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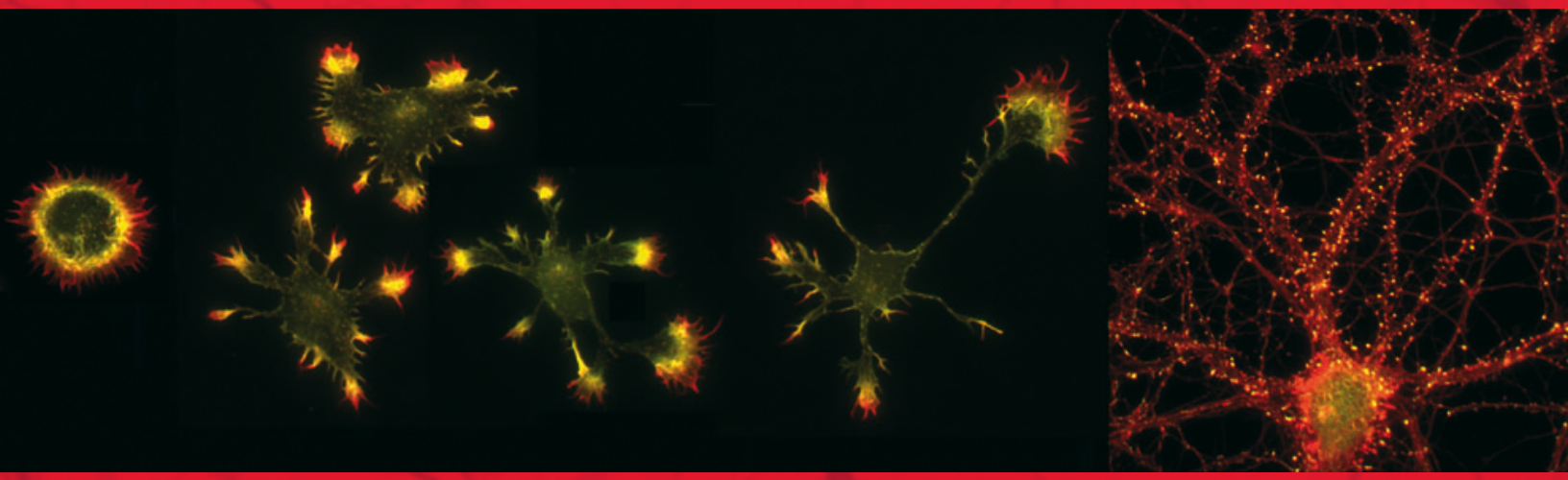

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23RD BIENNIAL MEETING OF ISN/ESN

Athens, Greece
28 August–1 September 2011

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Sunday Oral Sessions
Plenary Lecture 1

PL1

DECONSTRUCTING SMELL

Buck, L.B.

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We have explored how mammals detect odorants and pheromones and how the brain translates those chemicals into diverse perceptions and instinctive behaviors. We found that odorants are detected in the nasal olfactory epithelium (OE) by ~1000 different odorant receptors (ORs) whereas pheromones are detected in the vomeronasal organ (VNO) by two smaller receptor families. Our studies showed that ORs are used combinatorially to encode odor identities, thereby allowing the discrimination of a multitude of odorants. Exploring the patterning of OR inputs, we found that each sensory neuron in the OE expresses a single type of OR and that neurons with the same OR are scattered in one zone, but synapse in a stereotyped fashion in OR-specific glomeruli in the olfactory bulb. The code for an odor in the OE is thus a dispersed ensemble of neurons, each expressing one OR component of its receptor code whereas in the bulb it is a specific combination of glomeruli that receive input from those ORs and whose spatial arrangement is similar among individuals. To explore how pheromones alter reproductive physiology and behavior, we made mice expressing a transneuronal tracer in gonadotropin releasing hormone (GnRH) neurons. These studies revealed that GnRH neurons receive pheromone signals from the OE as well as the VNO. We subsequently discovered a second class of chemosensory receptors in the OE, called TAARs, which might permit the detection of pheromones and other social cues in the OE. Our recent experiments suggest that TAARs are also likely to be expressed in the human OE.

Symposium 1
History of Neurochemistry

S01-01

GREEK CONTRIBUTION TO NEUROSCIENCE - FROM ALKMAION TO ECONOMO

Paxinos, G.

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The natural philosophers of the 5th century BC were the first to reject supernatural causes as explanation of the physical world and the nature of the soul, including the relation between psychological events and the body. After a number of false attributions of cognitive processes to the air, blood, or heart, some of these philosophers and physicians concluded that the mind is the product of the brain. Circa 500 BC, the physician Alkmaion of Kroton in Magna Grecia might have been the first to attribute consciousness and perception to the brain. For Plato, the 'immortal' soul was associated with the brain. Hippocrates of Kos (circa 400 BC) had a strikingly modern view of the brain - the seat of the intellect and the cause of neurologic diseases. Aristotle (384–322 BC) opted for the heart as the seat of the soul (vegetative, sensory and intellectual soul). Galen of Pergamon (216–129 AD) fought against the cardiocentric view, stating that the brain receives all sensation, produces images and understands thoughts. Galen thought that the ventricular system was the place that harbored the mind. Encephalocentric and cardiocentric views battled each other until the modern scientific endeavor at which time also the seat of the mind passed from the ventricles to the cerebral cortex. The most notable of modern neuroscientists with some Greek connection is von Economo who together with Koskinas published in 1925 an atlas of the human cerebral cortex, a masterpiece of neuroscience in which they identified 107 cortical areas on the basis of cytoarchitecture.

S01-02

FROM JEAN CRUVEILHIER AND ROBERT CARSWELL TO JEAN MARTIN CHARCOT: THE INITIAL DESCRIPTION OF MULTIPLE SCLEROSIS

Zalc, B.[1,2,3]

[1]*University Pierre & Marie Curie, CRICM, Paris, France*
[2]*Inserm U975, Paris, France*
[3]*CNRS UMR7225, Paris, France*
[4]*Pitié-Salpêtrière, AP-HP, Paris, France*

Who was first? The eternal question in Science (and Medicine included)! It all started with pathologists describing lesions, without knowing it was Multiple Sclerosis (MS). And already here, there is a debate: The English will favor Carswell while the French have a tendency to prefer Cruveilher, of course!!! And this happened in 1835–38, 30 years before these characteristic descriptions of lesions in the brain and spinal cord were attributed to the typical association of a series of clinical signs constituting MS as a clinical entity. Here there is a general consensus on the inalienable and funding contribution of Jean Martin Charcot at the hospital de la Salpêtrière. However, a careful review of the 19th century medical literature illustrates the additional amazing contributions of the German clinicians, pointing to the fact that as usual, a scientific discovery only seldom arises from nowhere, but occurs only on a fertilize soil. In addition, here as in many occasions, credit is often attributed not to the true discoverer, in as much as such an individual is not simple to identify, but to the one who has both the talent to summarize scattered findings into a solid new concept and even more importantly the know-how to advertize internationally this new concept.

S01-03

THE FALCK-HILLARP FLUORESCENCE METHOD: A BREAKTHROUGH IN MONOAMINE RESEARCH

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In 2012 we will celebrate the 50th anniversary of the initial publications of the Falck-Hillarp method, which for the first time allowed the demonstration of a transmitter, in this case dopamine, noradrenaline and serotonin, in individual cell bodies and nerve endings in the microscope. Developed primarily in Lund, but also in Gothenburg together with Arvid Carlsson's team, the method was then exploited not only in Lund but also at Karolinska in Stockholm, where Hillarp moved from Lund in 1962. Within a few years the monoamine systems in brain, spinal cord and periphery were mapped. For example, Fuxe and Dahlström in 1964/1965 published their now classic description of the brain monoamine neurons, defining the catecholamine groups as A1–7 (noradrenaline) and A8–14 (today -17) (dopamine), as well as the serotonin neurons (B1–9), and their wide spread terminal ramifications throughout the brain and spinal cord. This method and its applications were not only a breakthrough in monoamine research but, in fact, was essential for the establishment of a new discipline, chemical neuroanatomy, in research on the nervous system, or as we today call it in 'neuroscience'. The brilliant neuroanatomist Walle J. H. Nauta phrased it as follows: 'Classical neuroanatomy provided us with the blue print of the nervous system, while chemical anatomy added the red-print to the construction plan'. It came in parallel with many other exciting developments in the monoamine field, e.g. the possibility to monitor the levels of the above mentioned monoamines in peripheral and central tissues with biochemical assays, the characterization of the enzymes involved in synthesis and breakdown of the monoamines, and novel pharmacological tools which allowed dissection of monoaminergic mechanisms, leading to understanding of, and new treatment strategies for, various diseases. It was, indeed, a golden age.

S01-04

GLUTAMATE. FROM ITS ISOLATION TO NEUROTRANSMITTER FUNCTION

Fonnum, F.

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Glutamate was first identified and isolated in 1866 by Karl Heinrich Leopold Ritthausen in 1866. It has gained importance as responsible for the taste umami (K Ikeda, 1907), as a major neurotransmitter in the brain and as responsible for excitotoxic

effects in the brain. Five early observations were important for the focus on glutamate in the brain. Firstly, it was present in brain in much higher concentration than in any other tissue. Secondly, Weil-Malherbe (1936) showed that glutamate was the only amino acid which could oxidised by brain tissue. Thirdly, intravenous injections of glutamate allowed a rapid recovery of patients treated by insulin coma (Mayer Gross and Walker 1949). Fourthly, in 1943 Price et al successfully used glutamate in the treatment of petit mal type of seizures and noted ' mental and physical alertness have been increased in the patients'. This initiated a series of studies on uptake and metabolism of glutamate. This has lead to the concepts of metabolic compartments, of Chinese restaurant syndrome and of excitotoxic effects. Scientists such as H Waelsch, HW Berl, DD Clarke and JW Olney, among many others played an important part in this work. In 1952 Hayashi showed that glutamate induced convulsions and in 1959 Curtis et al showed that micro electrophoresis of glutamate resulted in fast excitation on spinal neurons. But there were many objections before glutamate were accepted as being a neurotransmitter, mainly because it appeared to lack of specificity. The early work by S Snyder, GAR Johnston, A Lajtha, HH Jasper and KA Elliot on the uptake and release mechanisms and synthesis of specific receptor agonists like NMDA by JC Watkins should be remembered in this respect.

Monday Oral Sessions
Plenary Lecture 2

PL2

TOWARDS A MOLECULAR UNDERSTANDING OF THE MOLECULAR IDENTITY OF OLIGODENDROCYTES

Casaccia, P.

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Myelin, the membrane extension of a specialized cell called oligodendrocyte, has recently gained tremendous interest, due to its involvement in a wide variety of neurological and psychiatric disorders, ranging from neonatal ventricular leukomalacia to metabolic syndromes of genetic origin, from schizophrenia to inflammatory demyelination, such as Multiple Sclerosis. Our laboratory has been working towards an understanding of the molecular mechanisms underlying the acquisition of oligodendrocyte identity, which is necessary for myelin formation in the developing and adult brain. Early ultra-structural studies in the brain of developing animals had reported that the progression of oligodendrocyte progenitors towards myelinating cells was characterized by increased chromatin compaction. We have reproduced these changes *in vitro*, in primary cultures of oligodendrocyte progenitors from the developing rodent brain, thereby validating this culture system as an important model for the study of chromatin during oligodendrocyte differentiation. The basic unit of chromatin is the nucleosome, which is composed of an octamer of four core histones and 146 base pairs of DNA wrapped around it. The structural and functional state of chromatin is modulated by post-translational modifications of residues at the tails of histones. We shall review evidence that histone modifications and other epigenetic events are essential for the acquisition of a myelinating phenotype. The implications for disease states will be discussed. (Supported by grants RO1-NS024295 and NS52738.)

ISN Young Scientist Lecture 1

YSL1

PRO-SURVIVAL AND PRO-DEATH MOLECULAR EVENTS DOWNSTREAM OF NMDA RECEPTOR ACTIVITY

Hardingham, G. E.

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NMDA receptors are a subtype of ionotropic glutamate receptor with an important role in the physiology and pathophysiology of central neurons. Inappropriate levels of Ca^{2+} influx through the NMDA receptor can contribute to neuronal loss in acute trauma such as ischemia and traumatic brain injury, as well as certain neurodegenerative diseases such as Huntington's. However, normal physiological patterns of NMDA receptor activity can promote neuroprotection against both apoptotic, oxidative and excitotoxic insults. As a result, NMDA receptor blockade can promote neuronal death outright or render them vulnerable to secondary trauma.

There is a growing knowledge of the molecular mechanisms underlying both the neuroprotective and neurodestructive effects of NMDA receptor activity, as well as the factors that determine whether an episode of NMDA receptor activity is harmful or beneficial. The coordinated transcriptional changes that underlie NMDAR-dependent neuroprotective effects will be discussed, both in terms of the molecular mechanisms by which they are initiated, as well as the basis for their effect. Furthermore, we will discuss the factors that determine whether an episode of NMDA receptor activity is toxic to neurons, including synaptic vs. extrasynaptic localization, and subunit-specific signalling by the NR2 C-terminus. Increased understanding in these areas of NMDAR signalling is leading to new potential therapeutic targets and strategies for excitotoxic disorders, as well as a growing appreciation of the harmful consequences of NMDA receptor blockade.

Symposium 2
Synaptic Dysfunction in Mental Disorders and Addiction

S02-01

PLASTICITY OF AMPA RECEPTOR TRANSMISSION DURING COCAINE WITHDRAWAL

Wolf, M. E.

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The nucleus accumbens (NAc) is a key brain region for motivation, reward and drug addiction. NAc neurons are excited primarily by AMPA-type glutamate receptors. This is required for cocaine seeking in animal models of cocaine addiction, suggesting AMPA receptor transmission in the NAc as a key control point for cocaine-related behaviors. Using rodent models, we have shown that cell surface and synaptic expression of AMPA receptors on NAc neurons is persistently increased after withdrawal from repeated cocaine exposure. GluA2-containing AMPA receptors are added to NAc synapses after withdrawal from experimenter-administered cocaine (repeated i.p. injections). In contrast, GluA2-lacking AMPA receptors (high conductance, calcium-permeable AMPA receptors that are normally excluded from NAc synapses) are detected after prolonged withdrawal from extended access cocaine self-administration. We hypothesize that the incorporation of additional AMPA receptors increases the reactivity of NAc neurons to glutamate inputs from cortical and limbic brain regions, facilitating the ability of these inputs to trigger cocaine seeking and thus contributing to the persistent vulnerability to relapse that characterizes addiction. My presentation will focus on two current lines of investigation. First, we showed recently that stimulation of group I metabotropic glutamate receptors (mGluR) can remove calcium-permeable AMPA receptors from NAc synapses, providing a potential strategy for reducing cocaine craving. In addition, we have observed decreased mGluR1 surface expression in the NAc after cocaine withdrawal, suggesting that a long-term decrease in group I mGluR tone may be one factor that contributes to the accumulation of calcium-permable AMPA receptors in NAc synapses. Second, we are investigating the role of kalirin-7 in mediating cocaine-induced increases in synaptic AMPA receptor levels and associated changes in dendritic spine density in the NAc. Supported by DA009621, DA015835 and DA029099.

S02-02

THE ROLE OF THE X-LINKED MENTAL PROTEIN IL1RAPL1 IN REGULATING EXCITATORY SYNAPSE STRUCTURE AND FUNCTION

Sala, C., Valnegri, P. and Passafaro, M.

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At excitatory synapses postsynaptic sites is localized a specialized protein complex called the postsynaptic density (PSD) which functions include the regulation of adhesion, the control of receptor clustering, and the regulation of synapses functions. The PSD contains neurotransmitter and transmembrane receptors, various scaffold proteins, cytoskeletal elements and regulatory enzymes, all of which are assembled together in a disc-like structure that is 30–40 nm thick and a few hundred nanometers wide. In the PSD are also localized proteins which deletion or mutation cause severe form of mental retardation. We recently found that Interleukin-1-Receptor Accessory Protein Like 1 (IL1RAPL1), which gene mutations is associated to non-syndromic X-linked mental retardation, interacts with PSD-95, another major scaffold protein of the PSD. Using gain and loss of function experiments in neurons, we demonstrate that IL1RAPL1 regulates the synaptic localization of PSD-95 by controlling JNK (c-Jun terminal Kinase) activity and PSD-95 phosphorylation. Mice carrying a null-mutation of the mouse Il1rapl1 gene show a reduction of both dendritic spines density and excitatory synapses in the CA1 region of the hippocampus. These structural abnormalities are associated with specific deficits in hippocampal long-term synaptic plasticity. Interestingly the extracellular domain of IL1RAPL1 regulates also excitatory synapse formation. In conclusion, the interaction of IL1RAPL1 with PSD-95 discloses a novel pathophysiological mechanism of cognitive impairment associated with alterations of the JNK pathway leading and abnormal synaptic formation and function.

S02-03

SYNAPTIC DEFICITS IN PSYCHIATRIC DISORDERS

Penzes, P., Cahill, M., Xie, Z. and Russell, T.

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Dendritic spines are the sites of most excitatory synaptic connections in the brain, and are crucial for cognitive functions. Consequently, dendritic spine morphology has been found to be altered in several psychiatric disorders, including schizophrenia. Schizophrenia is a severe mental disorder that affects 0.5% of the world population. Reduced connectivity in cortical circuits is seen as closely associated with information processing deficits, including cognitive and working memory deficits, which are core features of schizophrenia. The protein kalirin is a critical regulator of dendritic spine formation, plasticity and maintenance, as well as of dendritic morphology. Its mRNA was found to be significantly and specifically underexpressed in the dorsolateral prefrontal cortex of patients with schizophrenia, and recent studies identified missense mutations in KALRN gene associated with schziophrenia. Furthermore, kalirin interacts with the schizophrenia susceptibility gene DISC1, and is modulated the 5HT2A receptor, a target of atypical antipsychotics. Kalirin-7 directly activates Rac1 and is concentrated in the postsynaptic densities. Based on these findings, we generated and characterized a kalirin-deficient mouse model and examined the interaction of kalirin with the schizophrenia susceptibility molecules NRG1 and erbB4. KALRN knockout mice have specific reductions in cortical, but not hippocampal, Rac1 signaling and spine density. These mice exhibit robust deficits in working memory, sociability, and pre-pulse inhibition, paralleled by locomotor hyperactivity reversible by clozapine in a kalirin-dependent manner. We found that the behavioral profile of KALRN KO mice was similar to WT mice during early postnatal development, while behavioral

dysfunctions were apparent during young adulthood. Similarly, cortical spine deficits were robust in post-adolescent mice and absent in young mice. In addition, we found that NRG1 and erbB4 modulated kalirin function in cortical interneurons, and together, they regulated interneuron dendritic morphology. Our data suggest that kalirin functions in a common pathway with several schizophrenia susceptibility molecules, and controls cellular and behavioral phenotypes relevant for schizophrenia.

S02-04

FUNCTIONAL SCREEN FOR SYNAPTIC ORGANIZERS: IDENTIFICATION OF TRKC-PTPσ AND SLITRK, CANDIDATE GENES IN NEUROPSYCHIATRIC DISORDERS

Takahashi, H., Arstikaitis, P., Prasad, T., Bartlett, T. E., Wang, Y. T., Murphy, T. H. and Craig A. M.

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Dysfunction of a number of molecules implicated in synapse development is linked to many neuropsychiatric disorders. Synapse development requires not only physical adhesion between axons and dendrites but also chemically matched pre- and post-synaptic differentiation. The Neuroligin-Neurexin complex has been noted as a trans-synaptic adhesion complex that organizes pre- and post-synaptic differentiation, and as a genetic determinant predisposing to autism. However, synapse diversity and disease variety suggest many further trans-synaptic adhesion complexes that organize excitatory and/or inhibitory synapses. For global identification of synaptic organizers, we developed a functional expression screen based on a neuron-fibroblast coculture assay combined with full-length cDNA library, and then isolated several novel synaptogenic adhesion molecules. Here, we report neurotrophin receptor TrkC non-catalytic isoform as an adhesion molecule that triggers differentiation of glutamate release sites in axons. All TrkC isoforms including catalytic ones, but not TrkA or TrkB, function directly in excitatory glutamatergic synaptic adhesion by neurotrophin-independent high-affinity trans-binding to axonal PTPσ tyrosine phosphatase receptor. PTPσ triggers and TrkC mediates clustering of excitatory postsynaptic molecules such as NMDA receptors and PSD-95 in dendrites, indicating bidirectional synaptic organizing functions. Effects of a TrkC neutralizing antibody that blocks TrkC-PTPσ interaction and TrkC knockdown in culture and *in vivo* reveal essential roles of TrkC-PTPσ in glutamatergic synapse formation. These results indicate that postsynaptic TrkC trans-interaction with presynaptic PTPσ generates bidirectional adhesion and recruitment essential for excitatory synapse development. In addition to the TrkC-PTPσ complex, we discovered Slitrk2 as another synaptogenic adhesion molecule through the coculture screen combined with bioinformatics. Given that TrkC and the Slitrk family are linked to several neuropsychiatric disorders including panic disorder, obsessive-compulsive disorders and Tourette's syndrome, our findings suggest that the dysfunction of synaptic organizing complexes may underlie the basic pathogenesis of neuropsychiatric disorders.

Symposium 3
Dynamics Interactions Underpinning Secretory Vesicle Fusion

S03-01

CAPS UTILIZES A LIPID-LINKED MECHANISM FOR PRIMING VESICLE EXOCYTOSIS

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In the regulated secretory pathway of neuroendocrine cells, the fusion of vesicles with the plasma membrane is dependent upon Ca^{2+} elevations. After vesicle delivery to the plasma membrane, a rate-limiting process of priming enables a subset of vesicles to undergo Ca^{2+}-triggered exocytosis. Our studies are directed at determining the molecular mechanisms that underlie priming. Priming is thought to involve the assembly of loose trans SNARE complexes and requires Munc13 and CAPS proteins. Consistent with this, Ca^{2+}-dependent secretion in PC12 cells is strongly inhibited by the down regulation of CAPS or Munc13-2. Priming also requires the lipid PI(4,5)P2. Consistent with this, Ca^{2+}-dependent secretion in PC12 cells is strongly inhibited by the overexpression of PLCη2 or PIP2 5-phosphatase. Previous studies found that CAPS and Munc13-2 both bind PI(4,5)P2 via their PH and C2B domains, respectively; the latter but not the former is Ca^{2+}-dependent. A mechanism for CAPS and PI(4,5)P2 function in priming was suggested by *in vitro* studies. CAPS was found to bind each of the membrane-integrated SNARE proteins (VAMP-2, syntaxin-1, SNAP-25) required for vesicle exocytosis even in the absence of PI(4,5)P2. With PI(4,5)P2 present on acceptor liposomes, CAPS stimulated the assembly of trans SNARE complexes and promoted the fusion of VAMP-2 donor and syntaxin-1/SNAP-25 acceptor liposomes. The CAPS stimulation of fusion was dependent upon a functional PI(4,5)P2-binding PH domain. SNARE binding by CAPS was localized to a C-terminal MHD1 domain that is present in all CAPS/Munc13 family proteins. MHD1 was shown to be required for CAPS function in Ca^{2+}-dependent secretion. The results suggest that CAPS functions in vesicle priming by coupling its PI(4,5)P2-mediated membrane association with SNARE protein binding to promote the assembly of trans SNARE complexes that render vesicles competent for Ca^{2+}-triggered fusion. Similarities and differences between CAPS and Munc13-2 function are under investigation.

