bmi1 via its helix-turn-helix domain. Necdin relieved Bmi1-dependent transcriptional repression at the 16Ink4a promoter as determined by luciferase reporter assay. Bmi1 incorporation analysis showed that Bmi1 relieved necdin-induced suppression of S-phase population of transfected HEK293A cells. Furthermore, lentivirus-mediated overexpression of Bmi1 increased the S-phase population in NSCs but failed to increase it in necdin-deficient NSCs. These data suggest that embryonic NSC proliferation is regulated through the antagonistic interplay between necdin and Bmi1.

TU01-23
EFFECT OF PESTICIDE CARBOFURAN ON REGULATORY DYNAMICS OF NEUROGENESIS

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During brain development new neurons are generated throughout lifetime from NSC (neural stem cells) through neurogenesis. These immature cells (NSC) line ventricles of neural tube, and they migrate long distances to their assigned locations, differentiating into all three lineages such as neurons, astrocytes and oligodendrocytes generated. Several recent animal studies indicate that there is strong association between environmental contaminants and variety of neurological disorders on their prolonged exposure. These environmental neurotoxicants includes pesticides, endocrine disruptors, heavy metals, and industrial and cleaning solvents. Carbofuran (2,3-Dihydro-2,2-dimethyl-7-benzofuranol N-methylcarbamate), a Carbamate pesticide has incidents of accidental and occupational poisoning in humans worldwide. In the present study, we hypothesize that Carbamate pesticide, such as Carbofuran (CFN) exposure during childhood and adulthood may affect ongoing neurodevelopmental process and neurogenesis. We have carried out studies to assess the effect of CFN on neurons and astrocytes in the rat brain and effects on neurobehavior. Young and adult rats were chronically treated with CFN from gestational day 5 to post-natal day 28 and 90. Effect of CFN on rat’s neurobehavior (locomotion, muscle strength, learning and memory) was assessed by measuring spontaneous locomotor activity, grip strength, and conditioned active avoidance response. We found significantly decreased BrdU positive cell number in the hippocampus and sub-ventricular zone in CFN treated animals at both time points studies. We observed significantly decreased NeuN positive neuron number and increased GFAP positive astrocytes in the CFN treated group. We found a significantly altered expression of Wnt and Notch pathway related genes such as wnt, disheveled, Beta catenin, Axin, TCF and GSK-3, Notch beta in the CFN treated group. We observed significant changes in neurobehavioral performance in CFN treated rats as compared to...
TU01-24
INTRICATED GENOMIC AND NON-GENOMIC RETINOIC ACID SIGNALLING MECHANISMS IN REGENERATING NEURONS IN CULTURE
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A potent gene transcription regulator, retinoic acid (RA) is known to influence more than 500 genes, whose functions include regulation of neuronal differentiation and patterning of the developing nervous system. It has been previously proposed that RA synthesis takes place only in the cytoplasm mediated by three retinaldehyde dehydrogenases (Raldhs) namely Raldh1, Raldh2 and Raldh3. From this cytoplasmic location RA is then transported to the nucleus where RA receptors (RAR) α, β and γ initiate gene transcription. RA is then removed from the nucleus and degraded by microsomal cytochrome 450 (Cyp26) A1, B1 or C1 enzymes. New studies have pointed to additional roles for RARs in the cytoplasm to regulate kinase function. The aims of the study were to determine if the synthesis/degradation of RA is localized within subregions of regenerating neurons and subsequently to establish if RA signalling is genomic (RAR control of nuclear transcription) or non-genomic (RAR control of cytoplasmic kinases). We tested three types of neurons cultivated from hippocampus, cortex and retina. Our findings showed that RA may have a pivotal role in neuronal regeneration in culture and that it exerts that function by combining its genomic function with a transcription independent mechanism. We base that conclusion on the subcellular location of the metabolic enzymes Raldh 1, Raldh2, and CYP26A1 and nuclear receptors RAR α, RAR β and RAR γ in neuronal cultures. We also show that both RA synthetic enzymes Raldh1 and Raldh2 and the degrading enzyme CYP26A1 previously considered only cytosolic can also have a nuclear location. Our results imply that besides the expected location of RA synthesis and degradation, it can also be metabolized locally in different cell compartments most likely to regulate differing processes.

TU01-25
HUMAN NEURAL STEM CELLS ENCODING CHAT GENE IMPROVE COGNITIVE FUNCTION OF AGED MICE
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Aging is commonly associated with progressive, functional and structural deterioration of neural systems, affecting both cognitive and motor functions. In this study, male 18-month-old mice were intracerebroventricularly transplanted with human neural stem cells (NSCs; F3.ChAT, 1x10⁶ cells/mouse) overexpressing human choline acetyltransferase (Chat). Four weeks later, learning/memory and motor functions were evaluated via passive avoidance and Morris water-maze tests and locomotor activity, respectively. Transplantation of F3.ChAT near-fully improved the cognitive function, in parallel with the recovery of brain acetylcholine (ACh) levels, which were superior to the original F3 NSCs. Locomotor activity was also recovered by transplantation of F3 and F3.ChAT cells. Transplanted F3.ChAT cells were found to migrate to cortices and hippocampus, and differentiate into neurons and astrocytes. The present study demonstrates that human NSCs expressing Chat restore learning and memory deficits as well as decreased locomotor activity associated with natural senescence by increasing ACh production.

TU01-26
CELLULAR PRION PROTEIN IS REQUIRED FOR NCAM-DEPENDENT NEURONAL DIFFERENTIATION OF SUBVENTRICULAR ZONE-DERIVED NEURAL PRECURSOR CELLS
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KP and FP contributed equally

Cellular prion protein PrPc is a ubiquitous glycoprotein prominently expressed in the brain, in differentiated neurons but also in neural stem/precursor cells (NSCs). The misfolding of PrPc is a central event in prion diseases, yet the physiological function of PrPc has remained elusive. PrPc-/- mouse show no obvious abnormalities, however recent studies have associated PrPc with neurite outgrowth as well as with peripheral myelin maintenance. Additionally, PrPc has been implicated in the proliferation and differentiation of NSCs. As PrPc has been previously reported to co-immunoprecipitate with the neural cell adhesion molecule NCAM, we asked if PrPc interacts with NCAM to influence the proliferation and/or differentiation properties of NSCs. To this end, we used neurosphere cultures derived from wild-type and PrPc-/- mice grown in the absence or presence of NCAM-Fc, the chimeric soluble form of NCAM. We observed that NSCs derived from PrPc-/- mice show decreased neuronal differentiation in comparison with wild-type NSCs, as estimated by a reduction in the percentages of double-cortin (DCX+/Ki67+) proliferating neuronal progenitors, DCX+ early neuroblasts and TUJ-1+ differentiated neurons, without changes in the percentage of GFAP+ astrocytes. Addition of recombinant NCAM-Fc in wild-type NSCs results in decrease of proliferation and increase of neuronal differentiation (DCX+ and TUJ-1+ cells), without affecting the number of GFAP+ astrocytes. Interestingly, NSCs derived from PrPc-/- mice do not respond to NCAM-induced neuronal differentiation. Moreover, upon NCAM-Fc addition in PrPc-/- NSCs, DCX+ neuronal progenitors accumulate at the proliferating stage without proceeding to differentiation. Taken together these results suggest that PrPc affects the differentiation program of NSCs in an NCAM-dependent manner. We are running ex vivo and in vivo experiments to further assess the NCAM-dependent role of PrPc in neurogenesis and migration of newborn neurons in the SVZ-RMS-OB pathway.

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TU01-27
MICROGlia INSTRUCT NEUROGENESIS AND OLIGODENDROGENESIS IN THE EARLY POST-NATAL SVZ
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Microglia are the immune effector cells of the central nervous system (CNS) and exist in three distinct forms known as amoeboid, ramified and reactive/activated microglia which serve different functional roles according to brain circumstances. Owing to their drastic phenotypic changes in the pathological conditions, many reports have focused on their characteristics in the pathological conditions. However, we recently obtained some data suggesting that microglia have important roles in the early post-natal brain development. We discovered that activated microglia accumulated inside the subventricular zone (SVZ) from P1 to 10, and such accumulation was no longer observed at P30. When microglial activation was suppressed by i.p. administration of minocycline from P1 to P4, the number of cells positive for Kl67 (a marker for proliferating cells), Doublecortin (a marker for neuronal progenitors), or O1 (a marker for oligodendrocyte progenitors), significantly decreased. Minocycline also decreased the concentration of IL-1alpha, IL-1beta, IL-6, IFN gamma, and TNFalpha in SVZ extract. When neurospheres derived from rat cortical stem cells were treated with these cytokines, IFN gamma and IL-4 enhanced neurogenesis, and IL-1beta enhanced both of neurogenesis and oligodendrogenesis. Taken together, our data strongly suggest that activated microglia instruct neurogenesis and oligodendrogenesis by releasing cytokines in the early post-natal SVZ.

TU01-28
INVESTIGATION OF THE ROLE OF THE TRANSCRIPTION FACTOR KLFl4 IN THE PROLIFERATION OF RETINAL PROGENITORS
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Transcription factors are essential for the regulation of nervous system development. We are interested in evaluating the role of KLF4, a member of Sp/KLF family of transcription factors, on retinal development and, more specifically, on the effect of the Pituitary Adenylyl Cyclase Activating Polypeptide (PACAP) in cell proliferation. We have previously observed that PACAP inhibits cell proliferation in rat retina (Njaine et al. 2010). In the current study, we aimed to determine the expression pattern of KLF4 and to investigate the hypothesis that KLF4 mediates the antiproliferative effect of PACAP. We demonstrated using standard RT-PCR that KLF4 is expressed in embryonic, neonatal and mature retinas. Quantitative RT-PCR (qRT-PCR), in-situ hybridization, immuno-fluorescence and western-blot were performed to evaluate mRNA level, protein content and which cell types express KLF4. Our results suggest that protein and mRNA content of KLF4 increases at the post-natal days. Moreover, KLF4 is present either in cytoplasm or nucleus both in progenitors and post-mitotic cells in early development and in various neurons and glia later. Using qRT-PCR and Western-blot we also demonstrated that KLF4 expression increased after PACAP treatment for 1h in post-natal day 1 retinas. This effect was confirmed to be in the neuroblastic layer by immunofluorescence and in situ hybridization. Moreover, we observed by EMSA that nuclear KLF4 is induced in response to PACAP and binds to Sp1 motifs, which are present in rat cyclin D1 promoter. In fact, the expression of this cell cycle regulator is reduced after PACAP treatment as analyzed by qRT-PCR. We describe the presence and distribution of KLF4 throughout retinal development. Also, our data suggest that PACAP may modulate the proliferation of progenitor cells through KLF4-induced downregulation of cyclin D1. Further studies are in progress in our laboratory to depict KLF4 roles, based on overexpression and knockdown strategies.

TU01-29
HUMAN STEM CELLS DERIVED DIFFERENTIATING NEURONS HAVE XENOBIOTIC METABOLIZING CAPABILITIES
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Xenobiotic metabolism in adult brain is well documented now. However, the status in neural stem cells and developing brain cells is still not known and to be explored. We investigate the expression and inducibility of selected cytochrome P450s (CYP 1A1, 2B6, 2E1 and 3A4) in differentiating neuronal cells derived from human umbilical cord blood cells (hUCBSC). Following purification and characterization, hUCBSC were allowed to differentiate into neural subtypes for 16 days in specific growth conditions. At various points of differentiation (day 0, 2, 4, 8 and 16) cells were studied for mRNA expression of neuronal specific markers (96 genes) using taqman low density array. Genes showing significant alterations in the expression were further studied for translational changes. Significant expression of nestin and beta-III tubulin was detected by day 2 of differentiation, whereas maximum expression of other neuronal markers with morphological differentiation was achieved by day 8. In further differentiation, increase in the magnitude of these markers was insignificant except morphological differentiation. These differentiating cells were also studied for expression (mRNA and protein) and inducibility of selected CYPs (1A1, 2B6, 2E1 and 3A4) following exposure of known CYP1A1 inducer i.e., 3-methylcholanthrene (MC). Significant (p < 0.001) expression of CYP 1A1, 2B6, 2E1 and 3A4 was recorded even at day 0. A continuous increase in the expression of CYP1A1 and 3A4 was recorded all through the differentiation, whereas peak expression of CYP2B6 and CYP2E1 was observed by day 4 of differentiation. Expression of CYP1A1 was found to be up-regulated significantly against MC exposure (4 μM) for 3 h at all the differentiation points. Expression and inducibility of CYPs in differentiating hUCBSC suggest their applicability for developing specific biomarkers of exposure and effects for human specific neural developmental, injury and repair.
TU01-30
MELATONIN AUGMENTS HIPPOCAMPAL NEUROGENESIS THROUGH MELATONIN RECEPTORS BY ACTIVATING ERK SIGNALING CASCADES
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Melatonin, a major indoleamine produced by the pineal gland has recently shown to be favorable in promoting neurogenesis of neural stem/precursor cells obtained from different neurogenic regions. However, the mechanisms are still unknown. Recent investigations have revealed the expression of melatonin receptors in the hippocampal precursor cells. As a result, we hypothesized the involvement of the MAPK-ERK signaling cascades, which are one of the signaling cascades coupled to melatonin receptors 1A, to be involved with the neurogenic actions of melatonin. Adult mice were sub-chronically and chronically treated with melatonin for 4 and 8 consecutive days respectively, before the hippocampi were obtained for the analysis of phospho-c-Raf, phospho-ERK and phospho-mye levels. The results demonstrated a high rise in the levels of the signaling molecules in the melatonin treated group when compared to the control group whereby the rise was more in the chronic melatonin treatment group. The signaling molecules basically play significant roles in the activation of genes involved with cell proliferation and survival. Additionally, melatonin also caused a rise in the expression of melatonin receptor 1A. Nonetheless, further analyses are required using hippocampal cultures before explicit conclusions can be brought about.

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TU01-31
ACTIVATED ERK/MAPK PATHWAY BY MELATONIN INCREASES PROLIFERATION OF CULTURED PRECURSOR CELLS OBTAINED FROM ADULT MOUSE SVZ
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Melatonin, a circadian rhythm-promoting molecule secreted mainly by the pineal gland, has shown a variety of biological functions and neuroprotective effects. However, it is still unclear how the involvement of melatonin in adult neurogenesis. Our previous study showed melatonin can modulate precursor cells from adult mouse subventricular zone (SVZ) proliferation and differentiation. Traditionally, precursor cells isolated from SVZ and exposed to epidermal growth factor (EGF) in culture grow to form neurospheres that are self-renew and multipotent. In this study, we examined the effects of melatonin on signaling molecules using an in vitro culture system. By separately adding EGF and melatonin into culture, we found that melatonin alone can induce precursor cell proliferation and phosphorylation of p-c-Raf, ERK1/2, pERK1/2 and c-Myc, respectively. Our findings suggest that melatonin may stimulate proliferation of adult precursor cells via ERK/MAPK pathway. As stem cell replacement is thought to play an important therapeutic role in neurodegenerative diseases, understanding the regulatory mechanism of melatonin might be beneficially used for stimulating endogenous neural stem cells.

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TU01-32
GEMININ IS ESSENTIAL FOR THE GENERATION AND DIFFERENTIATION OF ENTERIC NERVOUS SYSTEM PROGENITOR CELLS
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Enteric Nervous System (ENS) mainly derives from vagal neural crest that colonizes the entire gut. In addition to that anterior trunk neural crest colonizes esophagus and part of the stomach, while sacral neural crest colonizes the hindgut. In mice this process starts at approximately embryonic day 9.5 (E9.5dpc) and the colonization of the entire gastrointestinal tract has been completed by E14.5dpc. The formation of a fully functional ENS depends on the extensive proliferation of these ENS progenitor cells and their progressive differentiation into neuron and glial cells. Our goal is to investigate how cell cycle control is integrated with signaling cues that promote differentiation in the developing enteric nervous system. Towards this direction we are studying the role of Geminin on the maintenance, migration and differentiation of self-renewing enteric progenitor cells (EPCs). Geminin is a coiled-coil protein that has been shown to inhibit replication and interact with transcription factors and chromatin modifying complexes affecting the balance between self-renewal and differentiation. We have generated and analysed mice that lack Geminin expression specifically in enteric nervous system and our findings show that Geminin is necessary for survival and maintenance of EPCs in a non differentiating state. As a result, the enteric nervous system of mice that lack Geminin expression is aganglionic reminiscent Hirschsprung disease.

TU01-33
ROLE OF THE ORPHAN NUCLEAR RECEPTOR NR5A2 IN NERVOUS SYSTEM DEVELOPMENT
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NR5A2/LRH-1 is an orphan nuclear receptor that has been associated with liver differentiation and function. It plays an important role in embryogenesis since knockout mice embryos die at E6.5. In addition, recent evidence suggests that NR5A2 can replace Oct4 in the reprogramming of murine somatic cells to pluripotent cells. We have recently shown that NR5A2 is involved in the Proxl-mediated suppression of Notch1 gene expression
during neuronal differentiation (Kaltezioti et al., 2010, PLoS Biol.). However, the physiological function of NR5A2 in the nervous system (NS) is still elusive. To this end, we examined the expression pattern of NR5A2 in the developing NS of mouse and chick embryo. NR5A2 was shown to be expressed throughout neuronal lineage in central and peripheral NS, both in ventricular (VZ) and mantle (MZ) zones, where neural stem (NSCs) and differentiated cells lie, respectively. Accordingly, in the spinal cord, NR5A2 is detected at higher levels in bIII-tubulin+ and NeuN+ post-mitotic neurons than in Nestin+ and Pax6+ NSCs, suggesting a correlation to neuronal differentiation. NR5A2+ cells are also found in various brain regions and ganglia as well as in the eye. To further understand the role of NR5A2 in the regulation of proliferation versus differentiation decisions of embryonic NSCs derived from mouse spinal cord, we performed in vitro gain-of-function experiments. Forced expression of NR5A2 in NSCs and in Neuro2A mouse neuroblastoma cell line, led to a significant decrease in the proportion of BrdU+ cells. Additionally, the proportion of Nestin+ NSCs was dramatically reduced, indicating a negative effect in their self-renewal capacity. Furthermore, in agreement with our in vivo and in vitro expression studies, which showed that NR5A2 is largely excluded from GFAP+ astrocytes, NR5A2 over-expression was sufficient to inhibit astroglogenesis. Interestingly, we observed a partial increase in the proportion of bIII-tubulin+ neurons. Taken together, these observations indicate an important function of NR5A2 in NS development, rendering it a candidate gene for therapeutic strategies in future regenerative medicine applications.

TU01-35
PROTEIN KINASE A ACTIVATION PROMOTES DIFFERENTIATION OF N2A CELLS MEDIATED BY A HORMETIC INCREASE IN REACTIVE OXYGEN SPECIES
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The development of the central nervous system requires the generation of hundreds of different cell types and their specialization. The understanding of this system is the fundamental goal of developmental neuroscience not only for the elucidation of the natural neuron development but also for the advancement of new regenerative strategies to treat degenerative diseases. A plethora of signaling molecules are known to affect neural differentiation. Between them, reactive oxygen species (ROS) are on the focus in this study. ROS have been widely considered as harmful for cell development and as promoters of cell aging by increasing oxidative stress. However, ROS have an important role in cell signaling and they have demonstrated to be beneficial by triggering hormetic signals, which could prevent the organism from later insults. N2a murine neuroblastoma cells were used as a paradigm of neural differentiation. Differentiation was triggered by two well known activators of PKA, forskolin (activator of adenylate cyclase) and db-cAMP (cAMP analog). A marked differentiation was detected by fixation and staining with coomassie brilliant blue after 48 h treatment. In line with these results, an increase in free radicals was detected by flow cytometry and fluorescence microscopy on cells treated for 48 h with both PKA activators. Nitric oxide and superoxide anion were selected for further study due to the fact that they are important signaling molecules in cellular processes including differentiation. Nitric oxide is known for increasing the number of mitochondria, which are the major ROS producers within the cells. Nitric oxide was found not to be increased in differently treated cells as detected by Griess reaction. However, superoxide anion, detected by a superoxide cell-based biosensor, was increased in cells treated for 48 h with forskolin and db-CAMP.

Our data suggest that differentiation triggered by PKA activation can be part of a hormetic response which is mediated by a modest increase of ROS within the cell.

TU01-36
STUDY ON QUALITY OF MOUSE PLURIPOTENT STEM CELLS INCORPORATED FIBROBLAST FEEDER LAYERS USING A MICRO-ASSAY
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Undifferentiated pluripotent stem cells (ES and iPS cells) are formed in colonies on a feeder cell layer of fibroblast. It is known that a feeder layer have activity on the growth and maintenance in the pluripotent state of the stem cells. They also support the stem cells attachment through extracellular matrix and adhesion molecules. However, fibroblasts are primary cultures with a limited mitotic potential and low cell yield which are observed after the initial embryo dissociation. Some cells may be recovered by passaging. During passaging the cell from several plates onto a single plate, feeder cells differ in their capacity to support the activity for stem cells. Whereas plate can still become confluent, the cell in size will change. Here, we focused on growth and
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undifferentiation of the stem cells and cell in size in fibroblasts. The supportive potential of the feeder cell culture conditions were indirectly evaluated by measuring expression of stem cell markers on co-cultured pluripotent stem cells. Flow cytometer is shown three different types (large, middle and small in size) in fibroblast cell suspension. After one passage, the morphology of some cells was changed into larger and huge spreading one and observed in fibroblast cell suspensions of which passage number 2–8. We sorted the three fibroblast groups by a FCM, mitotically inactivated and cultured them for making feeder layer, respectively. These 1000 cells were attached one microliter to the substratum of the culture dish (micro-assay). Mouse pluripotent stem cells were seeded on the three feeder layers. Results show that fibroblast cells in size affected the growth and colony property of the stem cells. On all fibroblast layers, some colonies were thin and others were thick. The thin colonies showed low expression of pluripotent marker, but thick colonies was stronger. Therefore small-type fibroblast which supported many thick colonies would be high quality as a feeder layer.

TU01-37

POSSIBLE INVOLVEMENT OF ACTIVATED MICROGLIA IN PROMOTION OF ENDOGENOUS NEUROGENESIS FOLLOWING NEURONAL LOSS IN THE DENTATE GYRUS

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Neurological injuries are widely known to promote endogenous neurogenesis in hippocampal dentate gyrus of adulthood. Our previous studies demonstrated that the granule cells in the hippocampal dentate gyrus are injured and disappeared by treatment with trimethyltin chloride (TMT), with being regenerated in the dentate granule cell layer (GCL) after neuronal loss. To evaluate the involvement of activated microglia in proliferation of neural stem/progenitor cells after dentate granule cell loss, we determined the expression of microglia-related factor in the hippocampus after TMT treatment. Mice were given TMT (2.8 mg/kg, i.p.) to prepare slices for immunohistochemical analyses of Iba1 (microglia marker), nestin (neural stem/progenitor marker), and brain lipid binding protein (BLBP, radial glial marker). Cells positive to Iba1, nestin, and BLBP markedly increased in the subgranular zone (SGZ)/GCL, molecular layer, and hilus on days 3–7 (regeneration stage) after TMT treatment. RT-PCR analysis revealed that a significant increase in the mRNA levels of TNFalpha, IL-1beta, and IL-6 was seen in the hippocampus on day 3 post-TMT treatment. Immunohistochemical studies revealed that TMT markedly augmented nuclear translocation of NF-kappaB p50 and p65 in cells of the SGZ. Double immuno-staining revealed that the majority of cells positive for nestin and BLBP had p65 immunoreactivity in the SGZ on day 3 after TMT treatment. Taken together, our results support the possibility that pro-inflammatory cytokines released from activated microglia may be involved in promotion of endogenous neurogenesis through activation of NF-kappaB signaling pathway following the dentate neuronal loss induced by TMT treatment.

TU01-38

IGF-II AND IR PROMOTE NEURAL PRECURSOR STEMNESS

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The insulin-like growth factor (IGF) system plays a critical role in brain development and growth. IGF-I and IGF-II both activate the IGF-1R. In contrast, IGF-II, but not IGF-I can activate a splice variant of the insulin receptor (IR) known as IR-A. We hypothesized that IGF-II will exert distinct effects on neural stem/progenitor cells (NSPs) than IGF-I. IHC revealed that IGF-II is expressed as a gradient in the neural stem cell niche. Q-PCR analysis revealed that IGF-II mRNA is highly expressed by the choroid plexus. Additionally, Q-PCR showed that the IGF-1R and the IR isoforms are differentially expressed between NSPs and more lineage restricted cells, with IR-A being predominant in NSPs. IGF-II promoted neural stem/progenitor cell expansion better than either IGF-I or standard culture medium (containing superphysiological levels of insulin). A combination of IGF-I and IGF-II mimicked standard neurosphere growth conditions in terms of neurosphere number and size; however, limiting dilution and differentiation analyses revealed that IGF-II was superior to IGF-1 in promoting neurosphere number. Knockdown of either the IR or IGF-1R using shRNAs supported the conclusion that the IGF-1R promotes progenitor proliferation whereas the IR is important for self-renewal. RT Q-PCR revealed that IGF-II increased Oct4, Sox2 and FABP7 mRNA levels in neurosphere cells. Altogether our data support the conclusion that IGF-II promotes the self-renewal of neural stem/progenitors via the IR. By contrast, IGF-1R functions as a mitogenic receptor that increases cell cycle progression of progenitors. Supported by a Dean’s Grant from NJMS awarded to SWL and TLW and F31NS065607 awarded to ANZ.