S03-02

ROLE OF PIP2 METABOLISM AT THE NEURONAL SYNAPSE

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Phosphoinositides, such as phosphatidylinositol-4,5-bisphosphate [PI(4,5)P2] are key regulatory phospholipids that control a myriad of cellular functions. At the neuronal synapse, PI(4,5)P2 levels are negatively regulated by a lipid phosphatase, synaptojanin 1, which has been implicated in the recycling of synaptic vesicles and the internalization of AMPA receptor. Our recent studies have shown that synaptojanin 1-mediated hydrolysis of PI(4,5)P2 is dependent upon membrane curvature and is particularly robust on highly curved membranes, where it is stimulated by BAR/SH3 domain protein endophilin. Additionally, we found that acute induction of PI(4,5)P2 hydrolysis on endocytic structures promotes their fragmentation, suggesting a role for the PI(4,5)P2 phosphatase in endocytic fission. Interestingly, synaptojanin 1 is encoded by a gene (SYNJ1) located on human chromosome 21. Work from our lab has demonstrated that overexpression of SYNJ1 in transgenic models of Down syndrome increases PI(4,5)P2 catabolism in the brain, which correlates with synaptic malfunction and cognitive deficits. In a separate study, we have found that amyloid-beta also decreases PI(4,5)P2 levels, suggesting that a deficiency of this lipid may occur in Alzheimer's disease as well. Furthermore, the heterozygous deletion of Synj1 protects against the synaptotoxic action of amyloid-beta both *in vitro* and *in vivo*. Altogether, our studies have expanded our knowledge on the physiological role of PI(4,5)P2 in synaptic function and identified synaptojanin 1 as a promising drug target in Down syndrome and Alzheimer's disease.

S03-03

FINE-TUNING OF NEUROEXOCYTOSIS BY TWO MEMBERS OF THE PI3-KINASE FAMILY: TYPE I PI3KDELTA AND TYPE II PI3K-C2ALPHA

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Phosphoinositides (PIs) are a class of phospholipids characterised by an inositol head group that can be phosphorylated on the 3, 4 and 5 position to generate 7 lipid isotypes (1, 2). PIs have emerged as key regulators of neuroexocytosis – the mechanism underpinning neuronal and hormonal communication (1).

We have characterised a novel regulation of neuroexocytosis by 3'-phosphorylated phosphoinositides in neurosecretory cells. We previously demonstrated that Type 2 PI3K-C2α is necessary for ATP-dependent priming of secretory vesicles (3), and that its enzymatic activity is tightly regulated by Ca^{2+} and responsible for producing PI3P on a subpopulation of secretory vesicles (4).

We have recently found that specific inhibition of the type I PI3Kδ significantly potentiates neuroexocytosis and have unraveled the molecular mechanism underpinning this regulation: through an activation of PTEN (5), inhibition of PI3Kδ promotes a transient increase in PI(4,5)P2 generated on the plasma membrane. Total Internal Refection microscopy revealed that this transient increase in PI(4,5)P2 is sufficient to mobilize and translocate secretory vesicles to the plasma membrane of neurosecretory cells – an effect completely inhibited by cytochalasin-D. Our results suggest that

PI(4,5)P2 acts as coordinator of the actin network thereby controlling the number of secretory vesicle delivery to the plasma membrane.

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S03-04

REGULATION OF PHOSPHATIDIC ACID SYNTHESIS AT THE EXOCYTOTIC SITE: IMPLICATION OF GTPASES AND KINASES

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Exocytosis of neurotransmitters and hormones occurs through the fusion of secretory vesicles with the plasma membrane. This highly regulated process involves key proteins such as SNAREs but also specific lipids at the site of membrane fusion. Phospholipase D (PLD) has recently emerged as a promoter of membrane fusion in various exocytotic events potentially by providing fusogenic cone-shaped phosphatidic acid (PA). Using molecular and pharmacological tools, we demonstrate that PLD1 plays a important role in neurosecretion. Overexpression of a probe for PA, allowed us to show that the fusogenic lipid accumulates at the plasma membrane facing chromaffin granules that appeared morphologically docked at the electronic microscopy level. Various monomeric GTPases are activated concomitantly to PLD as a result of cell stimulation. Silencing RNA experiments demonstrated that the GTPases ARF6, RalA, and Rac1, each contributes to the maximal activation of PLD and to the secretory activity in neuroendocrine cells. On the other hand, overexpression of a PLD mutant unable to interact with the protein kinase C, suggested that PKC also contributes to the optimal activation of PLD during exocytosis. I will also show here that PLD is regulated by ribosomal S6 kinase 2 (RSK2)-dependent phosphorylation. Hence the results presented here suggest that PLD acts as a conductor in the regulation of fusogenic lipid dynamic by integrating various signaling pathway from both GTPases and kinases during exocytosis.

S03-05

BOTULINUM NEUROTOXINS AND THE NEUROEXOCYTOSIS NANOMACHINE

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The seven botulinum neurotoxins cause botulism lasting for different periods of time. An analysis of their mode of action suggested a model for the nanomachine which mediates neurotransmitter release (neuroexocytosis) based on the formation of a rosette of 8-10 SNARE complexes touching each other via the C-terminal of SNAP-25. An analysis of SNAP-25 isoform sequences indicates that there is a highly conserved Arginine residue (198 in vertebrates, 206 in the Drosophila melanogaster, Dm) within the C-terminal region cleaved by botulinum neurotoxin A, with consequent blockade of neuroexocytosis. The possibility that this residue may play an important role in the function of the neuroexocytosis nanomachine was tested at the Dm neuromuscular junction by expressing SNAP-25 whose Arg206 was replaced by alanine in a wt background. Electrophysiological recordings of spontaneous and evoked neurotransmitter release under different conditions as well as testing for the assembly of the SNARE complex indicate that this residue, which is at the P1' position of the botulinum neurotoxin A cleavage site, plays an essential role in neuroexocytosis. Computer graphic modeling suggests that this Arginine residue mediates protein-protein contacts within a rosette of SNARE complexes and predicts other residues that make up the protein-protein contact regions of the rosette SNARE supercomplex. This model is in agreement with recent data obtained by others by mutation of Dm neuronal synaptotagmin.

Symposium 4
Highlighting the Molecular Basis of Purinergic Transmission

S04-01

P2X RECEPTORS IN THE POST-STRUCTURE ERA

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P2X receptors are non-selective cation channels gated by extracellular ATP. There are seven P2X receptor subtypes in mammals, which display widespread tissue distribution and are involved in a variety of physiological processes, including nerve transmission, pain sensation, control of smooth muscle tone and inflammatory signalling. Until recently, our understanding of P2X receptor structure was limited to data gathered from structure-function experiments, and a handful of low-resolution structures derived from electron and atomic force microscopy of purified protein. The recent report of the first crystal structure of a P2X receptor, zebrafish P2X4.1, represents a step change in our understanding of this unique family of ligand-gated ion channels. I will explore the impact of the crystal structure on our understanding of P2X receptor function, highlighting recent research on global receptor architecture, the ATP binding site, the conformational change induced by ATP binding, and the architecture of the channel pore. I will also outline some challenges that remain to be addressed as, armed with a crystal structure, we continue to explore the molecular basis of P2X receptor structure-function relationships.

S04-02

STRUCTURE AND CATALYTIC MECHANISM OF NTPDASES AND E5NT IN PURINERGIC SIGNALLING

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The action of ATP in purinergic signaling is terminated by ectonucleotidases. NTPDases catalyze the stepwise dephosphorylation of ATP via ADP to AMP, which is converted to adenosine by ecto-5'-nucleotidase (e5NT, CD73). To characterize the catalytic mechanism and the molecular basis for substrate specificity, we have determined crystal structures of these enzymes. The spatial structures of ectonucleotidases will also facilitate the rational development of specific inhibitors for pharmaceutical and biological studies. Following E. coli expression, refolding, purification and characterization of the ectodomains, the proteins could be crystallized.

The active site of the NTPDases is located at the interface between the two domains of the actin/hsp70/sugar kinase superfamily fold. Co-crystal structures of NTPDase2 with products and substrate analogs suggest a mechanism, in which a water molecule deprotonated by Glu-165 attacks the nucleotide's terminal phosphate group, which is positioned by coordination to a divalent metal ion. The specificity for ATP and ADP is achieved by an alternative binding mode of the α-phosphate group. An analysis of sequence diversity among different NTPDases in the active site region suggests that the development of type-specific inhibitors might be a feasible task.

For CD73, structures of related bacterial and yeast nucleotidases have been characterized in two conformations (open and closed), which differ in the relative orientation of the two domains by a rotation of up to 96°. The domain movement is unique in that the cleft between the domains does not open up, but the residues of the domain interface slide along the interface. A comparison of the crystal structures of the bacterial 5NTs with CD73 indicates, that the domain movement might also be involved in the specificity of the eukaryotic enzymes for AMP. In contrast, *E. coli* 5NT hydrolyzes ATP, ADP as well as ATP.

S04-03

MOLECULAR PROPERTIES AND TRANSPORT MECHANISM OF VESICULAR NUCLEOTIDE TRANSPORTER (VNUT)

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VNUT is a membrane proteins encoded by SLC17A9 gene and distributes throughout various endomembrane organelles such as secretory granules in some endocrine cells and synaptic-like vesicles in neurons and astrocytes. VNUT transports various nucleotides such as ATP and GTP at the expense of membrane potential established by vacuolar type proton ATPase, and is responsible for vesicular storage of nucleotides. Suppression of SLC17A9 gene expression causes decreased ATP secretion from ATP-secreting cells. VNUT and its orthologues are present in almost all animal species. Here, after showing some introductory information of SLC17 transporters, I will update our study on the structure and function of VNUT. In particular I will talk (i) molecular properties of VNUT, and (ii) inhibitors. The results further support its essential role in ATP secretion in the purinergic chemical transmission.

S04-04

MOLECULAR MECHANISMS OF NUCLEOTIDE RELEASE: FOCUS ON PANNEXIN1 CHANNELS

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ATP sensitive P2 receptors mediate several biological functions, including the transmission of calcium signals within the interconnected astrocyte network and the generation of spontaneous calcium oscillations seen in migrating neural progenitors. Two pathways, regulated secretion and diffusion through ion channels, have been implicated in the release of ATP from astrocytes. Among the ion channels, Pannexin1(Panx1) has been proposed to provide such a pathway. Pannexins (Panx1, 2 and 3) comprise a newly discovered group of proteins that share significant sequence homology with the invertebrate gap junction proteins, the innexins. Panx1 is

ubiquitously expressed and has been shown to form large conductance (500pS) plasma membrane channels that are voltage-dependent, mechanosensitive, activated following $P2X_7$ receptor stimulation, and permeable to relatively large molecules, such as ATP. The contribution of Panx1 channels to ATP release and calcium signaling in astrocytes was assessed using transgenic mice lacking Panx1. The outwardly rectifying Panx1 currents that display an activation voltage dependent on extracellular K^+ levels, and the BzATP-induced currents were absent in Panx1-null astrocytes. Compared to wild-type astrocytes, Panx1-null cells were impermeable to the dye YoPro1 and did not release ATP when stimulated with high levels of extracellular K^+, with BzATP or with the calcium ionophore ionomycin. Although Panx1-null astrocytes were able to transmit calcium signals following focal mechanical stimulation, these cells failed to display amplification of calcium waves as seen in wild-type cells exposed to low divalent cation solution. These results, together with our previous studies using Panx1 siRNA and pharmacological tools, clearly indicate that the release of ATP from Panx1 channels are necessary for the amplification but not for the transmission of calcium waves. Although vesicular release of ATP contributes to astrocyte calcium signaling, as we and others have previously shown, Panx1 channels provide an alternative route for nucleotide release, which can be triggered under specified conditions.

Symposium 5
Epigenetics and Histone Deacetylases in Neurodegenerative Disease, Aging, and CNS Repair

S05-01

ACETYLATION STATUS DURING NEURODEGENERATION, MEMORY FUNCTIONS AND AGING: USE OF EPIGENETIC MODULATORS IN ALZHEIMER'S DISEASES?

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Alzheimer's Disease (AD) is an aged-related neurodegenerative disease which, besides its two histopathological hallmarks (amyloid plaques and tauopathy), is also characterized by neuronal loss and memory impairments. There is no cure for AD, the benefit of current strategies being only modest. Our aim is to find new therapeutic options to mitigate cognitive dysfunctions in AD. On the one hand, important acetyltransferase enzymes, such as the CREB-Binding Protein (CBP), are primed to degradation in neurodegenerative contexts. On the other hand, recent evidence suggest that epigenetic regulations, including histone acetylations, are dynamically implicated in higher brain functions such as plasticity and memory formation. Our work is aimed at establishing major chromatin acetylation alterations during the formation of a memory in normal and pathological conditions, in order to define the potential therapeutic opportunity for the use of acetylation modulator molecules in AD.

As deficit of declarative memory is a primary AD symptom, we focused on a hippocampal-dependent task, such as the establishment of a spatial memory, in different animal models. In young adult rats, we found that the expression of different HATs (including CBP) was induced in the hippocampus as spatial memory forms and consolidates, an event associated with specific histone markings at several memory/plasticity genes. In a memory-deficient rat model bearing AD-related lesions, CBP and subsequent histone acetylation regulations were severely altered. In addition, critical components of this pathway presented different degrees of alteration in a model of aged rats.

Our data point to the use of molecules inducing a histone hyperacetylated state as therapeutic tools, potently able to increase memory-related transcription programs and reactivate synaptic plasticity. So far, some histone deacetylase (HDAC) inhibitors have been able to counteract neurodegeneration or enhance memory functions *in vivo*. As much progress is being made in understanding HDAC function in these processes, better HDAC inhibitors can be developed in the near future. However, it seems essential to envisage direct stimulation of the acetyltransferase function as a new therapeutic tool in neurodegenerative diseases.

S05-02

HISTONE DEACETYLASES: PROMOTERS AND INHIBITORS OF NEURODEGENERATION

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Histone deacetylases (HDACs) are a family of proteins that play an important role in regulating transcription as well as a variety of cellular processes. The 18 mammalian HDAC proteins are grouped into four sub-families: Class I (HDAC 1, 2, 3 and 8), Class II, (HDAC 4, 5, 6, and 10), Class III (Sirt 1 – 8), and Class IV (HDAC 11). Administration of pharmacological inhibitors against HDACs prevents neuronal loss and improves behavioral outcome in a variety of tissue culture and *in vivo* models of neurodegenerative disease. Although these results implicate one or more of the HDAC proteins in promoting neuronal death, because the commonly used inhibitors block the activities of all HDACs efficiently, the identity of the HDAC(s) responsible for neurodegeneration and that is targeted by the inhibitors has been unclear. Moreover, in some cell culture systems treatment with HDAC inhibitors actively promotes neuronal death. These results suggest that the regulation of neuronal survival by HDACs is complex and might involve the activities of both neuroprotective and neurotoxic members. In our presentation we will review our work on the role of individual HDAC proteins in the regulation of neuronal survival and death. We will describe that some members of the Class II family of HDACs including HDRP, HDAC4 and HDAC7 protect neurons from death. A brief description of the mechanism by which they exert their neuroprotective effect will be presented. On the other hand, some Class I HDACs such as HDAC3 have potent and selective neurotoxic effects. Pharmacological inhibitors designed to specifically target neurotoxic HDACs will be of great value to the treatment of neurodegenerative diseases.

S05-03

EPIGENETIC PROTEINS AS TARGETS FOR PROTECTION AND REPAIR IN THE CNS: HDACS AND BEYOND

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Oxidative stress, defined as an imbalance of cellular oxidants and antioxidants sufficient to mediate damage, has been implicated as an intiator or perpetuator of neurological disease. Despite intensive study, methods for abrogating aspects of oxidative stress in the central nervous system remain obscure. Our laboratory has utilized the experimental leverage of an *in vitro* model of oxidative stress

that involves cystine deprivation and glutathione depletion. We initially showed that cell death in this model has features of apoptosis and is transcription-dependent. Sp family transcription factors are robustly induced by glutathione depletion. Over a decade ago, we demonstrated that Sp1 and Sp3 induction was associated with increased acetylation of these proteins. We further demonstrated that broad, non selective histone deacetylase inhibitors augmented Sp1 acetylation, increased Sp1 transcriptional activity and prevented neuronal death not only *in vitro* but also *in vivo* in models of stroke, multiple sclerosis, and mitochondrial toxins. *In vitro* we noted that HDAC inhibition leads to durable protection but also a small and reproducible cell death Temporal manipulation of HDAC inhibition indicates that these two distinct aspects of HDAC inhibition can be separated. More recent data has shown that cell cycle related transcription factors such as Myc can be acetylated by oxidative stress and that Myc downregulation is sufficent to protect neurons from glutathione depletion. We are using this strategy to identify HDAC isoforms that interact with Myc as a strategy for identifying nuclear HDACs involved in neuroprotection and distinguishing those from HDACs involved in toxicity. Finally, we will introduce a novel HDAC-independent strategy for modulating salutary gene expression in the CNS.

S05-04

FROM BASIC MECHANISMS TO THERAPEUTIC TARGETS IN HUNTINGTON'S DISEASE

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Epigenetics at the level of protein post-translational modification has emerged as a critical modifier of numerous cellular processes including transcriptional control. Protein modification has also taken on a growing importance in human diseases ranging from cancer to neurodegeneration, therefore understanding the processes impacted by these modifications and the underlying mechanisms and enzymes involved represent key therapeutic approaches for these diseases. Hungtington's disease is a devastating autosomal dominant disease that strikes in mid-life with no disease-modifying treatments available. It is caused by a polyglutamine repeat tract within the Huntgintin (Htt) protein encoded by a CAG repeat repeat within the HD gene. While the mutant protein is expressed throughout the body, overt cellular dysfunction and cell death are confined to regions of the brain, primarily cortex and medium spiny neurons of the striatum. Beginning with data showing that Htt interacts with key transcriptional regulatory proteins, inhibits *in vitro* protein acetylation and that the presence of the mutation causes reproducible and early transcriptional dysregulation, we have investigated the potential therapeutic value of histone deacetylase inhibitors. HDAC inhibitors have now shown great promise in multiple models of polyglutamine diseases and a Phase I human clinical trial for HD has been carried out. We have extended these studies to investigating protein modification of the Htt protein itself and find that SUMOylation, acetylation, and phosphorylation are all critical modifications that contribute to subcellular localization, transcriptional regulation and protein turnover. Further, epigenetic mechanisms underlying gene expression changes are showing specific modulation of histone methyltransferases in brain. Enzymes involved in these processes have been investigated and current work will be presented.

Symposium 6
Molecular, Cellular and Behavioral Aspects of Mental Retardation and Autism

S06-01

ABNORMAL SYNAPTIC HOMEOSTASIS IN AUTISM SPECTRUM DISORDERS

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The diagnosis of autism spectrum disorders (ASD) is based on impairments in reciprocal social communication, and repetitive behaviors. But beyond this unifying definition lies an extreme degree of clinical heterogeneity. Our previous studies pointed at one synaptic pathway associated with the disorder. Among the causative genes, synaptic cell adhesion molecules (neuroligins and neurexins) and scaffolding proteins (SHANK3) are crucial for synapse formation/maintenance as well as correct balance between inhibitory and excitatory synaptic currents. In parallel, we identified genetic mutations that disrupt the melatonin signaling in a subset of patients with ASD. Melatonin is known to play a key role in the regulation of circadian rhythms such as sleep-wake cycles and was shown to modulate inhibitory currents, as well as neurite and memory formation.

In this presentation, I will present recent results from human genetics and animal models studies that shed new light on the complex inheritance of ASD and on the behavioral consequences of a synaptic defect. Based on these results, we propose that ASD could be caused by an alteration in the homeostasis of the synaptic currents in specific regions of the brain. In some cases, imbalance of excitatory/inhibitory currents could be revealed or amplified by an alteration of the serotonin-melatonin pathway and/or abnormal sleep homeostasis. To date, it is not clear how many genetic/epigenetic and environmental factors can modulate synaptic homeostasis and how these factors interact with each other to modulate the risk for ASD. A better knowledge of these interactions will be necessary to understand the complex inheritance pattern of ASD and to discover knowledge-based treatments.

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S06-02

TRANSLATION DYSREGULATION IN AUTISM SPECTRUM DISORDERS

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Mutations in several negative regulators of translation initiation cause developmental disabilities and autism spectrum disorders in humans, including fragile X syndrome and tuberous sclerosis complex. Mouse models have been developed for several of these disorders, and they exhibit altered translation control, abnormal synaptic function, and aberrant behavior. Findings from recent studies of mice that model fragile X syndrome, Wolcott-Rallison syndrome, and non-syndromic autism will be discussed in this presentation. These studies have revealed interesting links among the biochemical activities of translation factors, synaptic function, and behavior, and provide insight into the molecular basis of developmental disabilities and autism spectrum disorders.

S06-03

THE CYTOPLASMIC FMRP INTERACTING PROTEIN 1 CYFIP1 LINKS FRAGILE X SYNDROME TO OTHER NEURODEVELOPMENTAL AND PSYCHIATRIC DISORDERS

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Fine regulation of mRNA transport and translation at synapses underlies brain development, synaptic plasticity and spine morphology. One of the key molecules involved in this process is the Fragile X Mental Retardation Protein (FMRP), the protein lost in the mental retardation form called Fragile X Syndrome (FXS). We have previously demonstrated that FMRP represses translation initiation via its cytoplasmic interacting protein CYFIP1/Sra1, known as a regulator of the actin cytoskeleton. FMRP tethers a specific subset of neuronal mRNAs to CYFIP1, which can in turn block the translation initiation factor eIF4E. By combining brain fractionation into sub-cellular compartments, CYFIP1 immunoprecipitation and mass spectrometry, we found new interactors of the CYFIP1-FMRP particle assembled in specific molecular complexes according to their subcellular location. We provide evidence for a novel interplay between local translational regulation and cytoskeleton remodeling. Finally a gene wide association study of the novel CYFIP1 partners here identified revealed that these proteins are encoded by genes associated to Autism, Schizophrenia and other psychiatric disorders.