TU01-39

NUCLEOTIDE SIGNALLING IN ADULT NEUROGENESIS

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In the adult mammalian brain, the subgranular layer of the hippocampus (SGL) and the subependymal zone (SEZ) at the lateral ventricles harbour progenitor cells providing new neurons for the granule cell layer and the olfactory bulb, respectively. Nucleotides act via a multiplicity of receptors that differ regarding agonist specificity and the induced intracellular signal pathways. Nucleotide signalling is terminated or modulated by cell surface-located nucleotide-hydrolyzing enzymes (ectonucleotidases). We previously showed that ectonucleoside triphosphate diphosphohydrolase 2 (NTPDase2), an enzyme that hydrolyzes extracellular nucleoside triphosphates to the respective nucleoside monophosphates, is specifically expressed by neural progenitors in the SEZ and SGL (1–3). Both nucleotides and the growth factor EGF stimulated in vitro progenitor cell proliferation and migration and induced converging intracellular signalling pathways (3, 4), implicating a role of nucleotides in neurogenesis in vivo. In NTPDase2 knock-out mice proliferation of neural progenitors cells cultured as...
neurospheres was enhanced by a factor of two. Mice were subjected to time-controlled protocols of intraperitoneal BrdU application and short and long-term survival of labelled cells was investigated. In NTPDase2 knockout mice progenitor cell proliferation was increased twofold in both the SEZ and the dentate gyrus, whereas young neuron survival in the olfactory bulb and in the hippocampus was not altered. Our data suggest that NTPDase2 knockout increases extracellular nucleotide concentrations in the neurogenic niches, resulting in enhanced progenitor cell proliferation. These data provide first in vivo evidence for a contribution of purinergic signalling to the control of adult neurogenesis.

References:

TU02 Brain Bioenergetics

TU02-01

MODULATION OF CEREBRAL ASCORBATE LEVELS BY PARENTERAL ASCORBATE ADMINISTRATION

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Ascorbate (vitamin C) is present in the brain tissue, especially in neurons, at concentrations significantly higher than plasma [1–3]. Currently the mechanism of ascorbate transport into brain is controversial. With parenteral ascorbate administration of pharmacologic doses using intraperitoneal injection (i.p.), plasma concentrations are ~100 fold higher than with oral administration [4]. Such pharmacologic plasma ascorbate concentrations could overwhelm normal brain transport mechanisms, thereby leading to higher concentrations in brain. Here we investigated the effect of i.p. ascorbate administration on cerebral ascorbate levels in living animals. Nine Sprague-Dawley rats were studied before and after i.p. ascorbate injection. 1H MR spectra were acquired from a voxel (90 μl) in the hippocampus at 9.4T. Ascorbate concentrations at ~1 hour post infusion were significantly higher compared with those at the baseline by 20% and 60% at doses of 1 and 3 g/kg, respectively. Based on blood volume in rat brain of 3.4 ml/100 g [3] and ascorbate concentrations in plasma following i.p. injection [5], the potential influence of high plasma ascorbate to brain ascorbate signals was estimated to cause at most 9% and 25% signal increases for 1 and 3 g/kg doses, respectively. Thus the observed ascorbate signal increases of 20–60% cannot be explained by elevated plasma ascorbate levels. These data are the first to indicate that cerebral ascorbate accumulation in living animals can occur by the presence of sufficiently high levels of plasma ascorbate. This work is partly supported by NIH (R21DK081079 to Choi).

References:

TU02-02

ADENO-ASSOCIATED VIRUS MEDIATED DELIVERY OF PEPTIDE AGONISTS INTO THE RAT BRAIN

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Relaxin-3 is a highly conserved neuropeptide, present in ascending projections from the pontine nucleus incertus that target relaxin family peptide receptor 3 (RXFP3) expressing neurons, in limbic and hypothalamic areas. Relaxin-3/RXFP3 signalling has been implicated in metabolism, reproduction and stress by studies using an RXFP3 selective agonist (R3/I5) and antagonists. Central peptide administration is limited and precludes insights into chronic effects of RXFP3 modulation. This study was designed to develop recombinant adeno-associated viruses (rAAV) to chronically produce R3/I5 within RXFP3-rich brain areas of rats and assess the neuroendocrine and behavioural impact. R3/I5 was cloned into the viral vector, CB-TR-FIB, which contains a fibronectin secretory signal sequence facilitating constitutive peptide secretion in vivo. Constitutive secretion of peptide was tested by transfection of HEK293T cells with FIB-R3/I5 and analysis of media by specific immunoassay and activity assays in RXFP3 expressing cells. Both assays demonstrated that FIB-R3/I5 was able to direct the constitutive secretion of bioactive R3/I5 in vitro. Vectors (expressing GFP or FIB-R3/I5) were packaged into mosaic serotype 1/2 capsids to produce rAAV. rAAV1/2-GFP was demonstrated to efficiently transduce neuronal-like GT1-7 cells in vitro, the virus was titrated before stereotaxic bilateral infusion into the hypothalamus of Sprague–Dawley (SD) rats. rAAV1/2-GFP successfully transduced oxytocin expressing hypothalamic neurons in vivo. We have successfully produced a recombinant construct to direct the constitutive secretion of a specific RXFP3 agonist. Additionally we have shown that rAAV1/2 efficiently transduces hypothalamic neurons in vitro and in vivo. Therefore, rAAV-FIB-R3/I5 will now be utilised to demonstrate the effects of chronic RXFP3 activation in the hypothalamus. This novel approach will yield further insight into the role of relaxin-3/RXFP3 signalling in the brain.

TU02-03

CONSEQUENCES OF A TREATMENT OF CULTURED ASTROCYTES WITH IRON FROM IRON OXIDE NANOPARTICLES

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Magnetic iron oxide nanoparticles (Fe-NP) are considered for various diagnostic and potential therapeutic applications in the...
central nervous system. Although, Fe-NP are able to cross the blood-brain-barrier, little is known on the consequences of a treatment of brain cells with Fe-NP and on the fate of the iron in such particles incorporated. To address such questions, we exposed astrocyte-rich primary cultures with dimercapto succinate-coated Fe-NP. During a 4 h Fe-NP exposure, the cellular iron content of viable cultured astrocytes increased from initially 16 ± 3 nmol/mg to up to 1500 nmol/mg. Despite this 100-fold elevated cellular iron content the viability of the cells was not compromised. The cells remained viable after removal of exogenous Fe-NP and subsequent incubation for up to 7 d. During this time the cellular iron content remained almost constant. Compared to controls, neutral red uptake and the lactate release were not altered in Fe-NP-treated astrocytes. In addition, the ratio of cellular glutathione disulfide to glutathione remained very low and evidence for increased production of reactive oxygen species was not observed, suggesting that Fe-NP-treated astrocytes do not suffer from oxidative stress. However, the strong upregulation of the iron storage protein ferritin in Fe-NP-treated astrocytes demonstrates that these cells liberate iron from accumulated Fe-NP which subsequently induces ferritin synthesis. These results demonstrate that under the conditions used an Fe-NP treatment is not toxic for cultured astrocytes and that these cells liberate iron from Fe-NP and store it in ferritin.

TU02-04
GOOD OR BAD? - EXPOSURE OF OLIGODENDROGLIAL CELLS TO IRON OXIDE NANOPARTICLES
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Magnetic iron oxide nanoparticles (Fe-NP) are considered for various applications in neurobiology, for example for drug delivery or as contrast agent for magnetic resonance imaging. However, little is known so far about the biocompatibility and the fate of such particles in brain cells. We have used the oligodendroglial cell line OLN-93 to investigate these questions. Exposure of OLN-93 cells to dimercapto succinate-coated Fe-NP containing a total iron concentration of up to 1000 μM did not compromise cell viability, although the specific cellular iron content increases about 100-fold within 48 h reaching 976 ± 86 nmol iron/mg protein. Despite this high specific iron content hardly any generation of reactive oxygen species was observed and the specific cellular glutathione content and the cellular ratio of glutathione disulfide to glutathione were not altered. In the presence of Fe-NP, OLN-93 cells were able to partially bypass the inhibition of proliferation that is induced by the iron chelator deferoxamine. In addition, the exposure of OLN-93 cells led to a concentration dependent increase in the amount of the iron storage protein ferritin. Both, cell proliferation under iron restricted conditions and the increase of ferritin levels demonstrate that OLN-93 cells are able to liberate low molecular weight iron from accumulated Fe-NP. The results obtained demonstrate that accumulation of Fe-NP into OLN-93 cells does not induce oxidative stress and suggests that an application of Fe-NP could be a save approach to deliver iron to brain cells.

TU02-05
EFFECTS OF METHANOL ON ROTATIONAL MOBILITY OF N-(9-ANTHRHYLOXY) STEARIC ACID IN NEURONAL AND MODEL MEMBRANES
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To provide a basis for studying the molecular mechanism of pharmacological action of n-alkanols, we carried out a study of the membrane action of the methanol. Neuronal membranes (SPM) were isolated from fresh bovine cerebral cortex. Liposomes of total lipids (SPMVTL) and phospholipids (SPMVPL) extracted from the SPM. The set of n-(9-anthryloxy) stearic acid (n-AS) probes (n = 2, 6, 9, 12 and 16) have been used to examine gradients in fluorescence polarization. In a dose-dependent manner, methanol decreased the anisotropies of 6-AS, 9-AS, 12-AS and 16-AP in the hydrocarbon interior of SPMV, SPMVTL and SPMVPL, but the methanol increased the anisotropy of 2-AS in the membrane interface. The magnitude of rotational mobility in accordance with the carbon atom numbers of phospholipids comprising SPMV, SPMVTL and SPMVPL was in the order at the 16, 12, 9, 6, and 2 position of aliphatic chain present in phospholipids. The sensitivity of increasing or decreasing effect of rotational mobility of the hydrocarbon interior or surface region by methanol differed depending on the carbon atom numbers in the descending order of 16-AP, 12-AS, 9-AS, 6-AS and 2-AS. Furthermore, the sensitivity of increasing or decreasing effect of rotational mobility of the hydrocarbon interior or surface region by the methanol differed depending on the neuronal and model membranes in the descending order of the SPMV, SPMVPL and SPMVTL.

TU02-06
ACCUMULATION OF FLUORESCENT IRON OXIDE NANOPARTICLES BY CULTURED MICROGLIAL CELLS
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In the last years iron oxide nanoparticles came into focus for medical applications in brain and have been considered as cancer treatment or contrast agents for magnetic resonance tomography. However, little is known on the consequences of a treatment of brain cells with such particles. Microglial cells are the immune cells of the brain and may be affected strongly by the presence of nanoparticles. To address this question, we have investigated how cultured microglial cells react upon exposure to fluorescent magnetic iron oxide nanoparticles (ffFeNP). Exposure of microglial cells to up to 15 μM iron as ffFeNP for up to 3 hours did not result in an alteration of the distribution of intracellular activity of the cytosolic enzyme lactate dehydrogenase (LDH), indicating that these conditions did not compromise the membrane integrity. In
addition, the glutathione content and the ratio of glutathione to glutathione disulfide were not affected, nor was reactive oxygen species (ROS) production detected after treatment with FeNP. Incubation of microglia cells with FeNP caused a time- and concentration-dependent accumulation of iron in the cells. After 4 hours of incubation with 15 μM iron supplied as FeNP, 80% of the iron applied was accumulated by the cells. Staining for cellular iron by the Perls’ method showed a co-localization of iron and fluorescence. These data demonstrate that viable microglial cells efficiently accumulate FeNP. Absence of oxidative stress and co-localization of fluorescence and iron suggest that the accumulated FeNP remain stable within the cells under the conditions used.

TU02-07
ACUTE AND CHRONIC ETHANOL EFFECTS ON β-ENDORPHIN EXPRESSION IN THE RAT BRAIN
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The dopaminergic mesocorticolimbic system plays an important role in the reinforcing effects of ethanol. Opioid peptides modulate the activity of this system and have been suggested to mediate, at least in part, the reinforcing properties of ethanol. Thus, beta-endorphin (β-END) could participate in the development of ethanol reinforcement and addiction. The aim of this work was to investigate the acute and chronic ethanol effects on β-END content in regions of the mesocorticolimbic system and to examine if chronic ethanol treatment alters ligand binding to mu opioid receptor (μOR). Male Wistar rats received a single acute ethanol dose of 2.5 g/kg or water by intragastric administration. For chronic ethanol treatment experiments, one group of rats was given ethanol (10% v/v solution) to drink, two groups were given equivalent ethanol treatment experiments, one group of rats was given ethanol dose of 2.5 g/kg or water by intragastric administration. For chronic ethanol treatment experiments, one group of rats was given ethanol (10% v/v solution) to drink, two groups were given equivalent volumes of sucrose (14.14% isocaloric solution) or water, respectively, and a fourth group had ad libitum access to food and water. Treatment was followed for 4 weeks. Beta-endorphin content in brain regions was quantified by radioimmunoassay and ligand binding studies to μOR were performed by quantitative autoradiography using 8 nM [3H]-DAMGO, MePheγ, Gly-ol-[3H]-enkephalin (μ)-DAMGO as radioligand. Acute ethanol decreased β-END content in the hypothalamus (26%) 1 h after administration. No ethanol effects were observed in the midbrain, ventral tegmental area, substantia nigra, nucleus accumbens, nucleus accumbens-septum and prefrontal cortex. Chronic ethanol treatment neither changed β-END levels nor [3H]-DAMGO binding to mu opioid receptors in any of the regions studied. However, β-END levels in the sucrose group were significantly increased in the nucleus accumbens and substantia nigra, in comparison to all other groups. These findings suggest that different neural mechanisms and specific brain regions may be involved in the reinforcing effects of ethanol and sucrose.

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TU02-08
SUBstrate SELECTIVITY and KINETICS for GAMMA-HYDROXYBUTYRATE DEHYDROGENASE FROM RALSTONIA EUTROPHA
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The current report uses cloned iron (II)-dependent gamma-hydroxybutyrate dehydrogenase (GHB-DH; EC 1.1.1.61) originally from the bacterium Ralstonia eutropha to test 42 natural and synthetic compounds for substrate activity. GHB is a human drug of abuse recently accepted as a natural neurotransmitter. In order of descending efficacy, good substrates of GHB-DH are trans-4-hydroxyxylonate, gamma-hydroxybutyrate, trans-3-hydroxypropylsulfonate (synthetic), and (RS)-2-methyl-4-hydroxybutyrate (synthetic). They each contain a primary alcohol, a singly charged, negative functional group located 3 carbon atoms from the alcohol, and a conformaion presumably similar to that of substantially fixed trans-4-hydroxyxylonate. Ethanol at > 1% (v/v) also is a substrate. The GHB-like drug of abuse (RS)-4-hydroxypentanoate and other analogues, homologues, and metabolites of GHB are poor or non substrates. No other good natural substrate of GHB-DH is likely to exist. GHB and 3-hydroxypropylsulfonate exhibit initial-velocity kinetics diagnostic of random-order binding by the alcohol and NAD⁺ to the dehydrogenase. When testing otherwise normal human urine or blood under the prescribed conditions, GHB-DH from Ralstonia eutropha will detect only ingested GHB.

TU02-09
REGULATION OF AXONAL MITOCHONDRIA MOTILITY VIA AN INTERACTION BETWEEN MILTON AND O-GLCNAC TRANSFERASE
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Tight regulation of mitochondria distribution in neurons in response to local energy changes or metabolic demand is essential for cell survival. Axonal transport of mitochondria from the cell body toward axon terminals is generated by Kinesin-1, which interacts with the mitochondrial protein Miro through an adapter protein Milton. Milton has been shown to bind to a cytosolic enzyme O-GlcNAc Transferase (OGT) that is responsible for a post-translational modification called O-GlcNAcylation. Enzymatic activity of OGT is regulated by nutrient availability and metabolic state of the cell. We have studied the role of OGT in the regulation of mitochondria motility. We show that OGT overexpression in neurons leads to a motility arrest through a direct interaction with Milton. This motility arrest can be rescued by disruption of the OGT binding domain on Milton. We also show that OGT recruitment to Milton doesn’t disturb the Kinesin-Milton-Miro motor complex. Changes in OGT substrate level also change o-GlcNAcylaion levels and have a significant effect on mitochondria motility. Thus, OGT catalyzed post-translational modifications are likely to regulate mitochondria motility in axons.
TU02-10

PHYSICAL AND ANATOMIC COMPARTMENTALIZATION OF ASTROGLIAL GLUTAMATE TRANSPORTERS WITH GLYCOLYTIC ENZYMES AND MITOCHONDRIA

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Astroglial Na+-dependent transporters consume energy to maintain low synaptic concentrations of glutamate (25 nM) in an environment that contains millimolar concentrations of this excitatory neurotransmitter. Several studies have linked glutamate transport to changes in astrocytic glycolysis and to changes in mitochondrial function. We immunoprecipitated GLT-1 from rat brain and subjected the material to analysis by mass spectrometry. Subsequent forward and reverse immunoprecipitations from various brain regions or transfected cells were conducted. These studies suggest that the glial glutamate transporters (GLT-1 or GLAST) exist in a multi-protein complex with the Na+/K+ ATPase as well as several glycolytic enzymes and mitochondria. Several lines of evidence indicate that GLT-1 is not present in mitochondria. We examined co-localization of GLT-1 with the mitochondrial protein UQCRC2 in vivo (there was significant covariance of staining, IC0 = 0.11 ± 0.02, p < 0.005, n = 9). We co-transfected individual astrocytes in hippocampal slice cultures with fluorescently tagged variants of GLT-1 and the mitochondrial targeting sequence of cytochrome c oxidase subunit VIII. We find that 68 ± 3% of GLT-1 puncta overlap with mitochondria in fine astrocytic processes (51 processes, from 11 cells). Together these studies suggest that the glial glutamate transporters exist in a multi-protein complex with Na+/K+ ATPase as well as several glycolytic enzymes and mitochondria. We are currently mapping domains of GLT-1 required for these interactions and examining the potential metabolic implications of this compartmentalization. This anatomic compartmentalization of the glial glutamate transporters may spatially support energy production and ion buffering. In addition, it may influence glutamate disposition in astroglia.

TU02-11

NMDA RECEPTORS STABILIZE GLYCOLYTIC KEY-PROMOTING ENZYME PKFB3 IN CORTICAL NEURONS

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Neurons are known to have a very low glycolytic capacity when compared with astrocytes (1), and this explains the higher vulnerability of neurons to mitochondrial bioenergetic stress and hypoxia. Recently, we revealed (2) that the low glycolytic rate in neurons could be wholly ascribed for by continuous degradation of a key glycolytic-promoting enzyme, 6-phosphofructo-2-kinase/fructose-1,6-bisphosphatase-3 (Pfkfb3) which, by synthesizing fructose-2,6-bisphosphate, is an activator of 6-phosphofructo-1-kinase activity, the master regulator of glycolysis. Pfkfb3 protein degradation is induced by the E3 ubiquitin ligase anaphase promoting complex/cyclosome (APC/C) (2), which requires its adaptor protein Cdh1 for this activity. When Cdh1 is phosphorylated by Cdk5 upon N-methyl-D-aspartate (NMDA) receptor stimulation (3), APC/C becomes inactive; however, whether NMDA receptors control glycolysis in neurons through regulating the stability of Pfkfb3 remains unknown. Here, we addressed this issue and stimulated NMDA receptors in rat cortical neurons in primary culture with glutamate (100 micromolar for 15 minutes); this induced Cdh1 phosphorylation, as expected (3), as well as Pfkfb3 protein accumulation and mitochondrial reactive oxygen species (ROS) production. These events were prevented in the presence of NMDA receptor antagonist, MK801, and increased ROS was prevented by inhibition of glycolysis using small interfering RNA against phosphoglucose isomerase. We further show that glutamate induced the release from the nucleus to the cytosol of Pfkfb3, as it spontaneously occurred by expressing a Pfkfb3 mutant form lacking the Cdh1-recognising KEN motif. These results suggest that APC/C-Cdh1-mediated Pfkfb3 stabilization by NMDA receptor stimulation may be an important contributing factor in the control of neuronal bioenergetics, oxidative stress and excitotoxicity. [Funded by MICINN (SAF2010-20008; CSD2007-00020), FIS (PS09/0366) and JCyL. (GREX206)].

References:

TU02-12

DIFFERENTIAL EFFECTS OF CHLORINATED ACETATES ON GLUTATHIONE AND GLUCOSE METABOLISM OF ASTROCYTES

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The chlorinated acetates monochloroacetate (MCA), dichloroacetate (DCA), and trichloroacetate (TCA) are environmental toxins that are generated in water disinfection processes and are formed during metabolic detoxification of industrial solvents such as trichloroethylene. DCA gained interest as investigational drug for the treatment of metabolic acidosis and myocardial or cerebral ischemia. In order to test for the beneficial and/or toxic consequences of an exposure of brain cells to the different chlorinated acetates, glutathione levels and lactate production of primary astrocyte cultures were investigated as indicators for the potential of chlorinated acetates to disturb cellular detoxification processes and glucose metabolism, respectively. Exposure of the cells to MCA caused a time and concentration dependent deprivation of cellular glutathione, inactivation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity, and loss in cell viability with halfmaximal effects observed for MCA concentrations of 0.3 mM, 3 mM and 10 mM, respectively. In contrast, the presence of DCA, or TCA even in a concentration of 10 mM did not compromise cell viability nor affect cellular glutathione content or GAPDH activity. However, the presence of DCA and TCA significantly lowered the rate of cellular lactate production in viable astrocytes with TCA being by a factor of two more potent than DCA. These data demonstrate that the extent of chlorination strongly determines the potential of chlorinated acetates...
to affect glutathione and/or glucose metabolism of astrocytes and suggest that TCA rather than DCA could be used to inhibit lactate production by astrocytes to prevent metabolic acidosis.

**TU02-13**

**THE EFFECTS OF AGING AND DIETARY RESTRICTION ON CHOLESTEROL METABOLISM IN RAT CORTEX**

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Maintaining the cholesterol homeostasis is essential for normal CNS functioning and is accomplished by a series of interdependent processes that include synthesis, storage, transport and removal of excess of cholesterol. Enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) is involved in cholesterol biosynthesis, while enzyme responsible for cholesterol excretion from the brain is cholesterol 24S-hydroxylase (Cyp46). Cholesterol synthesis is strongly balanced with cholesterol excretion. Brain cholesterol is recycled by a very efficient apolipoprotein-dependent process involving apolipoprotein E (ApoE). Liver X receptors (LXRs) act as cholesterol sensors and regulate cholesterol homeostasis. It was shown that dietary restriction (DR) may enhance brain functions that diminish with aging, including learning and memory, synaptic plasticity, and neurogenesis. The aim of this study was to analyze the influence of aging and long-term dietary restriction on cholesterol homeostasis in the rat cortex. The experiments were performed on 3-, 12-, and 24-month-old male Wistar rats fed ad libitum (AL), or exposed to long term DR (100% every other day-EOD) starting from 3 months of age. The levels of cholesterol and its metabolite in the brain, 24S-hydroxycholesterol, were measured by gas chromatography/mass spectrometry. Expression of proteins involved in cholesterol metabolism (HMGCR, Cyp46, ApoE and LXRs) was determined using Western blot analyses. Aging induced slight but significant increase of cholesterol in the rat cortex, while the level of 24S-hydroxycholesterol remained stable throughout whole aging period. Expression of proteins involved in cholesterol metabolism was affected in different manner during aging. ApoE expression was increased, while HMGCR expression was decreased in aged rat cortex. There were no changes in Cyp46 and LXRs expression. DR has shown the most prominent influence on ApoE expression, maintaining it on control level during aging. Long-term DR maintained cholesterol homeostasis in aged rat cortex by affecting cholesterol trafficking via modulation of ApoE expression.

**TU02-14**

**ROLE OF GDH2, THE HUMAN ISOFORM OF GLUTAMATE DEHYDROGENASE, IN ASTROCYTE METABOLISM**

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Astrocyte processes encircle the synaptic area of glutamatergic synapses and have numerous obligations in the attempt to provide optimal conditions for neuronal function, such as maintenance of glutamate homeostasis. Glutamate dehydrogenase (GDH) catalyzes the reversible oxidative deamination of the excitatory neurotransmitter glutamate to α-ketoglutarate, predominantly in astrocyte mitochondria. It thus occupies a central position interlinking glutamate neurotransmitter homeostasis, ammonia and energy metabolism. GDH exists in two isoforms GDH1 and 2. GDH2 is a nerve-tissue specific form only expressed in humans and apes. They are both activated by ADP and leucine. However, the effect is 10-fold higher for GDH2 than for GDH1 and GDH2 is barely active in the absence of ADP. GDH2 is, in contrast to GDH1, insensitive to an inhibitory effect of GTP. To unravel the role of GDH2, mice transgenic for GLUD2 were generated. Two lines were examined expressing GLUD2 mRNA at levels approximately 5- and 24-fold times higher than in human brain. Energy and glutamate metabolism were investigated in cultured astrocytes using [14C] glutamate and monitoring of 14CO2. Carbon and nitrogen metabolism were further mapped employing [13C] glucose, [13C] glutamate, 15NH4+ and [15N]glutamate, and analyses of cell extracts and media were performed using mass spectrometry. Interestingly, the CO2 production from [14C] glutamate increased significantly in astrocytes from the transgenic mice when they were incubated with 500 μM [14C] glutamate in the absence of glucose. This effect was abolished when 2.5 mM glucose was added during the incubation. This indicates an increased capability in the transgenic animals to utilize glutamate to sustain metabolism in the absence of glucose. In addition, the cultured astrocytes obtained from the transgenic animals show a dramatic increase in the content and release of glutamate after exposure to 15NH4+ which point towards a different handling of ammonia in GDH2 expressing animals.