S06-04

THE RHO-LINKED MENTAL RETARDATION PROTEIN OLIGOPHRENIN-1 CONTROLS SYNAPSE FORMATION AND PLASTICITY

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Mutations in genes encoding regulators and effectors of Rho GTPases have been found to underlie various forms of mental retardation (MR). Oligophrenin-1 (OPHN1), which encodes a Rho-GTPase activating protein, was the first identified Rho-linked MR gene. It was initially identified by the analysis of a balanced translocation t(X;12) observed in a female patient with mild MR. Subsequent studies have revealed the presence of OPHN1 mutations in families with MR associated with cerebellar hypoplasia and lateral ventricle enlargement. All OPHN1 mutations identified to date have been shown, or predicted, to result in OPHN1 loss of function; however, the pathophysiological role of OPHN1 has remained poorly understood. By temporally and spatially manipulating OPHN1 gene expression, we obtained evidence that during early development postsynaptic OPHN1 plays a key role in activity-dependent maturation and plasticity of excitatory synapses, indicating the involvement of OPHN1 in normal activity-driven glutamatergic synapse development. More recently, we obtained evidence that OPHN1 also plays a critical role in mediating mGluR-LTD in CA1 hippocampal neurons. mGluR-LTD induction elicits rapid dendritic OPHN1 synthesis, which is dependent on mGluR1 activation. This response is essential for mGluR-LTD, as acute blockade of OPHN1 synthesis impedes LTD. mGluR-induced OPHN1 mediates LTD and associated persistent decreases in surface AMPARs via interactions with Endophilin-A2/3. Importantly, this role of OPHN1 is separable from its effects on basal synaptic strength. Thus, these data unveil a critical role for rapid OPHN1 synthesis in mGluR-LTD, providing not only novel insight into the mechanism and function of mGluR-LTD, but also into the cellular basis by which mutations in OPHN1 could contribute to the behavioral and cognitive deficits in OPHN1 patients.

Symposium 7
Novel Functions for Cell Cycle Proteins in Post-Mitotic Neurons

S07-01

REGULATION OF THE KINASE ACTIVITY AND FUNCTION OF CYCLIN-DEPENDENT KINASE 5 IN POST-MITOTIC NEURONS

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Cdk5 is a member of cyclin-dependent kinase family. While most Cdks function in proliferating cells, Cdk5 is predominantly expressed in post-mitotic neurons. Different from cycling Cdks, whose kinase activity is tightly coupled to cell cycle events, Cdk5 plays a role in various neuronal activities including neuronal migration, synaptic activity and neuron death, those unrelated to cell cycle. As the investigation on cycling Cdks has advanced greatly our knowledge on cell proliferation, elucidating the regulatory mechanism of the Cdk5 activity would be useful for understanding of the Cdk5-dependent neuronal activities. However, it is not known yet how the kinase activity of Cdk5 is associated with those neuronal activities. Cdk5 requires a p35 or p39 regulatory subunit for activation. While cycling Cdks work mainly in nucleus, Cdk5 is a cytoplasmic kinase anchored to membranes through myristoylation of p35 or p39. Cycling Cdks require the T-loop phosphorylation of Cdk for activation, but Cdk5 does not. In contrast to cycling Cdks, whose kinase activity is inhibited by Tyr15 phosphorylation of Cdk, Cdk5 is activated by its phosphorylation. The activation of Cdk5 by Tyr15 phosphorylation is reported to induce neurite retraction, spine shrinkage, and neuronal death. We re-evaluated the role of Tyr15 phosphorylation in Cdk5 activation. However, the conclusion we obtained was that the Tyr15 phosphorylation does not activate Cdk5-p35, but rather the p35 binding to Cdk5 suppresses the Tyr15 phosphorylation. One of Cdk5 functions is to regulate neurite outgrowth. However, it is not still clear how Cdk5 controls neurite outgrowth. We have recently reported that AATYK1, a Ser/Thr kinase expressed highly in neurons and a substrate for Cdk5, regulates recycling endosomal trafficking in CHO-K1 cells. We extended this study to neurons and found AATYK1 is an inhibitory factor for neurite outgrowth and its phosphorylation at Ser34 by Cdk5 suppressed the function. The difference in the activation mechanism and function between cycling Cdks and neuronal Cdk5 will be discussed.

S07-02

THE CYTOPLASMIC FUNCTION OF ATM IN NEURONS: BEYOND DNA BREAKS

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ATM (ataxia-telangiectasia mutated) is a member of the PI3 kinase family. It was discovered by virtue of its linkage to ataxia-telangiectasia (AT), a devastating neurodegenerative condition of childhood. The AT syndrome includes predilection towards cancer, radiation sensitivity and immune system defects. These non-CNS symptoms derive from the functions of ATM in the DNA damage response (DDR) system, including DDR protein phosphorylation and cell cycle checkpoint activation. While the CNS symptoms are assumed to be related to the DDR deficiencies, the specific mechanisms underlying this proposed linkage remain uncertain. We have found that the functions of ATM in brain are far more diverse than previously assumed. As in lymphocytes, ATM serves as a major neuronal cell cycle inhibitor. In brain, this inhibition is a constitutive activity, and its failure leads to ectopic neuronal cell cycling, a first step on the pathway to death. In neurons, far more than in other cells, ATM has a strong cytoplasmic presence and a major role in the regulation of two key synaptic vesicle proteins, VAMP2 (synaptobrevin) and synapsin I. ATM phosphorylates both proteins and without this activity, vesicle trafficking and release functions are reduced. Hippocampal LTP is also significantly impaired. As these functions are predominantly cytoplasmic, the findings emphasize the significance of ATM's cytological localization. ATM activity is also required for the cytoplasmic retention of the Class II histone deacetylase (HDAC), HDAC4. In ATM-deficiency, HDAC4 shifts to the nucleus where it reduces MEF2 and CREB occupancy of a number of neuroprotective genes, reducing their mRNA and protein levels. At the same time it stimulates the synthesis of message and protein for several cell cycle genes. In the aggregate our data strongly suggest that the DDR/cell cycle checkpoint function of ATM is only one of its many functions in neurons. This implies that the full spectrum of causes of the neurodegenerative phenotype may be more broadly based than DNA damage alone.

S07-03

FUNCTIONS OF APC/C-CDH1 IN POSTMITOTIC NEURONS

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The anaphase-promoting complex/cyclosome (APC/C) is a multisubunit E3 ubiquitin ligase that controls cell cycle progression by assembling polyubiquitin chains on regulatory proteins, such as mitotic cyclins, targeting them for proteasomal degradation. Being terminally differentiated cells, post-mitotic neurons actively repress proteins related with the cell cycle and DNA replication, and the aberrant expression of these proteins has been associated with neuronal cell death. APC/C is activated by Cdh1 in post-mitotic neurons, where it regulates axonal growth, synaptic plasticity, glucose metabolism and survival. The APC/C-Cdh1 substrate, cyclin B1, has been found to accumulate in degenerating neurons of patients suffering from neurological disorders, such as Alzheimer's disease and stroke. This highlights the importance of elucidating

cyclin B1 regulation by APC-Cdh1 in neurons under stress conditions relevant to neurological disease. We have demonstrated that stimulation of N-methyl-D-aspartate receptors that occurs in neurodegenerative diseases and ischemis promoted the phosphorylation of Cdh1, a condition sufficient to inhibit APC activity. This led to nuclear accumulation of cyclin B1 and to an aberrant attempt of post-mitotic neurons to re-enter the cell cycle. Moreover, we found that activation of cyclin-dependent kinase-1 by cyclin B1 promoted mitochondrial dysfunction and oxidative stress, leading to neuronal apoptosis. We identified Cdk5, a cyclin-dependent kinase important for synaptic plasticity and neurotoxicity, to be responsible for Cdh1 phosphorylation. These results reveal Cdh1 as a novel Cdk5 substrate that mediates cyclin B1 neuronal accumulation in excitotoxicity and neurological disease.

This work was funded by Fondo de Investigación Sanitaria (PS09/0366), RENEVAS (RD06/0026/1008; Instituto de Salud Carlos III) and Junta de Castilla y León.

S07-04

RB/E2F PATHWAY REGULATES NEUROGENESIS BY MODULATING THE COMPOSITION OF NEURAL PRECURSOR POPULATION

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Mutations within the pRb/E2F pathway are common events in tumourigenesis; we have described expanded roles for this protein family in ensuring the functional integrity of the developing and adult brain. To determine the mechanisms by which pRb/E2f controls neuronal function we have focused on identifying the properties of the E2f3 transcription factor, as it is one of the most highly deregulated E2fs in the Rb mutant brain. We have recently shown that E2F3 is an important regulator of neural precursors, whereby its loss in mice leads to a decreased progenitor pool but an expanded stem cell population. We now show that E2f3 controls the number of neurons generated in the cortex by employing two distinct protein isoforms to differentially regulate the balance between the number of proliferating precursors and differentiated neurons. E2f3 isoforms each modify the activities of unique neural precursor populations, with differential effects on neuronal generation. These intriguing observations prompted us to ask which genes are regulated by E2f3 in neural precursor cells. Using ChIP-on-chip technology, we found that E2f3 binds the promoter region of over 3000 genes with functions most markedly involved in neurogenesis and CNS development, including multiple known regulators of neural precursor renewal, proliferation, differentiation, death, and neuronal maturation. These results highlight E2f3 as an important regulator of neurogenesis and neuronal maturation in the mammalian brain.

Symposium 8
Astrocytic Contribution to Brain Diseases and Recovery

S08-01

MECHANISMS UNDERLYING GLIOTRANSMITTER ATP AND THEIR DYSFUNCTIONS

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ATP and other extracellular nucleotides are important chemical transmitters that mediate intercellular communications. In the CNS, these extracellular nucleotides are especially important for regulation of glial functions or for neuron-to-glia communications. Astrocytes could receive various stimuli, respond to them and produce out-put signal as a form of release of gliotransmitters, among which, ATP has a central role. Astrocytes release ATP both in the activity dependent- and independent-fashion, and regulate synaptic transmission dynamically. However, mechanisms underlying ATP release is a still matter of debate. Here we show exocytotic release of ATP from astrocytes and its contribution to neuronal functions and dysfunctions. Astrocytes express several machineries for exocytosis of ATP, i.e. ATP vesicles, SNAREs and vesicular ATP transporters. Astrocytes released ATP in a Ca^{2+} and SNARE-dependent mechanisms. Using a TIRF microscopy, we imagined exocytotic ATP release. Inhibition of vesicular ATP accumulation by bafilomycin, astrocytic ATP release disappeared. Knock-down of vesicular nucleotide transported also inhibited the ATP release. Regulation by of neuronal activities by astrocytes was dependent on exocytotic ATP release, by which neuronal baseline activities were finely tuned. Taken together, extracellular nucleotides have key roles for regulation of neuron-to-glia communications, by which the brain controls its normal functions.

S08-02

GLIAL MODULATION OF GLUTAMATERGIC NEUROTRANSMISSION AT ONSET OF INFLAMMATION

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Recent advances in the field of glia to neuron interaction have pointed out astrocytes as the glial cell subtype that could actively participate to synaptic transmission and synaptic processing of information. Because astrocytes have been found to modulate neuronal transmission and synaptic network through glutamate and purine release, their involvement in central nervous system (CNS) pathologies has been more and more investigated. In most CNS diseases, astrocytes have been found to become reactive. This astrogliosis was found to be concomitant with the activation of microglia another sub-type of glial cells. Microglia originate from the myeloid lineage, they are considered as the immune cells of the brain and are the main effectors of inflammatory responses. While most studies consist to investigate the modification of astrocytic behavior when they switch to their reactive form, very little is known on their interaction with microglial cells and in which extend they modulate neuronal activity upon inflammation. In order to better understand the modulation of gliotransmission at the onset of inflammation, we used the Toll like receptor (TLR) signaling pathway to activate microglial cells while recording neuronal activity of CA1 pyramidal neurons by patch clamp. We found that the bacterial compound lipopolysaccharide (LPS) a specific TLR-4 ligand used to activate microglia and mimic inflammation triggered an increase of glutamatergic transmission within the first minute of application. Further investigating microglia to neuron signaling, we found that purines were at the center of this signaling and that astrocytes were required for this neuronal modulation. Overall our data suggest that microglial activation modulates neuronal activity through a signaling that involves purines and astrocytes within the first minute of inflammation. These results indicate that in pathological conditions glial cells may cooperate to modulate neuronal activity.

S08-03

REPAIRING THE DISEASED CNS VIA THE EXPLOITMENT OF ADULT GLIAL PROGENITOR CELLS

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Three seminal findings have recently changed our view of astrocytes and of the mechanisms of CNS repair. First, both during development and adulthood, all the three CNS cell types (i.e., neurons, astrocytes and oligodendrocytes) originate from one single stem cell, which is an astrocyte in nature. Second, the generation of new neurons and new glia (neurogenesis) continues throughout life. Third, adult neurogenesis does not only occur in the well known 'neurogenic niches' (i.e., the subventricular zone and the hippocampus) but the entire brain's parenchyma is full of quiescent progenitors, that are activated after injury and can differentiate to new functional cells. Specifically, two main types of parenchymal stem-like cells have been identified: (i) proliferating reactive astrocytes; these cells remain within their lineage *in vivo*, but, as revealed by ex-vivo studies, re-acquire capacity for self-renewal and are potentially able to generate all the three CNS cell types (1); (ii) NG2-positive polydendrocytes, a type of neural precursors that can differentiate to mature oligodendrocytes and participate to re-myelination after injury; these cells retain some multipotency and, under some conditions, can also generate neurons and astrocytes (2). We have recently shown that, at early differentiation stages, NG2 cells express the new purinergic P2Y-like receptor GPR17. Activation of GPR17 by its endogenous ligands (e.g., uracil nucleotides) promotes cell differentiation to mature myelinating oligodendrocytes, while its inhibition by receptor antagonists or small interfering RNAs retain NG2 cells in an undifferentiated state (3, 4). GPR17 is thus a new key player in polydendrocytes maturation. We are currently assessing if GPR17 manipulation can also instruct NG2 cells to generate new neurons, thus opening new hopes for repairing the brain in both acute (trama, stroke) and

chronic disorders, as Alzheimer's, Parkinson's diseases and multiple sclerosis.

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S08-04

GLIAL REGULATION OF BLOOD FLOW IN THE NORMAL AND DIABETIC RETINA

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Local increases in neuronal activity lead to spatially and temporally coordinated increases in blood flow in the CNS. This response, termed functional hyperemia, serves to meet the increased energy demands of active neurons. We have investigated the role of glial cells in mediating functional hyperemia in the healthy and diabetic retina of the rat. We found that in the healthy, freshly isolated retina, both light- and glial-evoked vasodilations were mediated by glial production of the arachidonic acid metabolites PGE2 and EETs, and that vasoconstrictions were mediated by production of 20-HETE. When nitric oxide levels were raised, glial- and light-evoked vasodilations were reduced and vasoconstrictions increased. Similarly, glial- and light-evoked vasodilations were reduced when oxygen levels were raised from 21% to 100%. Interestingly, light-evoked vasodilations are reduced in patients with diabetes. We used a streptozotocin-induced rat model of type 1 diabetes to study the mechanism responsible for this reduction. We found that light-evoked arteriole dilation was reduced by 58% in the isolated, diabetic retina and that glial-evoked vasodilation was reduced by 60%. The diabetic retinas showed neither a decrease in the thickness of the retinal layers nor an increase in neuronal loss, although signs of early glial reactivity and an upregulation of inducible nitric oxide synthase (iNOS) were detected. Inhibition of iNOS with aminoguanidine (100 μM) or 1400W (1 μM) restored both light- and glial-evoked dilations to control levels. Functional hyperemia was also investigated *in vivo* by monitoring light-evoked arteriole dilation in rats. We found that light-evoked dilations were significantly reduced in diabetic animals and that the response was partially restored by inhibiting iNOS with aminoguanidine, delivered by IV injection or through the animal's drinking water. These findings suggest that high NO levels resulting from iNOS upregulation alters glial control of vessel diameter and may underlie the loss of functional hyperemia observed in diabetic retinopathy. Restoring functional hyperemia by iNOS inhibition may limit the progression of retinopathy in diabetic patients. Supported by the Leducq Foundation and NIH EY004077.

Symposium 9
Parkinson Disease Genes, Protein Degradation and Mitochondrial Quality Control

S09-01

ENDOPLASMIC RETICULUM STRESS IS ASSOCIATED WITH α-SYNUCLEINOPATHY IN TRANSGENIC MOUSE MODEL

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Accumulation of aggregated α-synuclein (αS) is a pathological hallmark of Parkinson's disease (PD) and other α-synucleinopathies. Cellular accumulation of misfolded proteins is often associated with activation of endoplasmic reticulum (ER) stress pathway or unfolded protein response (UPR). Recent studies implicate ER stress in αS toxicity in cellular context. Herein, we show that transgenic (Tg) mice overexpressing mutant human (Hu) αS exhibits ER stress with onset of neurodegeneration. With the disease A53T HuαS Tg mice exhibit increased levels of ER chaperons (BIP/Grp78, Grp94 and PDI) and activation of ER stress-related transcription factors, ATF6 and xbp1. However, induction of ER chaperons occurred in absence of the expected increase in the phosphorylation of eIF2α. This abnormal ER-stress response the A53T HuαS Tg mice was associated with increased levels of cleaved caspase 12, an ER stress-related caspase in mouse, and increased activation of caspase 9, a downstream target of cleaved caspase 12. The above signs of UPR coincide with disease and were not seen in areas that are not affected by α-synucleinopathy (e.g. Cortex). Thus, the ER stress and activation of caspase 12 and 9 are selectively associated with α-synucleinopathy.

Analysis of microsomal fractionations from spinal cords of A53T HuαS Tg mice shows that αS is associated with the ER/microsome fraction and the levels microsomal αS increases with onset of disease and induction of ER stress. In addition to the monomeric αS in the lumen of microsomes, oligomeric αS are associated with outside of microsomes. Co-immunoprecipitation and cross-linking studies show that ER chaperones are associated with microsomal αS. Analysis of human PD cases show increased microsomal αS in PD cases. We hypothesize that accumulation of misfolded/aggregated αS in the ER of A53T HuαS Tg mice causes ER-stress. abnormal UPR, and contributes to neurodegeneration.

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S09-02

PARKIN-MEDIATED UBIQUITINATION AND REGULATION OF SYNAPTIC PROTEINS

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Mutations in the Parkin gene cause an autosomal recessive juvenile-onset form of PD that account for a large fraction of familial cases. It is now well established that the parkin protein functions as an E3 ubiquitin (Ub)-ligase. Ubiquitination targets substrates to different cellular pathways depending on the length and architecture of the Ub chain. Typically, substrates modified with lysine 48 (K48) linked Ub chains are targeted to the proteasome for degradation, whereas substrates modified with Ub chains linked via K63 or mono-Ub influence cellular functions as diverse as signal transduction, transcription and membrane trafficking. In addition to assembling canonical K48-linked Ub chains, parkin has been shown, under certain circumstances, to assemble K63-linked Ub chains as well as the attachment of mono- and multi-mono-Ub, implicating it in proteasome-independent pathways. In particular, we have shown that parkin regulates cell-surface receptor trafficking and kinase signaling pathways via the mono-ubiquitination of adaptor proteins such as Eps15 and PICK1. More recently, we have been exploring the role of the N-terminal parkin Ub-like (Ubl) domain as a versatile interaction module, connecting parkin to proteins involved in ubiquitination and trafficking. In addition to the well-characterized Ub-Interacting Motif (UIM), we have identified the SH3 domain within proteins such as endophilin-A as a novel parkin Ubl-interacting module. The structural basis and functional consequences of the interactions will be discussed along with an attempt to link the findings to pathways relevant to neurodegeneration, including mitochondrial quality control.

S09-03

PARKINSON'S DISEASE: PINK1 AND MITOCHONDRIAL COMPLEX I FUNCTION

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The etiology of PD remains unknown, although clinical and experimental evidence implicate the involvement of mitochondrial dysfunction and oxidative stress. Exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or to rotenone, both Complex I toxins, caused parkinsonism in humans and in laboratory animals. Mutations in the mitochondrial kinase PINK1 cause recessive inherited early onset PD. Overexpression and loss of function studies have implicated PINK1 in apoptosis, abnormal mitochondrial morphology, impaired dopamine release and motor deficits. However, the underlying molecular mechanisms remain to be clarified. Using Drosophila and mouse models we show here that PINK1 deficiency or clinical mutations impact on the function of Complex I of the mitochondrial respiratory chain, resulting in mitochondrial depolarization and increased sensitivity to apoptotic stress in mammalian cells and tissues. In neurons we find that Pink1 deficiency affects synaptic function in Drosophila neurons as reserve pool of synaptic vesicles is not mobilized during rapid stimulation. The fundamental importance of Pink1 for energy maintenance under increased demand is further corroborated as this deficit can be rescued by adding ATP to the synapse. The clinical relevance of our

observations is confirmed by the fact that human wild type PINK1, but not PINK1 containing clinical mutations can rescue Complex 1 deficiency. Our work provides proof of concept that Complex I deficiency underlies the pathogenesis of a hereditary form of PD. As Complex I dysfunction is also implicated in sporadic PD, a convergence of genetic and environmental causes of PD on a similar molecular mechanism is emerging.

S09-04

PINK1 AND AUTOPHAGY IN MITOCHONDRIAL AND NEURITIC QUALITY CONTROL

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Alterations in autophagy regulation have been implicated in neurodegeneration associated with Parkinson's and related diseases (PD), including familial parkinsonism caused by mutations in PTEN-induced putative kinase 1 (PINK1) and leucine-rich repeat kinase 2 (LRRK2). Loss of endogenous PINK1 function adversely affects mitochondrial function and structure, promoting selective mitophagy. Overexpression of wild type, but not mutant, PINK1 reverses mitochondrial pathology and autophagic neurite degeneration in toxin and genetic models of PD, with some effects dependent upon mitochondrial targeting of PINK1 and others attributed to cytosolic activity. PINK1 regulates mitochondrial quality control and neuritic/synaptic health through multiple mechanisms.

Workshop 1
Advanced Strategies for Fate Mapping in Vivo

W01-01

AGE-DEPENDENT CHANGES IN FATE AND FATE POTENTIAL OF POLYDENDROCYTES (NG2 GLIAL CELLS)

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Glial cells that express the NG2 proteoglycan and the alpha receptor for platelet-derived growth factor represent a fourth glial cell population that is distinct from mature oligodendrocytes, astrocytes, or microglia. We have used NG2cre and NG2creER BAC transgenic mice crossed to various Cre reporter mouse lines to follow the fate of NG2 cells *in vivo*. In NG2cre:zeg double transgenic mice, NG2 cells generated oligodendrocytes in gray and white matter and a subpopulation of protoplasmic astrocytes in the gray matter of ventral forebrain. To determine the fate of NG2 cells at different developmental stages, Cre was induced by 4-hydroxytamoxifen in NG2creER:reporter double transgenic mice at different developmental time points. Analysis of reporter-expressing cells revealed that postnatal NG2 cells generated only of NG2 cells and oligodendrocytes, while NG2 cells in the embryonic ventral forebrain generated protoplasmic astrocytes in addition to oligodendrocyte lineage cells. By using a low induction of Cre recombination, the fate of single NG2 cells could be examined in isolated clusters of reporter-expressing cells. NG2 cells in the early postnatal brain generated more clusters consisting exclusively of mature oligodendrocytes compared with those in the mature brain which generated more clusters that contained only NG2 cells or a mixture of NG2 cells and mature oligodendrocytes. These observations suggest an age-dependent change in the fate of NG2 cells. Our recent observations on age-dependent effects of ablating the transcription factor Olig2 in NG2 cells and our findings using different Cre reporter lines will be also discussed.

W01-02

SPLIT-CRE MEDIATED ANALYSIS OF A PROGENITOR CELL POPULATION ACTIVATED BY BRAIN LESIONS

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Cre/LoxP recombination is the gold standard for conditional gene regulation in mice *in vivo*. To overcome the limitation of a single promoter used to drive Cre expression we added a second dimension of recombination control by designing a novel 'split-Cre' system based on the complementation of Cre protein fragments. Transgenic mice were generated which express NCre or CCre under the control of the Gfap- or Plp-promoter, driving the expression of split-Cre proteins in astrocytes and oligodendrocytes, respectively. In the brain of these transgenic mice, we thereby genetically defined a subgroup of glial progenitor cells in which the Plp- and the Gfap-promoter are simultaneously active, giving rise to a population of astrocytes and NG2-positive glia. Furthermore, during lesion of the cortex or the retina cells with simultaneously active Gfap- and Plp-promoters reappear, which end up as cells of the astrocytic lineage. Split-Cre is a versatile tool to study transient cell populations during development and disease processes and to precisely target cell populations by Cre/LoxP mediated DNA recombination.

W01-03

LINEAGE ANALYSIS OF GLIAL CELLS IN THE INTACT AND INJURED ADULT MOUSE CNS

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Glial cells in the adult brain are very diverse and some of them represent the stem and progenitor cells of the CNS. The only proliferating cells in the healthy, adult cerebral cortex are the NG2+-cells. As they can differentiate into oligodendrocytes *in vitro* they have often been named as oligodendrocyte precursor cells. However, whether NG2+-cells are multipotential, giving rise to neurons and astrocytes as well as oligodendrocytes, is highly debated. In order to reveal the origin and progeny of distinct glial cell types, we are using different experimental approaches.

By using tamoxifen inducible Cre-recombination in the Olig2 locus, we could show regional differences between NG2+/$Olig^{2+}$cells in the adult cortical white (WM) and grey matter (GM) with NG2+-cells in the WM to preferentially differentiate into mature, myelinating oligodendrocytes. When we compared these data with BrdU labeling-retaining experiments we could show that GM NG2+-cells with high levels of Olig2 are more prone to remain progenitors. We are currently performing transplantation experiments to study whether the difference in the progeny of NG2+-cells between GM and WM is due to environmental cues or due to intrinsic properties of the adult NG2+-cells. Interestingly, even after an acute injury, the progeny of NG2+-cells always remained within the oligodendrocyte lineage.

To overcome the low recombination efficiency in the Olig2::CreERTm mice, labeling mainly NG2+-cells with high Olig2-levels, we generated a new BAC transgenic mouse expressing the inducible form of iCre-recombinase (iCreERT2) in the locus of the transcription factor Sox10, allowing a precisely timed recombination in NG2+-cells as well as oligodendrocytes with a very high efficiency. Additionally to cells of the oligodendrocyte lineage this mouse model allows us to trace also neural crest derivatives during development and in the adult CNS. I am going to present data analyzing the progeny of Sox10-iCreERT2 labeled cells not only in the intact but also in the acute injured mouse brain.

W01-04

MAPPING LINEAGE IN THE DEVELOPING NERVOUS SYSTEM WITH BRAINBOW MULTICOLOUR TRANSGENES

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The mammalian brain develops through a series of complex events, starting with the division of progenitors at the ventricular surface of the embryonic neuroepithelium. Major advances in understanding these events have been made possible with lineage tracing techniques that label either one progenitor (such as retroviral injection), or a group of them (such as genetic fate mapping). Whether neural progenitors contribute equally to the brain architecture is however difficult to assess with a single type of label. Multiple markers would be required to distinguish adjacent progenitors and their lineage, to compare their fate and to analyse their interactions in developing and mature neural structures. Multicolor labeling can be created with Brainbow transgenes which rely on Cre/lox recombination to trigger the expression of random combinations of 3–4 distinct fluorescent proteins (such as CFP, YFP and RFP) in a cellular population. The resulting hues can be used as markers to track cell lineage through cell divisions. To develop this multiplex lineage tracing approach, we are: (i) generating Brainbow transgenes expressing an expanded palette of labels in order to unambiguously individualize groups of clonally related cells; (ii) improving expression strategies to restrict both the timing and spatial extent of the multicolor labeling; (iii) developing volume imaging methods amenable to study clones in their entirety. This multicolor lineage tracing approach will allow for resolving adjacent clones, comparing their migratory behavior and their fate and for analyzing their interactions at various steps of brain development. It should be broadly applicable to a variety of systems.

Workshop 2
Role of NCAM in Health and Disease

W02-01

PRESENTATION OF THE STRUCTURE OF NCAM

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The neural cell adhesion molecule (NCAM) promotes neuroprotection and cognition under normal and pathological conditions. The extracellular part of NCAM is composed of five immunoglobulin(Ig)-like modules and two fibronectin type III (FN3) modules. NCAM-180 and NCAM-140 are transmembrane proteins which differ in the length of their intracellular part, whereas NCAM-120 is attached to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor. NCAM has 6 N-glycosylation sites. In the polysialylated form of NCAM (PSA-NCAM), the N-glycans located at the 5th and 6th N-glycosylation site in the fifth Ig module are modified by one or more PSA chains. During the last decade by means of nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography, the structures of the majority of modules constituting the NCAM ectodomain have been determined and the mechanisms of NCAM-mediated cell adhesion and polysialylation, NCAM interactions with glial cell-line derived neurotrophic factor, and fibroblast growth factor (FGF)-receptor have been elucidated at the atomic level.

W02-02

THE POLYSIALYLATION OF NCAM

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In all vertebrate organisms investigated so far, the synthesis of polysialic acid (polySia) on the major acceptor molecule NCAM is mediated by two polysialyltransferases (polySTs; ST8SiaII and ST8SiaIV). The enzymes are individually able to catalyze the polymerisation reaction on NCAM, while more recently identified and less abundant polySia carriers seem to be recognized by only one polysialyltransferase. The process mediating acceptor specificity of polySTs is barely understood foremost, because studies suited to analyze potential enzyme-acceptor interactions are limited by the lack of stable recombinant enzyme. Bacterial expression has not been successful since both, acceptor proteins (foremost NCAM) as well as polySTs depend on the proper formation of disulfide bonds and on protein glycosylation. Here we describe the production of functional recombinant enzymes and acceptors in insect cells and their use in protein interaction studies aimed to deepen insight into the specificity of acceptor-recognition by polySTs.

W02-03

NCAM - A COMMON REGULATOR OF GROWTH FACTORS IN BRAIN

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The neural cell adhesion molecule (NCAM) promotes neuronal survival and synaptic plasticity including learning and memory consolidation making it an attractive therapeutic target. A number of NCAM mimetics with memory modulating, neuroprotective and neuroregenerative properties has been developed in recent years. NCAM is a multi-modular molecule expressed on neurons, and extracellularly various NCAM modules are not only involved in homophilic cis- and trans-interactions underlying the adhesion mechanism, but also in a variety of heterophilic interactions with other cell adhesion molecules, extracellular matrix proteins, and the FGF-receptor. In 2003 a new ligand of NCAM, the glial cell-line derived neurotrophic factor (GDNF) was identified, and recently we have identified two novel ligands of NCAM, neuropeptide Y (NPY) and erythropoietin (EPO). GDNF, NPY and EPO conventionally interact with their cognate receptors. Interaction of NCAM with GDNF, NPY and EPO is an entirely novel aspect of the role of this cell recognition molecule as an alternative receptor for known growth factors, thus establishing NCAM as one of the key modulators of the signaling properties of a variety of molecular cues in the brain.

W02-04

THE ROLE OF POLYSIALYLATION IN BRAIN DEVELOPMENT

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The neural cell adhesion molecule NCAM is modified with the unique carbohydrate polysialic acid (polySia), which is added to NCAM by the two polysialyltransferases ST8SiaII and ST8SiaIV. PolySia and NCAM are major determinants of cellular interactions during brain development. In humans, abnormal levels of NCAM or polySia as well as polymorphisms in the genes for NCAM and ST8SiaII have been linked to schizophrenia. In mice, the combined ablation of ST8SiaII and ST8SiaIV leads not only to a complete loss of polysialylation but also to a gain of polySia-free NCAM. The postnatally lethal phenotype of the polySia-negative, NCAM-positive mice reveals the vital role of the polySia modification. In the brain, these mice are characterized by severe defects of major axon tracts and altered densities of defined GABAergic neuron populations. In this presentation, the neurodevelopmental mechanisms leading to these defects as well as the striking parallels to structural brain pathology in schizophrenia will be discussed.

W02-05

RELATION BETWEEN IMPAIRMENT OF NCAM EXPRESSION AND NEUROPSYCHIATRIC DISORDERS

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The neural cell adhesion molecule (NCAM) plays important role in brain plasticity. Dysfunctional brain plasticity is implicated in the development of depression and cognitive impairment. Our studies on NCAM knockout mice have demonstrated that constitutive

deficiency in NCAM gene results in the impairment of cognition and depression-like behaviour. This behavioural phenotype is accompanied by the reduced phosphorylation of the fibroblast growth factor (FGF) receptor and impaired intracellular calcium-calmodulin kinase (CaMK) II and IV signalling pathways. NCAM heterozygous mice with partial reduction in the NCAM protein levels develop depression-like phenotype but their cognitive functions remain unchanged. The depression-like behavior observed in NCAM+/- mice is accompanied by the partial reduction in the phosphorylated FGF receptor levels in the hippocampus. In contrast to NCAM -/- mice, no changes in the CaMKII and CaMKIV signalling pathways or CREB phosphorylation levels were observed in NCAM+/- heterozygous mice. Both genotypes NCAM-/- and NCAM+/- had reduced expression of serotonin transporters. In conclusion, our data show that partial constitutive reduction in NCAM proteins results in the depression-like behaviour without impairment in the cognitive functions of animals. Partial reduction in NCAM affects the phosphorylation of FGF receptor and expression of serotonin transporters without major alterations in CaMKII and CaMKIV intracellular signaling pathways. It is proposed that partial reduction in the phosphorylation of FGF receptor and reduced levels of serotonin transporters at least in part might explain the depression-like phenotype in NCAM heterozygous mice.

Workshop 3
The Endocannabinoid System: Novel Therapeutic Opportunities in Brain Repair?

W03-01

NEURONAL PRECURSOR PROLIFERATION IS ENHANCED BY CANNABINOIDS VIA CB1/AKT/GSK-3BETA/BETA-CATENIN SIGNALING

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Endocannabinoid signaling pathways have been implicated in a broad range of neurobiological processes, including promotion of brain development. Recent studies have demonstrated the presence of a functional endocannabinoid system in neuronal progenitor cells of the ventricular (VZ) and subventricular (SVZ) zone and the subgranular (SGZ) zone of the hippocampal dentate gyrus, where it increases cell proliferation. Consistent with the proliferation-promoting function of CB1 receptors, impaired proliferation is observed during cortical development in the VZ/SVZ of CB1 knockout mice. Information on a possible role of the endocannabinoid system in the regulation of precursor proliferation outside the VZ/SVZ/SGZ is missing. The first goal of our study was to establish whether cannabinoids play a role in the modulation of cerebellar granule cell precursor (GCP) proliferation. Since very little is known on the mechanisms whereby endocannabinoids modulate neuronal precursor proliferation, the second goal of our study was to elucidate the molecular mechanisms underlying this effect. We found that the cannabinoid CB1 receptor was expressed by GCPs during early cerebellar development and that activation of the CB1 receptor enhanced proliferation of GCPs. Activation of CB1 receptors by the agonist HU-210 increased GCP proliferation both *in vitro* and *in vivo*. Consistent with this finding, in CB1-deficient mice cell proliferation was significantly lower than in wild type littermates, indicating that the endocannabinoid system plays a significant role in the regulation of GCP proliferation. We found that in neuronal precursors from both the cerebellum and SVZ, CB1 receptors promote proliferation through the PI3K/AKT/GSK-3β/β-catenin pathway, suggesting that this pathway plays a pivotal role in the CB1-dependent modulation of neuronal proliferation. In conclusion, our results show that the cannabinoid system participates in the control of neurogenesis during early phases of cerebellar development, which support the notion that endocannabinoids may represent important cues involved in the control of neuronal precursor proliferation.

W03-02

REGULATION OF NEURAL PROGENITOR CELL BIOLOGY BY THE ENDOCANNABINOID SYSTEM

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Endocannabinoids acting via CB1 and CB2 cannabinoid receptors have been shown to regulate neural cell proliferation and survival and thus play an important role in the regulation of adult neurogenesis. In addition, recent studies demonstrated the functional role of CB1 receptor signalling during cortical development. CB1 receptors regulate pyramidal neuronal specification and migration, in addition to their involvement in GABAergic interneuron morphogenesis. Thus, conditional CB1 receptor ablation in the glutamatergic lineage results in defective subcortical connectivity and axonal fasciculation due to their involvement in the regulation of neuronal specification and cortical positioning. Moreover, acute genetic and pharmacological gain and loss of function manipulation of cannabinoid signaling during development regulates neural progenitor cell cycle maintenance and neurogenesis. Our results identify CB1 and CB2 receptors signalling as critical regulators of the proneural transcription factor network involved in the specification of cortical neuron subpopulations. Altogether, these findings provide a rationale for the use of cannabinoid-targeting drugs as novel therapeutic approaches for neural progenitor fate manipulation in neurodegenerative disorders.

W03-03

EXPLORING THE ROLE AND THERAPEUTIC OPPORTUNITIES OF ENDOCANNABINOID SIGNALING IN ADULT NEUROGENESIS

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Neurogenesis persists in two regions of the adult brain, namely the hippocampus and subventricular zone (SVZ) of the lateral ventricles. In the dentate gyrus in the hippocampus, stem cells proliferate and generate neurons that integrate into the local circuitry. In the SVZ neural stem cells proliferate and generate neuroblasts that normally migrate along the rostral migratory stream (RMS) to the olfactory bulb, where they differentiate into neurons. However, recent evidence suggests that neuroblasts can migrate out of the RMS to an area of brain injury where they might mitigate the effects of the injury. It follows that understanding the cues that regulate stem cell proliferation and neuroblast migration might offer new therapeutic opportunities to enhance brain repair. Neural stem cells and migrating neuroblasts have the ability to synthesize endocannabinoids by the diacylglycerol lipases (DAGLs) and respond to them via cannabinoid receptors CB1 and CB2 (collectively termed endocannabinoid, eCB, signaling). This has led us to investigate the role of this system in stem cell proliferation and neuroblast migration using various models, including mice where the DAGLs have been knocked out. Pharmacological inhibition and/or knockout of the DAGLs substantially reduce neurogenesis in both the hippocampus and SVZ. Similar effects are seen when CB1 and/or CB2 cannabinoid receptors are inhibited. These results support the hypothesis that the eCB system drives proliferation of neural stem cells in both niches. We next asked if eCB signaling also drives the migration of the newly generated neuroblast along the RMS: – results from both *in vivo* studies and studies on SVZ explants provide evidence that a DAGL generated eCB tone operates on both CB1 and CB2 receptors to promote

neuroblast migration in the postnatal brain. Finally, we will review data that demonstrates that pharmacological activation of the eCB signaling can reverse the substantial decline in adult neurogenesis that is seen with aging.

W03-04

THE RESPONSE OF THE ENDOCANNABINOID SYSTEM TO SPINAL CORD INJURY

Molina-Holgado, E., Garcia-Ovejero, D., Sierra-Palomares, Y., Paniagua-Torija, B. and Arevalo-Martin, A.

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Endocannabinoids are lipid mediators that participate in a variety of physiological and patophysiological processes. Many of these effects depend on their binding to their canonical seven transmembrane receptors CB1 or CB2. Using a clinically relevant model of spinal cord injury in rats we have studied the function of the endocannabinoid system in the spinal cord. Our research has focused in the expression and regulation of several elements of the endocannabinoid system (endogenous ligands, cannabinoid receptors and enzymes of synthesis and degradation of endocannabinoids), in the therapeutic potential of 2-arachidonoyl glycerol, and finally, in the role played by cannabinoid receptors in the normal progression of the injury.Funded by grants from the Instituto de Salud Carlos III of Spain (08/1999) and Gobierno de Castilla-La Mancha (Fundación para la Investigación Sanitaria en Castilla La Mancha; PI-2008/37, PI-2008/40).

Workshop 4
Nanotechnology, Nanomedicine and Biomedical Targets in Neurodegenerative Disease

W04-01

BIOLOGICAL SYNTHESIS OF METAL NANOPARTICLES AND THEIR INTERACTION WITH BIOLOGICAL TARGETS IMPLICATED IN NEURODEGENERATIVE DISEASES

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Loss of cholinergic neurons is a significant feature of neurodegenerative disease and discovering mechanisms that can inhibit the progression of such disorders requires an understanding of the molecular causes responsible for such neurochemical deficits and neuronal loss. The deposits of neurofibrillary tangles and senile plaques perhaps as a consequence of fibrillogenesis of β-amyloid peptides has also been shown to be a hallmark in the etiology of this disease and we have been searching for biological markers and/or biomedical targets to identify the early onset of Alzheimer disease (AD). In this work we show that platinum, gold and silver nanoparticles interact strongly and inhibit nitric oxide synthase [NOS], superoxide dismutase [SOD] and acetylcholinesterase [AChE] three enzymes that are associated with the metabolism of arginine. Arginine accumulates in Alzheimer patients indicating a metabolic disturbance of enzymes that metabolise this amino acid while SOD is involved with oxidative stress leading to the formation of reactive oxygen species as peroxynitrite. AChE activity has also been shown to be influenced by arginine. All metal nanoparticles were synthesised inside the viral capsid of cowpea chlorotic mosaic virus. Their small size allows them to bind with the specific enzymes and not illicit any immune response by the host. The SOD activity was assayed, by measuring the competition with ferricytochrome c for superoxide radicals generated from the xanthine-xanthine oxidase system. A CPMV inoculum was used to infect plant leaves, the RNA removed from the viral capsid by sucrose ultracentrifugation and silver nanoparticles incubated with the empty virion, trapping the nanoparticles inside the capsids. SOD was obtained through competent E.coli cell transformed with kanamycin-resistant plasmid harbouring Plasmodium falciparum SOD gene. After complete expression the cells were lysed by sonication, clarified by centrifugation and the enzyme purified on a nickel based affinity chromatography column. nNOS was purified from bovine brain by standard biochemical processes. Michaelis Menten kinetics was used to establish respective inhibitor kinetics.

W04-02

COLLOIDAL METALLIC NANOPARTICLES AND BLOOD-BRAIN-BARRIER

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The purpose of the current studies was to determine what role the microvessel endothelial cells that comprise the blood-brain barrier (BBB) have in brain inflammation and neurotoxicity associated with colloidal metallic nanoparticles (NPs) like silver (Ag), gold (Au) and copper (Cu). A primary culture of rat brain microvessel endothelial cells (rMVEC) was isolated by enzymatic digestions and differential centrifugation for an *in vitro* model of the BBB. Confluent rMVEC monolayers were treated with various sized Ag (25, 40 or 80 nm) or Au (3, 5, 7, 10, 30 or 60 nm) or Cu (40 and 60 nm) NPs. The cellular accumulation of the NPs was determined spectrophotometrically. The cytotoxicity was evaluated by XTT in rMVEC following 24-hours of NPs exposure (0.7 to 50 μg/ml). The extracellular concentrations of proinflammatory mediators (IL-1B, IL-2, TNFα and PGE2) were evaluated by ELISA at various time intervals (0,2,4,6 & 8 h) following exposure to various sized NPs. The cytotoxicity of rMVEC following 24-hours of exposure to Ag-NPs (LD50; below 700 ng/ml (25 nm) and 10 ug/ml (40 and 80 nm)) was significantly increased, when compared to Au-NPs where as Cu-NPs LD50 was approximately 12.5 μg/ml for both sized. Au-NPs above 3 nm in size showed no significant cytotoxic effects. PGE2 release following Ag and Cu NPs exposure was significantly increased when compared to control at the end of the 8-h experiment. The basal levels of TNF and IL-1B were significantly increased following Ag or Cu NPs, but not with Au-NPs. These data suggest that the brain microvessel endothelial cells may play a significant role in the neurotoxicity associated with Ag, Au or Cu NPs. Similarly, Ag-NPs or Cu-NPs appear to be significantly more toxic to rMVEC compared to Au-NPs. The interactions of the NPs with rMVEC produce a cascade of proinflammatory mediators that can induce brain inflammation and neurotoxicity.

W04-03

NANOPARTICLES AGAINST ALZHEIMER'S DISEASE: PEG-PACA NANOPARTICLES LINK THE Aβ-PEPTIDE AND INFLUENCE ITS AGGREGATION KINETIC

Andrieux, K.[1], Brambilla, D.[1], Verpillot, M.[1], Taverna, M.[1], Le Droumaguet, B.[1], Nicolas, J.[1], Kona, J.[2], De Kimpe, L.[3], Canovi, M.[4], Gobbi, M.[4], Nicolas, V.[5], Salmona, M.[4], Scheper, W.[3], Tvaroka, I.[2] and Couvreur, P.[1]

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Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by progressive loss of cognitive functions and the formation of extracellular β-amyloid (Aβ) peptide aggregates. Despite all scientific efforts, at the moment, effective pharmacotherapeutic options for prevention and treatment of this dementia are

lacking. A possible solution could come from nanotechnology. Especially poly [(hexadecylcyanoacrylate)-co-poly(ethylene glycol cyanoacrylate)] (PEG-PHDCA) nanoparticles (NPs), developed in our laboratories, exhibit not only high *in vivo* stability but also the ability to reach the CNS. The aim of this study was to study ther ability of PEG-PHDCA NPs to link the Aβ peptide 1–42 and to influence its aggregation kinetic.

Capillary Electrophoresis, Confocal microscopy, Surface Plasmon Resonance, Thioflavine T assays and Molecular Modelling experiments clearly confirmed that our NPs bound Aβ peptides and suggested a pivotal role played by the PEG chains in this interaction. All these information allow us to anticipate a possible capture of soluble forms of the peptide by our NPs, both in the bloodstream and brain and a subsequent elimination. This property could open new routes in the field of AD therapy.