**TU02-15**

**FORMALDEHYDE STIMULATES MRP1-MEDIATED GSH EXPORT FROM CULTURED ASTROCYTES**

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Formaldehyde is an environmental toxin that is also endogenously produced in the brain. The level of formaldehyde in brain has been suggested to be elevated with age or in neurodegenerative disorders like Alzheimer’s disease. Since the tripeptide glutathione (GSH) plays an important role in the detoxification of xenobiotics by brain cells, we tested for the consequences of a formaldehyde exposure on the GSH metabolism of brain cells using astrocyte-rich primary cultures as model system. Treatment of these cells with formaldehyde resulted in a rapid time- and concentration-dependent depletion of the cellular GSH. Exposure of astrocytes to 1 mM formaldehyde for 3 hours almost completely deprived the cells of GSH. The decrease in cellular GSH levels on exposure to formaldehyde was accompanied by a matching increase in the extracellular GSH content, although the viability of the cells was not compromised. Analysis of the ratio of GSH to its disulfide GSSG has been suggested to be elevated with age or in neurodegenerative disorders like Alzheimer’s disease. Since the tripeptide glutathione (GSH) plays an important role in the detoxification of xenobiotics by brain cells, we tested for the consequences of a formaldehyde exposure on the GSH metabolism of brain cells using astrocyte-rich primary cultures as model system. Treatment of these cells with formaldehyde resulted in a rapid time- and concentration-dependent depletion of the cellular GSH. Exposure of astrocytes to 1 mM formaldehyde for 3 hours almost completely deprived the cells of GSH. The decrease in cellular GSH levels on exposure to formaldehyde was accompanied by a matching increase in the extracellular GSH content, although the viability of the cells was not compromised. Analysis of the ratio of GSH to its disulfide GSSG in both cells and media following formaldehyde treatment revealed that GSH was present almost exclusively. Deprivation of cellular GSH appears to be rather specific for formaldehyde, since its metabolites methanol and formate as well as acetaldehyde did not
Affect cellular GSH levels. Both cellular GSH deprivation and the increase in extracellular GSH content after formaldehyde exposure were completely prevented by the application of MK571, an inhibitor of the multidrug resistance protein 1 (Mrp1) which is known to mediate GSH efflux from cultured astrocytes. These data demonstrate that formaldehyde deprives astrocytes of GSH by stimulating Mrp1-mediated GSH export. This process could contribute to an altered GSH homeostasis in brain and subsequently lead to oxidative stress.

**TU02-16**

**ROLE OF MOTONEURON-DERIVED NT-3 ON SENSORY NEURON DEVELOPMENT**

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Motoneuron and sensory neuron interacts to form spinal neural circuit during development. However, molecular mechanism underlying their interaction is not fully understood. To investigate the role of motoneuron derived factor(s) on sensory neuron development, we analyzed sensory neuron phenotypes in the dorsal root ganglia (DRG) of Olig2 knockout (KO) embryos, which have no motoneurons in the spinal cord. We found increased number of apoptotic cells in the DRG. Furthermore, abnormal axonal projections of both the central and peripheral branch from sensory neurons were also observed. We focused on neurotrophin-3 (NT-3)/TrkC signaling, because NT-3 and its receptor, TrkC were strongly expressed in motoneuron and DRG neurons, respectively. Significance of motoneuron-derived NT-3 was investigated using conditional NT-3 knockout (NT-3 cKO) mice, in combination with Olig2-Cre driver mice. Our results indicated that motoneuron-derived NT-3 is important for sensory neuron development.
TU03-01
EFFECT OF DMARDS ON NEURONAL HYPERACTIVITY GENE: C-FOS, IN RESPONSE TO CHRONIC PAIN IN MODEL OF ADJUVANT-INDUCED ARTHRITIS
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Methotrexate and sodium aurothiomalate are one of the important drugs, used as a treatment for cancer suppression by acting on immune system. They are also used as preferential effective therapies for rheumatoid arthritis (RA) because they target the cause of pain and inflammation associated with arthritis and often referred as disease modifying anti-rheumatic drugs (DMARDs). In the present study, we have used four different DMARDs namely methotrexate and sodium aurothiomalate (gold salt), azothioprine, and chloroquine, and evaluated their role as a possible disease-modifying agents in the adjuvant-induced arthritis model of human RA in rats. Gait analysis was used to examine the role of these DMARDs in the development of pain. Body weights and paw volumes were also measured to monitor the progression of disease and the systemic anti-arthritis effects of the test DMARDs. Cellular immediate-early genes (c-fos) which reflects pattern of neuronal activity and can directly regulate the expression of pro-inflammatory agents including cytokines was used as a cellular marker to monitor the effect of the treatments on central pain processing. Our results showed that the tested DMARDs markedly inhibited the macroscopic inflammatory changes and significantly reversed the gait deficits seen in the control arthritic rats. Furthermore, the immunohistochemical analysis and RT–PCR analysis revealed that on the cellular level, the DMARDs showed a significant effect on c-fos mRNA and protein expression. Among the treatment groups, the maximum effect was seen with azothioprine followed by chloroquine when compared with arthritic control group. Our results suggest that among selected DMARDs treatment, azothioprine and chloroquine are most effective in controlling the pain related neuronal hyperactivity and may help in reducing the inflammation and have immunomodulatory activity and anti-arthritis properties.

TU03-02
ARACHIDONIC ACID IN RED BLOOD CELLS, IMMUNE CELLS AND PLASMA IN PATIENTS WITH MULTIPLE SCLEROSIS
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Arachidonic acid (C20:4n-6) is excised from the cell membrane phospholipids by phospholipase A2 (PLA2) and the free FA is used as a precursor for eicosanoid production, of which PGE2, a highly pro-inflammatory agent. PGE2 promotes inflammation by increasing vascular permeability and vasodilation and by directing the synthesis and migration of proinflammatory cytokines into the site of inflammation. The aim of the present study was to investigate the relationship between plasma and blood cell membrane fatty acids in patients with multiple sclerosis. The plasma, red blood cell and peripheral blood mononuclear cell membrane fatty acids were measured by gas chromatography. In red blood cells, C20:4n-6 was significantly decreased ($p < 0.05$), whilst in the immune cells the elongation product of C20:4n-6, C22:4n-6 was decrease and in plasma C18:2n-6 the parent fatty acid was decreased. In general PUFAs showed a positive correlation between plasma and RBC in both control and patient groups, however in patients C20:2n-6 did not correlate, between these compartments $p = 0.16$. In contrast, the relationship between plasma and PBMC PUFAs were completely changed in patients from that of controls; Controls showed highly significant positive correlations between PUFAs (both n-6 and n-3), MUFAs and SATS between these 2 compartments, while in MS no correlation was found between the n-6 fatty acids, including C20:4n-6 or SATS. Our findings suggest that fatty acids, particularly C20:4n-6, play a major role in the pathogenesis of multiple sclerosis, as shown by the disturbed relationship between C20:4n-6 between the blood compartment in MS patients.

TU03-03
POTENTIAL CONTRIBUTION OF RESIDENT MICROGLIA DURING INJURY-INDUCED NEUROGENESIS
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Adult neurogenesis occurs in the subgranular zone (SGZ) of the hippocampal dentate gyrus generating new dentate granule neurons. This process can be induced with brain injury suggesting a capacity for “self-repair” in the hippocampus. Both resident microglial cells and infiltrating macrophages produce inflammatory molecules in response to brain injury. While inflammation has been reported to be detrimental to hippocampal neurogenesis, other studies have suggested rather that the localized inflammatory response and stimulation of microglial cells can promote neurogenesis. Thus the question arises, what distinguishes beneficial versus adverse effects of inflammation on neurogenic “self-repair”? It is our groups working hypothesis that activated resident microglia may serve a supportive role during injury-induced neurogenesis in the hippocampus. To examine our hypothesis, we used the hippocampal toxicant, trimethyltin (TMT; 2.3 mg/kg, ip), as a tool to selectively target dentate granule cell death in adolescent CD-1 male mice. Within 48h post-TMT, neuronal death is accompanied by resident microglia activation, and elevations in tumor necrosis factor alpha (TNFα) and interleukin-1β (IL-1β) mRNA levels Bromodeoxyuridine (BrDU) incorporation identified the peak time of neurogenesis as coinciding with peak of neuroinflammation. BrdU+ cells were transiently in contact with process bearing microglia within the SGZ and inner granule cell layer (GCL). The proliferative response was sufficient to fully repopulate neurons in the GCL and provide functional recovery. Using laser-capture microdissection, SGZs...
Emergent seizures are common in Alzheimer’s disease (AD), although the mechanisms mediating this are unknown. We propose that N-methyl-D-aspartate receptor (NMDAr) agonist quinolinic acid (QA), a neurotoxic tryptophan metabolite of the kynurenine pathway, increases seizures and concurrently contributes to neuronal loss via excitotoxicity, including via QA impact on glutamate transport. We have found earlier that expression of pro-inflammatory interleukin-18 (IL-18, interferon-gamma inducing factor) is increased in the brain of AD-patients and it is detectable in microglia, neurons, astrocytes and amyloid-beta plaques. Interferon-gamma is a known inducer of indoleamine-2,3-dioxygenase (IDO), a key enzyme in induction of the kynurenine pathway. We clarified the role of stress inducible IL-18 in regulation of kynurenine pathway members with immunoblotting. Interferon-gamma was the strongest inducer of IDO in SH-SY5Y and NHA. IL-18, IL-1beta and TNF-alpha were able to modestly increase its expression, whereas IL-6 had only minor impact. In SH-SY5Y, IL-18 and IL-1beta dose-dependently increased the expression of kynureninase. QA increased expression of kynurenine aminotransferase II (KAT-II), producer of the alpha7-nicotinic receptor and NMDAr antagonist kynurenic acid (KynA) in both cell types. In conclusion, inflammatory cytokines can have a direct impact on neuronal and astrocytic kynurenine pathway enzymes and therefore on tryptophan metabolism. QA also increased KAT-II which converts kynurenine to KynA and may therefore contribute to suboptimal arousal induced deficits in cognition. As to whether the production of KynA reaches a high enough concentration to inhibit the NMDAr, and therefore negatively feedback on seizure susceptibility requires further investigation.

TU03-05
PLD4 WAS ASSOCIATED WITH THE PROLIFERATION OF MICROGlia VIA P38 PATHWAY
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Phospholipase D (PLD) family is known to be involved in various cellular functions including membrane trafficking, secretion and mitogenesis. Previously, we reported that the expression of a novel member of PLD family, PLD4, was specifically upregulated in amoeboid microglia in the white matter of mouse cerebellum, both in the developmental stage and the pathological conditions. In the analysis using cultured microglial cell line (MG6), we demonstrated that PLD4 was primarily located in the nucleoplasm. In addition, PLD4 was upregulated by LPS stimulation in nucleoplasm. However, function of PLD4 in the nucleoplasm and the signaling pathway of PLD4 during LPS treatment were totally unknown.

In this study, to clarify the function of PLD4 in nucleoplasm, we focused on the functional association of PLD4 with proliferation. We investigated proliferation of MG6 cells. We divided the 6 groups: LPS, PBS, PLD4-siRNA-LPS, PLD4-siRNA-PBS, control-siRNA-LPS and control-siRNA-PBS treatment groups. MG6 cells were transfected by either PLD4- or control-siRNA for 24 hours, after siRNA treatment, we added LPS or PBS into MG6 cells for 24 hours. As a result, the proliferation of the PLD4-siRNA-LPS treated group was significantly decreased compared with those in LPS and control siRNA-LPS treatment groups, but PLD4-siRNA-PBS treated groups were not significantly decreased compared with those in control and control siRNA-PBS treatment groups. As a result, PLD4 was associated with LPS stimulated proliferation.

Next, we investigated the PLD4 signal pathway, we used MAPK and PI3K inhibitors. The expression level of PLD4 was significantly changed by P38 MAPK inhibitor: SB202190. In addition, the treatment of SB202190 increased the uptake of BrdU by MG6 cells. These results suggest that the PLD4 in nucleoplasm may be regulated by P38 and associated with proliferation.

TU03-06
NOVEL MECHANISM OF N-ACETYL CYSTEINE AGAINST STREPTOZOTOCIN INDUCED MEMORY DYSFUNCTION
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Alzheimer’s Disease is a degenerative brain disorder characterized clinically by progressive loss of memory, cognition, reasoning, and judgment. Growing evidences indicate that oxidants and antioxidant defenses interact in a vicious cycle, which plays a critical role in the pathogenesis of AD. The present study was carried out to elucidate the neuroprotective effect of N-acetyl cysteine (NAC) against the intracerebroventricular infusion of streptozotocin (ICV STZ) induced cognitive impairment and oxidative damage in rats. Male adult Wistar rats were injected with
ICV STZ bilaterally (3 mg/kg) in first day and 3 days latter. NAC was applied in doses of 50 and 100 mg/kg, i.p., one day pre-surgery, 3 day surgery and continued for three weeks in post surgery. The rats were sacrificed on the 21st day following the last behavioral test and cytoplasmic fractions of hippocampus and cortex were prepared for the quantification of acetylcholine esterase, oxidative stress parameter, inflammatory mediator like tumor necrosis factor (TNF-\( \alpha \)), IL-6 activities and caspase-3. ICV STZ resulted in poor retention of memory in Morris water maze task and caused marked oxidative damage as compared to naïve group. It also caused a significant increase in the acetylcholinesterase enzyme activity, TNF-\( \alpha \), IL-6 and caspase-3 levels in hippocampus and cortex as compared to sham animals. Chronic treatment NAC significantly improved memory retention and attenuated oxidative damage parameters, inflammatory markers and acetylcholinesterase activity in colchicine treated rats. Therefore, these results demonstrate the effectiveness of NAC in preventing the cognitive impairment as well as the oxidative stress caused by ICV STZ in rats and its potential in the treatment of neurodegenerative diseases such as Alzheimer’s disease.

**TU03-07 DNA DAMAGE AND IMMUNE-ENVIRONMENTAL EVENTS IN AUTISM**

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Worldwide, the rate of autism has been steadily rising since a visible progress in many research areas have been found, including environmental, immunological and genetic, as a tool to explain some fisiopathological mechanism occurring in autism, all these factors contributing to dysregulation in autism spectrum disorder (ASD). Methods: We evaluated the influence of environmental factors, autoimmune response pattern and DNA damage in patients bearing autism and their probable interaction with learning development. We also explored the toxic metal body burden and the autoimmune response, IgE serum level and DNA damage in children’s diagnosed as ASD were also tested. The children (4 to 11-years-old), were also evaluated regard the severity of learning impairment following the Therman-Merrill test. Toxic metal (mercury and lead) body burden was assessed in blood by atomic absorption method while IgE serum levels was estimated using a microanitic assay. DNA damage was studied using both an electrophoretic method and 8-hydroxy 2 deoxyguanosine (8-OHdG) serum content determination following an ELISA kit. Results: Differences were observed regarding the severity of learning defects and the measurements of toxic metal, but it was not relevant to IgE serum level. At the same time, a no particular autoimmune pattern was observed in these patients, beside the DNA affection and toxic effect of metal evidenced in this study as an element influencing pathogenesis of ASD itself. Conclusions: This study may confirm the previous hypothesis of autism pathology, the association between the severity of autism and body burden of toxic metals from the severity of learning impairment analysis, and may conduct to a new way to understand the pathological events conducing to the stereotyped patterns of behavioral and interests as well as the genetic susceptibility in autism.

**TU03-08 INTERLEUKIN-18 TREATMENT ALTERS HUMAN SH-SY5Y NEUROBLASTOMA CELL LYSATE AND MEDIUM PROTEOMES: 2D-DIGE- ANALYSIS**

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Neuropathological changes in Alzheimer’s disease (AD) brain include extracellular Amyloid-\( \beta \) plaques and intraneuronal neurofibrillary tangles composed of hyperphosphorylated tau-protein. There are also signs of chronic inflammation. Interleukin-18 (IL-18) is an inflammatory cytokine largely produced in the brain by activated microglia. IL-18 is also detectable in the brains of AD patients. Our previous studies suggest that IL-18 can have an impact on tau and kinases related to tau hyperphosphorylation. However, the links between IL-18 and AD pathogenesis require further studies. This study aims to clarify the impact of IL-18 on neuronal protein expression and secretion. We used two-dimensional difference-gel-electrophoresis (DIGE) to examine proteome changes in differentiated human SH-SY5Y neuron-like cells after IL-18 treatments, and compared the results to untreated controls. Protein changes of cell lysate and culture mediums were examined and quantified using DIGE gels. Proteins exhibiting changes were matched to those in silver stained gels, cut-out, in-gel digested with trypsin, and identified using mass spectrometry and database searches. We found that IL-18 has a time-dependent (24–72 hours) effect on lysate proteome profile in SH-SY5Y cells. Altogether 57 altered lysate proteins were identified, and the changes were examined mainly for proteins involved in cell proliferation and/or differentiation, inflammation, regulation of oxidation and cell signaling. We are currently identifying proteins exhibiting quantitative changes in the secreteme, and will continue to further examination of these alterations using immunoblotting. Our results indicate that IL-18 can alter both SH-SY5Y cell lysate and medium proteome profiles.

**TU03-10 EFFECTS OF SECRETED \( \alpha \)-SYNUCLEIN ON CELLULAR HOMEOSTASIS - A FOCUS ON GLIAL CELLS**

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\( \alpha \)-synuclein is a neuronal protein that has been genetically and biochemically linked to the pathogenesis of Parkinson’s disease (PD). Although, the aberrant role of \( \alpha \)-synuclein still remains elusive, oligomeric intermediates of the protein are considered to be the toxic species. We have shown that \( \alpha \)-synuclein is normally secreted by neuronal cells with a mechanism that partly involves...
Exosomes. Neuroinflammatory mechanisms also seem to contribute to the cascade of events leading to neuronal degeneration in PD. Here we used conditioned media (CM) from SH-SY5Y cells inducibly overexpressing and secreting wild-type α-synuclein, under biologically relevant conditions to study the effect of secreted α-synuclein forms (monomeric, oligomeric & exosome-associated) in the inflammatory process. Using size exclusion chromatography we have isolated from the CM different high and low molecular weight secreted α-synuclein species and applied them on microglia cells. We show that neuronal; naturally secreted α-synuclein activates the NF-κB pathway in BV-2 murine microglia cell. We have also established primary mouse microglia cultures for the study of the same signaling pathways. We are also investigating the cytokines and chemokines produced following application of different α-synuclein species on microglia cells. Considering that the levels of α-synuclein seem to be critical in the pathogenesis of PD, we have sought to investigate factors and mechanisms that regulate the steady state protein levels of the naturally secreted α-synuclein. To this end we are studying the effect of the serine-protease kallikrein-related peptidase-6 (KLK6) in α-synuclein degradation. We are further investigating other mechanisms of extracellular α-synuclein clearance, including phagocytosis/ internalization by glial cells.

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TU04 Cellular Mechanism of Alzheimer’s Disease

TU04-01
IRS-1 INHIBITION LINKS IMPAIRED INSULIN SIGNALING IN ALZHEIMER’S AND TYPE-2 DIABETES: PROTECTION BY ANTI-DIABETIC DRUGS

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Alzheimer’s disease (AD) has been linked to defective brain insulin signaling, a proposed third type of diabetes. Although this intriguing connection between AD and diabetes has been suggested, a major unknown is the mechanism by which insulin resistance develops in AD brains. Here we show that serine phosphorylation of IRS-1 (IRS-1pSer) is a common denominator in these diseases. Alzheimer brain tissue was found to present elevated IRS-1pSer636/639, analogous to what occurs in peripheral tissue in diabetes. A molecular basis for this elevation was found in the ability of Aβ oligomers, toxins that accumulate in Alzheimer brain and instigate synapse damage, to activate the JNK/TNF-α pathway, induce IRS-1pSer636, and inhibit physiological IRS-1 tyrosine phosphorylation in maturecultured hippocampal neurons. Elevated levels of phosphorylated JNK were also verified in hippocampi of APPSwe, PS1deltaE9 transgenic mice. Significantly, intracerebroventricular injection of Aβ oligomers triggered JNK activation and enhanced IRS-1pSer levels in adult cynomolgus monkeys. Both insulin and exendin-4, a novel anti-diabetic drug, prevented oligomer-induced neuronal pathologies in vitro. Exendin-4 further rescued IRS-1pSer and phospho-JNK levels in transgenic mice hippocampi. By establishing molecular links between dysregulated insulin signaling in AD and diabetes, results open avenues for rapid implementation of novel and safe therapeutics in AD.

TU04-02
PRE-DISPOSITION OF DIABETES IN ACCENTUATING ALZHEIMER’S PLAQUE PATHOLOGY
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Objectives: Alzheimer’s disease (AD) is an age-dependent neurodegenerative disease currently afflicting > 5 million Americans and > 26 million people worldwide with vast majority of diagnosed AD cases being sporadic in nature. Therefore it is becoming increasingly important to uncover non-familial clues that contribute to this global epidemic. Emerging association of type 2 diabetes and insulin resistance with cognitive decline and dementia is currently being recognized, but has insufficient evidence to prove diabetes as a co-morbid predisposing factor in the development of non-familial AD. This study was undertaken to test if AD brains with a history of diabetes would exhibit aggravated plaque deposition than the AD brains without diabetes.

Methods: Human postmortem Alzheimer’s disease (AD) brain sections (Frontal cortex, Area 9), from AD (n = 4) and age-matched controls (n = 4) with diabetes; and AD (n = 4) and age-matched controls (n = 5) without diabetes were processed for semi-quantitative immunocytochemistry using 6E10 and 4G8 antibodies for detecting beta-amyloid (Aβ) plaques within the brain parenchyma. The number of plaques and density were quantitated with the use of ImagePro imaging program.

Results: Even with the small sample size, it was consistently observed that compared to age-matched controls, AD brains without diabetes had 32–36% more plaques while AD brains with diabetes had 46–48% more plaques, indicating an additional increase of ~10-12% plaque load in AD brains with diabetes.

Conclusions: Consistent with current epidemiological data, current findings substantiate that diabetes appears to be a strong predisposing co-morbid trigger for developing sporadic Alzheimer’s disease.

TU04-03
NOREPINEPHRINE REDUCES Aβ-MEDIATED CYTOTOXICITY AND MCP-1/CCL2 PRODUCTION BUT ENHANCES Aβ-INDUCED IL-1β PRODUCTION
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Degeneration of locus ceruleus (LC) neurons and subsequent reduction of norepinephrine (NE) in LC projection areas is an early pathological indicator of Alzheimer’s disease (AD). Evidence indicates that NE elicits antiinflammatory actions and plays a neuroprotective role where inflammatory events contribute to AD pathology. Here, we evaluated the effects of NE on amyloid beta (Aβ)-induced cytotoxicity and proinflammatory cytokine/chemokine production and determined the mechanisms through which NE exerts its actions in human THP-1-derived macrophages. NE
treatment reduced the Aβ-mediated cytotoxicity and production of the proinflammatory chemokine, monocyte chemotactic protein-1 (MCP-1/CC2L). However, NE treatment enhanced Aβ-mediated induction of IL-1β. Of note, the ability of NE to modulate the Aβ-mediated inflammatory response was mediated by β-adrenoceptors, as the aforementioned effects were replicated by the β-adrenoceptor agonist, isoproterenol, and blocked by β-adrenoceptor antagonist, propranolol. Moreover, the phosphatidylinositol 3-kinase (PI3K) inhibitor, LY294002, and the NADPH oxidase inhibitor, DPI, mimicked independent alterations of MCP-1 and IL-1β production provoked by NE, indicating that the NE-mediated effects were coupled with downregulation of PI3K or redox sensitive pathways. In contrast, both LY294002 and DPI had no effect on Aβ-mediated cytotoxicity. Overall, NE differentially modulates the innate inflammatory response by Aβ challenge through acting at β-adrenoceptors in human THP-1 macrophages. Our data also suggest that NE provides the protective effect against Aβ insult independent of downregulation of PI3K/ Akt or NADPH oxidase (supported by National Research Foundation of Korea, 2010-00226S).

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VASCULAR DYSFUNCTION IN A TRANSGENIC MODEL OF ALZHEIMER’S DISEASE: EFFECTS OF CANNABINOID AGONISTS

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Alzheimer’s disease (AD) is characterized by increased deposition of β-amyloid (Aβ), neurofibrillary tangles, loss of subsets of neurons and glial activation. Aβ accumulation occurs both in senile plaques and in cerebrovascular deposits. There is evidence of altered vascular function in AD and transgenic models of the disease. Cannabinoids, neuroprotective and anti-inflammatory agents, induce vasodilation both in vivo and in vitro. We have demonstrated a beneficial effect of cannabinoids in models of AD by preventing glial activation. Now we have studied the effects of these compounds in amyloid precursor protein (APP) transgenic mice, line 2576, and on Aβ altered vascular responses in isolated ring aortae. We have found an increased density of collagen IV positive vessels in AD frontal cortex and in 12 months old Tg APP mice. In APP Tg mice aortae the vasconstriction induced by phenylephrine and the thromboxane agonist U46619 was significantly increased, and no change in the vasodilation to acetylcholine (ACh) was observed. WIN 55,212-2, a CB1 and CB2 agonist, and JWH-133, a CB2 selective agonist, caused a dose-dependent vasodilation in wild type mice, which was significantly reduced in Tg APP. Aβ incubation reduced ACh-induced relaxation; cannabinoids counteracted this effect. At the ultrastructural level Tg APP aortae were similar (e.g. endothelial cells, mitochondria or muscle cells), although they had increased collagen. In summary, we have confirmed and extended the existence of altered vascular responses in Tg APP and in Aβ treated isolated vessels. Furthermore, Tg APP displayed decreased vasodilation to two pharmacologically different cannabinoid agonists, which were able to prevent decreased ACh vasodilation in the presence of Aβ. These results suggest that treatment with cannabinoids may ameliorate the vascular responses in AD-type pathology.