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 212043.

W04-04

TRANSLOCATION, RETENTION AND POTENTIAL NEUROLOGICAL LESION IN THE BRAIN AND FOLLOWING NANOPARTICLE EXPOSURE

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The increasing use of nanomaterials is likely to result in their release into the environment. We have shown previously that intranasally instilled copper nanoparticles could transport into the murine Central Nervous System (CNS) and accumulate in the brain. This presentation will describe analytical methods to detect and characterize nanomaterials in the brain and the use of these methods to assess central nervous system exposures to nanoparticles. Specific examples will consider metal oxide nanoparticles including Cu and TiO_2.

After nasal exposure to TiO_2 nanoparticles, the obvious morphological changes of hippocampal neurons and increased GFAP-positive astrocytes in the CA4 region were observed, which were in good agreements with higher Ti contents in the hippocampus region. Oxidative stress occurred obviously in whole brain of exposed mice such as lipid peroxidation, protein oxidation and increased activities of catalase, as well as the excessive release of glutamic acid and nitric oxide. After exposed to different dose of copper nanoparticles for 15 days, the body weight of middle- and high- dose treated group were obviously decreased compared with the control group. The Cu concentration was significantly increased and accumulated in olfactory bulb except the low dose group. The metabolism of monoamine neurotransmitters was disrupted in all the determined brain regions except the striatum.

To summarize, results provided the preliminary evidence that nasal instilled TiO_2 nanoparticles could be translocated into the central nervous system and cause potential lesion of brain, and the hippocampus would be the main target within brain. The underlying mechanism needs further study.

However, there are important differences between rodents and humans. How are there species differences for response to NPS? The drugs existing in the pharmaceutical market to treat brain diseases are limited due to blood-brain barrier. Can nasal administration be a new way for pharmaceutical treating neural diseases? These issues are being addressed and discussed further.

W04-05

POLYMER NANOPARTICLES FOR BIOLOGICAL SENSING & BRAIN TUMOR THERAPY

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PEBBLE nanoplatforms are self-assembled, fluorescent probes with conserved cores that contain multiple elements for the selective localization and measurement of single analytes in a living cell. Biocompatible matrices are hydrophobic or hydrophilic and include plasticized polyvinyl chloride, decylmethacrylate, polyacrylamide or sol gels with diameters of 20%AD 200 nm. Elements that may be embedded within the co-polymer matrix include a fluorescent sensing molecule, enzymes, sensitizers, antioxidants, dendrimeric antenna supermolecules, and magnetic/superparamagnetic chelates or nanoparticles. These possible combinations provide for a wide variety of probe functions in a range of biological specimens. Available probes include sensors for Ca^{2+}, Mg^{2+}, Zn^{2+}, K^{+}, Na^{+}, pH, Cl^{-}, O_2, glucose, NO and E-fields. Incorporation of sensing and other elements into the biocompatible matrix provides for separation of the sensor chemistry from the biological environment, permitting the use of highly toxic, but selective sensor molecules. PEBBLE and other related nano-scale optical sensors provide for analysis of physiological processes in living cells and biological media in real time. Histologic examination of tissues from rats exposed to intravenous doses of stable/insoluble hydrogel PEBBLE nanoparticles did not reveal evidence of an adverse effect for up to 42 days. These results are supported by the lack of plasma indicators of liver, renal and other organ function. However, loading of biocompatible hydrogel nanoparticles with embedded monocrystalline iron oxide nanoparticles produces hemorrhagic changes in situ. Development of biodegradable polymers significantly reduces toxicity and is consistent with the findings of others with respect to persistent insoluble mineral fibers with high toxic potential. More recent advancements in PEBBLE nanoplatforms (Dynamic NanoPlatforms %AD DNPs) have enabled MR imaging of orthotopic experimental brain tumors and for enhancement of visual contrast for neurosurgical resection. This work was supported by grants from the National Cancer Institute, the WM Keck Foundation, the Department of Defense and the National Institute of Environmental Health Sciences.

Monday Poster Sessions
MO01 Glia

MO01-01

COMBINED ACTION OF CEND1 AND NEUROGENIN-2 IN DIRECTING CULTURED ASTROCYTES TOWARDS A RADIAL GLIA AND NEURAL STEM CELL PHENOTYPE

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Recent studies demonstrate that besides the well-documented neural stem cell properties of astrocytic populations of the two neurogenic regions of the adult brain, astroglial cells isolated from non-neurogenic adult brain regions have the potential to be reprogrammed into synapse-forming neurons through forced expression of specific transcription factors known to instruct neurogenesis during embryonic development. Based on our previous studies on the potential of the neurogenic gene Cend1 in directing neural stem/precursor cells to exit the cell cycle and acquire a neuronal phenotype, in parallel with evidence demonstrating direct activation of Cend1 expression by the bHLH proneural genes of the neurogenin family, we aimed to explore their combined effect on the proliferation and differentiation properties of postnatal cortical astrocytes. To this end, forced expression of either Cend1, Neurogenin-2 (Ngn2) or both, resulted in an important increase of two subpopulations of morphologically distinct GFAP(-) cells with elongated morphology that strongly expressed the radial glial marker Glast, 24 h and 48 h following transfection. More specifically, Cend1-overexpressing radial glia cells were bipolar, elongated cells, while Ngn2 overexpressing radial glia cells were mostly triangular ''kite'' like cells. This enhancement of radial glial phenotype amounted to a 50–70% increase in Cend1 or Ngn2 overexpressing cells, while it was doubled in Cend1/Ngn2 transfected cells as compared to controls. A parallel 3-fold decrease in the number of GFAP(+) astrocytes overexpressing both Cend1 and Ngn2 was noted. Surprisingly, in the double-transfected cultures, colonies of small round Cend1(+)/Ngn2(+)/Glast(+)/nestin(+) cells were detected after 24 h, which, a day later, formed round three-dimensional spheres of high proliferative potential attached to the culture dish. Studies using live cell imaging for longer time periods and neural stem cell-culture conditions are in progress to further investigate the proliferation and differentiation potential of these cells, as well as the combined role of Cend1 and Ngn2 on astrocytic reprogramming.

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MO01-02

LIF TREATMENT REDUCES NOGO-A DEPOSITS IN SPINAL CORD INJURY MODULATING RHO-GTPASE ACTIVITY AND CRMP-2 PHOSPHORYLATION

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We have previously shown that exogenous leukemia inhibitory factor (LIF) following overhemisection spinal cord injury, prevents oligodendrocyte death and secondary demyelination. However, the question of whether this may lead to potentiation of axonal regeneration is not clear. Here, we report that the prevention of oligodendrocyte death by LIF treatment reduces Nogo-A deposits in the spinal cord. This finding is associated with modulation of Nogo-A-dependent signaling in white matter axons whereby active RhoA is decreased. Furthermore, the levels of Rho-kinase specific phosphorylation of the downstream target of RhoA, collapsin response mediator protein-2 (p-Thr555-CRMP-2), are reduced near the site of the lesion. This is associated with upregulation in the expression of the axonal growth-related molecules GAP-43 and GTP-Rac1. These findings suggest a mechanism by which exogenous LIF can promote axonal growth in the mammalian spinal cord following injury, by providing a permissive tissue environment for axonal regrowth.

MO01-04

CAMP/PKA SIGNALING PATHWAY MODULATES OLIGODENDROGLIAL MORPHOLOGY *IN VITRO*

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Oligodendroglial differentiation depends on coordinated changes in the cytoskeleton and on its relationship with the plasmatic membrane, a critical site regarding the formation of the myelin sheath. 2'3'cyclic nucleotide 3' phosphodiesterase (CNPase) is related to cytoskeleton modulation, anchoring microtubules to the plasmatic membrane. *In vitro*, CNPase composes, together with F-actin and microtubules, the vein-like structures or radial components in myelin sheath. Its phosphorylation and gene expression are regulated by changes in cAMP levels. In order to evaluate the effects of the cAMP/PKA pathway modulation on oligodendroglial differentiation, 5-day cultures of cerebral hemispheres were treated for 30 min or 24 h with the adenylyl cyclase inhibitor SQ22536 – SQ [1 μM] or with its activator forskolin [10 μM], or with the PKA inhibitor H-89 [1 μM]. Cells were identified using anti-CNPase antibody. At 30 min, no apparent differences between control (C) cultures or cultures submitted to any treatment were observed. At 24 h, cultures treated with forskolin showed a predominance of cells with a more mature phenotype when compared to C cultures. Cultures treated with H-89 showed a predominance of immature oligodendrocytes and SQ treatment showed more cells with a mature phenotype and bigger membranous vellum when compared to control cultures. Furthermore, forskolin treated oligodendroglia showed an apparent increase in processes' length, however, with decreased thickness and secondary branching. H-89 treated cells showed a decrease in cell bodies' size and a reduction in membranous vellum. SQ treatment caused variable changes in oligodendroglial morphology. The total number of cells did not change suggesting that treatment did not affect oligodendroglial survival. These results suggest a role for the cAMP/PKA pathway in oligodendroglial maturation and maintenance of oligodendroglial morphology.

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MO01-05

FUNCTIONAL MODULATION OF MICROGLIAL P2X4 RECEPTOR-CHANNELS BY UDP-ACTIVATED P2Y6 RECEPTORS

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P2X receptors are ATP-gated cation channels contributing to diverse physiological mechanisms including pain signalling and inflammatory response. The P2X4 subtype has been shown to be directly involved in microglial activation and central sensitization of nociceptive neurons, making its functional regulation a crucial process in chronic pain pathologies. The P2Y6 receptor is positively coupled to phospholipase C via Gq/11 proteins and is also expressed in microglia, where it plays a pivotal role in the initial response to nerve injury, triggering phagocytosis upon UDP-mediated activation. Interestingly, recent reports have shown that expression of both P2X4 and P2Y6 is upregulated in activated microglia following nerve injury. Here, we show that in primary mouse microglia, activation of P2Y6 modulates P2X4 function as P2Y6 activation by its agonist UDP induced a significant decrease in P2X4-mediated calcium entry. We recently observed that microglial P2X4 channels can dilate into a macropore upon prolonged ATP stimulation; this property was also inhibited by simultaneous activation of P2Y6 as measured via YO-PRO-1 uptake assay. We reproduced this modulation in the Xenopus oocyte expression system, where P2Y6 activation induced a decrease in P2X4 current amplitude, activation and desensitization rates, as well as an inhibition of P2X4 macropore formation. This interaction was blocked by U73122, a phospholipase C inhibitor, but was unaffected by blocking protein kinase C (PKC) with staurosporine. This suggests that the functional modulation of P2X4 relies on the hydrolysis of PI(4,5)P2, a membrane-bound phosphoinositide recently shown to be a direct positive regulator of P2X4 channel function. These data indicate that metabotropic P2Y6 receptors can significantly affect both P2X4 current and macropore dilation, representing a novel cross-talk between purinoceptors in microglia. High extracellular levels of ATP and UDP are observed in conditions of nerve injury, therefore interactions between receptors sensitive to these agonists are critical in regulating pain-inducing microglial responses.

MO01-06

NITRIC OXIDE SYNTHASE III IS EXPRESSED IN HUMAN OLIGODENDROCYTES

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Nitric Oxide Synthase III (NOS3, NOS-3, eNOS) has not been formerly characterized in oligodendrocyte, in contrast to the other NOS isoforms. We present converging data of NOS3 expression at the molecular and protein levels in primary culture of oligodendrocytes. NOS3 was detected at the protein level by immunocytochemistry, and at the messenger level by quantitative PCR. We used antibodies from different companies that were strictly specific to NOS3 and did not recognize the other isoforms NOS1 (nNOS) and NOS2 (iNOS), as assessed by Western Blot. Immunostaining for NOS3 was found across species in human, rat and baboon primary oligodendrocyte cultures. NOS3 protein was found restricted to the cytoplasm, in the cell body and the thick processes branching out from the cell body. NOS3 was never present in the flat membrane extensions. NOS3 protein and mRNA were found at any time points during the 3 weeks culture necessary for human oligodendrocytes to regenerate large membranes expressing galactocerebroside, myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein. NOS3 protein was found expressed early in the lineage in bipolar oligodendrocyte progenitors, and its expression was maintained at the mature stage of membrane-bearing human oligodendrocytes in long-term culture up to 2 months. NOS3 was also found in mouse oligospheres derived from E13 cells. NOS3 colocalized with caveolin-1, a protein bound to NOS3 and one of its main regulator in endothelial cells. Inhibition of NOS3 by the specific ligand N-[imino(methylamino)methyl]-L-ornithine (L-NMMA) led to a disruption of the organization pattern of MBP and the cytoskeleton component actin, that were accompanied by marked changes in oligodendrocyte arborization. NOS3 appears to be a signaling molecule for the cytoskeleton which is involved in intracellular trafficking, process extension and myelin production in oligodendrocytes.

MO01-07

MTOR-MEDIATED REGULATION OF OLIGODENDROCYTE DIFFERENTIATION

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Our previous study demonstrated that inhibition of mammalian target of rapamycin (mTOR), a downstream target of Akt, arrested oligodendrocyte differentiation at the O^{4+}/GalC- late progenitor stage (Tyler et al., 2009). [[AUTHOR: Please provide full punblication details for Tyler et al., 2009 in the reference list.]] Recent proteomic analysis revealed novel mTOR targets and a more complex picture of mTOR function in differentiating oligodendrocyte progenitor cells (OPCs). To further this analysis and search for direct targets of mTOR during oligodendrocyte differentiation we performed iTRAQ-MS analysis of protein samples isolated following 2d and 4d of differentiation in the presence or absence of rapamycin, an mTOR inhibitor. The changed proteins at 2d of differentiation, either increased or decreased in the presence of rapamycin, were further sorted based on molecular function. Cytoskeletal proteins represented the largest group downregulated by mTOR inhibition consistent with the hypothesis that mTOR regulates morphological complexity at the transition from the late progenitor to the immature oligodendrocyte. Proteins that were upregulated in the presence of rapamycin included those involved in regulating protein translation and translation initiation. As a major function of the mTOR/raptor complex is to positively regulate protein translation, the upregulation of translation factors in the presence of rapamycin suggests that the ability of mTOR signaling to finely tune the expression of specific transcripts via translational control plays a central role in regulating oligodendrocyte differentiation. Ongoing studies are directed towards validating identified targets in the mTOR-regulated proteome and elucidating direct nuclear targets of mTOR necessary for mediating oligodendrocyte differentiation.

MO01-08

MECHANISMS UNDERLYING ATP RELEASE FROM CULTURED ASTROCYTE

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It has been considered that astrocytes play merely a supportive role for neurons but recently it has been shown that astrocytes regulate neural network more directly by releasing gliotransmitter, such as glutamate and ATP. However, spatial and temporal ATP release and the releasing mechanism are not obvious. We have developed a new system to visualize ATP release from cultured astrocytes using a high sensitive camera. Intracellular calcium level elevated rapidly in astrocytes after hypotonic stimulation followed by the oscillation of calcium concentration. On the other hand ATP release from astrocytes was much slower and showed longer duration. The peak of ATP release was about 200 s after hypotonic stimulation. All astrocytes showed intracellular calcium elevation in response to hypotonic stimulation. However, only a small percentage of astrocytes exhibited ATP release after hypotonic stimulation. Next we examined the possible mechanism underlying this ATP release through pharmacological analysis. We treated astrocytes with a maxi-anion channel inhibitor or a hemichannel blocker before stimulation. Both agents partially suppressed the frequency of ATP release. These results suggest that several distinct machineries are used for the ATP release from astrocytes.

MO01-09

ASTROCYTE PRECURSOR CELLS IN THE DEVELOPING MOUSE CEREBELLUM

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It is generally considered that neural stem cells are committed to become precursor cells before terminally differentiating into either neurons or glial cells during neural development. Neuronal precursor cells and oligodendrocyte precursor cells have been identified in several areas in the murine central nervous system. Presence of astrocyte precursor cells (APCs), however, has been reported rarely so far. We hereby provide several lines of evidence that CD44-positive cells are APCs in early postnatal mouse cerebellum. In developing cerebellum, CD44-positive cells, most of which resided mainly in the white matter, were positive for the markers of the astrocyte lineage, but negative for the markers of mature astrocytes. We purified CD44-positive cells from postnatal cerebellum by fluorescence-activated cell sorting, and characterized them *in vitro*. In the absence of any signaling molecule, many cells died by apoptosis. The surviving cells, however, gradually expressed glial fibrillary acidic protein, a marker for mature astrocytes, indicating that differentiation into mature astrocytes is the default program for these cells. They produced no neurosphere in the presence of basic fibroblast growth factor (FGF) and/or epidermal growth factor. Neither did they produce neurons nor oligodendrocytes under any condition we examined, indicating they are not neural stem cells. Leukemia inhibitory factor greatly promoted astrocytic differentiation of CD44-positive cells, while bone morphogenetic protein 4 (BMP4) did not. Basic FGF was a potent mitogen for these cells, but was insufficient for their survival. BMP4 inhibited activation of caspase-3 and greatly promoted their survival, suggesting a novel role for BMP4 in the control of development of astrocytes.

MO01-10

UPREGULATION OF GLIA-DERIVED NEUROTROPHIC FACTOR EXPRESSION IN ASTROCYTES EXPOSED TO NICOTINE

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We have previously shown the functional expression of nicotinic acetylcholine receptors (nAChRs) endowed to increase intracellular free Ca^{2+} levels upon exposure to nicotine in primary cultured rat astrocytes. This study was aimed to elucidate the possible role of astrocytic nAChRs in mechanisms underlying neuroprotection by nicotine. Cellular viability was significantly decreased in cultured cortical neurons exposed to H_2O_2, while the decreased vitality was rescued after the culture with conditioned medium from astrocytes previously exposed to nicotine. Similarly significant protection was seen against the neurotoxicity mediated by A23187, 2,4-dinitrophenol and tunicamycin in neurons cultured with astrocytic culture medium previously conditioned by nicotine. Nicotine was found to selectively induce mRNA expression of glia-derived neurotrophic factor (GDNF) amongst different neurotrophic factors in cultured astrocytes, while exposure to nicotine significantly increased the luciferase activity in cortical astrocytes transfected with the luciferase reporter plasmid linked to GDNF promoter. Similarly, nicotine not only increased expression of corresponding protein for GDNF in astrocytes, but also promoted the release of immunoreactive GDNF in culture medium. These results suggest that nAChRs may be functionally expressed by rat cortical astrocytes to induce upregulation of GDNF expression through transactivation toward protection against the neurotoxicity mediated by different cytotoxins.

MO01-11

CRITICAL ROLE OF HISTONE METHYLATION IN OLIGODENDROCYTE DEVELOPMENT

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Failure to remyelinate after demyelinating lesions contributes to clinical disability observed in patients with demyelinating disorders, such as multiple sclerosis. Oligodendrocytes are the myelin-forming cells of the central nervous system. The generation of of oligodendrocytes involves cross-talks between transcription factors and epigenetic regulation, including histone modifications, DNA methylation and microRNAs. We have previously demonstrated that the differentiation of oligodendrocyte progenitor cells (OPCs) requires histone deacetylation, to decrease the levels of oligodendrocyte differentiation inhibitors. Here we focus our study on the role of

histone methylation in OPC differentiation. We detected increasing levels of histone methylation during the differentiation of OPC into myelinating cells. We also showed that inhibition of histone methylation in cultured oligodendrocyte progenitors led to decreased expression of myelin genes and decreased number of mature oligodendrocytes during a specific temporal window. Furthermore, using chromatin immunoprecipitation, we detected enrichment of methylated histones at the promoters of genes encoding for transcriptional inhibitors of differentiation. Together, these data suggest a critical role of histone methylation in OPC differentiation and myelination. The potential role of histone methylation for remyelination is being investigated in the brain of mice with animal models of demyelination and of patients with multiple sclerosis (Supported by Fellowship FG1874-A-1 from National Multiple Sclerosis Society and by NINDS-R01-NS42925-S01 ARRA funds).

MO01-12

GOLLI MYELIN BASIC PROTEINS STIMULATES OLIGODENDROCYTE SURVIVAL AND PROLIFERATION IN THE REMYELINATING ADULT BRAIN

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Golli proteins are necessary for normal myelination, acting via voltage and store-dependent Ca^{++} entry at multiple steps during oligodendrocyte progenitor cell (OPC) development. To date nothing is known about its role in demyelination or remyelination events. Cuprizone (CPZ) intoxication has been used as a model to study de- and re-myelination. Feeding CPZ to mice results in demyelination and damage to oligodendrocytes (OLs) without damage to other cell types in the CNS. When CPZ is terminated an almost complete remyelination takes place. In this work the effects of golli ablation and overexpression in myelin loss and recovery during and after the CPZ treatment was examined. CPZ induced a progressive decrease in OLs and an increase in OPCs, which returned to near normal levels when the control animals were returned to a control diet. However, after CPZ intoxication, a significant increase in the number of proliferating and differentiating OPCs was found in the corpus callosum (CC) of golli-overexpressing (JOE) brains compared to controls. This increase in the number of early OPCs in JOE brains during an acute demyelination/remyelination event suggests that golli modulates the induction and/or survival of early OPCs. Histological examination after CPZ withdrawal revealed a greater number of mature OLs repopulating the CC in JOE animals compared to controls. Myelin production gradually increased during the recovery phase reaching normal levels after 4 weeks of CPZ withdrawal. In contrast, reduced MBP and PLP expression was seen in large areas along the CC of golli-KO mice during the recovery phase, reflecting irregular recovery of the OL population and myelin sheath formation. In summary, our findings indicate golli may be an inducer of OPCs in adult mouse brain in acute demyelination caused by CPZ. Therefore this work is relevant to developing means to induce remyelination in myelin degenerative diseases and for myelin repair in damaged nervous tissue.

MO01-13

GAS6 IS A SURVIVAL AND MATURATION FACTOR FOR HUMAN OLIGODENDROCYTE/HUMAN DORSAL ROOT GANGLION (DRG) CO-CULTURES

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We have developed a model system that supports MBP+ oligodendrocyte maturation in association with axons, and have explored the effect of recombinant human growth-arrest-specific protein 6 (GAS6) on oligodendrocytes when co-cultured with DRG explants. Oligodendrocytes were enriched from mixed glial cultures derived from brain at 14–17 gestational weeks. After 14 days, GAS6-treated co-cultures plus and minus several growth factors were evaluated for increases in the number of myelin basic protein-positive (MBP+) oligodendrocytes and the extent of contacts between the MBP+ processes and axons. The addition of 2.6 nM GAS6 increased the number of MBP+ oligodendrocytes 3–6-fold relative to co-cultures maintained in the absence of GAS6; ($p = 0.016$). Platelet-derived growth factor (PDGF)+GAS6+ co-cultures had 3-fold more MBP+ oligodendrocytes relative to PDGF+GAS6- co-cultures; however, few of the MBP+ oligodendrocytes were in direct contact with axons. The length of the MBP+ segments parallel to the axons was unchanged in the absence or presence of GAS6 (0.56 ± 0.48 mm^2 vs. 0.33 ± 0.15 mm^2). GAS6 in defined-medium containing insulin-like growth factor-1 (IGF-1) and brain-derived neurotrophic factor (BDNF) increased the number of MBP+ oligodendrocytes 6-fold relative to co-cultures without GAS6. IGF-1+BDNF+ supplemented medium did not increase the overall number of MBP+ oligodendrocytes relative to co-cultures treated with PDGF; but it did increase the number of oligodendrocytes in contact with axons 3-fold. Further, the length of MBP+ processes parallel to axons was significantly increased in the BDNF+IGF-1+GAS6+ co-cultures relative to the PDGF+Gas6+-treated co-cultures (4.0 ± 1.1 mm^2 vs. 0.33 ± 0.16 mm^2; $p = 0.014$) demonstrating that IGF-1 and BDNF enhance MBP+ process elongation along the axon. The length of the oligodendrocyte processes was not significantly different in the IGF1+BDNF+ co-cultures plus and minus GAS6. However, there were twice as many MBP+ oligodendrocytes with processes parallel to axons in GAS6+ co-cultures. MBP immunofluorescence showed MBP+ processes wrapping axons. Ongoing electron microscopic studies will determine whether GAS6 enhances myelination within the co-cultures. In summary, our data support a role for rh gas6 in survival and maturation.

MO01-14

THE ROLE OF BERGMANN GLIA IN CEREBELLAR DEVELOPMENT

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Bergmann glia are in astrocyte-lineage and their cell bodies locate in the cerebellar Purkinje cell layer. They are characterized by the Bergmann fiber that extends from the cell body to pial surface

through the molecular layer. In the early postnatal cerebellum, granule cell progenitors proliferate in the outer granular cell layer and migrate through Purkinje cell layer to reach inner granular cell layer. It is well accepted that Bergmann glia guide the granule cell migration. In the course of study aiming at altering the expression of Mlc1 in astrocyte-specific manner, we found a unique phenotype in their cerebellar development. Mlc1 is predominantly expressed in astrocyte-lineage cells and its mutation causes 'Megalencephalic leukoencephalopathy with subcortical cyst' in human. we found disturbance of Purkinje cell and granule cell alignments in the cerebellum, which should have been caused by Mlc1 over-expression in Bergmann glia. Moreover, many Bergmann glia mislocalized in the cerebellar molecular layer and showed reactive astrocyte-like shape. In this mouse, Mlc1 is over-expressed since embryonic day 14 in radial/ Bergmann glia. When the Mlc1 over-expression level was returned to the normal level after postnatal day 0, there was no abnormality in the cerebellar architecture. Therefore, Mlc1 overexpression postnatally should have caused this abnormality.

MO01-15

THE ASSEMBLY OF CNS MYELIN: A NOVEL HYPOTHESIS

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Myelin is a multilayer membrane that envelops axons. Its best characterized function is that of a facilitator of rapid nerve conduction. The assembly of CNS myelin is the work of oligodendrocytes (OLGs). The prevailing assumption is that an OLG extends a process that circumnavigates an axon to generate the multilamellar structure - a hallmark of myelin. Given that OLG plasmalemma and myelin are compositionally distinct, questions concerning myelin synthesis, transport and deposition are highly pertinent and, yet, remain largely unanswered. We have developed an *in vitro* model consisting of pure cultures of OLGs, isolated from post-myelinating brains, and have shown that such OLGs can be reprogrammed to re-enact the ontogenic development of myelin - a process we call myelin palingenesis. We have used such cultures in conjunction with immuno-electronmicroscopy and three-dimensional electron tomography to address the issue of myelinogenesis. We hypothesize that myelin membranes are assembled into specific organelles, translocated to the sites of delivery and deposited in response to a signal. In support of this hypothesis we provide three-dimensional electron tomographic evidence that myelin membranes are packaged as vesiculotubular structures – named here 'myelin membrane carrier organelles'. Such organelles move to the cell periphery and to the sides and tips of processes. Membranes within these organelles contain proteolipid protein, myelin basic protein, 2', 3' cyclic nucleotide phosphodiesterase, and galactocerebroside as shown by immuno-electronmicroscopy. These membranes self-assemble into typical myelin structures. Supported in part by Grant # UL1 RR024999 from NCRR, NIH.

MO02 Gene Regulation and Genetics

MO02-01

A GENOME WIDE APPROACH TO STUDY THE MOLECULAR MECHANISM OF PAIN AND RELIEF LEARNING

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Animals and man form two opposing kinds of memory from a traumatic experience. For example, fruit flies learn to avoid an odour as a signal for 'pain' if it preceded an electric shock during training; whereas they approach an odour as a signal for 'relief' if it followed the shock during training (Tanimoto et al. 2004). I take a genome wide approach to study the molecular mechanisms of such pain and relief learning. To this end, I am currently characterizing 40 inbred fly strains in either kind of learning. These strains have already been analyzed in terms of the genome-wide transcript abundance and transcript sequence polymorphisms (Ayroles et al. 2009) . I will combine my learning data with these transcriptomic data to look for transcripts whose abundance and/or sequence polymorphisms are associated with either pain or relief learning. Having obtained a list of such transcripts I will pick few initial candidates to further scrutinize using reverse genetic methods. Once their roles have been verified, the respective genes can be used for analyzing the neural circuits underlying either kind of learning. Furthermore, their human homologues may make good therapeutic targets for psychiatric conditions.

MO02-02

TRANSCRIPTIONAL REGULATION OF α-SYNUCLEIN

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α-Synuclein (SNCA) is an abundant neuronal protein linked to the development of neurodegenerative diseases, and in particular Parkinson's disease (PD). Genetic overexpression or missense point mutations of SNCA lead to PD in humans, and its overexpression is sufficient to cause PD in some animal models. We previously identified elements in the 1st intron of SNCA that are important in its transcriptional regulation in PC12 cells in response to treatment with NGF and bFGF (Clough and Stefanis, 2007). Using a small-scale exonuclease deletion approach we aimed to identify the transcription factor (TF) binding sites in intron 1 responsible for this induction. Multi-species sequence alignment and promoter analysis identified 2 putative binding sites for TFs ZSCAN21 and HNF4. We further characterized the role of ZSCAN21 in the regulation of SNCA. A luciferase construct lacking the ZSCAN21 binding site exhibited a 40% reduction in activity when compared to control sequence. Electromobility shift assay with PC12 nuclear extract identified a specific shift for a biotin-labeled ZSCAN21 probe. RT-PCR verified the expression of ZSCAN21 in naïve and NGF-treated PC12 cells, as well as in different areas of the rat brain, including the ventral midbrain. siRNA against ZSCAN21 reduced the activity in the luciferase assay to the same levels as for constructs lacking this sequence, and significantly inhibited the protein expression of SNCA in PC12 cells and cortical neurons, thus establishing a role for ZSCAN21 in the transcriptional control of SNCA in these model systems. In order to extend these results *in vivo*, we intend to use stereotactic injections of lentivirus expressing shRNA against ZSCAN21 in the rat substantia nigra. Such studies may cement ZSCAN21 as an important regulator of SNCA transcription, and may provide potential therapeutic targets not only for PD but also for other synucleinopathies.

MO02-03

EXPRESSION OF THE CLOCK GENE BMAL2 IN PATIENTS WITH PARKINSON'S DISEASE

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Bmal1 is one of the central regulators of the clock machinery. Recently, we examined the expression profile of Bmal1 in total leukocytes for a 12 h duration during the evening, overnight, and the morning, in subjects with Parkinson's disease (PD) and healthy controls. The results indicate that the relative abundance of Bmal1 is significantly lower in PD patients versus control subjects. However, it is still unclear whether other key regulators of the clock machinery, especially Bmal2, the paralog of Bmal1, are also expressed differently in PD. To address this issue, the expression profiles of Bmal2, Clock, and Dec1 were examined in the same samples using real-time RT-PCR assay. The results show a difference in the expression pattern of Bmal2, but not Clock and Dec1. The relative abundance of Bmal2 is also significantly lower in PD at 21:00h ($p = 0.005$) and 00:00h ($p = 0.025$). These results together with our previous findings suggest that the molecular clock in total leukocytes is disturbed in PD patients.

MO02-04

MODULATION OF GLUTAMATE TRANSPORT BY RUNT RELATED FACTOR-2 EXPRESSED BY ASTROCYTES

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We have previously shown marked expression of the master regulator of osteoblastogenesis runt related factor-2 (Runx2) in cultured rat astrocytes and C6 glioma cells. This study was thus aimed at elucidation of the possible functional role of Runx2 in astrocytes. Cultured rat astrocytes were transiently transfected with Runx2, followed by extraction of total RNA and subsequent microarray analysis. Among a variety of genes scanned, marked upregulation was seen for Runx2 and its osseous target genes, such as osteopontin and matrix metalloproteinase 13, whereas significant downregulation was found for particular astrocyte-relevant genes including excitatory amino acid transporter-1 (EAAT1). Cultured astrocytes were next infected with recombinant adenovirus for either Runx2 (AdV-Runx2) or green fluorescent protein (GFP) (AdV-GFP) for 48 h, followed by Western blotting analysis, along with the determination of [3H]L-Glu accumulation. In astrocytes infected with AdV-Runx2 at 20 MOI, markedly elevated expression was seen for Runx2 protein compared to cells infected with AdV-GFP. In

contrast, a significant reduction was observed in EAAT1 protein expression, in addition to a significant decrease in temperature-dependent [3H]L-Glu accumulation, in astrocytes infected with Runx2 adenovirus. These results suggest that Runx2 may be functionally expressed by astrocytes to play a role in the regulation of the extracellular levels of glutamate through the modulation of EAAT1 expression.

MO02-05

IDENTIFICATION OF THE TRANSCRIPTION FACTOR(S) RESPONSIBLE FOR PIMT GENE EXPRESSION

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The spontaneous conversion of L-asparaginyl and L-aspartyl (L-Asp) residues in proteins to L-isoAsp or D-Asp occurs during aging. These atypical Asp residues can interfere with the protein function and lead to malfunction of cells. Protein L-isoaspartyl methyltransferase (PIMT) functions as a repair enzyme that promote repair of L-isoAsp or D-Asp residues to normal L-Asp residues. PIMT knock-out mice exhibit brain enlargement and fatal epileptic seizures. PIMT expression and activity are reduced by half in human epileptic hippocampus. These lines of evidence suggest that the expression of PIMT plays important roles in brain. However, little is known about the regulatory mechanisms of the PIMT gene expression. In this study, we cloned and functionally characterized the 5'-flanking (promoter) region of the human PIMT gene. About a 1 kbp fragment of the putative promoter region of the human PIMT gene was isolated from HEK293 cells and ligated into a luciferase-expression vector, pGL3-basic, to generate the pGL3-PIMT reporter plasmid. The putative promoter activity was confirmed using dual luciferase reporter gene assay system. The minimal region required for basal activity of the PIMT promoter was determined by generating a series of deletion and point mutation constructs. It consists of a sequence around -190 relative to the translation initiation codon. The binding protein(s) to the minimal region was briefly purified using Dynabeads M-280 streptavidin and biotin-labeled DNA probe, and subsequently LC-MS/MS analysis was performed to identify the transcription factor(s) involved in the regulation of PIMT gene expression. Binding activity of the identified protein(s) to the minimal region was confirmed using electrophoretic mobility shift assay and chromatin immunoprecipitation assay.

MO02-06

DOPAMINE D4 RECEPTORS, RETINAL FUNCTION, AND SYNCHRONIZATION OF CIRCADIAN RHYTHMS IN THE MOUSE RETINA

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The vertebrate retina contains autonomous circadian clocks that regulate gene expression, photoreceptor outer segment turnover, and visual sensitivity. Dopamine is a neuromodulator that is secreted from inner retinal neurons in response to light exposure. In the current study, we examined the role of dopamine D4 receptors (D4R) in the circadian organization of the mouse retina. Targeted disruption of the D4R (*Drd4-/-* mice) abolishes circadian rhythms of retinal cAMP accumulation, *Adcy1* mRNA, and Ca^{2+}/calmodulin-stimulated adenylyl cyclase activity. Administering a D4R agonist 4 h prior to the time of light onset in the morning phase advanced the circadian rhythm of *Adcy1* mRNA in WT mice, indicating that dopamine acts as an entrainment stimulus. Microarray analysis of wild type (WT) and *Drd4-/-* retinas indicated that ~4% of the retinal transcriptome is differentially expressed,, including genes encoding photoreceptor-enriched proteins, and proteins involved in enzyme regulation and chromatin remodeling. Many of the differentially expressed transcripts represent circadian clock-controlled genes; more than 50% of the top 30 differentially expressed transcripts were previously identified as clock-controlled genes. The circadian rhythms of these transcripts were damped or abolished in retinas of *Drd4-/-* mice. Visual function testing indicated a dramatic decrease in contrast sensitivity in *Drd4-/-* mice, with no significant effect on visual acuity. Electroretinographic (ERG) analysis demonstrated a circadian rhythm with higher light-adapted b-wave amplitudes in the subjective daytime compared to subjective night. In retinas of *Drd4-/-* mice, the ERG rhythm was damped on the first day of constant (24 h/day) darkness and was abolished on the second day of constant darkness, indicative of a progressive loss of synchrony of the circadian clocks controlling the ERG rhythm. Similar results were observed in a conditional retina-specific knockout of tyrosine hydroxylase. Collectively, these results suggest that dopamine and the D4R play a major role in the circadian organization of the mouse retina by entraining and synchronizing circadian rhythms in retinal neurons.

MO02-07

MOLECULAR STUDIES IN EVIDENCING ON INCREASING LEVELS OF IRON AND COPPER AND ITS CORRELATION TO DNA INTEGRITY IN AGEING HUMAN BRAIN

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Genomic stability based on the conformation of DNA play a significant role in brain function. Previous studies reported that alterations in DNA integrity exist in the brain regions of neurological disorders like Parkinson and Alzheimer's disease. However, DNA stability in ageing brain and the factors responsible for genomic instability still remains elusive. In this present investigation, we assessed the levels of Copper (Cu), Iron (Fe) and Zinc (Zn) in three age groups (Group I: below 40 years), Group II: between 41–60 years) and Group III: above 60 years) in hippocampus and frontal cortex of aged human brain subjects (n = 8 in each groups). Genomic DNA was isolated and its integrity was studied by nick translation study and presented as single and double strand breaks. We observed that the levels of Cu and Fe were significantly elevated while Zn significantly depleted from Group I to III. The increase in the level of metal ions was high in frontal cortex compared to hippocampus region. During the process of ageing, the amount of single strand breaks increases compared to double strand breaks. Nick translation analysis revealed that the amount of single strand breaks was high in frontal cortex compared to hippocampus region. Lucid correlations between Cu and Fe levels versus strand breaks in ageing brain regions were observed. The results indicate that genomic instability is progressive with ageing and later alter the

gene expression. To the best of our knowledge, till date this is a new comprehensive database on the accumulation of Cu and Fe and induction of DNA strand breaks in DNA in the brain regions of ageing human brain. The biological significance of these findings relevance to mental health has been further elucidated.

MO02-08

ADENOVIRUS-MEDIATED EXPRESSION OF THE FRAGILE X MENTAL RETARDATION PROTEIN IN NEURONS

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Fragile X Syndrome, the most common form of inherited mental retardation, results from the absence of Fragile Mental Retardation Protein (FMRP), an RNA binding protein implicated in translation modulation in neurons. While it is well established that FMRP is mainly associated with the translation machinery in the cell body, a small population is also found in RNA-granules that are transported in dendrites to be delivered at the synapses. An inherent difficulty in studying FMRP function in dendrites is the lack of available expression vectors to study FMRP since transfection techniques using classical vectors lead to over-expression and induce formation of Stress Granules. In addition, classical transfection of neurons in cultures is below 0.1%. We have developed a GFP-FMR1 gene transfer system using an adenovirus recombinant vector containing different specific promoters. This allowed us to study the fate, dynamics, kinetics and biochemistry of FMRP in RNA granules, using combined approaches such as time-lapse videomicroscopy and sub-cellular fractionation technics. We present new insights into the mechanisms underlying the regulation of dendritic FMRP-RNA transport and local synaptic translation.

MO02-09

ATP7A MUTATIONS CAUSING DISTAL HEREDITARY MOTOR NEUROPATHY: IS COPPER A MISSING LINK IN MOTOR NEUROPATHIES?

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Distal hereditary motor neuropathies (distal HMNs) are a clinically and genetically heterogeneous group of disorders affecting the peripheral motor nerves. We have identified two missense mutations (P1386S and T994I) in the copper transport gene ATP7A that cause X-linked distal hereditary motor neuropathy (distal HMNX). Male patients with these mutations present with variable age onset distal muscle wasting of both upper and lower limbs but show no clinical or biochemical abnormalities of Menkes Disease or occipital horn syndrome, disorders typically associated with ATP7A gene mutations. We investigated the possibility that the mutations causing distal HMN disrupted the copper transport functions of ATP7A. Patient fibroblasts were incubated in 200 μM Cu which causes the wild type ATP7A to traffic from the transGolgi network (TGN) to the plasma membrane, however, the mutant ATP7A protein failed to traffic normally, leaving a substantial proportion in the TGN. This suggests that copper efflux from the mutant cells was defective, and this result was consistent with elevated copper concentrations in the fibroblasts. Our unexpected findings that copper transporter mutations cause an inherited motor neuropathy suggest an important role for copper in the function and maintenance of motor neurons. Previous workers had provided a clue to the possible the mechanism involved: copper was released from ATP7A-containing vesicles upon activation of the NMDA receptor and the released copper was neuroprotective. It is possible that other neurodegenerative diseases also involve a similar copper-related mechanism. Our future work will involve analysis of the effect of the distal HMN mutations on the glutamate-induced trafficking of ATP7A in neuronal cell types.

MO02-10

TRANSCRIPTIONAL ACTIVATION OF LINE1 ELEMENTS IN RESPONSE TO HEAVY METAL INDUCED STRESS

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Objective: The goal of our study is to examine the effect of heavy metals on the transcriptional activation of long interspersed element (L1Rn) in the rat tissues.
Method: Male Swiss albino wistar strain rats of different age groups were given intra peritoneal subleathal dose of various heavy metals ($CdCl_2$, $HgCl_2$, $PbCl_2$, $NiCl_2$, and $AlCl_2$). Total RNA was isolated from heart, whole brain, liver, kidney and testis and used in experiment. We performed Real time RT-PCR to quantitate L1Rn expression, using GAPDH as internal control.
Results: Present results show upregulation of LINE1 retroelement (L1Rn) transcripts in response to heavy metal induced stress. Tissue specific transcriptional upregulation of L1Rn was observed in different organs in response to different heavy metal treatment.
Conclusion: Our results showed that L1Rn activation occurs in response to various heavy metals which form a part of environmental pollutants and their regulation is dependent on tissue specific factors. LINE1 elements may play an important role in the genetic damage associated with environmental exposures.

MO02-11

THE ROLE OF ATP IN EXPRESSION OF CLOCK GENES IN MICROGLIAL CELLS

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Clock genes are shown to play a pivotal role in the mechanism relevant to the oscillation of circadian rhythm in the suprachiasmatic nucleus in the mammalian brain, whereas recent studies have demonstrated the possible functional expression of several clock genes in eukaryotic cells other than neurons. In this study, we have investigated the possible functional expression of particular clock genes by microglial cells. Microglial cells were prepared from whole brains of 1-day-old ddY mice, followed by culture in DMEM/F12 medium containing FBS for 2 weeks with medium change twice a week and subsequent aspiration of culture medium containing detached cells with trypsin DMEM/F12 solution. In these primary cultured microglial cells and in the mouse microglial cell line BV-2 cells, which were both highly positive to isolectin-b4 staining, but not to either MAP2 or GFAP, an RT-PCR analysis

revealed mRNA expression of different clock genes including Per1, Per2, Per3, Cry1, Cry2, Dec1, Dec2, DBP, Bmal1, NPAS2 and Clock. Sustained exposure to ATP led to a significant but transient increase in Per1 mRNA expression without significantly affecting other clock gene mRNA expression in primary microglia and BV-2 cells. However, exposure to lipopolysaccharide failed to significantly alter expression profiles of different clock gene mRNA in BV-2 cells. The increase by ATP was significantly prevented by PPADS, isoPPADS and oxATP, but not by MRS2500, while adenosine was ineffective in significantly inducing Per1 mRNA expression. These results suggest that clock genes may be functionally expressed by microglial cells with responsiveness to activation of the P2X7 purinergic receptor subtype.

MO02-12

STUDIES ON ALTERED DNA INTEGRITY IN THE BRAIN REGIONS OF SUICIDAL VICTIMS OF BIPOLAR DEPRESSION

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Deoxyribonucleic acid (DNA) integrity plays a significant role in cell function. There are limited studies with regard to the role of DNA damage in bipolar affective disorder (BP). In the present study, we have assessed DNA integrity, conformation, and stability in the brain region of bipolar depression (BD) patients ($n = 10$) compared to age-matched controls ($n = 8$). Genomic DNA was isolated from 10 postmortem BD patients' brain regions (frontal cortex, Pons, medulla, thalamus, cerebellum, hypothalamus, Parietal, temporal, occipital lobe, and hippocampus) and from the age-matched control subjects. DNA from the frontal cortex, pons, medulla, and thalamus showed significantly higher number of strand breaks in BD ($p < 0.01$) compared to the age-matched controls. However, DNA from the hippocampus region was intact and did not show any strand breaks. The stability studies also indicated that the melting temperature and ethidium bromide binding pattern were altered in the DNA of BD patients' brain regions, except in the hippocampus. The conformation studies showed B-A or secondary B-DNA conformation (instead of the normal B-DNA) in BD patients' brain regions, with the exception of the hippocampus. The levels of redox metals such as Copper (Cu) and Iron (Fe) were significantly elevated in the brain regions of the sufferers of BD, while the Zinc (Zn) level was decreased. In the hippocampus, there was no change in the Fe or Cu levels, whereas, the Zn level was elevated. There was a clear correlation between Cu and Fe levels versus strand breaks in the brain regions of the BD. To date, as far as we are aware, this is a new comprehensive database on stability and conformations of DNA in different brain regions of patients affected with BD. The biological significance of these findings is discussed here.

MO02-13

THE ROLE OF THE KH DOMAINS OF DROSOPHILA MELANOGASTER FRAGILE X PROTEIN IN LEARNING AND MEMORY

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Fragile X syndrome (FXS), the most common known cause of inherited mental retardation, is caused by alterations of the FMR1 gene. The gene encodes an apparent RNA binding protein, which appears to be involved in the regulation of translation, transport, and stability of target mRNAs, especially locally at particular synapses. In addition, FMR protein (FMRP), appears also to be involved in developmental decisions at the level of neurite extension, guidance and branching. However, the precise role of FMRP in these processes has not yet been defined with certainty. Individuals with the syndrome display a range of developmental and behavioural deficits with mild to severe mental retardation, attention deficit disorder, autistic behavior, sleep disorders, memory deficits and problems related to anxiety such as hyperactivity among others.