TU04-05
DETERMINATION OF CSF BIOMARKER LEVELS IN PATIENTS WITH EARLY AND CLASSIC ONSET OF ALZHEIMER’S DISEASE

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The aim of our study was to compare CSF levels of beta-amyloid 1-42 (Abeta1-42), total tau (T-tau) and tau phosphorylated at threonine 181 (P-tau181) between AD patients with different age onset of the disease and controls. We analyzed CSF samples from 98 AD patients (age 74.29 ± 5.41 years) with classic onset of the disease (> 65 years), 35 AD patients (age 54.66 ± 5.38 years) with early onset (< 65 years) and 35 control subjects (age 70.15 ± 11.10 years) using the Innotest (Innogenetics-Belgium) ELISA sandwich tests. Our results showed (using ANOVA, with age as covariate), that all three biomarkers showed highly significant diagnostic value (p < 0.001). Still, no difference was noted among AD patients with classic and early onset of the disease (Abeta1-42 449 ± 209 vs. 460 ± 206 pg/mL; T-tau 697 ± 603 vs. 549 ± 298 pg/mL; P-tau181 130 ± 88 vs. 131 ± 73 pg/mL). Based on the obtained results, the optimal cut-off values for the previously mentioned biomarkers were calculated. The cut-off value for Abeta1-42 was 563.1 pg/mL, while for both T-tau and P-tau values were determined according to the age groups. In the group under age 65, values determined were P-tau > 83.4 pg/mL, T-tau > 244.7 pg/mL; between ages 65 and 74, values were P-tau > 150.5 pg/mL, T-tau > 541.95 pg/mL; whereas in patients older than 75, cut-off values were P-tau > 146.2 pg/mL and T-tau > 713.2 pg/mL. The cut-off value for Abeta1-42/P-tau ratio was determined to be < 7.1. The obtained results suggest that CSF biomarkers may have an important role as supportive diagnostic tool in the diagnosis of AD in routine clinical practice.

TU04-06
MATHEMATICAL MODELING OF CHANGES IN COPPER, IRON, CERULOPLASMIN AND FERRITIN IN ALZHEIMER’S DISEASE

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The etiology of Alzheimer’s Disease (AD) is unknown and many theories including the possible role of trace elements such as copper and the related oxidative stress have been debated. The trace metal Copper has been attributed as a major risk factor and therapies have been centered on metal chelation concept. The transporter of copper, ceruloplasmin, is a multifunctional enzyme and sporadic literature in associating ceruloplasmin to neurodegeneration. The increase in
brain metal concentration is associated with normal aging and a variety of degenerative diseases including AD. We studied serum copper, Fe and ceruloplasmin and Ferritin levels in 20 early and 15 severe patients with AD, mean age 68, and 30 control samples with age matching. The patient classification as early and severe is done by Psychiatrist. Serum metal levels are estimated by ICP-AES and ceruloplasmin by chemoluminescence method and Ferritin by ferrooxidase technique. We report here differential increase of copper and iron levels samples in early and severe AD associated with increased level of ceruloplasmin only, but not with ferritin. We developed data mining studies to understand the interrelations of metal and metalproteins in AD and proposed its inter-relations.

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TU04-07

THE AMYLOID PRECURSOR PROTEIN FAMILY: DISTRIBUTION AND SUBCELLULAR LOCALISATION IN THE ADULT MOUSE BRAIN

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The pathological role of the amyloid precursor protein (APP) in Alzheimer disease has been intensively studied, although our knowledge about its physiological function still remains rudimentary. APP belongs to the APP family, which in mammals additionally includes the amyloid precursor-like protein 1 and 2 (APLP1, APLP2). All three proteins consist of highly conserved domains and are similarly processed by the γ-, β-, α-secretases, although APLP1 and APLP2 do not express the pathogenic Aβ region. APP and APLP2 mRNAs are ubiquitously expressed in mouse tissues; in contrast, the APLP1 mRNA is restricted to the nervous system. Assigning APP family members essential functions in mouse development, loss of function studies of APP mutants revealed lethal phenotypes in double-knockout mice whereas single knockouts showed milder phenotypes. These data are indicative for compensatory mechanisms of the three proteins with partially redundant physiological roles – thus prompting the question of the specific endogenous physiological role of each of the proteins. We compared the expression of APP, APLP1 and APLP2 proteins in the adult mouse brain by Western blot and immunohistochemical analysis. All three mammalian APP family members including their corresponding splice variants were detected in homogenates of the hippocampus, the cortex, the olfactory bulb and the cerebellum by Western blot analysis. Moreover applying confocal laser scanning microscopy, we evaluated the localization and the distribution of the three amyloid precursor proteins. Immunohistochemical staining revealed high expression levels for all three proteins in the mitral cells of the olfactory bulb, the purkinje cells of the cerebellum, and in cell populations of the medulla oblongata, the cortex and the hippocampus. Immunohistochemical data indicate that all APP family members are contained in membranes of intracellular organelles with predominant perinuclear localization. In order to provide additional information on amyloid precursor proteins we here show a comparison of the distribution and the cellular localization of APP, APLP1 and APLP2 in adult mouse brain.

TU04-08

SCAVENGER RECEPTOR TYPE B CLASS I REGULATES PERIVASCULAR MACROPHAGES AND MODIFIES AMYLOID PATHOLOGY IN AN AD MOUSE MODEL

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Scavenger receptor class B type I (SR-BI) is a High Density Lipoprotein (HDL) receptor that regulates cholesterol efflux from the peripheral tissues to the liver. SR-BI has been identified on astrocytes and vascular smooth muscle cells in Alzheimer’s disease (AD) brain and has been shown to mediate adhesion of microglia to fibrillar amyloid-β (Aβ). Here we report that SR-BI mediates perivascular macrophage response and regulates Aβ related pathology and memory deficits in an Alzheimer mouse model. Reduction or deletion of SR-BI gene in heterozygous or homozygous deficient mice (SR-BI±, –/–) resulted in a significant increase in perivascular macrophages in the brain. SR-BI deletion had no effect on APOE or ApoAI levels in the mouse brain. Our analysis revealed increased levels of SR-BI expression in the brains of huAPP (Swe Ind) transgenic mice (J20 line). To evaluate the role of SR-BI in AD pathogenesis, we inactivated one SR-BI allele in J20 transgenic mice. SR-BI reduction in J20/SR-BI± mice enhanced fibrillar amyloid deposition and cerebral amyloid angiopathy and also exacerbated learning and memory deficits compared to J20 littermates. Immunohistochemical analysis revealed localization of SR-BI on perivascular macrophages in tight association with Aβ deposits. Our data suggest that SR-BI reduction impairs the response of perivascular macrophages to Aβ and enhances the Aβ related phenotype and CAA in the J20 mice. These results reveal for the first time that SR-BI, a scavenger receptor primarily involved in HDL-cholesterol transport plays an essential role in AD and CAA.

TU04-09

POTENTIAL ROLE OF PROTEIN SUMOYLATION IN THE PROTECTIVE EFFECT OF CURCUMIN AGAINST AMYLOID β-INDUCED TOXICITY

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Alzheimer’s disease (AD) is an irreversible, progressive neurodegenerative disorder, which is the most common cause of dementia among older people. New treatments to manage this complex disorder require full understanding of its pathophysiology that involves amyloid β (Aβ)-induced toxicity. Substantial evidence indicates that curcumin has protective properties in AD; however, the molecular mechanisms remain far from established. Recently, it has been suggested that protein SUMOylation, a post-translational modification where small ubiquitin-like modifiers (SUMO) is conjugated to target proteins, might play a role in several neurodegenerative diseases, including AD. We are currently investigating whether protein SUMOylation is involved in the neuroprotective effects of curcumin in an in vitro model of AD, based on...
treatment of dispersed cell and organotypic slice cultures with Aβ1-42. Firstly, we are analysing the effects of curcumin on the global levels of SUMOylation, by SUMO-1 and SUMO-2/3, and protein levels of the main SUMO-specific conjugating (UBC9) and deconjugating enzymes (SENP-1) in our AD model. To test the hypothesis that SUMO conjugation contributes to the protection mediated by curcumin, we aim to transfet cells with a combination of SUMO-1, SUMO-2, UBC9 and SENP-1. Using multi-electrode array (MEA) recordings of action potentials we intend to analyze the effects of curcumin on Aβ-treated cultures. With this study, we hope to gain new insights into the molecular and cellular basis of AD by defining mechanisms underlying curcumin protection. Support: CNPq and Royal Society.

TU04-10
EVALUATION OF MONOCYTE FUNCTION IN ALZHEIMER’S DISEASE PATIENTS
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The aim of our study was to assess the characteristics of monocytes isolated from peripheral blood of AD patients, compared to the control subjects. The patients selected were the ones with probable AD, according to NINCDS/ADRDA criteria. Control subjects were age- and sex-matched and cognitively unimpaired. The flow cytometric analysis of PBMCs cultivated with 1-42 FITC-labelled Abeta for 24 h showed that the frequency of monocytes containing phagocytosed Abeta-FITC was significantly lower in the cells from AD patients compared to the Abeta uptake by monocytes from the control subjects (p < 0.01). AD monocytes also showed lower expression of surface markers important for immune function, CD44 in particular. Flow cytometric analysis revealed significant difference in CD44 expression between monocytes from AD patients versus controls (p < 0.05). The evaluation of monocytes’ apoptosis level after 24 h in culture (using the Annexin V-FITC/propidium iodide staining and subsequent FACS analysis) showed difference in percentage of monocytes undergoing early (Ann+;PI-) and, particularly, late apoptosis (Ann+/PI+) between AD monocytes, and monocytes derived from control subjects. These results suggest change in functional properties of the AD patients’ monocytes, implicating that further investigation of monocyte function may contribute to better understanding of AD pathogenesis.

TU04-11
MESENCHYMAL STEM CELLS THERAPY ABOLISHED THE CELL DEATH INDUCED BY MISFOLDED TRUNCATED TAU
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We have developed cell model for Alzheimer’s disease (AD cells) expressing human misfolded truncated tau protein (AT tau). We have showed that truncated tau slowed down the cell proliferation and reduced the metabolic activity. Moreover, truncated tau induced the increased release of the adenylate kinase from the cells and caused cell shrinkage, nuclear and DNA fragmentation that AT tau reduced the metabolic activity of the AD cells. The aim of this study was to test whether mesenchymal stem cells (MSCs) have the potency to prevent Alzheimer’s disease cell model from cell death induced by human truncated tau. We found that MSCs significantly promoted survival and increased the metabolic activity of the AD cells (p < 0.0001). Moreover stem cells induced cell differentiation and formation of AD cell neurites with numerous varicosities. These data clearly indicate that mesenchymal stem cell have significant impact on tau cell death cascade and can ameliorate toxic effect of misfolded truncated tau. We suggest that the cell neuroprotective therapy rather than cell replacement therapy represent prospective strategy for treatment of Alzheimer’s disease and related tauopathies.

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TU04-12
CDK5-REGULATED INTERACTION BETWEEN PEPTIDYL-PROLYL ISOMERASE PIN1 AND MICROTUBULE-ASSOCIATED PROTEIN TAU
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Tau is a microtubule-associated protein predominantly expressed in neurons. Hyper-phosphorylated Tau is a major component of neurofibrillary tangles in Alzheimer’s brains. Tau is phosphorylated by Cyclin-dependent kinase 5 (CdK5). CdK5 is a Ser/Thr kinase that is hyper-activated by p25. CdK5-p25 is suggested to induce the hyper-phosphorylation of Tau. However, it is not known yet why the hyper-phosphorylation of Tau occurs in disease brains. We reported previously that dephosphorylation of Tau phosphorylated by CdK5 was enhanced by Ptn1 (Yotsumoto et al., J. Biol. Chem, 2009). Ptn1 is a peptide-aryl isomerase catalyzing the cis/trans isomerization of phospho-Ser/Thr-Pro sequences, stimulating dephosphorylation by protein phosphatase 2A (PP2A). We analyzed interaction between Ptn1 and Tau phosphorylated by CdK5-p25 using the GST-pulldown assay and Biacore. We firstly confirmed Ser202, Thr205, Ser235, and Ser404 as major CdK5 phosphorylation sites.
using two-dimensional phosphopeptide map analysis. Pin1 bound to Cdk5-phosphorylated Tau but not Tau Ala mutants at four Cdk5 phosphorylation sites, indicating that Pin1 binds one of above Cdk5 phosphorylation sites. We examined Pin1 binding site using Ala mutant of Tau at phosphorylation sites, and found that Pin1 bound to any of Cdk5 phosphorylation sites. Interestingly, however, dephosphorylation was enhanced in Tau with phosphor-Ser202 and phosphor-Ser404. FTDP-17 mutant Tau, P301L or R406W, showed slightly weaker binding to Pin1 than WT Tau. Based on these results, we discuss how FTDP-17 mutant Tau is highly phosphorylated in patient’s brains.

TU04-13
FOLLOW UP OF COGNITIVE DEFICITS AND INSULIN-DEGRADING ENZYME EXPRESSION IN A RAT MODEL OF SPORADIC ALZHEIMER’S DISEASE
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Introduction: Growing body of evidence suggests the involvement of insulin degrading enzyme (IDE) in sporadic Alzheimer’s disease (sAD) pathophysiology. IDE degrades also amyloid β (Aβ) peptide, found pathologically accumulated in sAD. Rats treated intracerebroventricularly with streptozotocin (STZ-icv) have been recently proposed as an experimental sAD model. We have done a long-term follow-up of cognitive deficits and hippocampal IDE pathology in STZ-icv rat sAD model.

Methods: Wistar rats were given STZ-icv (3 mg/kg) while controls received vehicle only. Cognitive functions were tested by Morris Water Maze Test (MWM) and Passive Avoidance Test (PA) at different time points (one week to 6 months following the STZ-icv treatment). IDE protein and mRNA expression was measured in hippocampus (HPC) by SDS-PAGE electrophoresis/immunoblotting and RT-PCR, respectively, and data analysed by Mann–Whitney test (p < 0.05). Aβ accumulation was visualised by Congo red staining.

Results: Learning and memory deficits was found as early as two weeks following the STZ-icv treatment (-25.03%), and persisted up to six months after STZ-icv (-28.61% MWM; -94.36% PA). IDE protein expression was found decreased one month after the STZ-icv administration (-55.88%), persisting decreased till 6 months (-26%), while IDE mRNA expression remained unchanged until three months, when it started to decrease (-18.9%), further deteriorating up to 6 months after STZ-icv administration (-38.20%). Aβ accumulation in meningeal capillaries was found not earlier than three months after STZ-icv injection. Cdk5-phosphorylated Tau showed slightly weaker binding to Pin1 than WT Tau. Based on these results, we discuss how FTDP-17 mutant Tau is highly phosphorylated in patient’s brains.

Conclusion: The onset of cognitive deficits in sAD model does not correlate with IDE protein and mRNA changes in HPC which appear later on in the time-course of disease model. The appearance of cerebral amyloid angiopathy seems to correlate with IDE protein changes in HPC which appear later on in the time-course of disease model. The appearance of cerebral amyloid angiopathy seems to correlate with IDE protein changes.

Acknowledgement: Supported by MZOS and DAAD

TU04-14
THE HUMAN TRUNCATED TAU PROTEIN DOES NOT CAUSE NEURONAL LOSS IN TRANSGENIC RAT MODELS OF HUMAN TAUOPATHY
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Neuronal loss is one of major pathological hallmarks of Alzheimer’s disease and related tauopathies. Both neuronal loss and neurofibrillary tangles (NFT) increased in parallel with the duration and severity of illness. The question whether aberrant tau can induce neuronal death was addressed in several animal studies. However, the results coming from these studies yielded contradictory results. In order to analyze the neuronal loss induced by human misfolded truncated tau, we stereologically quantified the neuronal numbers in three independent transgenic rat models of tauopathy (SHR318, SHR24, SHR72). The stereological study was performed in 7.5-month-old transgenic (SHR72), 10.5-month-old transgenic rats from the line SHR318, 15-month-old transgenic rats (SHR24) and age-matched wild-type SHR control rat males. Analyses of potential neuronal loss induced by human misfolded truncated tau were carried out in the rat brain areas highly affected by NFT. Mean estimated total number of brainstem neurons in 7.5-month-old transgenic males SHR72 was 12 342 ± 884 (mean ± SEM) in transgenic and 12 676 ± 497 neurons in SHR control males. This difference was not significant (t-test, p = 0.7697). Similarly, 10.5-month-old transgenic rat males of line SHR318 did not differ in total neuron numbers in brainstem (9985 ± 1168 neurons) from nontransgenic controls (10 240 ± 1270 neurons). The mean estimated total number of cortical neurons in transgenic males SHR24 was 1.1x10⁷ ± 379,600, whereas in SHR control males was 1.16x10⁷ ± 872,500 neurons. This difference was also not significant (t-test, p = 0.5373). Our results demonstrated that the expression of truncated tau protein in three independent rat models of tauopathy did not cause neuronal loss in the highly vulnerable brain areas. On the basis of these findings we suggest that the truncated tau is able to switch off the apoptotic cascade which seems to be the prerequisite for tangle formation. Acknowledgement: This work was supported by research grant VEGA 2/0205/11.

TU04-15
CELL CYCLE ABERRATIONS MEDIATE NEUROTOXICITY INDUCED BY SOLUBLE AMYLOID-BETA Oligomers
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Accumulating evidence suggests that aberrant neuronal cell cycle re-entry precede the selective neurodegeneration observed in Alzheimer disease (AD). While the causal role of cell cycle alteration in the pathogenesis of AD remains to be determined, our recent animal model study clearly demonstrated that dysregulation of cell cycle re-entry results in neurodegeneration in vivo suggesting the causal link between cell cycle re-entry and neuronal cell loss in AD. Therefore, the re-activation of cell cycle in the vulnerable neurons in AD might be an essential part of mechanism leading to neuronal cell death. However, the signaling mechanism(s) associ-
ated with cell cycle re-entry and neuronal cell death in AD is unclear and needs to be identified. In this study, we investigated the intracellular signaling mechanisms triggered by the amyloid-beta oligomers and determined the causal relationship among these pathways with the amyloid-beta oligomers-mediated neurotoxicity in the organotypic hippocampal slice cultures. We found that amyloid-beta oligomers cause cell cycle re-entry in hippocampal slice cultures and subsequent neuronal cell death. Initially, amyloid-beta oligomers increase intracellular Ca²⁺ through NMDA receptor and this event activates calcium/calmodulin-dependent protein kinase (CaMKII), which subsequently activates the extracellular signal-regulated protein kinase (ERK1/2) signaling pathways. The activated ERK1/2 induces p27Kip degradation, which leads to cell cycle re-activation. Importantly, inhibition of ERK/CaMKII signaling pathway and cell cycle re-entry significantly attenuate neuronal cell death induced by oligomeric amyloid-beta. Our data strongly support the hypothesis that cell cycle re-entry is an important mechanism of the neurodegeneration of AD. We also find that the CaMKII-ERK1/2 signaling pathway, by mediating the degradation of p27Kip, is a key mechanism for neuronal cell cycle re-entry and cell death induced by oligomeric amyloid-beta.

TU04-16

NNEUROVASCULAR COUPLING IN ALZHEIMER’S DISEASE: IN VIVO AND REAL-TIME MEASUREMENT OF NITRIC OXIDE AND CEREBRAL BLOOD FLOW

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Following neuronal activation the increased need for O₂ and glucose require a tight controlled mechanism by which an information is sent from neurons (producer site) to pericytes/endothelial/smooth muscle cells (transducer site) in order to achieve an increase in cerebral blood flow (CBF). Any impairment in this process, the neurovascular coupling (NVC), may be translated into dysfunction associated with toxic phenomena and disease. The mechanistic details of such a process have remained controversial, largely because of experimental difficulties in addressing the problem in a real time, quantitative and dynamic fashion in vivo.

Using a tri-component microsensor array consisting of NO-selective microelectrode, an ejection pipette and a laser Doppler sensor inserted stereotaxically in the brain of anesthetized rat (Wistar) we firstly established that NO, blood flow and O₂ transitory elevations in hippocampus are coupled in terms of time, space and amplitude. To assess whether NVC is affected in AD we have used a selective microsensor array consisting of NO-selective microelectrode, an ejection pipette and a laser Doppler sensor inserted stereotaxically in the brain of anesthetized rat (Wistar) we firstly established that NO, blood flow and O₂ transitory elevations in hippocampus are coupled in terms of time, space and amplitude. To assess whether NVC is affected in AD we have used a

TU04-17

ALZHEIMER’S TOXIC AMYLOID-BETA OLIGOMERS INDUCE ENDOPLASMIC RETICULUM STRESS IN MATURE HIPPOCAMPAL NEURONAL CULTURES

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Alzheimer’s disease (AD) is a devastating neurodegenerative disorder associated with cognitive impairment and memory deficits. Present knowledge strongly suggests that soluble amyloid-beta-derived oligomers (ADDLs) specifically bind to neuronal synapses and trigger diverse neurotoxic effects, leading to neuronal dysfunction. These findings unveil ADDLs as central neurotoxins in AD. However, the mechanisms through which these oligomers exert their damaging role are still not fully understood. Endoplasmic reticulum (ER) stress is a cellular condition caused by excessive misfolded protein accumulation, reduced proteasome activity and altered Ca²⁺ homeostasis. ER stress is accompanied by activation of the unfolded protein response (UPR) and cell death pathways. Recently, it has been shown that ER stress markers are present in the brains of AD patients and that different aggregates of the amyloid-beta peptide induce this event, although the role of soluble and diffusible amyloid-beta oligomers is not clear. Here we report that ADDLs markedly increase the phosphorylation of eIF2α, an ER stress marker, in dendrites of rat hippocampal cultures at 18 days in vitro, inhibiting its critical activity in normal local translation after a 3-hour treatment. These results suggest that local protein synthesis is impaired by ADDL signaling and corroborate that ER stress may be a prominent feature in Alzheimer’s disease.

TU04-18

HOMOCYSTEINE-INDUCED TOXICITY IN HUMAN NEUROBLASTOMA CELLS: ROLE OF CHOLESTEROL METABOLISM AND ROS PRODUCTION

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Abnormally high blood homocysteine (Hcy) levels have been associated with an increased risk of developing Alzheimer’s disease (AD). Previous studies have shown that Hcy generates oxidative stress, increases brain amyloid-β protein (Aβ) levels and potentiates its toxicity. However, the molecular and cellular mechanisms by which Hcy contributes to development of AD are not completely understood. In addition to Hcy implications in AD it has been largely recognized its role on the accumulation of cholesterol in

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several cell types. Association between Hcy and cholesterol accumulation in the CNS is important because it has been suggested that cholesterol can increase the production of Aβ. Moreover, high cholesterol also contributes to oxidative stress trough chemical interaction between Aβ and metals such as copper (Cu^{2+}). We used human neuroblastoma cells (MSN) to study the Hcy effects on reactive oxygen species (ROS) production and cholesterol accumulation. In differentiated MSN cells with retinoic acid and NGF, Hcy increased 20% cholesterol content compared with untreated neurons. We found also that Hcy induced ROS formation and enhanced the Cu^{2+}- and cholesterol-mediated neurotoxicity. Co-incubation of cholesterol with Aβ-Cu^{2+} complexes additionally increased cell death. These results indicate important relationships between Hcy, cholesterol and Aβ-Cu^{2+} complexes, which promoted ROS production and caused neuronal death. Currently we are analyzing the effect of Hcy on cholesterol metabolism examining changes in the expression of the rate-limiting enzyme for cholesterol biosynthesis, HMGCoAR in MSN. Supported by PAPIIT IN19509

TU04-19
PHOSPHORYLATION DIFFERENTIATES TAU-DEPENDENT NEURONAL TOXICITY AND DYSFUNCTION
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Tauopathies are a heterogeneous group of neurodegenerative dementias involving perturbations in the levels, phosphorylation status or mutations of the microtubule-binding protein tau. Using the established fruit fly model of Tauopathies, we study the biochemical alterations on tau that result in dysfunction and/or toxicity and the consequent defects in learning and memory. Briefly, toxicity of hyperphosphorylated tau is manifested specifically in fly brain neurons functionally analogous to vertebrate hippocampus, the mushroom bodies (MB). The MB aberrations depend, at least in part, on occupation of two novel phosphorylation sites: Ser238 and Thr245. Significantly, replacing these residues with non-phosphorylatable alanines yields animals with structurally normal but profoundly dysfunctional MBs, as animals accumulating the mutant protein exhibit strongly impaired associative learning. Importantly, these data indicate that phosphorylation on both or one of these sites is required for toxicity and they demonstrate that MB toxicity is clearly dissociable from dysfunction. Furthermore, we have generated phosphospecific antibodies to each of these sites and tested whether pathogenic tau is actually phosphorylated in these residues. Moreover, we investigate how phosphorylation of individual disease-associated phosphorylation sites impacts tau-induced neurotoxicity and/or dysfunction. Our objective is to understand the functional roles of such characteristic tau phosphorylations with respect to neuroplasticity deficits associated with Alzheimer’s disease and other Tauopathies. Our collective results support the notion that phosphorylation at particular sites rather than hyperphosphorylation per se mediates toxicity or dysfunction in a cell-type-specific manner.