FMRP has the ability to bind RNA through two ribonucleoprotein K homology (KH) domains and a cluster of arginine and glycine residues (RGG box). The phenotype of Fragile X syndrome is more severe when the patient's dfmr gene is mutated in a very conserved hydrophobic residue in the KH2 domain.

In this study we focus on the effects of mutated KH domains of dFMRP (the FMRP homolog in Drosophila). We study the phenotype, associative and non-associative learning and memory of wild type flies and flies heterozygous for dfmr1 when they overexpress mutated KH dFMRP domains. In this way we try to model the severe learning disabilities of FXS patients with mutation in FMRP KH2 domain in flies.

MO02-14

A GENETIC STUDY TO INVESTIGATE POSSIBLE LINK BETWEEN TPH1 GENE AND AUTISM SPECTRUM DISORDER (ASD) IN THE INDIAN POPULATION

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Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder. It is characterized by impaired communication skills, social interaction, with stereotypic behaviors and interests. A latest report shows that 1 out of 91 individuals is affected by ASD indicating a highly increasing trend. However, the root cause is still a mystery and a specific molecular marker to use as diagnostic tool is yet to be discovered. High platelet serotonin content in ASD, the most consistent finding has revealed platelet hyperserotoninemia as one of the promising endophenotype for ASD. This has kindled the interest of researchers to look further into serotoninergic regulation of autistic phenotype. TPH1 (tryptophan hydroxylase1), the rate limiting enzyme in serotonin biosynthesis is an important target molecule to be examined for investigating the serotonin system abnormality in autism etiopathology. Recent studies have shown possible involvement of TPH1 in ASD through haplotype analysis. In the present study, we analyzed three SNP markers (rs211106, rs684302 and rs682580) of TPH1 to examine possible link with ASD. Genotyping was performed using 473 samples from West

Bengal, India. DSM-IV criteria and CARS were used as diagnostic and assessment tools. DNA was extracted from blood cells of each volunteer and genotyping was carried out employing RFLP and sequencing analyses following PCR. Our results from population- and family-based approaches do not show any association of the three makers with ASD in the Indian population. Therefore we suggest that these markers are unlikely to be involved in the etiology of ASD in the Indian population.

MO02-15

GENE POLYMORPHISMS, PERSONALITY TRAITS AND ALCOHOL CRAVING IN ALCOHOL-DEPENDENT SUBJECTS

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Studies have shown that stress and alcohol-cue exposure can induce alcohol craving with increased cortisol level, hence stress response may be critical in regulating craving and predict relapse. Dopaminergic and serotonergic neurotransmissions play indispensible roles to modulate addictive behaviours through personality and stress response. This study explored the relationships among personality traits, salivary cortisol and alcohol craving in alcohol-dependent subjects, and their associations with gene polymorphisms. Alcohol-dependent subjects ($n = 156$) were recruited during a five-day detoxification treatment. Personality traits were assessed by NEO PI-R and Tridimensional Character Inventory. Alcohol craving was measured by Alcohol Urge Questionnaire and saliva sample for cortisol measurement. Genotyping was performed by PCR-RFLP. Results showed that alcohol craving correlated with salivary cortisol level in female subjects only (Spearman's rho = .430, $p = 0.022$). 5HTTLPR S-allele carriers had significantly higher cortisol level than LL counterparts (Student t test $p = 0.019$), and a significant correlation between salivary cortisol level and state alcohol craving in females (Spearman's $\rho = 0.466$; $p = 0.014$). Alcohol craving is significantly ($p < 0.05$) correlated with Neuroticism, Conscientiousness, Novelty-seeking and Reward-dependence personality scores for males with Extraversion, Conscientiousness, Novelty-seeking and Persistence scores for females. Significant correlation between alcohol craving and personality traits was only observed in DRD2 *Taq*IA A1-allele carriers, whose craving level was negatively correlated with Reward-dependence scores (adjusted $r^2 = 0.191$, $p < 0.001$). Structural Equation Modeling revealed the effect of DRD2 *Taq*IA on alcohol craving is mediated through Reward-dependence trait. In conclusion, correlations between alcohol craving, salivary cortisol level and certain personality traits are gender specific. 5HTTLPR S-allele may be associated with higher salivary cortisol level in female alcoholics, which is positively correlated with state alcohol craving. DRD2 A1-allele is associated with alcohol craving level through mediation of the Reward-dependence trait: alcohol-dependent A1-carrying patients with this trait may benefit from a detoxification treatment environment in which alcohol craving level is low.

MO02-16

AMPHETAMINE-INDUCED CHANGES IN CLOCK GENE EXPRESSION PATTERN IN THE RAT STRIATUM

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The mammalian circadian rhythms can be entrained by non photic stimuli. Drugs addicts have severe disruption in many rhythm of physiological and behaviors for example sleep/wake cycle. Interestingly, amphetamine is able to oscillate many circadian patterns which are independent of the suprachiasmatic nucleus (SCN). In order to gain more information of the circadian regulation of amphetamine on circadian genes expression, rats were injected with amphetamine subcutaneously (5 mg/kg) and vehicle (saline) for six consecutive days. The animals were sacrificed and removed brains every 6 h and the mRNA level of clock genes was analyzed by real-time PCR to obtain a daily profile. In pineal gland, the important output of the SCN to control the body circadian clock which the rhythm of melatonin play an important role to the sleep/wake behavior, showed that amphetamine had no effect on the rhythm of Per1 and Per2 expression similar to the report in the SCN. Also the expression of arylalkylamine-N-acetyltransferase (Aa-nat), a gene that produces the rate-limiting enzyme of melatonin synthesis showed unaffected by amphetamine treatment as well as the circulating melatonin rhythms. The daily injection of amphetamine shifted the phase of Per1 and Per2 expression from nocturnal to diurnal pattern. In contrast, rhythm of Bmal1 was shifted from diurnal to nocturnal pattern whereas amphetamine altered the Rev-erb alpha mRNA level of expression. These results suggested that amphetamine regulated the expression of clock genes within the specific region of the brain. The alteration of molecular clock components pattern may involve in the circadian rhythmicity by altering the behavior caused by drug addiction.

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MO02-17

CIRCADIAN E-BOXES AND SURROUNDING CPG ISLANDS ARE FREE FROM METHYLATION THROUGHOUT THE DAY

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Circadian E-boxes in the promoters of mPer1, mPer2, mCry1 and mDBP, play a key role in regulating rhythmic gene expression by recruiting the BMAL1/CLOCK heterodimer. However, each of these circadian E-boxes seems different from one another. In the mouse liver, BMAL1/CLOCK binds to E-boxes in the mPer1 and mPer2 promoters constantly, but binds with dynamic daily changes to mCry1 and mDBP promoters. It is unclear whether DNA methylation of these circadian E-boxes and the surrounding CpG islands would affect protein/DNA interactions and lead to distinct

binding features. In the present study, bisulfite sequencing analysis indicated that all E-boxes and their surrounding CpG islands were free from methylation, suggesting that DNA methylation does not determine distinct binding features. The methylation status of the E-box in the MAP kinase phosphatase 1 (MKP1) promoter was also examined. Our results indicated that DNA methylation does not regulate the tissue specific clock control of MKP1.

MO03 Neuroinflammation

MO03-01

THE RELATIONSHIP BETWEEN CCL-1 AND NEURONS/ GLIAL-CELLS IN THE NEUROPATHIC PAIN MODEL

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It has been reported that glial cells and cytokines are involved in the development of neuropathic pain. We focused on chemotactic cytokine ligand-1 (CCL-1), and investigated the relationship between CCL-1 and neurons/glial-cells. The protein level of spinal CCL-1 was increased by partially sciatic nerve ligation (PSNL). Intrathecal (i.t.) injection of recombinant CCL-1 to naïve mice induced allodynia. The CCL-1-induced allodynia was prevented by i.t. injection of the N-methyl-D-aspartate (NMDA) receptor antagonist MK-801. Prophylactic and chronic i.t. injection of anti-CCL-1-neutralizing antibody prevented the PSNL-induced allodynia. CCR-8, the receptor for CCL-1, was detected in the neurons and microglia *in vivo* and *in vitro*. Moreover, we identified that CCL-1 mRNA was expressed in neurons and glial cells. *In vitro*, CCL-1 induced chemotaxis and membrane ruffles of microglia. Our results strongly support the view that CCL-1-induced activation of NMDA receptor in the spinal cord together with involvement of microglia contributes to the development of neuropathic pain.

MO03-02

GENISTEIN INHIBITS APOPTOTIC DEATH IN VSC4.1 MOTONEURONS EXPOSED TO ACTIVATED MICROGLIAL CYTOKINES

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Pro-inflammatory cytokine production by microglia may be responsible for neuronal death and neurological deficits associated with neurodegenerative disorders. Estrogen is capable of ameliorating motoneuron death following SCI, but has a number of deleterious side effects. Genistein (GEN), an estrogen receptor-beta agonist and potent antioxidant, may be an alternative to estrogen therapy in neurodegenerative disorders. However, the neuroprotective effects of GEN are not well-established. We therefore tested whether GEN would prevent motoneuron apoptosis following exposure to pro-inflammatory cytokines released from IFN-γ activated microglia. Fulvestrant (ICI 182,780), an ER antagonist was also utilized to determine the role of ERs in neuroprotection. Exposure of VSC4.1 motoneurons to microglial cytokines resulted in significant apoptosis and reduced mitochondrial membrane potential. Increases in ROS, intracellular Ca^{2+}, calpain, caspases, cytochrome c, and the bax:bcl-2 ratio were also noted. GEN treatment reversed cytokine-induced neuronal damage and was associated with increased expression of ERβ, suggesting that receptor-mediated pathways may be responsible for the neuroprotective effects of GEN. The addition of ICI following GEN treatment attenuated neuroprotection, suggesting that GEN may act mainly via ERβ to protect VSC4.1 motoneurons. These findings indicate that GEN may be beneficial in the treatment of neurodegenerative disorders by reducing inflammatory insult to the CNS and thereby decreasing neuronal damage. This work was supported in part by funding from the National Institutes of Health-National Institute of Neurological Disorders and Stroke (NIH-NINDS) and the State of South Carolina Spinal Cord Injury Research Fund (SCIRF).

MO03-03

TREM2-DEPENDENT NEUROPROTECTIVE SYNAPTIC RESPONSES TO SYSTEMIC INFLAMMATION IN THE DEVELOPING HIPPOCAMPUS

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Nasu-Hakola's disease is a rare recessive genetic disorder characterized by early onset cognitive dementia, generalized seizures and abnormal EEGs. Individuals with Nasu-Hakola disease lack a functional Triggering Receptor Expressed on Myeloid cells (TREM)-2. Although this is a neurologic disorder, microglia are the only cells within the rodent CNS that express this orphan receptor. During early post-natal development, CNS neurons express high levels of TREM2 binding activity (putative TREM2 ligand) while microglia express nearly 20-fold higher levels of TREM2 than in the mature adult CNS. We hypothesized that in the absence of functional TREM2 pathways, microglial responses to immune challenges are maladaptive for neuronal development. We found that CNS responses to systemic immune challenges are not only developmentally regulated but are TREM2-dependent. Specifically, we find that intraperitoneal LPS injection leads to a transient elevation in microglial expression of TREM 2 as well as a transient influx of blood derived pro-inflammatory monocytes/macrophages. However, we also observe a selective prolonged elevation in microglial expression of TREM2 for one week post-injection in mice receiving LPS injections at post-natal day 14. A significant delay in the normal developmental increase in the numbers of excitatory Vglut1+ synapses occurred within the hippocampus co-incident with this age-specific prolonged microglial activation. Strikingly, mice that lack TREM2 not only exhibit chronic neuroinflammation even in the absence of an immune challenge, they have fewer numbers of Vglut1+ synapses than age-matched wild-type mice. Furthermore, TREM2KO mice have decreased numbers of inhibitory GAD65+ synapses within the hippocampus following LPS injection at P14. Consistent with these histologic data, LPS-challenged TREM2KO mice display a longer latency for PTZ-induced seizures, but the seizures are of longer duration. Taken together, our data suggest that the TREM2 pathway may be a novel neuronal-microglia regulatory pathway required to promote neuroprotective immunity during ordinary systemic inflammatory challenges encountered during childhood.

MO03-04

EFFECTS OF MAGNOLIA POLYPHENOLS ON OXIDATIVE STRESS AND INFLAMMATORY RESPONSES IN NEURONS AND GLIA

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Magnolol and honokiol are the active phenolic compounds isolated from the bark of various Magnolia species, and have been used in traditional Chinese medicine. Both polyphenols are known to have antioxidant and anti-inflammatory properties. In this study, we examined the neuroprotective effects of magnolol and honokiol on mitochondrial function and oxidative damage in neurons exposed to excitatory neurotransmitter agonists, and on cytokine-induced inflammatory and oxidative responses in glia. Besides primary rat cortical neurons, two types of immortalized glial cells, namely the rat DITNC astrocytes and mouse BV-2 microglial cells were used. The BV-2 microglial cells have been shown to respond to the proinflammatory cytokines (mixture of TNFalpha, IL-1beta, and IFNgamma) and lipopolysaccharides (LPS) in induction of inducible nitric oxide synthase (iNOS). DITNC cells respond to cytokines and LPS in the induction of secretory phospholipase A2-IIA (sPLA2-IIA). Results demonstrated that magnolol and honokiol inhibited NMDA-induced ROS production and mitochondrial dysfunction in primary culture of rat cortical neurons. Addition of magnolol and honokiol alone (up to 30μM) to primary neurons did not alter cell viability and mitochondrial function but both compounds could protect neurons from excitotoxic effects due to NMDA treatment. Pretreatment of DITNC astrocytes with magnolol and honokiol resulted in a dose-dependent inhibition of cytokine-induced sPLA2-IIA protein expression. When BV-2 cells were challenged with LPS, magnolol and honokiol were also able to inhibit LPS-induced nitric oxide (NO) production and iNOS expression. In summary, our results demonstrate that Magnolia polyphenols possess the neuroprotective effects by inhibition of neuronal excitotoxicity and glial inflammatory responses.

MO03-05

IMMUNE RESPONSES MEDIATE AXONAL SPROUTING IN THE INJURED SPINAL CORD

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Spinal cord injury leads to robust inflammatory processes, termed Wallerian degeneration, along tracts distal to the injury site. Inflammatory processes include the activation of astrocytes and microglia, and the recruitment of peripheral macrophages and T cells. The infiltrated T cells are thought to have both beneficial and detrimental effects in the injured CNS. Previously, we reported that over-expression of Neurotrophin-3 (NT-3) promoted axonal sprouting in the injured rat spinal cord but only when acute inflammatory responses were present. No axonal sprouting was observed when NT-3 over-expression was delayed 4 months after the injury when the inflammation had subsided. Axonal sprouting coincided with the activation of $CD4^+$ T cells in the spinal cord suggesting that they might participate in this neuroplasticity. To test this, we compared NT-3-induced neuroplasticity in nude rats (rnu/rnu) lacking functional T cells, rats heterozygous for the mutation (rnu/+) that had functional T cells, nude rats grafted with $CD4^+$ T cells, and nude rats grafted with $CD8^+$ T cells. After a unilateral lesion of the corticospinal tract (CST) NT-3 was over-expressed in lesioned side of the lumbar spinal cord by transducing motoneurons with an adenoviral vector carrying the NT-3 gene. Three weeks later we measured the number of axons that sprouted from unlesioned CST into the denervated side toward the source of NT-3. Axonal sprouting was significantly greater in rats with functional T cells and in nude rats that were grafted with $CD4^+$ T cells compared to nude rats or nude rats grafted with $CD8^+$ T cells. Additionally, $CD4^+$ T cells were polarized towards a Th2 phenotype when the CST was lesioned. These findings suggest that $CD4^+$ T cells activated by trauma-associated antigens play a role in NT-3-induced axonal sprouting. Supported by the Christopher and Dana Reeve Foundation; Craig H. Neilsen Foundation, and the U.S. Department of Veterans Affairs.

MO03-06

THE EFFECT OF THROMBOPOIETIN FOR FORECASTING THE RISK OF CEREBRAL PALSY

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Thrombopoietin (TPO), which is expressed in the central nervous system (CNS), acts neuroprotective properties by reducing cerebral injury and improving functional reconstruction. The dynamic change of TPO levels in different hypoxic-ischemic degrees and the correlation between TPO levels and cerebral injury will be helpful for understanding the biological function of TPO in cerebral palsy (CP). Serum samples of 23 fetuses with hypoxic-ischemic encephalopathy (HIE), 34 neonates with HIE and 31 CP children were obtained respectively. Thrombopoietin levels were measured by the enzyme-linked immunosorbent assay double sandwich method (ABC-ELISA). We found that TPO levels in HIE fetuses and HIE neonates were significantly higher than in normal controls ($p < 0.01$, respectively), but no difference in CP children ($p > 0.05$). TPO levels among mild, moderate and severe HIE were significantly different ($p < 0.01$). The more severe HIE was, the lower TPO levels the neonates were. In conclusion, TPO level was related to the severity of cerebral injury. TPO levels in CP indicates that it may participate in pathogenesis of CP. TPO can be used for early clinical monitoring of CP, and the measurement of TPO level is benefit for the classification of HIE.

MO03-07

EFFECT OF ARTESUNATE TREATMENT ON SOME BRAIN BIOMOLECULES AND ITS BEHAVIORAL IMPLICATION

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Artesunate (AS) is an artemisinin antimalarial drug used as a single drug or in combination with other antimalarials. This study

was to evaluate its effects on some brain biomolecules and behavioural activities in Wistar rats. Forty adult male Wistar rats weighing between 150–180 g were divided into four groups of A, B, C and D with 10 animals each. Group A served as the control that received tap water, while groups B, C and D served as the experimental groups that received 2.85 mg/kg (therapeutic dose-TD) and 5.71 mg/kg (high pharmacologic dose-HPD) of AS per day for 3 days, and 2.85 mg/kg (long duration therapeutic dose -LDTD) of AS per day for 6 days respectively. Half of the dose was administered twelve hourly (twice a day), and twelve hours after the last treatments, behavioural test using the 'open field maze' was carried out. Immediately after, the animals were sacrificed using chloroform anaesthesia and the whole brain removed and weighed. Whole brain homogenates were used to determine brain total protein (TP), triacylglycerol (TAG) and cholesterol (CH). Data were analyzed statistically by ANOVA with post-hoc Tukey-Kramer Multiple Comparative Test. There were no difference ($p < 0.05$) between the experimental groups and the control group in the anthropometric parameters and behavioural activities. In the brain biomolecules concentration, TP was lower in concentration in the HPD group, TAG was lower in concentration in the LDTD group, while the HPD and LDTD groups had lower CH concentration compared to the control. In all the parameters studied no difference was found between the TD group and the control. We therefore state that, AS at recommended dose may not affect some behaviour and brain biomolecule concentration, unlike when taken in excess of dose and or time. Even at these doses/time, there may be no behavioural manifestation.

MO03-08

GARCINIA KOLA IS NOT NEUROPROTECTIVE AGAINST THE NEUROTOXICITY OF COMBINED ISONIAZIDE AND RIFAMPICIN IN RATS

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A study has reported that combined Isoniazide (INH) and rifampicin therapy regime used in the treatment of tuberculosis induced neurotoxicity in an animal model in the absence of overt clinical signs. This study examined the potentials of the extracts from Garcinia Kola seed, a regular chewing refreshment in Nigeria, for neuroprotection against INH/rifampicin induced neurotoxicity in rats. Adult wistar rats (average weight - 200g) were maintained under standard laboratory conditions and randomly grouped into four (n - 7). Group A received 5% DMSO in normal saline (0.5 ml, i.p.) as the control; Group B received combined INH + rifampicin (50 mg/kg each, i.p) only; Group C received same dose of combined INH + rifampicin as Group B plus Garcinia Kola seed extract (60 mg/kg, p.o) while Group D received the Garcinia seed extract (60 mg/kg, p.o) only, daily at 1600 hours for 15 days. At the end of the administrations, a set of animals were sacrificed by cervical dislocation for brain weight determination while another set by whole body perfusion following anaesthesia for neurohistology (Nissl staining), flourescent GFAP immunoreactivity and TUNEL method for the detection of apoptosis from each group. Combined INH/rifampicin treatment induced highly reactive intracortical and subpial GFAP positive astrocytes, mostly localized as clusters in groups B and C, compared to the controls (groups A and D); the administration of Garcinia extracts in group C did not reduce the intense darkly stained discrete cells, positive for the TUNEL signals, when compared to group B. These findings suggest that the extracts from Garcinia Kola seed is not neuroprotective against combined INH/rifampicin induced neurotoxicity.

MO03-09

DIMETHYL FUMARATE REDUCES GLIAL INFLAMMATION VIA EFFECTS ON NRF2 AND NFKB

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Dimethyl fumarate (DMF) exerts anti-inflammatory and prometabolic effects in a variety of cell types, and a formulation (BG0012) is being evaluated for monotherapy in multiple sclerosis patients. DMF modifies glutathione (GSH) levels which can induce expression of the anti-inflammatory protein heme oxygenase-1 (HO-1). In primary astrocytes and C6 glioma cells, BG0012 dose-dependently suppressed nitrite production induced by either LPS plus IFNg (LI) or a mixture of pro-inflammatory cytokines, with greater efficacy in C6 cells. BG0012 reduced NOS2 mRNA levels and activation of a NOS2 promoter, reduced nuclear levels of NFkB p65 subunit, and attenuated loss of IkBa in both cell types although with greater effects in astrocytes. In astrocytes, LI decreased mRNA levels for GSH-reductase (GSHr) and glutamyl-cysteine synthetase (GCL), and slightly suppressed GSH-synthetase (GSHs) mRNAs. Co-treatment with BG0012 prevented those decreases and increased levels above control values. In contrast, LI reduced GSH-peroxidase (GSHp) and GCL in C6 cells, and BG0012 had no effect on those levels. BG0012 increased nuclear levels of Nrf2, an inducer of GSH-related enzymes, in astrocytes but not C6 cells. In astrocytes, GSH was reduced by BG0012 at 2 h, and increased at 24 h. Prior depletion of GSH using buthionine-sulphoximine increased the ability of BG0012 to reduce nitrites. In astrocytes, BG0012 increased HO-1 mRNA levels and effects on nitrite levels were blocked by an HO-1 inhibitor. These results demonstrate that BG0012 suppresses inflammatory activation in astrocytes and C6 glioma cells, but with distinct mechanisms, different dependence upon GSH, and different effects on transcription factor activation.