TU04-20
THE OVEREXPRESSION OF CPG15 ATTENUATES THE DEFICITS IN COGNITIVE FUNCTIONS IN ALZHEIMER’S DISEASE ANIMAL MODELS
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Candidate plasticity-related gene 15(CPG15) was first identified as an activity-related gene involved in synaptic plasticity and encodes a small, highly conserved protein. It is abundantly expressed in developing as well as adult brains. In a membrane bound form linked by GPI (glycosylphosphatidylinositol) anchor, CPG15 has been reported to function non-cell autonomously to coordinately regulate growth of opposing dendritic and axonal arbors, and to promote synaptogenesis. Interestingly, secreted form of CPG15 rescued cortical progenitor cells from apoptosis by preventing activation of caspase 3 during early development. Here, we examined a potential role for CPG15 in Alzheimer’s disease (AD). AD is the most common dementia of neurodegenerative disease and begins with memory deficits in short term memory in total loss of cognition and executive functions. We found that CPG15 expression was significantly down-regulated in the hippocampus and cerebral cortex of AD patients, compared to age-matched control subjects. The overexpression of CPG15 in the dentate gyrus of 12-months-old Tg2576 mice rescued learning and memory deficits when assessed by Morris water maze test. Furthermore, Overexpression of CPG15 increased synaptophysin expression. We conclude that CPG15 ameliorates the cognitive function impairments and would provide a therapeutic avenue for AD by improving cognitive functions.

TU04-21
NMDA RECEPTOR ANTAGONIST IN ALZHEIMER’S DISEASE TREATMENT: MORE OPTIONS THAN MEMANTINE?
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Alzheimer’s disease (AD) is a complex neurodegenerative and progressive dementia. AD is characterized neuropathologically by extracellular amyloid β plaques and intracellular neurofibrillary tangles, associated with loss of cholinergic function. NMDA receptors stimulation implicated in AD. Our specific study objective is to develop hypothetical model to understand whether, Aβ plaques can induce the excitotoxicity of glutamate due to over activation of NMDAR? We developed working computational model to understand the cross reaction of Aβ NMDAR through an excessive increase in intracellular calcium, activating signal cascades, the induction of cholinergic neurons death, and cognitive impairments. The results from our model indicates that NMDAR with NR2B subunit activation participate in Tau levels and NMDAR inhibition has a neuroprotective effect. The targeted treatment may include acetylcholinesterase inhibitors and Memantine that reduce cognitive
and memory loss. Memantine is a low affinity channel blocker of NMDARs. Memantine is well tolerated, but may induce aggressiveness, depression, anxiety behavior, hallucinations, seizures. We developed interaction model between Memantine and NMDR. We also developed a working hypothesis on NMDA as drug target. In our complex working model, we targeted reducing amyloid load and simultaneously increasing memory and cognition. In brief, the selective NMDAR antagonist may be useful in the early stages of AD, which may prevent the progression of neurodegeneration.
TU05 Therapeutic Approaches of Parkinson’s Disease

TU05-01
RECOVERY OF HYPOTHALAMIC DOPAMINE NEURONS TO NEUROTOXIC INSULT IS PROLACTIN INDEPENDENT AND CORRELATED WITH AN INCREASE IN PARKIN
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Central dopamine (DA) neurons show differential responsiveness to the mitochondrial Complex I inhibitor, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Nigrostriatal (NS) DA and tuberoinfundibular (TI) DA neurons both have an initial loss of terminal DA stores by 4 hours post-MPTP, but only TIDA neurons recover. The goal of the current study was to determine if TIDA recovery is due to extrinsic (activation by prolactin; PRL) or intrinsically mediated mechanisms. Mice were pretreated with the D2 agonist, bromocriptine (3 mg/kg; sc) or vehicle (4% ethanol in saline; 10 mL/kg; sc) prior to toxicant administration in order to suppress circulating PRL, and sacrificed 4 or 24 hours after treatment with MPTP (20 mg/kg; sc) or saline (10 mL/kg; sc). Bromocriptine suppressed plasma PRL but had no effect on ME DA. ME DA concentrations were significantly decreased at 4 h but fully recovered by 24 hours post-MPTP in both vehicle- and bromocriptine-treated mice. Recovery of ME DA was correlated with an increase in parkin expression in the mediobasal hypothalamus suggesting that resistance of TIDA neurons may be due to intrinsic up-regulation of parkin. A significantly positive correlation \( r^2 = 0.927 \); psurgery, mice were administered saline or MPTP and sacrificed 24 hours later. ME DA concentrations were unchanged following MPTP treatment and inhibition of proteasome function did not alter recovery from MPTP. Together, these results suggest that TIDA neuronal resistance to neurotoxic insult is independent of the activating effects of PRL and is, at least in part, due to an increase in parkin protein expression.

TU05-02
DIFFERENTIAL EXPRESSION OF PROTEINS IN CELLULAR AND ANIMAL MODELS OF PARKINSON’S DISEASE
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Parkinson’s disease (PD) is the most consistent movement disorder resulting from the demise of dopamine producing A9 substantia nigra pars compacta (SNpc) neurons. Amongst several neurotoxin-induced animal models, rotenone-induced Parkinsonism is shown to be progressive in nature and produce intracellular protein aggregation. Unilateral rotenone infusion into SNpc caused oxidative and nitrosative stress, progressive loss of striatal dopamine, and ubiquitine/a-synuclein positive neurons in this region. SN and striata were investigated for differential gene expression employing semi-quantitative PCR in the hemiparkinsonian rats that received unilateral stereotaxic infusion of rotenone in the medial forebrain bundle. Significant up-regulation of two genes, such as metastasis associated protein 1 (MTA1) and Bel-2-interacting mediator of cell death (Bim) was observed in both SN and striatum, whereas p53 up-regulated modifier of apoptosis (PUMA) was found to be over-expressed only in SN. SH-SY5Y neuroblastoma cells treated with rotenone (500 nM) for 48 hours resulted in > 1.5 fold up-regulation of different chaperone proteins like HSP70, heat shock 42 KD protein, dnaK type molecular chaperone, protein disulphide isomerase ER60, endoplasmic precursor, and calumenin, whereas /-tubulin, /-tubulin, vimentin, mitochondrial ATPase / chain precursor, 14-3-3 zeta/delta, transitional endoplasmic reticulum, Rho GDP dissociation inhibitor 1 and calreticulin precursor were significantly down-regulated. Protein aggregation (ubiquitin/a-synuclein) was also observed in SH-SY5Y neuroblastoma cell lines following exposure to rotenone. The over-expression of chaperones may result from oxidative stress and protein misfolding as evident in rotenone model of PD. It is suggested that under oxidative stress condition, MTA1 over-expression stabilizes p53 that promotes apoptosis mediated by PUMA and Bim.

TU05-03
DOSE- AND TIME-DEPENDENT THERAPEUTIC AND ADVERSE EFFECTS OF MUCUNA PRURIENS EXTRACT IN THE 6-OHDA RAT MODEL OF PARKINSON’S DISEASE
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Levodopa (L-DOPA)/carbidopa therapy is the most effective symptomatic drug treatment for Parkinson’s disease (PD), but after chronic use serious motor complications (e.g. dyskinesias) may develop. In traditional Ayurvedic Indian medicine, preparations of Mucuna pruriens (MP) seeds are used in the treatment of PD. Alcoholic extracts of MP seeds are rich in L-DOPA, but other, as yet unknown compounds may contribute to its therapeutic effects. The objectives of this study are (1) to determine the lowest, therapeutically effective dose of a methanol extract of MP as compared to synthetic L-DOPA and (2) to assess the severity of abnormal involuntary movements (AIMs) after chronic treatment in the unilateral 6-hydroxydopamine (6-OHDA) lesioned rat. Different batches of MP extracts were prepared containing ca. 20% L-DOPA (dry weight extract) and similar chemical profiles as assessed by HPLC and mass spectrometry. In two series of 6-OHDA rats we tested 3-6-9 mg L-DOPA/kg ip + benserazide (15 mg/kg ip) on the same day or on subsequent days and found that MP extract was more effective in normalizing contralateral forelimb akinesia than L-DOPA alone (two-way ANOVA, p < 0.01, n = 16 vs. n = 15), but the dose-dependent increase in AIMs was similar in both
Parkinson’s disease (PD) is a multifactorial syndrome often associated with mitochondrial dysfunction and oxidative stress. Cells have different enzymatic systems preventing the oxidative damage promoted by the reactive oxygen species (ROS) derive from mitochondrial activity. Certain post-mortem studies have reported an altered activity for superoxide dismutase (SOD) and catalase (CAT) in patients suffering PD. 6-Hydroxydopamine (6-OHDA) is a neurotoxin widely used to mimic PD through an oxidative stress-mediated process. 6-OHDA autoxidation yields some ROS and dopamine-derivates causing oxidative stress. In addition, some authors have reported the toxicity of 6-OHDA to inhibit complex I and to trigger apoptotic pathways. The aim of the present work was to study the ability of both SOD and CAT to protect against oxidative alterations caused by 6-OHDA on mitochondrial respiration. Respiratory control ratios were assessed by high-resolution respirometry and used to estimate the toxic effects caused by 6-OHDA on electron transport system (IC50 = 200 nM). Different concentrations of both SOD and CAT were used to evaluate the ability of these enzymes to prevent the uncoupling caused by 6-OHDA. The results obtained showed a differential capacity of both enzymes to protect mitochondrial uncoupling caused by 6-OHDA. The results obtained showed a differential capacity of both enzymes to protect mitochondrial function. Furthermore, our results appear to support the idea of differential capacity of both enzymes to protect mitochondrial uncoupling caused by 6-OHDA. The results obtained showed a differential capacity of both enzymes to protect mitochondrial function. Furthermore, our results appear to support the idea of differential capacity of both enzymes to protect mitochondrial function.
times, or supplementing the neurohormone at 2 hours intervals day or night. However, low dose of L-DOPA (5 mg/kg, by gavation) was administered alone or along with melatonin (10 mg/kg, i.p.) twice everyday for two days, 10 hours apart, after the second dose of MPTP significantly attenuated striatal dopamine levels. Catelepsy and akinesia caused due to the administration of MPTP was not affected by L-DOPA treatment, but significantly attenuated by melatonin, or by the combination therapy. These results strongly suggest that in Parkinsonism, adjuvant therapy with melatonin provides dual benefits to the patients, to obtain better therapeutic outcome, and to lower the dosage of L-DOPA.

TU05-07
MELATONIN ATTENUATES METHAMPHETAMINE-INDUCED α-SYNucleIN AGGREGATION BY BLOCKING CHAPERONE MEDIATED AUTOPHAGY (CMA)
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Parkinson’s disease (PD) is an age-related disorder that characterized by a progressive degeneration of dopaminergic neurons. The hallmark of PD is the accumulation of alpha-synuclein (α-syn) protein in lewy bodies that often organize into intra- or extracellular aggregates. In vitro studies have demonstrated that α-syn modulates dopamine (DA) toxicity, which was associated with reactive oxygen species ROS from DA oxidation. Methamphetamine (METH) is a commonly abused drug that involves in neurotoxicity and α-syn aggregation in PD. Normally, several proteins were degraded by ubiquitin-proteasome system (UPS), however; in some long-lasting proteins including α-syn that is degraded by UPS in the first and also possibly degraded by chaperone-mediated autophagy (CMA), a selective targeting of proteins to lysosomes. The typical form of α-syn could be binding with a lysosomal protein called the lysosome-associated membrane protein (Lamp 2A). It has a sequence “KFERQ motif” that similar with the sequence on α-syn. In the other hand, when cell was exposed to toxic substance toxicity, α-syn has been conformational changed to oligomer form and can not bind with Lamp2A Therefore, we hypothesized that METH-induced toxicity associated with CMA signaling cascade in α-syn degradation. We found that METH-induced α-syn aggregations by inhibiting α-syn degradation on CMA pathway and these effects were diminished by melatonin. These results implicated α-syn-dependent CMA degradation pathways in the processes of METH-induced toxicity and also indicated that melatonin has capacity to reverse this effect in SH-SY5Y cultured cells.

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TU05-08
AN IDENTIFIED TETRAHYDROISOQUINOLINE FROM AYURVEDA MEDICINE, PROTECTS NIGROSTRIATAL DOPAMINERGIC NEURONS IN EXPERIMENTAL MODELS OF PARKINSON’S DISEASE
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Parkinson’s disease (PD) is the most common neurodegenerative movement disorder, resulting from the loss of dopaminergic neurons in the A9 substantia nigra region of the brain. There exists no cure for the disease, but the disease syndromes are controlled by replenishment of the neurotransmitter, dopamine as its precursor or by inhibiting its catabolism using monoamine oxidase inhibitors. In the present study a component plant seed used for treating PD in Ayurveda, a traditional Indian medical system, has been extracted and fractionated to obtain a tetrahydroisoquinoline (TIQ) molecule. TIQ has been investigated for its neuroprotective role in MPTP-, MPP⁺- and 6-OHDA-mediated nigral dopaminergic lesion as seen in PD. TIQ administration per-orally in parkinsonian mice or i.p. in hemiparkinsonian rats provided significant attenuation of behavioral dysfunctions viz., akinesia, catalepsy and swimming ability and striatal dopamine depletion caused by the neurotoxins. TIQ was found to possess significant MAO-B inhibitory potential in vivo and acted as a potent hydroxyl radical scavenger in isolated mitochondria. MPP⁺-induced inhibition of mitochondrial NADH-ubiquinone oxidoreductase (Complex I) activity was reversed by TIQ at nanomolar concentrations. MPP⁺-induced loss in the mitochondrial membrane potential and cell death in SH-SY5Y cells were significantly reversed by TIQ treatment. These results strongly imply tetrahydroisoquinoline derivative as a potential antiparkinsonian drug with action at multiple biochemical target sites. Apparently the therapeutic efficacy of the Ayurveda medicine results from endogenously present natural compounds such as TIQ.

TU05-09
LACK OF NEUROPROTECTION IN THE ABSENCE OF P2X7 RECEPTORS IN TOXIN-INDUCED ANIMAL MODELS OF PARKINSON’S DISEASE
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Previous studies indicate a role of P2X7 receptors in processes that lead to neuronal death. The main objective of our study was to examine whether genetic deletion or pharmacological blockade of P2X7 receptors influenced dopaminergic cell death in various models of Parkinson’s disease (PD). mRNA encoding P2X7 and P2X4 receptors was up-regulated after treatment of PC12 cells with 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP). P2X7 antagonists protected against MPTP and rotenone induced toxicity in the LDH assay, but failed to protect after rotenone treatment in the MTT assay in PC12 cells and in primary midbrain culture. In vivo MPTP and in vitro rotenone pretreatments increased the mRNA expression of P2X7 receptors in the striatum and substantia nigra of wild-type mice. Basal mRNA expression of P2X4 receptors was higher in P2X7 knockout mice and was further up-regulated by MPTP treatment. Genetic deletion or pharmacological inhibition of
P2X7 receptors did not change survival rate or depletion of striatal endogenous dopamine (DA) content after in vivo MPTP or in vitro rotenone treatment. However, depletion of norepinephrine was significant after MPTP treatment only in P2X7 knockout mice. The basal ATP content was higher in the substantia nigra of wild-type mice, but the ADP level was lower. Rotenone treatment elicited a similar reduction in ATP content in the substantia nigra of both genotypes, whereas reduction of ATP was more pronounced after rotenone treatment in striatal slices of P2X7 deficient mice. Although the endogenous amino acid content remained unchanged, the level of the endocannabinoid, 2-AG, was elevated by rotenone in the striatum of wild-type mice, an effect that was absent in mice deficient in P2X7 receptors. We conclude that P2X7 receptor deficiency or inhibition does not support the survival of dopaminergic neurons in an in vivo or in vitro models of PD.

TU05-10
EFFECT OF AMPHETAMINE IN SUBSTANTIA NIGRA OF NEONATAL RAT BRAIN AND DOPAMINERGIC CELL LINES
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Amphetamine and its derivatives are the most widely abused drug in the world. Amphetamine is able to cause the direct effect to dopaminergic pathway in the brain and leads to induce the parkinsonian-like symptoms in drug abusers. The presence of Lewy bodies in the mid-brain has been recognized as a pathological hallmark of Parkinson’s disease, which contain alpha-synuclein as a major component. Alpha-synuclein (α-syn) is a protein that is highly enriched in presynaptic terminals of neurons. Despite of its unknown function, it is involved in the regulation of vesicles pool and dopamine in dopaminergic neurons including the clearance of dopamine from cytoplasm through vesicular monoamine transporter2 (VMAT2). VMAT2 is one of the transporter families that sequester dopamine and monoamines into the vesicles. The present study aims to investigate the levels of α-syn, tyrosine hydroxylase (TH), the rate limiting enzyme of dopamine, and VMAT2 mRNA expression in chronic amphetamine-treated substantia nigra of neonatal rat. To further confirm the mRNA expression, we also investigate the α-syn in dopaminergic SH-SY5Y cell line by semi-quantitative reverse transcription polymerase chain reaction. Our study showed that chronic amphetamine treatment induced the reduction of alpha-synuclein, tyrosine hydroxylase and VMAT2 mRNA expression in substantia nigra of neonatal rat. We also found that amphetamine is able to induce changes in alpha-synuclein expression in dopaminergic cell line. This study suggests that amphetamine administration can produce the alteration tyrosine hydroxylase and VMAT2 mRNA expression in neonatal rat brain as well as the alpha-synuclein mRNA expression both in neonatal rat brain and dopaminergic cell line.

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TU06 Myelination and Demyelination

TU06-01
HUMAN NEURAL STEM CELLS TRANSDUCED WITH OLG2 TRANSCRIPTION FACTOR AMELIORATE MOG-INDUCED EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Multiple sclerosis (MS) is featured with widespread demyelination and axonal loss caused by autoimmune damage and delayed remyelination. In the present study, we investigated the improving effects of human neural stem cells (F3 NSCs) transduced with Olig2 transcription factor (F3.OLG2) on myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Six days after MOG administration, female C57BL/6 mice were intravenously injected with F3.OLG2 (1 × 106 cells/mouse), and monitored neurobehavioral abnormalities up to 44 days. Transplantation of F3.OLG2 cells greatly recovered the clinical scores of EAE. Around the brain lesions, much more F3.OLG2 cells were detected by 44 days post-transplantation than parental F3 NSCs, and most of F3.OLG2 cells differentiated into mature oligodendrocytes with thick myelin sheaths surrounding the axons. Moreover, F3.OLG2 cells significantly attenuated autoimmune-mediated demyelination and axonal loss of host neurons. These results suggest that F3.OLG2 cells could be a promising candidate for prevention and restoration of MS via immunomodulation and increased remyelination.

TU06-02
CELL-AUTONOMOUS FUNCTION OF FGF-RECEPTOR SIGNALING IN OLIGODENDROCYTES IS NOT INHIBITORY FOR REMYELINATION, BUT PROMOTES REPAIR

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A common feature of demyelinated lesions in Multiple Sclerosis is astrogliosis, microglial activation, damage to oligodendrocytes (OLs) and axons, and failure of OL progenitors (OLPs) to differentiate, together contributing significantly to failed remyelination. The mechanistic basis for this is not well understood. FGF-2 is highly upregulated in demyelinated lesions. An inhibitory role of FGF-2 for OLP differentiation during remyelination has been suggested based on observations that following cuprizone-induced acute and chronic demyelination in the FGF-2-null mice there is enhanced repopulation of lesions by OLs (Armstrong et al., 2002; 2006). However, since FGF-2 can perturb the functions of not only OLPs but of other cell types including astrocytes and microglia also present in the lesion environment and express FGF receptors (Fgfrs), it remains unresolved whether the effect on OLP differentiation in the FGF-2-null mice is direct or occurs indirectly through other cell-types. To answer this question, we investigated the cell-autonomous function of FGF-signaling in OL-lineage cells during remyelination by conditionally inactivating FGF-receptors in these cells using two Cre-driver mouse lines (CNP-Cre and Olig1-Cre). Both acute and chronic cuprizone-induced models of demyelination were established in the Fgfr1/Fgfr2-double cKO. Recovery was evaluated by quantification of the numbers of PLP mRNA + and APC + OLs and staining of myelin with MOG and Luxol Fast Blue in the corpus callosum. In contrast to FGF-2-null mice we did not find an increase of OL numbers or remyelination in Fgfr1/Fgfr2-cKO mice in either of the models, on the contrary observed an inhibition of remyelination in the chronic lesions of the cKOs. This suggests that the enhancement of OL differentiation observed previously in the FGF-2-null mice was most likely indirect via other cell-types. More importantly, it suggests that the cell-autonomous function of FGF-receptor signaling in OLs is not inhibitory for remyelination but stimulatory and promotes repair of chronically demyelinated lesions. Supported by NIH grant NS38878 and NMSS, RG 4087-A-3.

TU06-03
OMICS BASED APPROACHES TO UNDERSTAND MYELIN BIOLOGY: IMPLICATION OF PERTURBED AXOGLIAL-APPARATUS IN PEDIATRIC MULTIPLE SCLEROSIS

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Myelin loss in the CNS due to genetic abnormalities, immune attacks (in Multiple Sclerosis [MS]) or trauma also causes subsequent disassembly of the node of Ranvier. This results in impaired nerve conduction and neurological dysfunction. In order to understand the biology of myelin and to identify novel molecules that may act as auto-antigens or disease-initiating targets in Multiple-sclerosis (MS) we analysed

(i) the protein composition of human myelin and axoglial-apparatus
(ii) the regulation of microRNAs (219, 338 and 17-92) during human OL differentiation
(iii) the protein composition of cerebrospinal-fluid samples obtained from children during the initial presentation of CNS-inflammation.

Results: We identified over one thousand proteins in myelin and the axoglial-apparatus with reciprocal protein representation of several molecules in the two fractions. In primary human OLs, we found regulation of these miRs during differentiation. In the CSF samples, we identified 563 proteins of which 67 were differentially expressed in children who later developed MS. By comparing the differentially expressed proteins to our myelin proteome maps, we found overrepresentation of axoglial-apparatus proteins, indicative of perturbed nodal organization.

Conclusions: (i) Several membrane proteins identified in the myelin proteome are regulated during oligodendrocyte differentiation. Currently, we are studying the role of some of the proteins in myelination and examining their role in MS by screening CSF samples for the presence of auto-antibodies against these targets. (ii)
Annexin 2 (AX2) is a calcium-dependent phospholipid binding protein, and is thought to be a modulator of the pathological processes through inhibition of cytosolic phospholipase A2 (cPLA2) or promotion of local fibrinolysis through binding of tissue plasminogen activator. Previously, we reported that AX2 level was up-regulated in the paranoid region and Schmidt-Lanterman incisure (SLI) in normal appearing myelin in surrounding area of the demyelinating region of lysolecithin-injected sciatic nerves. Reduction of AX2 by siRNA caused marked expansion of the demyelinating area, suggesting that increase of AX2 in the surrounding area may be important for limiting the progression of demyelination. Similar increase of AX2 in myelin was found in the peripheral nerves of two myelin mutants, sulfatide-deficient mice and shiverer mice, in which no demyelination was observed. To know the role of AX2 in the mutant myelin, we examined the sciatic nerves of these mutants and compared the results with lysolecithin-induced demyelinated nerves. Lysolecithin was injected intraneurally in the left sciatic nerve of each 8-week-old mice. The sciatic nerves were removed at 3, 5, 7, 9 and 14 days after injection for immunohistological or western blot analysis. In lysolecithin-treated nerves, AX2 and cPLA2 were significantly increased. Particular increase of phosphorylated cPLA2 (p-cPLA2), suggest that activation of cPLA2 is one of the key event for lysolecithin-induced demyelination. Amounts of AX2 were also increased in both mutant nerves. However, in contrast to lysolecithin-induced demyelination, no apparent increases of cPLA2 and p-cPLA2 were observed in the mutants. These results suggest that upregulation of AX2 is associated with various myelin conditions, but AX2 may function differently with diverse target molecules depending on pathological states of myelin.

TU06-07
ASSessment of Virus DNA in Blood from Patients with Multiple Sclerosis
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Multiple sclerosis is a chronic inflammatory disease of the central nervous system in which an infectious component has been implicated. Epstein Barr virus presence in patients with multiple sclerosis has been investigated by a number of research groups, and reports range from 0% to 100%. The aim was to investigate viral presence in patients with multiple sclerosis. Genomic DNA from 31 patients with multiple sclerosis and 30 control persons were used to establish presence of Epstein Barr virus latent genes EBNA-1, LMP-1 and Bam H-1W, as well as Human Herpes virus-6 gene U83, using PCR assays. We found a low prevalence of virus DNA in blood from both patients and controls. Epstein Barr virus in blood from both patients and controls was present in 6.5%. On the other hand Human Herpes virus-6 gene U83 was found in only 1 patient with multiple sclerosis. EBV presence in patients with MS has been investigated by a number of researchers without any consensus on percentage viral presence in patients. Similar to some research studies, our results indicated a very low presence of this virus in DNA from blood of both patients and controls.
TU06-08
ROLE OF CYSTATIN F IN DEMYELINATING DISEASES
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Myelin is a membrane structure enabling saltatory conduction of action potential and is formed by oligodendrocytes in the CNS. Multiple sclerosis (MS) is one of the demyelinating diseases. In chronic demyelinating lesions of the MS patient brain, oligodendrocyte precursor cells are found abundantly, and moreover pre-myelinating oligodendrocytes are also found, but they somehow fail to achieve terminal differentiation into myelin-forming oligodendrocytes. It has been reported that the TNF-alpha pathway is implicated in MS susceptibility through the observations in human clinical studies. Thus, we focused on the behavior of microglia, the major TNF-alpha producing cells in the CNS, in the demyelinating brain. We found that cystatin F (CysF), a cysteine protease inhibitor, is expressed in microglia during remyelinating stage and the expression level decreases when chronic demyelinated lesions appeared. CysF mRNA expression was induced when microglia phagocytosed myelin debris. Interestingly, CysF expression was not induced during normal development or in the hypoglial nerve injury, which results in the Wallerian degeneration. CysF is expressed in some immune cells but not in infiltrating T cells in the demyelinating lesions of MOG induced EAE model. In addition, the expression pattern of cathepsin C (CatC) which is the target of CysF was similar to that of CysF in remyelinating regions but we found CatC+/CysF- regions in chronic demyelinated lesions. Additionally, we found that CatC is expressed in microglia and CatC co-localized with that of CysF in primary cultured microglia. It is reported that CatC is involved in the production of pro-inflammatory cytokines. Together, we propose that CysF, expressed in microglia, dominates the activity of CatC that directs the production of pro-inflammatory cytokines during the re-myelinating phase in demyelinating lesions.

TU06-09
ALTERNATIVE IRON UPTAKE PATHWAY IN THE PERIPHERAL NERVOUS SYSTEM
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Although the molecular identity of the axonal signal that induces Schwann cell’s phenotype (SCs) is unknown, there is significant information on the intracellular signals and transcription factors that are involved in the myelination of SCs. Loss of axonal contact in isolated SCs in vitro or after nerve injury in vivo leads to de-differentiation of SCs. We have described that holotransferrin (hTf), (iron bound apo transferrin (aTf)) prevented this de-differentiation, while aTf was unable to avoid such effect. We analyzed the effect of iron on cultured SCs where we show that iron (as ferric ammonium citrate), in the absence of Tf or serum, also prevented SCs from de-differentiating. Furthermore, we demonstrated that intracellular signals towards differentiation become activated or stabilized through cAMP release, PKA activation and CREB phosphorylation. The prodifferentiating effect of iron and hTf suggests their participation in the axonal signal that occurs during the maturation of SCs, which enables their survival. Whereas Tf-mediated iron uptake is considered the main route of iron uptake, there is evidence for Tf-independent mechanisms. In the present work we demonstrate the existence of a divalent metal transporter (DMT1) highly described in literature as an iron metabolism key player, but never before within the PNS context. The presence of DMT1 was demonstrated in nerve homogenate, isolated adult-rat myelin and cultured SCs by Western Blot analysis and confirmed through its colocalization with S-100 (SC marker) by immunocytochemistry. Furthermore, the existence of its messenger was verified by RT-PCR both in the contralateral and ipsilateral nerves of rats submitted to sciatic nerve crush. Moreover, DMT1 mRNA was found along the SC progeny (SC precursors (E14), immature SCs (E16, E18, E20) and mature myelinating SCs (P4)). These data allow us to confirm the existence of a Tf independent iron uptake mechanism in SCs, validating the role of iron in the axonal signal.

TU06-10
LIMITING NOGO-A RECEPTOR 1-DEPENDENT PHOSPHORYLATION OF CRMP-2 REDUCES AXONAL DEGENERATION IN EAE
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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) characterized by demyelination in the relapsing-remitting form and axonal degeneration in the progressive form. The molecular mechanisms that underpin axonal degeneration are relatively unexplored in both MS and its mouse model, experimental autoimmune encephalomyelitis (EAE). We have previously reported that the axonal growth inhibitor, Nogo-A, plays a role in the pathophysiology of EAE. We now show that the phosphorylated form of the collapsin response mediator protein (pThr555-CRMP-2) is elevated during EAE. Localization of pThr555-CRMP-2 is demonstrated in degenerating axons near EAE lesions. Following the MOG35-55-induction in NgR1 knockout (ngr1-/-) mice, a significant delay in EAE onset and blunted progression was evident when compared to wild-type littermates (ngr1+/+). Furthermore, EAE-induced ngr1-/- mice displayed reduced axonal degeneration, myelin pathology and inflammation without abnormalities in immune activity. However, the limitation of axonal degeneration/loss in EAE-induced ngr1-/- mice was associated with lower levels of pThr555-CRMP-2 in the spinal cord and optic nerve, during the course of EAE. The levels of tubulin-bound CRMP-2 in ngr1-/- mice were similar to those demonstrated prior to the onset of EAE. Therapeutic administration of the anti-Nogo(623-640) antibody during the course of EAE demonstrated prior to the onset of EAE. Therapeutic administration of the anti-Nogo(623-640) antibody during the course of EAE could also abrogate the levels of pThr555-CRMP-2 in the spinal cord and was associated with an improved clinical outcome. We conclude that phosphorylation of CRMP-2 downstream of NgR1 activation may play a role in potentiating axonal degeneration in EAE and this mechanism is limited by inhibiting Nogo-A/NgR1 interaction.
TU06-11
TAG-1 EXPRESSION IN GLIAL CELLS IS SUFFICIENT FOR THE FORMATION OF JUXTAPARANODES AND THE PHENOTYPIC RESCUE OF TAG-1 MUTANTS
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Myelinating fibers are organized into specialized domains that ensure the rapid propagation of action potentials and are characterized by protein complexes underlying axoglial interactions. TAG-1 (Transient Axonal Glycoprotein-1), a cell adhesion molecule of the immunoglobulin superfamily (IgSF), is expressed by neurons as well as by myelinating glia. It is essential for the molecular organization of myelinating fibers as it maintains the integrity of the juxtaparanodal region, through its interactions with Caspr2 and the voltage-gated potassium channels (VGKCs) on the axolemma. The Tag-1-/- animals show important deficits such as impaired learning and memory as well as sensorimotor gating and gait coordination defects. Since Tag-1 is the only known component of the juxtaparanodal complex expressed by the glial cell, it is important to clarify its role in the molecular organization of juxtaparanodes. For this purpose, we generated transgenic mice that exclusively express Tag-1 in oligodendrocytes and lack endogenous gene expression [Tag-1-/-; plpTg(rTag-1)]. Phenotypic analysis clearly demonstrates that glial Tag-1 is sufficient for the proper organization and maintenance of the juxtaparanodal domain in the central nervous system. Biochemical analysis shows that glial TAG-1 physically interacts with Caspr2 and VGKCs. Ultrastructural and behavioral analysis of Tag-1-/-; plpTg(rTag-1) mice shows that the expression of glial Tag-1 is sufficient to restore the axonal and myelin deficits as well as the behavioral defects observed in Tag-1-/- animals. Taken together, these data highlight the pivotal role of myelinating glia on axonal domain differentiation and organization.

TU06-12
A ROLE FOR AUTOTAXIN IN MULTIPLE SCLEROSIS PATHOPHYSIOLOGY
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Autotaxin (ATX, Enpp2), originally isolated from the supernatant of melanoma cells as an autocrine motility stimulation factor, is a secreted lysophosphatidyl choline (LPC). Significant ATX expression has been detected in various types of cancer, and more recently in chronic inflammatory disorders. Increased amounts of ATX were also detected in the cerebrospinal fluid of multiple sclerosis (MS) patients, almost completely lacking in control fluids, suggesting a role for ATX/LPA in maintenance of cerebrospinal fluid homeostasis during pathological/demyelinating conditions. Moreover, ATX mRNA is highly upregulated during oligodendrocyte differentiation, with ATX protein transient expression and LPA signalling via the LPA1 receptor being implicated in myelination.

In this work, experimental autoimmune encephalomyelitis (EAE), was induced in C57BL6 mice, and immunohistochemistry of spinal cord sections revealed significant ATX upregulation in inflamed lesion areas, co-localized with inflammatory cells. ATX expression was also observed in activated astrocytes (GFAP+) in the periphery. To examine the role of ATX in MS pathophysiology, ATX expression was specifically ablated in oligodendrocytes through the mating of our proprietary conditional (LoxP) knock out mouse for ATX with a transgenic mouse line expressing the Cre recombinase under the control of the myelin oligodendrocyte glycoprotein (MOG) promoter, expressed specifically in oligodendrocytes. ATX oligodendrocyte ablation resulted in delay in EAE onset and significant reduction in clinical score, indicating a major role of ATX and LPA signalling in MS pathogenesis. In accordance, pharmacological inhibition of ATX also resulted in attenuation of EAE development, suggesting ATX as a promising therapeutic target in MS.

TU06-13
OLIG2 POSITIVE CELLS TAKE BRAIN REGION-SPECIFIC FATES IN RESPONSE TO PHYSIOLOGICAL OR PATHOLOGICAL STIMULI IN THE ADULT BRAIN
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Olig2 positive cells compose a subpopulation of oligodendrocyte precursors and are widely distributed in the adult brain. These cells are thought to have potentials to differentiate into multiple lineages of neural cells, namely neurons, astrocytes, and oligodendrocytes. We have been tracing the fates of the Olig2 positive cells using double transgenic mice harboring olig2 promoter driven CreERTM and ROSA-loxP-stop-loxP-GFP43-EGFP (Tatsumi et al. 2009). In the cryo-injured cerebral cortex, the GFP positive (once Olig2 promoter active) cells were abundantly found in the region surrounding a necrotic core. The GFP positive cells had a bumpy appearance under light microscopic observation with GFAP colo-
TU06-14
STRUCTURE DETERMINATION OF N-GLYCANS ON A FEW PMOL GLYCOPROTEIN AND ITS APPLICATION TO THE STRUCTURAL ANALYSIS OF N-GLYCANS ON P0
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N-linked glycans harbored on a glycoprotein affect its character by altering the protein structure or by altering the binding to other proteins. However, knowledge on their role in this alteration is limited because it has been difficult to identify precise carbohydrate structures on one glycoprotein. This is mainly due to the requirement of a large amount of glycoproteins to achieve structural determination of N-glycans. SDS-PAGE is widely used to separate proteins and has been used to identify protein structure by mass spectrometry (MS). However, this procedure is not successful for structural analysis of N-glycans because the recovery rate of N-glycans from an excised gel have been too low for the direct analysis by MS. We have refined an analytical method to detect a trace amount of N-linked sugar chains using three-dimensional HPLC system. We also developed a method to recover N-glycans from proteins separated by SDS-PAGE with a high recovery rate. These methods allowed us to determine N-glycans on a glycoprotein of pmol level. Myelin protein zero (P0) is the major myelin protein expressed by Schwann cells, comprising approximately 50% of all peripheral nervous system myelin proteins, and is necessary for normal myelin function and structure. P0 contains a single N-glycosylation site and heterogeneity in its glycosylation pattern has been reported. It was thought that the glycan heterogeneity on P0 might be regulated by alterations in physiological conditions. Here we applied our new method to analyze the porcine P0 N-glycans. Structure of 6 main N-glycans, including those with the HNK-1 epitope, was identified.

TU06-15
THE ROLE OF THE CELL ADHESION MOLECULE TAG-1 IN THE MYELINATED FIBER ORGANIZATION UPON DE- AND REMYELINATION
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Multiple Sclerosis (MS) is an autoimmune demyelinating disease of the CNS affecting mostly young adults. The myelin sheath of the myelinated fibers, responsible for the rapid propagation of action potentials is severely impaired during the course of disease. The pathology and the mechanisms implicated in MS are mostly studied in the animal model of experimental autoimmune encephalomyelitis (EAE) although the established Cuprizone model of toxic demyelination provides important insights, excluding the involvement of the immune system. TAG-1 is a cell adhesion molecule expressed both by axons and glial cells. In the adult nervous system, TAG-1 is responsible for the molecular organization of the juxtaparanodal domain of the myelinated fiber, where it interacts with Caspr2 and the potassium channels. Recently TAG-1 was identified as an autoantigen in MS patients and subsequent experiments in EAE animals have revealed its implication in white and grey matter pathology. The above and other data suggest that the molecular organization of the myelinated fiber is crucial during the onset and the progression of MS. In this study RNA samples and spinal cord cryosections from EAE rats were analyzed via quantitative real time PCR and immunohistochemistry. The expression levels of the myelinated fiber proteins differ between the different stages of the disease while their localization is excluded from the site of the lesion. Moreover, Tag-1 +/+; Tag-1 +/- and Tag-1 -/-; plp1Tg(fTag-1) animals were subjected in a 6 week treatment with Cuprizone neurotoxin which causes reversible demyelination of the CNS. Their analysis is still in progress whereas the upcoming results will provide a detailed characterization of the role of TAG-1 and more specifically of the glial form during de- and re-myelination.
TU07-01
REVERSIBLE INHIBITION OF H₂O₂ ELIMINATION BY CALCIUM IN BRAIN MITOCHONDRIA
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In the present work the Ca²⁺-dependence of mitochondrial H₂O₂ elimination was investigated. Mitochondria isolated from guinea pig brain were energized by glutamate and malate and incubated with micromolar concentrations of Ca²⁺ in the presence of ADP preventing permeability transition pore formation. After the completion of Ca²⁺ uptake, mitochondria were challenged with H₂O₂ (5 μM), then at various time points residual H₂O₂ was determined using the Amplex red method and compared to that in mitochondria incubated with H₂O₂ without Ca²⁺ addition. Dose-dependent inhibition of H₂O₂ elimination by Ca²⁺ was detected, which was prevented by the Ca²⁺-uptake inhibitor Ru 360. Stimulation of Ca²⁺ release from Ca²⁺-loaded mitochondria by a combined addition of Ru 360 and Na⁺ decreased the Ca²⁺-evoked inhibition of H₂O₂ removal. Following Ca²⁺-uptake (50 μM) mitochondrial aconitase activity was found to be decreased, partially attributable to the impaired elimination of endogenously produced reactive oxygen species. We found that the effects of Ca²⁺ and H₂O₂ on the activity of aconitase were additive.

These results confirm that Ca²⁺ inhibits elimination of H₂O₂ in mitochondria and demonstrate that this effect is concentration-dependent and reversible. The phenomenon described here can play a role in the modulation of ROS handling under conditions involving excessive cellular Ca²⁺-load.

TU07-02
GLUCOSE MODULATES GABA RELEASE AND NEURONAL CELL DEATH IN THE MATURE VERTEBRATE RETINA: A MATTER OF TIME AND CONCENTRATION
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Recurrent hypoglycemic episodes are considered a worrying condition for insulin-treated diabetic patients’ health. Once diabetes may cause vision loss, and glycemic oscillations may interfere with retinal function, this study aimed to analyze the effects of glycemic fluctuations on morphological and neurochemical aspects of the retina. Ex vivo chick retinas were kept under continuous perfusion with 95%O₂/5%CO₂ in physiological medium containing different glucose concentrations (in mM): 0, 5.6 and 35 for 30 min. The consequences of hypoglycemia (0 mM glucose) after 60 min were also analyzed. The samples were processed for Nissl staining, immunohistochemistry for γ-aminobutyric acid (GABA) and lactate dehydrogenase (LDH) activity. Hypoglycemia promoted a time-dependent progressive loss (50% for 30 min and 75% for 60 min) of GABA content from amacrine cells in the inner nuclear layer (INL) and ganglion cell layer (GCL) and its processes. This effect was due to GABA release in the first 30 min of hypoglycemia, since it could be blocked by type-1 GABA transporter (GAT-1) inhibitor (NO-711). However, a longer exposure (60 min) of retinas to hypoglycemic conditions induced swelling of the inner plexiform layer (two fold), increased LDH release (indicating cell death) and irreversible loss of GABA content from amacrine cells. In contrast, hyperglycemia (35 mM) during 30 min augmented the number of GABA-positive horizontal cells and amacrine cells. These data indicate that retinal exposure to 0 mM or 35 mM glucose during 30 min induces opposite effects on GABA release both in horizontal and amacrine cells. Furthermore, a longer period (60 min) of hypoglycemia induces retinal cell death.

TU07-03
EFFECT OF SENSORY-MOTOR COUPLING RETRAINING PROGRAM
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Stroke is the most common indication of neurological disability in adults. Re-education of joint position sensation and Motor-sensory coupling training is one of the promising new techniques to improve hand and arm functions of severely affected stroke patient. We conducted a randomized study comparing such therapy with conventional occupation therapy techniques. The newly published result showed hand function of the special program trained patients. In this paper we aim to look at functional re-organization of the patients.

All the patients underwent a fMRI scan while they attempt to move the wrist up and down repeatedly, during the first week of training and then one month after. All patients received 3 h per week arm training, other therapies given to the patients were all the same.

Visual analysis of the fMRI results showed that 55% of all the patients demonstrated a persistent recruitment pattern, in which the primary sensory motor area on the affected side together with other uni-lesional and ipsi-lesional cortices areas are activated in both the pre and post-training scans. This finding is equally found in control and experimental group at 56% and 53% respectively.

27% of all the patients showed no observable activation of the ipsi-lesional primary motor sensory cortex in the first, pre-training scan. The frequency are again, not different between groups. However All of the experimental groups and 40% of the control group patients that initially demonstrated this low activation pattern had later showed increased recruitment of the cortical areas. This would suggest that the sensory motor training therapy may induce a more physiologic cortical activation pattern, resemble to what one would expect to see in normal person.
TU07-04
DEXAMETHASONE TOXICITY INDUCES ALTERATION IN MITOCHONDRIAL FUNCTION AND CELL DEATH IN SH-SY5Y CELLS

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It has been assumed that higher levels of stress hormone are toxic to neurons or glia cells. Recent evidence shows that the addition of glucocorticoid analogue, dexamethasone increases apoptosis in neuronal cells. In addition, cell death stimuli such as excitotoxic and oxidative stress can cause mitochondrial dysfunction. However, the role of mitochondrial function in stress hormone-induced neuronal cells degeneration remains largely unknown. Here, we report the toxic effect of dexamethasone on cell viability and mitochondrial dynamics in SH-SY5Y cultured cells. Dexamethasone significantly increased reactive oxygen species formation but decreased cell viability. PTEN-induced putative kinase 1 (PINK1) is a tumor suppressor and primarily located in mitochondria, was significantly increased in dexamethasone treated cells. These results may emphasize possible role of oxidative stress-induced alteration in mitochondrial function and cell death, are associated with dexamethasone toxicity in neuronal cells.

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TU07-05
THE ARG72P53 POLYMORPHIC VARIANT INCREASES NEURONAL VULNERABILITY TO ISCHEMIA-INDUCED APOPTOSIS BY A MITOCHONDRIAL MECHANISM

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Tp53 encodes the tumor suppressor protein p53, an important transcriptional regulator of apoptosis, naturally occurs in humans in two variants with single nucleotide polymorphisms resulting in Arg (Arg72p53) or Pro (Pro72p53) at residue 72. Neurons in primary culture were transfected with minimum amount of Pro72-p53-IRESEGFP or Arg72p53-IRESEGFP cDNA not altering neuronal survival and were exposed to oxygen and glucose deprivation (OGD) for 1 h. The results showed that neurons expressing human Arg72p53 were more vulnerable against OGD-triggered apoptotic death and mitochondrial membrane potential disruption than those expressing the Pro72p53 one. The experiments were confirmed by expressing bacterial artificial chromosomes containing the endogenous promoter driven human p53 gene harboring either the Arg72 or the Pro72 allele in p53-null mice primary neurons. Interestingly, pifithrin-α, an inhibitor of p53-mediated transcriptional activation, fully prevented the modest Pro72p53 induced apoptosis without affecting that of Arg72p53. Thus, neuronal apoptotic death by the human specific Arg72p53 occurs through a transcriptional-independent mechanism not resembling rodent cell death caused by p53. In good agreement with this notion, we further show that Arg72p53, but not Pro72p53, is localized in the mitochondria and promotes cytochrome c release from the mitochondria to the cytosol. Furthermore, Arg72p53, but not Pro72p53, interacted directly with mitochondrial Bcl-XL and activated the intrinsic apoptotic pathway, increasing vulnerability to ischemia-induced apoptotic cell death. This work was supported by Instituto de Salud Carlos III (PS09/ 0366 and RD06/0026/1008), Junta de Castilla y Leon (GRS244/A- 08, GREX206), Ministerio de Ciencia e Innovacion (SAF2010- 20008), and Caja de Burgos.

TU07-06
CURCUMIN TO TREAT THE NEUROMUSCULAR DISORDER DYSFERLINOPATHY

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The limb girdle muscular dystrophy, dysferlinopathy, is an inherited neuromuscular dystrophy characterized by progressive limb muscle wasting. It is due to a defect of dysferlin which is involved in muscle membrane repair. There are no specific treatments for inherited neuromuscular disorders as their pathomechanisms remain largely unexplored. Among suggested treatments of pharmaco-therapy, gene and cell therapies, drugs appear the most feasible and best tolerated. Oxidative and nitrosative stress, implicated in several muscle wasting disorders, may underlie wasting of dysferlinopathy. The polyphenol curcumin is an antioxidant and nitric oxide scavenger that induces tissue regeneration.

Aim: To determine the potential of curcumin to treat dysferlinopathy.

Approach: (i) Determine if oxidative and nitrosative stress contribute to protein degradation in dysferlinopathic muscle by assay of reduced glutathione, nitrite, ubiquitinylated proteins and non-collagen protein in dysferlinopathic and normal muscle biopsies, obtained with consent. (ii) Determine if curcumin increases cell survival in cultured rat myoblasts subject to oxidative stress by exposure to hydrogen peroxide. (iii) Determine if curcumin affects muscle force generation, as a good drug should not interfere with function, by measurement of mechanical force in intact frog skeletal muscle treated with 1–3 µM curcumin.

RESULTS: Reduced glutathione, nitrite and ubiquitinylated proteins were significantly elevated twofold, 1.9 fold and 46% respectively and protein content reduced 40% in dysferlinopathic muscle compared to normal. Curcumin prevented oxidative stress induced cell death of rat myoblasts and increased cell number. Curcumin did not affect muscle force between 0–1 µM (167 ± 27 kPa and 172 ± 32 kPa respectively) but decreased force and induced myoblast death at 3 µM.

Conclusion: Muscle wasting may occur through oxidative and nitrosative stress induced protein ubiquitinylation in dysferlinopathy. Curcumin protects myoblasts against oxidative stress, prevents cell death and induces cell proliferation while preserving muscle function at low dose. Curcumin exhibits potential for treatment of muscle wasting induced by oxidative stress, as may occur in dysferlinopathy.
TU07-07
MITOCHONDRIAL DAMAGE IN THE RAT BRAIN
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Mitochondrial theory of ageing, a hypothesis of free radical theory of ageing, suggests that accumulation of mitochondrial oxidative damage leads to human and animal ageing because mitochondria are the main source and target of reactive oxygen species. All organisms live in an environment containing reactive oxygen species and mitochondrial respiratory chain which generate reactive species. There is increasing evidence that bioenergetic function of mitochondria and mitochondrial antioxidant pool decrease with advancing age in several tissues. We observed different kind of mitochondrial oxidative changes in the brain and in the heart during ageing. Brain mitochondria from three groups of Wistar rats (6, 15 and 26 months old) were investigated. To evaluate the effect of ageing on membrane we used fluorescen probe 1-anilino-8-naphthalenesulfonate and observed changes were not significant. There was a significant increase in lipid peroxidation products and in protein modification mediated by lipid peroxidation end products. Protein conjugates with lipid peroxidation end products increased to 121% in 15 months old animals and to 122% in 26 months old animals compared to 6 months old rats. Levels of conjugated dienes did not change during ageing. Oxidative damages in mitochondria with advancing age may contribute to cellular dysfunction and to neurodegenerative diseases. This work was supported by project “Center of translational medicine” co-financed from EC sources and European Regional Development Fund and by Ministry of Health 2007/57-UK-17.

TU07-08
TRANSGENIC LINES FOR GLUTATHIONE DEFICIENCY: RELEVANCE FOR NEURODEGENERATIVE DISEASES
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The brain is a highly susceptible target to pro-oxidant agents damage, and glutathione is the most important antioxidant system in this tissue. In fact, nigrostriatal glutathione deficiency is the earliest pre-symptomatic sign in sporadic Parkinson Disease (PD), though it is yet unknown whether its deficiency, alone, can trigger PD. Experimental evidences obtained in our laboratory have shown that glutathione deficiency, obtained by specifically interfering with the glutamate-cysteine ligase (GCL), the rate limiting enzyme in the biosynthesis of glutathione, in primary cultured neurons causes spontaneous degeneration, even in the presence of astrocytes in coculture (J. Biol. Chem. 280:38992-39001, 2005). Here, we present preliminary results of the establishment of a transgenic mouse line, that we have generated from a LoxP-based construct, designed to specifically interfere in the biosynthesis of glutathione in a tissue-specific and temporally controlled manner. This technique, implemented in vivo, has rendered mice whose cultured fibroblasts show a decrease in GCL protein content. Cross-breeding this line with several Cre lines we are currently developing in which Cre is driven by tissue-specific promoters (tyrosine-hydroxylase, CamKIIα or GFAP) will provide answers for the role of oxidative stress in neurodegenerative diseases, such as PD and Alzheimer’s diseases. This model could also represent a novel tool for the search of new pharmacological, genetic and cellular strategies for the treatment of such diseases.

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TU07-09
NITRATED HSP90 DECREASES MITOCHONDRIAL MEMBRANE POTENTIAL AND OXYGEN CONSUMPTION
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Production of peroxynitrite and formation of nitrotyrosine are associated with several pathologies, including neurodegenerative and inflammatory conditions, central nervous system trauma and stroke. Although there is evidence indicating that tyrosine nitration is directly involved in the induction of cell death, the nitratated targets mediating cell death remain unknown. Out of 17 proteins that are major targets for peroxynitrite, nitratated heat shock protein 90 (Hsp90) was the only able to induce cell death in PC12 cells and motor neurons through a toxic gain-of-function. Subcellular fractionation of PC12 cells treated with peroxynitrite showed that a fraction of nitratated Hsp90 was associated to the mitochondrial outer membrane. These results were confirmed using high content imaging. Incubation of Hsp90 with peroxynitrite resulted in 50% decrease in Hsp90 ability to interact with other proteins. Accordingly, the association of nitratated Hsp90 to isolated mitochondria was also decrease by 50%, either in the presence or absence of cytosolic proteins. However, wild type Hsp90 was not able to compete with nitratated Hsp90 (ratio 10:1) for the binding to isolated mitochondria, suggesting an increased affinity of nitratated Hsp90 for a previous mitochondrial client or binding to a new client. The intracellular delivery of nitratated Hsp90 to PC12 cells decreased the basal mitochondrial oxygen consumption rate by ~40% after 18 h in culture, as determined using the Seahorse Bioscience XF Analyzer. Similarly, the mitochondrial membrane potential was decreased by ~40% in isolated mitochondria from a PC12 cell homogenate incubated with nitratated Hsp90 for 45 min, as measured by JC-1 staining. The results suggest that peroxynitrite-treated Hsp90 decreases the mitochondrial membrane potential by altering interactions with mitochondrial clients.

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TU07-10  
NEURONAL NITRIC OXIDE SYNTHASE EXPRESSING CELLS AND MELATONIN CONCENTRATIONS IN THE ISCHEMIC SPRAGUE DAWLEY RAT BRAIN  
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In ischemic brain injury, nitric oxide can be either neuroprotective or neurotoxic depending on which isoform is expressed. The expression of neuronal nitric oxide synthase (nNOS) cells in the brain following global cerebral ischemia and effect of exogenous melatonin was studied in Sprague-Dawley rats. Global cerebral ischemia was induced by bilateral common carotid artery occlusion for 10 min followed by reperfusion. The pre-ischemia melatonin group received 5 mg/kg melatonin 30 min before ischemia; the post-ischemia melatonin group received the same dose of melatonin after ischemia and a third sham control group did not receive melatonin. Melatonin concentration was measured in duplicate using a commercially prepared radioimmunoassay ELISA procedure for the quantitative measurement of melatonin in the serum samples (IBL, Hamburg, Germany). All animals were euthanized 72 h post-ischemia, perfusion-fixed with 4% paraformaldehyde in phosphate buffer and the brains removed and sectioned at 50 μm. Putative NOS positive cells were observed in the cerebral cortex, putamen, caudate nucleus, substantia reticularis, olfactory bulb, nucleus caudatus, hippocampus and subcallosal cortex. No NOS positive cells were observed in the cerebellum in any group. The mean NOS positive cells number was highest in the sham control group (220), followed by the post-ischemia melatonin (179) and lowest (148) in the pre-ischemia melatonin group with the corresponding mean melatonin concentrations as 266.94, 291.58 and 272.96 pg/mol. Neuronal NOS positive cells were more in the subcallosal cortex, olfactory bulb, substantia reticularis and least in the nucleus caudatus. A neuroprotective role by melatonin in the post-ischemia phase seems to be the mechanism of action associated with NOS activity in ischemic brain injury.

TU07-11  
HYPERHOMOCYSTEINEMIA AND ISCHEMIC PRECONDITIONING IN RAT BRAIN: RESPONSE OF SPCA CA2+ ATPASE GENE EXPRESSION  
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Hyperhomocysteinemia is recognized risk factor of brain ischemia. Ischemic pre-conditioning (IPC) represents adaptation of the CNS to sub-lethal ischemia, resulting in increased brain tolerance. This study determines whether hyperhomocysteinemia alone or in combination with IPC affects the gene expression of secretory pathways Ca2+-ATPase (SPCA1). Hyperhomocysteinemia was induced by administration of homocystine (Hcy) (0.45 μM/g) for 14 days. Rats were pre-conditioned by 5 min of ischemia and 2 days later, 15 min of global ischemia was induced by four vessel occlusion. We observed that hyperhomocysteinemia significantly decreased level of SPCA1 mRNA in the cerebral cortex. This also led to non-significant decreases in expression levels in the hippocampus. Ischemic challenge did not significantly alter level of mRNA SPCA1 in comparison to controls. However, gene response to pre-ischemia was noticeable in homocysteine ischemia in both brain areas. In cortex, IPC in homocysteine group led to the abrupt stimulation of the mRNA expression level by 249% within the hyperhomocysteinemic ischemic group and by 321% in the hyperhomocysteinemic control. Values further exceeded those observed in the naive control. In the hippocampus, the differences between naive and homocysteine groups were not observed, however, IPC initiated significant elevation of mRNA expression to 159% (p < 0.05) of control with homocysteine and significant elevation of mRNA expression to 131% (p < 0.01) of ischemia with homocysteine. No effect of IPC challenge was observed in the naive groups. We conclude that hyperhomocysteinemia initiates suppression of the SPCA1 gene expression in both brain regions. Documented response of SPCA gene to IPC in hyperhomocysteinemic group might suggest a correlation of SPCA expression consistent with the role of cross-talks between intracellular Ca2+ stores including secretory pathways in the phenomenon of ischemic tolerance. 

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TU07-12  
PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-γ (PPAR-γ) INHIBITS NADPH OXIDASE ACTIVATION UPON ISCHEMIC INSULT  
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Background and Purpose: It has been shown that 15d-PGJ2 and thiazolidinedione attenuated reactive oxygen species (ROS) production through a PPAR-γ-dependent pathway against brain injury. However, it is not entirely clear how PPAR-γ reduces ROS production. Recent studies indicate that NADPH oxidase is one of the major sources for ROS production in brain. In the present study, we aim to study whether PPAR-γ interacts with NADPH oxidase, which then regulate the ROS formation upon ischemic insult. Methods: Oxygen-glucose deprivation followed by reoxygenation (H-R) was used to study the interaction between PPAR-γ and NADPH oxidase in cerebral endothelial cells (CECs) in vitro; and 3- vessel occlusion stroke model in vivo. MTT was used to detect cell viability. Flowcytometry and fluorescence microscopy were used to monitor apoptosis, MMP and cellular H2O2 levels. Reporter assay was used to detect the transcriptional activity. Confocal microscopy was used to dissect the subcellular localization. A transgenic mice with heterozygous knock-in of a PPAR-γ dominant-negative mutant, P465L (L/+), was used in this study.

Results: PPAR-γ agonists (15d-PGJ2) significantly reduced ROS production and NADPH oxidase activity in CEC cells. This antioxidative effect was abrogated by GW9662 or PPAR-γ siRNA. Over-expression PPAR-γ also showed anti-oxidative effect. p22-phox reduction probably attributed to this beneficial effect, since p22-phox siRNA significantly decreased cellular H2O2 production. 15d-PGJ2 significantly reduced NADPH oxidase levels in ischemic brain. This antioxidative effect was mimicked by PPAR-γ over-expression and wiped out in the presence of PPAR-γ siRNA. Interestingly, transgenic mice with only 50% of the PPAR-γ activity showed higher basal level of p22-phox.

Conclusion: We have demonstrated that PPAR-γ inhibited NADPH oxidase activity by down-regulated p22-phox subunit level, which
led to the reduction of ROS formation, and subsequently attenuated ischemic induced apoptotic cell death.

**TU07-13**

**CYCLIN B1 MEDIATES MITOCHONDRIAL DYSFUNCTION IN EXCITOTOXICITY**

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Anaphase-promoting complex/cyclosome (APC/C), an E3 ubiquitin ligase that destabilizes cell cycle proteins, is activated by Cdh1 in post-mitotic neurons, where it regulates axonal growth, synaptic plasticity and survival. The APC/C-Cdh1 substrate, cyclin B1, has been found to accumulate in degenerating brain areas in Alzheimer’s disease and stroke. Recently, we have reported that stimulation of N-methyl-D-aspartate receptors (NMDAR) that occurs in neurodegenerative diseases promotes the phosphorylation of Cdh1 by the Cdk5/p25 complex, a condition necessary and sufficient for its translocation to the cytosol and APC/C-Cdh1 inactivation. This led to the stabilization of cyclin B1 in cortical neurons and cell death. These results highlight the importance of elucidating the role of cyclin B1 in neurons under excitotoxic conditions relevant to neurodegenerative diseases. Cortical neurons in primary culture were stimulated with glutamate (100 µM) and mitochondrial function and generation of radical oxygen species were measured by flow cytometry. Here we described that glutamate promoted oxidative stress and mitochondrial membrane potential depolarization by a mechanism involving cyclin B1 accumulation. Moreover, expression of either cyclin B1 or hEmi1, a well-known APC/C inhibitor, induced oxidative stress, mitochondrial dysfunction and neurodegeneration. Our results suggest that NMDAR stimulation increased Cdk5 kinase activity leading to Cdh1 phosphorylation, APC/C inactivation and cyclin B1 stabilization. As a consequence, cyclin B1 promoted oxidative stress, mitochondrial dysfunction and neuronal apoptotic death. These results reveal Cdh1 as a novel Cdk5 substrate that mediates cyclin B1 neuronal accumulation in excitotoxicity.

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**TU07-14**

**CONTRIBUTION OF CALPAIN TO NEURONAL DEATH INDUCED BY GLUCOSE DEPRIVATION IN CULTURED NEURONS**

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Glucose is the main energy substrate in brain. Whenever blood glucose concentration declines to levels below 20 mg/dl, brain activity ceases leading to the hypoglycemic coma, and irreversible neuronal damage can take place in vulnerable brain regions, such as the hippocampus. Excitotoxicity triggered by the release of excitatory amino acids and oxidative stress, have been suggested to contribute to hypoglycemic neuronal damage. In the present study we have investigated the role of the calcium-dependent cysteine protease, calpain, a well-known mediator of excitotoxic damage, in neuronal death induced by glucose deprivation (GD) in hippocampal cultured neurons. Calpain activity, as assessed by the cleavage of the cytoskeletal protein, alpha-spectrin, is progressively activated during GD (from 15 min to 2 h) and neuronal survival is 50 percent reduced 2 h after 2 h of GD. The NMDA receptor antagonist MK-801, the calcium chelator EDTA and the calpain inhibitor MDL-28170, prevent calpain activity and cell death, suggesting that calcium influx is involved in calpain activation. Calcium release from the endoplasmic reticulum also contributes to calpain activity, since blockade of the ryanodine and the IP3 receptors reduces alpha-spectrin cleavage. We have previously demonstrated that reactive oxygen species (ROS) are rapidly produced during GD, by the activation of calcium-dependent enzymes and the superoxide-producing enzyme, NADPH oxidase (NOX). We have monitored the fluorescent signal of the oxidation-sensitive marker, dihydrodextrin (Et), to evaluate ROS production. Results show that blockage of ryanodine and IP3 receptors reduces the Et signal, suggesting that calcium release from the endoplasmic reticulum also contributes to ROS production. Moreover, the NOX inhibitor, apocynin, also reduces calpain activity. These results suggest a relationship between ROS production, intracellular calcium increase, calpain activation and neuronal death induced by GD.

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**TU07-15**

**QUANTITATIVE PROTEOMIC ANALYSIS OF S-NITROSYLATED PROTEINS IN MICROGLIAL BV-2 CELLS: EFFECTS OF EGCG UNDER NITROSATIVE STRESS**

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Nitric oxide (NO) is a signaling molecule involved in modulating many cellular functions. Emerging evidence suggests that nitrosative stress induces the NO-related protein modifications that contribute to neuroinflammation in neurodegenerative disorders. Akin to phosphorylation, S-nitrosylation (covalent adducts of NO to specific cysteine residues) is a prototypical and redox-based mechanism for NO signaling in cells. To characterize such redox-specific cysteine residues, is a prototypical and redox-based mechanism for NO signaling in cells. To characterize such redox-based S-nitrosylation relevant to pathological conditions, it is necessary to implement efficient methods to quantify S-nitrosylated proteins, identify their modification sites, and determine how protein S-nitrosylation impacts into health and disease. Herein we reported a gel-based proteomic approach to identify and quantify S-nitrosylated proteins by integrating the benchmark biotin switch assay and Differential In Gel Electrophoresis (DIGE). This approach, termed NitroDIGE as to a DIGE-like method for analysis of protein S-nitrosylation, is a ‘top-down’ screening strategy to identify specific protein modification under nitrosative stress. Using this approach, we investigated neuroinflammation-induced nitrosative/oxidative stress by endotoxin lipopolysaccharide (LPS) in...
immortalized murine microglial BV-2 cells, and evaluated the antioxidant effects of the green tea polyphenol (-)epigallocatechin-3-gallate (EGCG). We identified 47 proteins spots affected by the exposure of BV-2 cells to S-nitrosocysteine (SNOC), a physiological NO donor, or LPS. We found that EGCG could exert antioxidant effects by inhibiting NO production induced by LPS and preventing proteins from S-nitrosylation under nitrosative stress. Among these proteins, EGCG was shown to attenuate S-nitrosylation of peroxiredoxins, a family of antioxidant enzymes. Taken together, NitroDIGE is an effective proteomic approach for screening protein S-nitrosylation, and the effects of EGCG suggest its therapeutic potential for neurodegenerative disorders.

TU07-16
NOX2 MEDIATES APOPTOTIC DEATH INDUCED BY STAUROSPORINE, BUT NOT BY POTASSIUM DEPRIVATION IN CEREBELLAR GRANULE NEURONS
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Several studies have suggested that reactive oxygen species (ROS) are involved in neuronal apoptotic death. It has been recently suggested that one of the sources of ROS in neurons is one NADPH oxidase. This is a multimeric enzyme that generates superoxide anion, which was originally identified in phagocytic cells. This complex is constituted by two membrane (NOX and p22phox) and three cytosolic (p47phox, p67phox, and p40phox) subunits. Recent studies have shown that NADPH oxidase has several homologues expressed in non-phagocytic cells, termed NOX1 to 5. On the other hand, cerebellar granule neurons (CGN) die apoptotically when they are treated with staurosporine (ST) or when cells are transferred from a depolarizing medium (25 mM KCl; K25) to a medium containing 5mM KCl (K5). Several studies have shown that apoptosis of CGN is mediated by ROS. However, the source of ROS implicated has not yet been identified. In the present study, we evaluated the participation of NADPH oxidase, particularly the homologue NOX2, in the apoptotic death induced by K5 and ST of CGN. We found that CGN express NOX 1–4 and that different NOX inhibitors markedly reduced cell death induced by both ST and K5. However, we observed that NOX 2 deficient CGN were protected from death induced by ST, but not by K5. In addition, all the apoptotic parameters evaluated, including caspase-3 activation, nuclear condensation and apoptotic volume decrease, were markedly reduced in NOX 2 deficient CGN treated with ST, but not with K5. These results suggest that cell death induced by ST seems to involve NOX2 and that K5-induced death of CGN requires the participation of a NADPH oxidase different from NOX 2.

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TU07-17
COULD INCREASED DOPAMINE SYNTHESIS IN VASOPRESSIN NEURONS DUE TO PERINATAL HYPOXIA INDUCE DIABETES INSIPIDUS IN THE HUMAN NEONATE?
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Magnocellular neurosecretory neurons -in addition to vasopressin (VP) and oxytocin (OXY)- synthesize other peptide and non-peptide neurotransmitters under experimental activation. We previously showed that VP neurons of the human neonate express tyrosine hydroxylase (TH) -first and rate limiting enzyme in catecholamine synthesis- under perinatal hypoxic conditions. Increased TH expression was considered as a neuropathological marker of prolonged perinatal hypoxia in autopsy material (J Neuropath Exp Neurol, 69:1008–1016, 2010). Purpose of the present study was to immunohistochemically study the expression of neurophysin (NP), VP and OXY in parallel with TH induction in relation to the neuropathological grading of perinatal hypoxia. We studied the dorsolateral supraoptic nucleus (dl-SON) of 13 infants (aged 34–47 weeks) obtained from the Greek Brain Bank, after parental written consent for use of brain material for diagnostic and research purposes. Based on neuropathology three grades of hypoxic injury were recognized: grade 1 as severe/abrupt, grade 2 as moderate/prolonged and grade 3 as very severe with long duration. Computerized image analysis showed increased cellular and nuclear size in VP neurons of hypoxia grade 2 and 3 cases, indicating selective activation of VP neurons due to prolonged perinatal hypoxia. The optical density of VP and OXY presented a slight reduction in hypoxia grade 2 cases -probably due to their increased secretion in the periphery. In these cases, all the VP neurons of dl-SON were found to synthesize TH, indicating massive dopamine synthesis under prolonged hypoxic conditions. Dopamine can act as VP-inhibiting factor in man (Clin Endocrinol, 12:39–46, 1980) and therefore, through massive inhibition of VP release could cause central diabetes insipidus reported to occur in severe hypoxic encephalopathy (J Formos Med Assoc, 105:536–41, 2006).

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TU07-18
PERINATAL HYPOXIA: AN UNDERESTIMATED FACTOR FOR DOPAMINE DYSREGULATION IN NEUROLOGICAL AND PSYCHIATRIC DISORDERS
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Hypoxia during the last trimester or during the intrapartum period could cause long-term damage to the central nervous system leading to behavioral and/or neurological deficits later in development. Evidence from animal studies suggest that hypoxia to the
fetus - a consequence common to many birth complications in humans - results in long-term disturbances of central dopaminergic (DA) systems, that persist in adulthood (1). Immunohistochemical studies in the rat showed that perinatal hypoxia cause time-dependent changes in the number of DA cell bodies in substantia nigra (SN) and ventral tegmental area (VTA) that innervate basal ganglia and prefrontal cortex respectively. We studied the expression of tyrosine hydroxylase (TH) - the first and rate limiting enzyme in catecholamine synthesis - in SN and VTA of 18 infants, obtained from the Greek Brain Bank, after parental written consent for use of brain material for diagnostic and research purposes. Based on neuropathology three grades of hypoxic injury were recognized: grade 1 as severe/abrupt, grade 2 as moderate/prolonged and grade 3 as very severe with long duration. Computerized image analysis showed a striking loss of TH-immunoreactivity in SN and VTA in hypoxia grade 2 and 3 cases that suffered from prolonged perinatal hypoxia. This phenomenon was specific for mesencephalic DA neurons, since our previous studies (2) showed dramatically increased TH-immunoreactivity in magnocellular neurosecretory neurons of the same cases. Since dysregulation of DA systems is involved in many neurological and psychiatric disorders, such as Parkinson’s disease, schizophrenia and ADHD, the contribution of perinatal hypoxia in the pathophysiology of these disorders should be further investigated.

Supported by University of Athens, Special Account for Research Grants.

References:

TU07-19
MEMORY ENHANCING EFFECTS OF SAFFRON IN ADULT & AGED MICE ARE CORRELATED WITH THE ANTIOXIDANT PROTECTION: IN VITRO & IN VIVO STUDY
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In the present study, we examined: (a) the antioxidant, anti-amyloid and anti-cholinesterase properties of C. sativus styles extract (saffron) in vitro and the effects of its constituents on the oxidative status and Aβ-fibrillogenesis in various cell culture systems (SH-SY5Y, CHOAPP770) and (b) the effect of saffron on learning & memory, brain oxidative status (ascorbic acid, glutathione, malondialdehyde) and acetylcholinesterase activity (AChE) in aged, male Balb-c mice after intraperitoneal administration (7 days, 60 mg/Kg B.W.). Results in vitro showed that saffron possesses good antioxidant properties and the thioflavine T-fluorescence based assay showed a concentration and time-dependent inhibition of Aβ-fibrillogenesis. Kinetic analysis of acetylcholinesterase activity in the presence of the tested phytochemicals, showed a dose-dependent, non-competitive type of inhibition for crocetin (CRT) and safranal. In cell culture systems, both saffron and crocetin provided strong protection in rescuing cell viability, repressing ROS production and decreasing caspase-3 activation against H$_2$O$_2$-induced toxicity in SH-SY5Y cells, while only moderate effects were observed on Aβ-fibrillogenesis in CHOAPP770 cells. Saffron-treated mice exhibited significant improvement in learning & memory, accompanied by significantly lower brain lipid peroxidation and higher antioxidant parameters. AChE activity remained unchanged.

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TU07-20
ANTIOXIDANT AND ANTIAPOTPTIC FUNCTIONS OF MITOCHONDRIAL TARGETED SYNTHESIS OF GAMMA-GLUTAMYL-CYSTEINE IN CORTICAL NEURONS
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Mitochondria are the main source of reactive oxygen species (ROS) in the cell, being also the most important target of their effects. Although the exact mechanisms remain elusive, mitochondrial ROS can regulate different signal pathways through the interaction with mitochondrial proteins. In this work, our aim was to design and characterize a system capable of downregulating the formation of ROS specifically and persistently in the mitochondria. For this purpose, the mitochondrial-targeting domain of ornithine transcarbamylase was fused to the N-terminal domain of glutamate-cysteine ligase, catalytic subunit (GCL). This mitoGCL cDNA was expressed in neurons and HEK293T cells, which resulted in the efficient targeting of GCL to the mitochondria, as confirmed by subcellular fractionation, western blotting and fluorescence microscopy. The protein was proved to be functional within the organelle, as assessed by γ-glutamylcysteine formation in isolated mitochondria, measured by HPLC with electrochemical detection. The production of hydrogen peroxide and superoxide anion detected with the Amplex Red and MitoSOX probes, respectively, was found to be lower in mitochondria isolated from cells expressing mitoGCL when compared with those expressing cytosolic GCL. MitoGCL also supported protection against excitotoxic damage-mediated caspase 3 activation and apoptotic cell death. These effects could be wholly accounted for by the presence of GCL in the mitochondria, being fully independent on the formation of glutathione in the cytosol, which did not differ between controls and mitoGCL-expressing cells. In conclusion, our results show that γ-glutamylcysteine can act as an antioxidant that persistently down-regulates the formation of ROS specifically in the mitochondria. This strategy represents a novel tool for the study of signaling pathways modulated by mitochondrial ROS, as well as a new defense system against oxidative stress in the central nervous system. Funded by MICINN (SAF2010-20008; CSD2007-00020), FIS (PS09/0366), and JCyL (GREX206).
TU07-21
MECHANISM OF P53-DEPENDENT MITOCHONDRIAL APOPTOSIS INITIATION AFTER GLOBAL BRAIN ISCHEMIA IN RAT HIPPOCAMPUS

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Apoptosis is an evolutionarily conserved process of cell death that is crucial for development and tissue homeostasis in CNS. Deregulation of apoptosis, disrupting delicate balance between cell survival and death, plays a major role in the development of malignant brain diseases but can also elicit inappropriate cell death. Transient global brain ischemia represents a form of severe metabolic stress that culminates in selective delayed neuronal death. Induction of intrinsic (mitochondrial) apoptotic pathway after global brain ischemia was documented in several previous studies.

Using 4-vessel occlusion model, we have documented that global brain ischemia induces transcription-independent mitochondrial pathway since translocation of p53 to mitochondria was observed in hippocampus of rats subjected to global ischemia in duration of 15 min and consequent 3, 24 and 72 h of reperfusion. The level of other key players of mitochondrial apoptosis, Bax and Bcl-XL, was high in control mitochondria and was not affected by ischemia and consequent reperfusion. Finally, ischemia did not induce transcriptional activation of p53, expression of p53 regulated proteins, Bax and Bcl-2, were not affected by ischemia/reperfusion. Detection of genomic DNA fragmentation as well as Fluoro-Jade C staining showed that ischemia induces apoptosis in vulnerable pyramidal neurones of CA1 layer of rat hippocampus. Finally, ischemia-induced translocation of p53 to mitochondria was abolished by pretreatment of rats with sub-lethal ischemia two days before lethal ischemia. Ischemia-induced translocation of p53 to mitochondria inversely correlates with expression of heat shock protein 70 (HSP70) after naïve and preconditioned ischemia. Therefore we conclude that the elevated level of HSP70 might represent plausible explanation of inhibition of both translocation of p53 to mitochondria and ischemia-induced apoptosis observed after preconditioned ischemia.

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TU07-22
IS SUMOYLATION OF CALCIUM CHANNELS INVOLVED IN BRAIN ISCHAEMIA?

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Brain ischaemia has a huge impact on the afflicted individuals, their families and society in general. New treatments to manage this debilitating and life-threatening condition require a full understanding of the underlying pathogenic molecular mechanisms. These mechanisms include post-translational protein modifications such as Small Ubiquitin-like MOdifier (SUMO) conjugation. SUMOylation modifies protein-protein interactions, altering their subcellular localization, activity, and stability. SUMOylation of the majority of proteins is transient and is readily cleaved by the SUMO-specific family of SENP proteases, allowing cells to respond rapidly to varying cellular demands. Recently, it has been shown that protein SUMOylation plays a role in the dynamic regulation of presynaptic Ca²⁺ influx and glutamate release; however, the target proteins and the molecular mechanisms involved remain unknown. Among the important candidate proteins known to regulate neurotransmitter release are the presynaptic CaV2.2 calcium channel subunits, this subunit contains a high probability consensus SUMOylation motif that we are currently investigating. Using a range of complementary, biochemical, electrophysiological and cellular and molecular biology techniques, we are currently investigating if SUMOylation-dependent down-regulation of calcium channels provides a means to reduce ischaemia-induced excitotoxicity and subsequent neuronal death. Support: RETF PhD studentship and Royal Society.

TU07-23
EFFECTS OF THIAMINE PYROPHOSPHATE DEFICITS ON ACETYL-COA COMPARTMENTATION AND CHOLINERGIC PHENOTYPE OF SN56 NEUROBLASTOMA CELLS

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It is known that thiamine pyrophosphate (TPP) deficits inhibit pyruvate (PDHC) and ketoglutarate dehydrogenase complexes, and suppress cholinergic transmission in the brain, yielding cognitive, vegetative and motor deficits. In several cases this deficiency has subclinical course. General pathomechanisms of TPP deficiency are well recognized. However, no data exist on relationships between degree of TPP deficit and alterations in intracellular distribution of acetyl-CoA, in the cholinergic compartment of the brain. Therefore, the aim of this study was to investigate how amprolium-induced TPP deficits (TD) affect intracellular distribution of acetyl-CA in cholinergic neuroblastoma cells, originating from murine septum. In low thiamine medium, amprolium (0.5–5.0 mM) caused similar concentration-dependent decreases in TPP levels (40%) in nondifferentiated (NC) and differentiated cells (DC). In such conditions DC displayed significantly greater loss of viability (12%) than the NC ones (5%), despite of similar suppressions of PDHC and tetrazolium salt reducing activities in the former. Significant correlations were found between decrease of cellular TPP and inhibition of PDHC (r = 0.922, p = 0.026) and MTT reduction rates (r = 0.981, p = 0.003) in DC, but not in NC. Intramitochondrial levels of acetyl-CoA in DC were 73% lower than in NC, what explains greater susceptibility of the former to TD. Choline acetyltransferase activity and acetylcholine content in DC were two times higher than in NC. TD altered choline acetyltransferase activities neither in NC nor in DC. However, TD caused 50% decrease of cytoplasmic acetyl-CoA levels that correlated with 44% reduction of acetylcholine content in DC (r = 0.914, p = 0.029) but not in NC. These data indicate that particular vulnerability of DC to TD may result from relative shortage of acetyl-CoA in their mitochondria due to its higher utilization for acetylcholine synthesis. In addition, loss of acetylcholine in modestly TPP-deficient DC would result from limited provision of acetyl-CoA to cytoplasmic compartment and not from impairment their structural elements. Supported by M.N.S.W. projects NN401 1029937, IP 2010 035370 and GUMed fund St-57.

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TU08-01

ACTIVITY-DEPENDENT REGULATION OF ARC IN A MODEL OF THE AUTISM SPECTRUM DISORDER TSC

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Proper neural circuit function is dependent upon the ability of neurons to adapt to chronic changes in network activity during early brain development. Neurons achieve this by bidirectionally scaling excitatory synapses to maintain firing in an optimal range while preserving relative differences in the strength of individual synaptic inputs. This homeostatic plasticity is thought to be important for maintaining balanced excitation and inhibition in neural networks, a process proposed to be disrupted in neurodevelopmental disorders. The immediate early gene Arc has been identified as a putative effector of homeostatic plasticity; however, the signaling pathways which link changes in activity to the regulation of Arc are incompletely understood. Using dissociated hippocampal cultures we identified a signaling cascade which regulates Arc in response to increased network activity. This pathway required the co-activation of NMDA receptors and L-type voltage-gated calcium channels resulting in increased phosphorylation of Erk1/2 and rapid transcription of new Arc mRNA. The regulation of Arc in this context was largely at the level of transcription and did not require translational control through the mTOR pathway. Since activity-dependent modulation of gene transcription is thought to be important during early brain development, we investigated whether this pathway was perturbed in a model of the autism spectrum disorder Tuberous Sclerosis Complex (TSC). We found that Erk1/2 phosphorylation, Arc mRNA, and Arc protein levels were all basally increased in Tsc1 knock-out (KO) cultures. We monitored network activity levels using multi-electrode arrays and found that Tsc1 KO cultures displayed increased spontaneous spiking frequency compared to control cultures. This indicates that loss of Tsc1 leads to increased network activity and constitutive activation of the homeostatic pathway. Such perturbations could alter neural circuit dynamics and may contribute to the neurological dysfunction observed in TSC.

TU08-02

mGLU5 AND ADENOSINE A2A RECEPTOR INTERACTIONS REGULATE THE CONDITIONED REINFORCING EFFECTS OF COCAINE

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The striatum is known to play a crucial, integrative role in processes such as reward, motivation and drug-seeking behaviour. Adenosine A2A receptors and metabotropic glutamate type 5 (mGlu5) receptors are co-localised both presynaptically and postsynaptically in the striatum and have been shown to functionally interact to regulate drug-seeking. In the present study this interaction was explored using antagonism of mGlu5 receptors with 3-[2-methyl-1,3-thiazol-4-yl] ethynyl]-pyridine (MTEP) in combination with genetic deletion of A2A receptors. The conditioned rewarding and locomotor activating properties of cocaine were evaluated using the conditioned place preference (CPP) paradigm. Adenosine A2A receptor knockout (n = 16) and wildtype (n = 26) mice were subjected to alternating daily conditioning injections of cocaine (20 mg/kg, i.p.) or saline. 20 min prior to cocaine administration mice were pre-treated with either MTEP or vehicle. CPP was assessed following 8 days of conditioning. During each session the time spent in each compartment (sec) as well as locomotor activity (distance moved in cm) was measured. Vehicle-treated mice of both genotypes expressed a CPP to cocaine while MTEP abolished cocaine CPP in wildtype, but not A2A knockout, mice. These results were mirrored when conditioned hyperactivity was assessed. In contrast, MTEP attenuated the acute locomotor activating properties of cocaine similarly in both genotypes. These data provide evidence for a functional interaction between adenosine A2A and mGlu5 receptors in mediating the conditioned effects of cocaine (either directly or via modulation of incentive learning) but not direct cocaine-induced hyperactivity. This functional interaction is supported by modulation of 4-[2-[7-amino-2-[2-furyl][1,2,4]triazolo[2,3-a][1,3,5]triazin-5-yl-aminol]ethyl]phenol ([125I]ZM241385) binding to the A2A receptor by MTEP.

TU08-03

SYNAPTIC ACTIVITY-INDUCED FLUCTUATIONS IN ASCORBIC ACID CONCENTRATION COULD DRIVE CHANGES IN THE AVAILABILITY OF PLASMA MEMBRANE

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Ascorbic acid is an important water-soluble antioxidant and cofactor in various enzyme systems. It is concentrated in brain and other organs. Sodium vitamin C transporters (SVCTs) are able to translocate ascorbic acid across the plasma membrane. SVCT2 is the only ascorbic acid transporter expressed in brain. SVCT2 is highly expressed by neuronal cells showing an intracellular and plasma membrane localization. It has been described that synaptic activity triggers the release of ascorbic acid from intracellular reservoirs. Indeed, glutamate is able to stimulate ascorbic acid release from astrocytes. Fluctuations in brain ascorbic acid were described over 15 years ago. However, there is no data about the possible changes in SVCT2 localization induced by acute exposure to ascorbic acid. Immunofluorescence analyses showed SVCT2 colocalization with endosomal and plasma membrane markers. After ascorbic acid exposition we observed an increase in SVCT2 at plasma membrane. This increase was abolished in presence of an exocytosis inhibitor, Cytochalasin D. Fluorescence recovery after photobleaching (FRAP) analyses demonstrated a decrease in the relative mobility of SVCT2-EGFP when cells were previously exposed to ascorbic acid. Using total internal reflection microscopy (TIRM) we observed an increase of SVCT2-EGFP at plasma membrane level in ascorbic acid treated cells. The same was also
observed in presence of an endocytosis inhibitor. On the other hand, this effect was not seen in presence of Cytochalasin D. These studies were supported by biotinylation assays and kinetic assays using 14C-ascorbic acid. Therefore, an increase of extracellular ascorbic acid is able to stimulate an increase in plasma membrane availability of SVCT2. Mechanisms for acute modulation of SVCT2 could be relevant in brain where ascorbic acid fluctuates with synaptic activity. FONDECYT1110571.

TU08-04
DOPAMINE AND TYROSINE PHOSPHORYLATION OF NR2B (Y1472) IN THE HIPPOCAMPUS IS FUNDAMENTAL FOR ERK2 ACTIVATION AND NOVEL LEARNING

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We have previously shown that dopamine and NMDA (N-methyl-D-aspartate) converge on Extra cellular Regulated Kinase - Mitogen-Activated Protein Kinase signalling in the rat hippocampus and that ERK activation by dopamine is NMDA receptor dependent (Kaphzan et al., 2006). The complex interaction between dopamine and NMDA receptors is significant for different normal and abnormal learning processes. Here, we tested the hypothesis that dopamine interacts with NMDA receptors via tyrosine phosphorylation of the NR2 subunits A and B and that this interaction is upstream to MAPK cascade activation. We found that dopamine induces tyrosine phosphorylation of NR2A Y1325 (1.38 ± 0.09, p < 0.001, n > 10 vs. control) and NR2B Y1472 (1.47 ± 0.12, p < 0.001, n > 10 vs.control). Phosphorylation of NR2B Y1472 correlated with ERK2 activation (r = 0.41, p < 0.05, n = 14). Moreover, dopamine leads to induction in the phosphorylation of Src Y418 and the Src-protein tyrosine kinase inhibitor4-nitrophenylmethy-L-arginine (PP2) inhibits the dopamine effect on ERK2 (1.11 ± 0.05, p < 0.05, n = 20 vs. dopamine) and NR2BY1472 (1.01 ± 0.07, p < 0.01, n = 18 vs. dopamine), but not on NR2A Y1325 (1.39 ± 0.14, p < 0.05, n = 20 vs.control). In order to test causality between NR2B Y1472 phosphorylation and ERK2 activation by dopamine, we carried out similar pharmacological manipulations in hippocampal slices of WT and NR2B 1472 KI mice and detect clear induction in the WT, but no changes were observed in the KI mice. Since dopamine signaling is known to play key role in novelty learning, we tested the KI mice in different behavioral paradigms of novelty and found clear attenuation in the KI compared with the WT mice in novel place, novel object and novel taste learning for 0.5% saccharin. These results demonstrate that dopamine signaling via tyrosine phosphorylation of NR2B subunit is playing pivotal role in novel learning and ERK activation. It is plausible that the specific sites of post-translation modifications of the NMDA receptor can serve as new targets for therapy of psychiatric diseases such as Schizophrenia.

TU08-05
PROPERTIES OF ACQUIRED NMDAR CHANNELS EXPRESSED IN HEK293 CELLS

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N-Methyl-D-aspartate receptor (NMDAR) is a heteromeric complex between the essential NR1 subunit and one of NR2A-D subunits toward functional cation channels permeable to Ca2+ rather than Na+ ions. Although recent studies identified the dominant negative NR3A and NR3B subunits, whether these subunits inhibit Ca2+ influx across functional NMDAR channels is not clarified so far. In this study, therefore, we investigated Ca2+ influx across acquired NMDAR channels composed of different NR subunits artificially expressed in HEK293 cells. Cells were transfected with different NR subunit expression vectors, followed by loading of the fluorescent dye Fluo-3 and subsequent exposure to NMDA at different concentrations for determination of intracellular free Ca2+ levels. The addition of NMDA markedly increased the fluorescence intensity in cells transfected with either NR2A or NR2B subunit together with NR1 subunit. Further addition of dizocilpine completely inhibited the increase by NMDA in both types of acquired channels, while the NR2B subunit selective antagonist ifenprodil drastically increased the amount of NMDA in cells expressing NR1/NR2B, but not NR1/NR2A, subunits. Similar pharmacological profiles were invariably seen with cell death induced by NMDA. Introduction of both NR3A and NR3B subunits significantly inhibited the increase by NMDA in intracellular free Ca2+ levels in both acquired channels, while introduction of either NR3A or NR3B alone was ineffective. Introduction of both NR3A and NR3B subunits was also required for the prevention of increased mitochondrial free Ca2+ levels determined by Rhod-2, as well as decreased cellular viability, in cells expressing NR1/NR2A or NR1/NR2B subunits upon exposure to NMDA. These results suggest that expression of both NR3A and NR3B subunits is essential for the dominant negative properties on Ca2+ influx through acquired functional NMDAR channels.

TU08-06
LIMK1 AND PCREB AT LEARNING PROCESS IN INDUCED AND SPONTANEOUS MUTANTS OF THE DROSOPHILA MELANOGASTER LIMK1 GENE

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One of the crucial regulators of cytoskeleton remodeling is LIMK1 which phosphorylates cofilin and thereby affects actin filament dynamics leading to dendritic spine reorganization. LIMK1 also phosphorylates transcriptional factor CREB which initiates gene expression during memory formation, but little is known about a level of pCREB at adult neuromuscular junctions (NMJs) before and after learning. Using Western blot analysis we estimated the ratio D and C isoforms of LIMK1 in the heads of Drosophila males from strains with induced – agnts3 and spontaneous mutations of limk1 gene - Berlin, Oregon-R, Canton-S served as a control strain.
November environment increases plasticity-related protease neuropsin gene without change in tPA gene

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An enriched environment composed of novel, complex and stimulating surroundings promotes structural changes in the brain and enhances learning and memory performance in mammals. Recent studies using rodents have revealed that exposure to an enriched environment correlates with increased neurogenesis, dendritic fields and expression of neurotrophic factors, neurotransmitter receptors and synaptic proteins. Accumulating evidences have proven that secretory proteases modulate synaptic plasticity in activity-dependent manner. These proteases change synaptic microenvironment by multiple functions such as degrading extracellular matrix and cell adhesion molecules and activating other proteins. Kalikrein-related peptidase S/neuropsin and tissue-type plasminogen activator (tPA), are highly expressed in the hippocampus and participate in LTP formation, a component of learning and memory. It has not been well understood how these proteases function when animals engage in learning process from their living environments.

In this study, we investigated the influence of an enriched environment on the expression of neuropsin and tPA in the mouse hippocampus. We housed three littersmates in an enriched environment consisting of a large transparent cage with paper bedding, a metallic running wheel and a plastic tunnel. Both neuropsin and tPA expression increased significantly after 7 days’ exposure to the enriched environment and returned to basal expression levels, similar to those of control mice, after 28 days. Therefore, the enriched environment may induce synaptic modulations via these plasticity-related genes in the hippocampus.

Moreover, we kept mice in two different enriched environments, a familiar environment and then a novel environment. After 21 days’ housing of littermates in the previous enriched environment, they were exposed to the novel environment: an opaque cage with bedding of wood shavings, plastic platforms or a ball-shaped toy and a plastic horizontally-tilted running wheel. After 7 days of exposure to the novel environment, neuropsin gene expression increased significantly, but tPA gene expression did not change.

TU08-08
EFFECTS OF NEONATAL HANDLING ON AMPA RECEPTOR SUBUNIT EXPRESSION OF RAT BRAIN
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Neonatal handling, an experimental model of early life experiences, is known to affect the hypothalamic-pituitary-adrenal axis function thus increasing adaptability, coping with stress, cognitive abilities and in general brain plasticity-related processes. Previous study has shown selective effects of neonatal handling on rat brain NMDA receptors (Stamatakis 2009). AMPA receptors (AMPARs), which are crucial during neuronal development, synaptic plasticity and structural remodeling, mediate fast synaptic transmission at excitatory synapses in the CNS. AMPARs are composed of four types of subunits, designated as GluR1, GluR2, GluR3 and GluR4, which combine to form tetramers. Most AMPARs are heterotetramers made of at least two of the four proper subunits GluR1-4. AMPA receptors that are permeable to Ca2+ lack the GluR2 subunit.

The present study addressed the question of whether neonatal handling might have an effect on AMPARs, since it has been shown that the subunit composition and thus the Ca2+ permeability of AMPARs changes in response to sensory experience. According to the current neonatal handling protocol, each pup of a litter was removed from the nest for 15 min daily from the first postnatal day (PND1) until weaning (PND22). In situ hybridization was used in order to localize and quantify subunit mRNA expression, with specific cDNA oligonucleotides. AMPAR subunit expression was studied in specific brain regions that are involved in emotions, learning, memory and sensory perception, such as the hippocampus, cerebral cortex and amygdala of adult male and female rats. Differential changes were observed in AMPA receptor subunit expression depending on the brain region and the subunit, which imply an early experience-dependent selective modulation of brain circuits. Supported by Polembros Shipping Limited.

Reference:

TU08-09
INHIBITION OF CAMKII IN DORSAL CA1 AFTER NON-REINFORCED RETRIEVAL HINDERS SPATIAL MEMORY PERSISTENCE
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Well-consolidated spatial memories become vulnerable upon retrieval, requiring a protein synthesis dependent process in order to persist. Our aim was to analyze the role that Ca2+-CAM-dependent kinase type II (CaMKII) has on this process. Male Wistar rats were trained for five days in the Morris Water Maze task, and submitted
to a non-reinforced test 24 h after the last training day (PT1). The CaMKII inhibitor autocamtide-2-related inhibitory peptide (AIP, 1 nmol/side) or vehicle were infused in the CA1 region of the dorsal hippocampus at specific times after PT1. A second non-reinforced test was carried out either 24 or 120 h later (PT2). The infusion of AIP immediately, but not 30 or 90 min after PT1 hindered spatial memory when PT2 was carried out five days, but not 24 h after PT1. These findings suggest that early CaMKII activity after retrieval is required for the trace to persist over long, but not short time periods. The exact mechanism remains to be determined, and might involve regulation of the targeting of newly synthesized proteins to weakened synapses.

TU08-10 QUANTITATIVE ANALYSIS OF GLUTAMATE RECEPTOR SUBUNITS IN THE MOUSE BRAIN
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Ionotropic glutamate receptors (GluR) are classified into three subfamilies, AMPA type (AMPAR), kainate type (KAR) and NMDA type receptors (NMDAR), and each subtype is further composed of multiple subunits; four subunits (GluA1-4) in AMPAR, five subunits (GluK1-5) in KAR, and GluN1 and GluN2A-D in the main NMDAR subunits. The combination of these subunits determines the receptor function. Although it is very important to know the quantity of each subunit in various brain regions for the understanding of GluR function, no quantitative analysis has been made. We first determined antibody titers of four AMPAR subunits with analytical western blot using three chimeric AMPAR subunits (GluA2&GluA1, GluA3&GluA1, and GluA4&GluA1). Each titer was corrected by the titer of GluA1 C-terminal antibody, and used for quantitative analysis of four GluA subunits. Analysis showed that amounts of four AMPAR subunits were different in each brain region and subcellular fraction. In the crude fractions, there were abundant GluA2 and GluA3 subunits in the cerebral cortex (A1: A2: A3 = 1.0: 4.0: 3.0), whereas GluA1 and GluA2 subunits were abundant in the hippocampus (A1: A2: A3 = 1.0: 2.0: 0.5). There were no quantitative differences between the four subunits (A1: A2: A3: A4 = 1.0: 1.2: 1.2: 1.0) in the cerebellum. As in the case of AMPAR subunits, KAR subunits fused with GluA2 chimera (GluK1&GluA2, GluK2&GluA2, etc.) and NMDAR subunits with GluA2 chimera (GluN1&GluA2, GluN2A&GluA2, etc.) were generated and each titer was corrected by the titer of GluA2 C-terminal antibody, and used for quantitative analysis of KAR and NMDA subunits. It was shown that GluK2 subunit was much more abundant than other GluK subunits in the forebrain, but its expression level was much lower than GluA2, and that the amount of GluN1 subunit was lower than GluA2.

TU08-11 VOLTAGE-SENSITIVE DYE IMAGING OF GABA B-RECEPTORS MEDIATED RESPONSES IN THE LATERAL NUCLEUS OF THE MICE AMYGDALA
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The lateral nucleus of the amygdala (LA) is an ‘input’ nucleus of the amygdaloid complex where cortical inputs and thalamic inputs are associated and integrated. Although the timing of these two inputs is critical for establishment of fear conditioning, how excitatory and inhibitory responses processes temporal information is unknown. GABAergic interneurons in LA complicately control activities of principal neurons through feed forward and feedback inhibition. Using voltage-sensitive dye imaging, we monitored electrical activity of neurons at multiple sites in the coronal mouse slice to investigate how inhibitory responses regulate excitatory responses in LA. There was a clear relationship between the position of stimulating electrode and the topographical pattern of optical signals. Electrical stimuli to the external capsule (EC) caused optical signals propagating to LA, the amygdalostriatal transition area (Astr) and the basolateral nucleus (BLA). When a stimulating electrode was placed on EC at the upper part of LA, strong and long-lasting hyperpolarization (LLH) that spread throughout LA was observed. LLH was weaker in BLA than in LA and it was not observed in Astr. LLH in LA lasted for about 800 ms, and was mediated by GABAA receptors. Synchronous inhibitory response in LA has first been detected with voltage-sensitive dye imaging. Our results suggests that LLH is related to time window for detecting coincidence of cortical and thalamic inputs, and that postsynaptic GABAA receptors strongly participate in the information processing in LA.

TU08-12 REGULATORY ROLE OF DREBRIN IN HIPPOCAMPUS-DEPENDENT LEARNING
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Drebrin is a side-binding protein of F-actin that plays a pivotal role in intercellular communication, such as transmission across GAP junctions and immune synapses. Drebrin A is a neuron-specific isofrom that is highly concentrated in dendritic spines of mature neurons. In this study, we generated drebrin A-specific knockout (Dako) mice by deleting the drebrin A-specific exon from the drebrin gene, and sought to elucidate the role of drebrin conversion. In Dako mice, a ubiquitous-isoform drebrin E is expressed instead of drebrin A even in the adult brain. Adult Dako mice shows impairment of contextual fear learning, in spite of no apparent change in general behavioral profile, and shows impairment of synaptic accumulation of the NMDA receptor shortly after the blockade of the receptor activity. Then we compared hippocampus-dependent learning paradigms and hippocampal synaptic plasticity between young and adult Dako mice. We found that the
impairment of contextual fear learning in DAKO mice was age-dependent: the phenotype was evident in mice older than 6 month old, but not in mice younger than 2 month old. Further we found that hippocampal CA1 long-term potentiation was significantly attenuated in DAKO mice older than 6 month old. Then we examined whether the conversion of drebrin isofrom from drebrin E to drebrin A plays a role in drebrin dynamics by fluorescence recovery after photobleaching (FRAP) analysis, and found that stable fraction of GFP-tagged drebrin E were significantly smaller than those of GFP-tagged drebrin A. The difference of drebrin dynamics between isoforms might explain the impairment of synaptic plasticity in DAKO mice.

TU08-13

STRIATAL GLUTAMATE RELEASE IN SUPERFUSION: ROLES OF PHOSPHATASES, KINASES, CAMP AND CALMODULIN

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Striatum is considered the major connection hub in brain motor control, receives massive glutamatergic afferences from cortex and thalamus which represent a potential site for drugs treating Parkinson’s disease. This investigation employed rat striatal tissue in superfusion releasing preloaded 3H-glutamate (or 14C-aspartate) to evaluate how drugs affecting protein phosphorylation, calcium signaling and cAMP. Basal and KCl 35mM-stimulated releases were measured under control and drug superfusion using a Brandel 2500 Suprafusion system. Results demonstrate that Cav2.x type VSCC measured under control and drug superfusion using a Brandel 2500

Several studies have shown the role of Adenosine A2A receptors in learning and memory processes as well in LTP induction in hippocampus. In order to further understand the molecular basis of Adenosine/Glutamate interactions, we have investigated in the present study the effect of the 'in vitro' mGluR5 and A2A receptor activation on NMDA receptor phosphorylation as well as on ERK ½ kinases activation in hippocampal slices. Our experimental approach used the western-blotting analysis and specific antibodies against pNR2B at ser1303, pNR2B at tyr1472 and pERK1/2. Our preliminary results showed that 'in vitro' incubation of rat hippocampal slices with the mGluR5 receptor agonist CHPG : assignificantly increased, in a dose dependent manner, the phosphorylation state of NR2B subunit (ser-1303) of NMDA receptors and b) CHPG significantly increased, in a dose dependent manner, the phosphorylation state of the ERK1/2 kinases compared to control. Interestingly, our preliminary results showed that when CGS 21680, a selective agonist of A2A receptors, was co-administered at the concentration of 50nM, decreased the CHPG evoked phosphorylation of NR2B subunit (tyr-1472) of NMDA receptors as well as of ERK1/2 kinases. In conclusion, the mGluR5 receptor mediated phosphorylation of NR2B subunit at tyr-1472, probably through PKC kinase, might underly the enhancement of mGluR5 receptor evoked currents of NMDA receptors, shown by electrophysiological studies in rat hippocampus. The significance of the CHPG evoked activation of ERK1/2 signal transduction pathway could be related to synaptic plasticity phenomena in hippocampus, which must be further investigated.

TU08-15

PKC EPSILON REGULATES ERK ACTIVATION AND RECOGNITION MEMORY IN THE RAT

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ERK1/2 activity is an essential requirement for synaptic and neuronal plasticity and mammalian learning and memory. Considering the role of PKC isoforms as upstream effectors of the Raf/MEK/ERK1/2 pathway, several studies have suggested that PKC activity is important for memory processes. We and others have previously shown that expression and activation of PKCε, an abundant isoform in the brain, induces neuronal differentiation. Yet direct evidence for the role of PKCε in memory mechanisms is lacking. Hence, we sought to evaluate this role of PKCε in memory mechanisms using two PKCε-selective peptides, the inhibitory εV1-2 and the activator εζRACK (both conjugated to a TAT carrier
First, we demonstrated with immunohistochemistry that pyramidal neurons in the CA3 region of the rat hippocampus expressed an 8-fold higher PKCε immunoreactivity over other neurons and regions; equally increased was the expression of P (phosphorylated)-MARCKS, a PKC-specific substrate, indicating increased basal activity of PKCε. Indeed, when dissected rat hippocampi were incubated with εRACK and proteins were analyzed by Western blotting or immunoprecipitations, P-MARCKS expression was detected increased with time of treatment almost to levels seen after phorbol esters, while preconditioning with εV1-2 abolished effects of εRACK and phorbol esters. Moreover, with similar analyses we found that εRACK activated Src, Raf, and ERK1/2 in a time-dependent manner, establishing the role of PKCε in ERK activation in hippocampal neurons. We then investigated the putative role of PKCε in recognition memory in rats, after intraperitoneal injection of εV1-2, and we found that this selective PKCε inhibition impaired memory in the object recognition tasks. Most importantly, this amnesiac effect of εV1-2 could be eliminated when εRACK was co-administered. Taken together, these findings present the first direct evidence that PKCε activity is an essential molecular component of nonspatial recognition memory.