MO03-10

CANNABINOID INDUCED NEUROPROTECTION AGAINST CELL DAMAGE CAUSED BY HYPOXIA IN CULTURED RAT NEURONS

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Neuronal cells have been shown to be more susceptible to the injurious effects of hypoxia in that they may begin to die when oxygen supply is reduced or completely eliminated. Cannabinoid (CB1) receptor agonists have been shown to elicit several central nervous system effects, mediated via G protein-coupled receptors. This study examined the effects of cannabinoids on hypoxia-induced cell damage in rat cortical neuronal cells (B50) in culture.

The B50 cells in hypoxia (5%O_2; 5% CO_2), were treated with cannabinoid agonists to study their effects using downstream cellular activities such as morphology, proliferation, differentiation, lactate dehydrogenase (LDH) leakage, second messenger (cAMP) and extracellular signal-regulated kinases (ERK1/2) quantification compared to normoxic cells (21%O2; 5% CO_2). Three cannabinoid agonists [Win55, 212-2 mesylate (WIN), arachidonoylethanolamide (AEA) and 2-arachidonylglycerol (2-AG)], were administered to the cells as treatment for 48 h after 48 h of culture at concentrations of 10 nM, 50 nM and 100 nM. Neuronal viability, proliferation and second messenger activity were assessed using same-field assessment, LDH leakage, cellular proliferation, second messenger (cAMP), and phospho-ERK1/2 assays. The results showed that hypoxia induced a 4-fold increase in LDH leakage from B50 cells compared to normoxic cells ($p < 0.05$). Cannabinoid agonist treatment induced a 2- to 4-fold decrease in LDH release in hypoxic cells ($p < 0.05$). There was reduction in cAMP concentration in hypoxic cells compared to normoxic cells ($p < 0.05$). The cannabinoid treated hypoxic cells showed increases in cAMP concentration, phospho-ERK1/2 and cell proliferation, compared to untreated hypoxic cells ($p < 0.05$). The results of cannabinoid agonist treatments suggest they have some potential therapeutic and protective benefits in the treatment of hypoxia-induced toxicity in neuronal B50 cells in culture.

MO03-11

COMPARATIVE NEUROPROTECTIVE PROFILE OF STATINS IN QUINOLINIC ACID INDUCED NEUROTOXICITY IN RATS

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A possible neuroprotective role has been recently suggested for 3H3MGCoA reductase inhibitors (statins). Here, we sought to determine neuroprotective effect of statins in quinolinic acid induced neurotoxicity in rats. Rats were surgically administered quinolinic acid and treated with Atorvastatin (10, 20 mg/kg), simvastatin (15, 30 mg/kg) and fluvastatin (5, 10 mg/kg) once daily up to 3 weeks. Atorvastatin (10, 20 mg/kg), simvastatin (30 mg/kg) and fluvastatin (10 mg/kg) treatment significantly attenuated the quinolinic acid induced behavioral (locomotor activity, rotarod performance and beam walk test), biochemical (lipid peroxidation, nitrite concentration, SOD and catalase), mitochondrial enzyme complex alterations in rats suggesting there free radical scavenging potential. Additionally, atorvastatin (10, 20 mg/kg), simvastatin (30 mg/kg) and fluvastatin (10 mg/kg) significantly decrease the TNF-α level and striatal lesion volume in quinolinic acid treated animals indicating there anti-inflammatory effects. In comparing the protective effect of different statins, atorvastatin is effective at both the doses while simvastatin and fluvastatins at respective lower doses were not able to produce the protective effect in quinolinic acid treated animals. These modulations can account, at least partly, for the beneficial effect of statins in our rodent model of striatal degeneration. Our findings show that statins could be explored as possible neuroprotective agents for neurodegenerative disorders such as HD.

MO03-12

UPREGULATION OF KRUEPPEL LIKE FACTOR 4 IN MICROGLIA IS ASSOCIATED WITH ITS ACTIVATION AND SUCCESSIVE NEURO-INFLAMMATION

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Activation of microglia is the hallmark of neuroinflammation observed in several neurodegenerative diseases as well as pathological conditions associated with CNS infection. Our work focuses on understanding the role of Krueppel like factor 4 (Klf4), a zinc-finger transcription factor in mediating neuro-inflammation. Earlier reports have suggested that Klf4 is implicated in several cell processes including differentiation, cellular growth and peripheral inflammation. For our studies, we have used lipopolysaccharide (LPS) and IL-1β to treat mouse microglial BV-2 cell line and primary microglia isolated from BALB/C mice. For immunohistochemistry, brain tissues were isolated from BALB/c mice administered with 5mg/kg body weight of LPS. Expressions of various proteins were evaluated using Western blotting and reverse transcriptase polymerase chain reactions (RT-PCRs) along with quantitative real time PCR (qT-PCR) and cytokine analysis was carried out using Cytokine Bead Array (CBA). For understanding the role of Klf4 in mediating neuroinflammation, we have carried out Klf4 knockdown using SiRNA while its binding to iNOS promoter was confirmed by Electromobility Shift Assay (EMSA). Our studies have shown that lipopolysaccharide (LPS) increases Klf4 expression in a time-dependent manner. Our findings suggest that Klf4 is associated with the expression of pro-inflammatory cytokines as knockdown of Klf4 resulted in decreased levels of pro-inflammatory cytokines, TNF-α, MCP-1 and IL-6 along with a significant decrease in iNOS and Cox-2 expression. NO production also decreased as a result of Klf4 knockdown. Luciferase-promoter assays have shown that Klf4 interacts with iNOS and Cox-2 promoters by potentially interacting with pNF-κB as confirmed by immunoprecipitation studies. Our ongoing studies have also shown that IL-1β, a potent pro-inflammatory cytokine causes a significant increase in the expression of Klf4 in BV-2 cells which also correlated with the expression of HIF-1α. This has prompted us to further understand the interplay between Klf4 and HIF-1α expression in IL-1β stimulation.

MO03-13

CHRONIC PARACETAMOL USAGE INDUCES PROINFLAMMATORY CYTOKINES AND VASCULAR CELL ADHESION MOLECULES EXPRESSION VIA NF-KB SIGNALING

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The present study aims to investigate the effect of chronic paracetamol treatment on the cortical spreading depression (CSD)-induced the expression of pro-inflammatory cytokine IL-1 and vascular cell adhesion molecules (ICAM-1/VCAM-1) in the cerebral cortex by immunohistochemistry. Male Wistar Furt rats were divided into control, control with CSD, paracetamol treated and paracetamol treated with CSD groups. Paracetamol (200 mg/kg

BW, intraperitoneally) or vehicle was injected daily for a period of 30 days. Subsequently, the CSD was induced by the topical application of 3 mg solid KCl on the parietal cortex while 3 mg NaCl was applied at the non-CSD induced groups. The results showed that the induction of CSD alone had no effect on the expression of pro-inflammatory cytokines and vascular cell adhesion molecules. However, the CSD activation in combination with chronic paracetamol treatment could induce an increase in the number of IL-1 immunoreactive cells and the ICAM-1/VCAM-1 immunopositive vessels in the cerebral cortex than those observed in control and CSD group. The NF-kB expression was significantly enhanced in the chronic paracetamol treated with CSD group. The results of the present study suggest that CSD activation in chronic paracetamol exposure leads to an increase in the proinflammatory cytokines production, resulting in abnormality in cerebral vessels. The activation of NF-kB signaling may at least in part be the mechanism underlying this abnormality.

MO03-14

$P2Y_{12}$ RECEPTORS MEDIATE CHEMOKINES EXPRESSION IN PRIMARY RAT MICROGLIA

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Microglia are immune cells in the central nervous system and have many important roles on several CNS disorders including Alzheimer's disease, Parkinson's disease and neuropathic pain. Chemokine release is one of the important microglial functions in such pathological conditions. Furthermore accumulating evidences indicate that chemokines play an important role in the pathology of neuropathic pain. We have previously reported that $P2Y_{12}R$ is also crucially involved in the pathogenesis of neuropathic pain. However its detail mechanism remains to be elucidated. Here we demonstrate that the levels of CCL3 and CCL2 mRNA expression were markedly increased by $P2Y_{12}R$ agonists, ADP and 2MeSADP, in primary microglia. This increase was suppressed by pretreatment of $P2Y_{12}R$ antagonists. Additionally, pertussis toxin also inhibited increased CCL3 and CCL2 gene expression. Furthermore APDC, an NF-κB inhibitor, completely blocked increased expression of these genes. These results indicate $P2Y_{12}R$ regulates chemokine production via Gi coupled protein and NF-κB-involved signaling in primary microglia.

MO03-15

LENTIVIRAL EXPRESSION OF THE HUMAN NEURAL CELL ADHESION MOLECULE L1 FOR *EX VIVO* AND *IN VIVO* GENE THERAPY OF THE LESIONED CNS

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Regeneration failure after CNS trauma results in severe functional impairment and is attributed to inhibitory molecules creating a non-permissive environment for axonal regrowth. An emerging approach for improving regeneration is the use of gene therapy to manipulate cell adhesion molecule expression *ex vivo* or *in vivo*. Additionally, experimental transplantation in rodent and primate models of CNS injuries has led to the idea that Schwann cells are promising candidates for autologous transplantation to assist myelination of lesions and to deliver therapeutic agents in the CNS. L1 cell adhesion molecule promotes axonal growth and myelination during nervous system development and regeneration. Moreover, we have previously shown that transplantation of mouse Schwann cells transduced to express L1, accelerates remyelination and motor recovery in a mouse model of spinal cord injury (Lavdas 2010). As a next step towards prospective therapeutic application in humans, we aimed at generating an efficient, replication deficient lentiviral vector for expression of human L1 (hL1). To this end, FLAG-tagged hL1 cDNA was cloned into the pTRIP.ΔU3.CMV plasmid. A high-titer lentiviral vector Trip.hL1-FLAG was generated that efficiently transduced 293T cells (human origin), COS-7 cells (non-human primate origin) and primary mouse SC. Furthermore, Trip.hL1-FLAG and the control vector Trip.GFP transduced with >99% efficiency primary Schwann cells isolated from human peripheral nerve biopsies. Immunofluorescence and immunoblotting experiments confirmed hL1 expression in all cases. Experiments are in progress using the generated Trip.hL1-FLAG lentiviral vector for *ex vivo* and *in vivo* gene therapy approaches in mouse and non-human primate models of spinal cord injury. Supported by FP7 REGPOT Project 264083 Neurosign - Development of a Center of Excellence in Neurosignalling.

Reference:

1. Lavdas et al., (2010). Exp Neurol, **221**:206–216.

MO03-16

ROLE OF OVEREXPRESSED PROINFLAMMATORY CYTOKINES IN THE PATHOGENESIS OF CEREBRAL PALSY

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Cerebral palsy is one of the most common cause of severe physical disability in childhood. The pathophysiological mechanisms underlying cerebral palsy remains poorly understood and is thought to be multifactorial. The accumulating evidence indicates that inflammatory cytokines released during the course of brain damage caused by hypoxic-ischemic injury and/or perinatal infection may play a central role in the pathogenesis of cerebral palsy. To investigate the role of proinflammatory cytokines in the mechanisms of cerebral palsy. Thirty-one patients diagnosed as cerebral palsy, twenty healthy controls and neonates ($n = 37$) who suffered hypoxic-ischemic injury and/or perinatal infection, twenty healthy neonates who were used as controls were studied retrospectively. Enzyme-linked immunosorbent assays (ELISA) were performed for TNF-α and IL-6 in serum from all subjects. (1) TNF-α and IL-6 were significantly higher in cerebral palsy patients than in controls ($p < 0.05$); (2)TNF-α and IL-6 were significantly higher in suffered neonates than in controls ($p < 0.05$); (3) TNF-α in serum is significantly higher in patients than in suffered neonates ($p < 0.05$). However, there was no statistical difference of IL-6 levels between the two groups ($p > 0.05$). (i) TNF-α and IL-6 were significantly higher in suffered neonates than in controls, this showed that pronflammatory cytokines were relation to brain injury caused by

hypoxinc-ischemic and perinatal infection; (ii) Overexpressed pronflammatory cytokines may play an important role in the pathogenesis of cerebral palsy; (iii) Early detection of pronflammatory cytokines of neonates will be helpful to diagnosis and prognosis of cerebral palsy.

MO03-17

CHRONIC STRESS IN RAT INDUCES A LONG-LASTING INFLAMMATORY RESPONSE ACCOMPANIED BY CHANGES IN GLUCOCORTICOID DELIVERY AND SIGNALING

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Chronic stress induces a variety of maladaptive mental changes, such as anxiety and various forms of depressive disorders. However, the mechanisms underlying these phenomena are still poorly understood. Mental disorders are believed to be accompanied by both selective changes in hippocampus and neuroinflammatory response. Thus, we suggested that chronic stress can induce inflammatory changes in hippocampus triggering cerebral pathologies. We measured the expression of IL-1β mRNA in rats subjected to acute (6 h) or chronic (2 weeks) emotional painful stress. To test whether chronic stress induced irreversible alterations, we also evaluated IL-1β expression a month after the termination of chronic stress. In an effort to elucidate mechanisms involved in stress-induced inflammatory response, we further examined parameters of glucocorticoid system.

Results: Acute and particularly chronic stress induced higher increases of IL-1β mRNA in hippocampus as compared with cerebral cortex. Moreover, IL-1β expression in hippocampus remained significantly increased a month after the termination of chronic stress, suggesting a link between stress and stress-induced disorders. Acute stress increased, whereas chronic stress decreased corticosterone levels in both serum and cerebral cortex. The hippocampal corticosterone, however, remained significantly elevated under the chronic stress despite the decreased level of circulated hormone. Chronically elevated corticosterone is known to become proinflammatory due to alterations in glucocorticoid signaling. In an effort to check whether chronic stress changes glucocorticoid signaling, we measured the expression of two glucocorticoid targets, igf1 and crf. Acute stress induced respective changes in glucocorticoid target genes expression in cerebral cortex and hippocampus, however, no changes were observed in hippocampus under the chronic stress despite elevated level of corticosterone.

Conclusion: The results suggest that chronic stress induces selective inflammatory changes in hippocampus but not cerebral cortex, providing a link between stress and stress-inducing disorders. The maladaptive inflammatory response in hippocampus is accompanied by local alterations in glucocorticoid delivery and signaling, presumably underlying this response.

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MO03-18

REGULATION OF NFKAPPAB ACTIVITY IN ASTROCYTES: EFFECTS OF FLAVONOIDS AT DIETARY-RELEVANT CONCENTRATIONS

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Activation of the NFkappaB system in astrocytes can initiate a number of processes which contribute to neurodegeneration, while suppression of astrocyte NFkappaB offers a potential route to neuroprotection. We have previously shown that flavonoids, major components of a diet rich in fruit and vegetables, at dietary-relevant concentrations are able to regulate several signaling pathways in both neurons and astrocytes which are neuroprotective. In this study, we tested the hypothesis that flavonoids are able to suppress NFkappaB activation in astrocytes. All experiments were carried out using primary cultures of mouse cortical astrocytes prepared from E14 Swiss mouse embryos. To monitor regulation of the NFkappaB pathway, astrocytes were transfected at 12DIV with plasmids encoding a kappaB luciferase reporter construct and renilla luciferase as an internal control and monitored 48–72 h later using the Dual-Glo luciferase assay system. Astrocytes showed a functional NFkappaB response which could be regulated by proinflammatory stimuli. TNFα (150 ng/mL) produced an increase in luciferase activity which was abolished by co-transfection with a dominant negative IκBα construct. In line with this observation, TNF enhanced the levels of phosphorylated IκBα as determined by Western blotting and increased nuclear localization of p65 subunit of NFkappaB as determined by fluorescence microscopy and live cell imaging. To investigate the potential of dietary-relevant concentrations of flavonoids to modulate NFkappaB activity, cortical astrocytes were treated with flavonoids from different classes; Flavan-3-ols ((-)-epicatechin and (+)-catechin hydrate), flavones (luteolin and chrysin), flavonol (kaempferol) or flavones (naringenin and hesperetin) for 18 h. Flavonoids were tested at 100 nM, 300 nM and 1 μM, corresponding to dietary relevant concentrations. None of the flavonoids regulated NFkappaB activity in their own right, nor were they able to suppress TNFα regulation of NFkappaB activity. Thus we conclude that the astrocyte NFkappaB pathway is not a major target for flavonoids in astrocytes at dietary-relevant concentrations.

MO03-19

VIA PHOSPHOLIPASEA2 EXPRESSION AFTER PROINFLAMMATORY LIPOPOLYSACCHARIDE TREATMENT OF ASTROCYTES RESULTS IN ENHANCED CA^{2+} SIGNALING

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Many Ca^{2+}-regulated intracellular processes are involved in the development of neuroinflammation. However, the changes of Ca^{2+} signaling in brain under inflammatory conditions were hardly studied. ATP-induced Ca^{2+} signaling is a central event of signal transmission in astrocytic networks. We investigated primary astrocytes after pro-inflammatory stimulation with lipopolysaccharide. We reveal that Ca^{2+} responses to purinergic ATP stimulation are significantly increased in amplitude and duration after stimulation with LPS. We detected that increased amplitudes of Ca^{2+} responses to ATP in LPS-treated astrocytes can be explained by

substantial increase of Ca^{2+} load in stores in endoplasmic reticulum. The mechanism implies enhanced Ca^{2+} store refilling due to the amplification of capacitative Ca^{2+} entry. The reason for the increased duration of Ca^{2+}responses in LPS-treated cells is also the amplified capacitative Ca^{2+} entry. Next, we established that the molecular mechanism for the LPS-induced amplification of Ca^{2+} responses in astrocytes is increased expression and activity of VIA phospholipase A2 (VIA iPLA2). Indeed, both gene silencing with specific siRNA and pharmacological inhibition of VIA iPLA2 with S-bromoenol lactone reduced the load of the Ca^{2+} stores and caused a decrease in the amplitudes of Ca^{2+} responses in LPS-treated astrocytes to values, which were comparable to those in untreated cells. Our findings highlight a novel regulatory role of VIA iPLA2 in development of inflammation in brain. We suggest that this enzyme might be a possible target for treatment of pathologies related to brain inflammation.

MO03-20

EFFECT OF 5-HT DEPLETION ON THE CSD INDUCED THE RELEASE OF NITRIC OXIDE AND CGRP IN THE TRIGEMINOVASCULAR NOCICEPTIVE SYSTEM

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Serotonin (5-HT) and nitric oxide (NO) is an important neurotransmitter involved in both mediating and modulating pain process. The low level of 5-HT could induce the hyper-excitability of the cortical neurons after CSD activation. However the mechanism underlying this increase is unclear, an alteration of the NO system is one possible explanation. In this study, we aimed to determine the effect serotonin depletion on the CSD induced the expression of neuronal nitric oxide synthase (nNOS) and calcitonin gene related peptide (CGRP) in the trigeminovascular nociceptive system. Male Wistar rats were divided into control, CSD, and 5-HT depleted with CSD group. Serotonin was depleted by administration of para-chlorophenylalanine and CSD was induced by application of KCl on the cortical surface. In this experiment, the immunoreactivity of nNOS and CGRP and were investigated in the trigeminal ganglion (TG) and trigeminal nucleus caudalis (TNC). Fos expression was also examined in the TNC. The results showed that, the trigeminal nociception in 5-HT depleted with CSD group was enhanced as indicating by the increase in the number of Fos immunoreactivity cells and Fos expression in TNC. Furthermore, nNOS and CGRP expression were demonstrated in the same area (laminar I-II of TNC). Both nNOS and CGRP immunoreactivity were increased in 5-HT depleted with CSD group as compared with CSD and control group. Taken together, our results demonstrated that this phenomenon might be the mechanism underlying the hyper-excitability of the trigeminovascular nociceptive system in serotonin depleted condition.

MO03-21

CYTOKINE-INDUCED EXPRESSION OF SPLA2-IIA IN ASTROCYTES AND MICROGLIAL CELLS

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Secretory phospholipases A2 are small molecule proteins (~14 kDa) that are secreted and many of them exhibit inflammatory properties. Among some 12 isoforms of these proteins, the sPLA2-IIA has been most studied due to its link to inflammatory and infectious diseases. Although recent studies showed upregulation of sPLA2-IIA in neurodegenerative diseases including Alzheimer's disease and stroke, the mechanism and signaling pathways for induction of this enzyme in different cell types have not been studied in detail. In previous studies, upregulation of this protein was found in cultured astrocytes upon stimulation by pro-inflammatory cytokines and lipopolysaccharides (LPS). Due to a missense mutation of this gene in many mouse strains, studies with rodent models have been restricted mainly to rats. In the immortalized rat astrocytes (DITNC), cytokines such as TNFalpha and IL-1beta can individually stimulate expression of sPLA2-IIA mRNA and protein, and the induced protein is subsequently secreted to the culture medium. Although induction of this protein has been shown to involve the NF-kB pathway, whether other signaling molecules, such as NADPH oxidase and MAPKs, also regulate the induction pathway remains to be further investigated. The rat immortalized microglial cells (HAPI) respond to cytokines in induction of iNOS. Surprisingly, cytokines could not induce the expression of sPLA2-IIA mRNA and protein in these cells. We further tested the microglial cells that are present in rat primary astrocyte culture. Double staining with sPLA2-IIA and CD11b showed immunoreactivity of sPLA2-IIA in microglial cells, even prior to treatment with cytokines. Furthermore, primary microglial cells isolated from the primary astrocyte preparation also showed expression of this protein, both prior to and after cytokine treatment. Our study demonstrated for the first time the presence of sPLA2-IIA expression in rat microglial cells and its induction by cytokines. Understanding the mechanism for sPLA2-IIA induction in specific glial cell types is important to clarify the role of this inflammatory protein in neurodegenerative diseases.

MO03-22

THE CHOROID PLEXUS EPITHELIAL CELL LINE ECPC-4 CELLS EXPRESSED NO SYNTHASES BY LPS

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Objective: The choroid plexus epithelium constitutes the structural basis of the blood-cerebrospinal fluid barrier. However several lines of evidence had shown that choroid plexus epithelium had an inflammatory response in CNS. We investigated by which mechanisms the choroid plexus epithelial cell line ECPC-4 cells expressed NO synthases (NOSs) by LPS.

Methods: The LTRs and cytokines mRNAs were detected by RT-PCR using specific primers. The NOSs, NFkB and IkB were identified by Western blotting. The NFkB gene silencing were using RNA interference method.

Results: The toll like receptor, LTR-2 and -4 were expressed in ECPC-4 cells. The pro-inflammatory cytokines, interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and cyclooxygenase-2 mRNA increased within 4–8 h after LPS addition. The addition of IL-1β or TNF-α could remarkably induce iNOS expression in ECPC-4 cells. ECPC-4 expressed the three types NOSs, eNOS, iNOS and nNOS, and enhanced iNOS and eNOS within 24–72 h by LPS treatment. Then icv LPS administration in mice, iNOS was expressed in choroid plexus. Furthermore, to elucidate the mechanism of iNOS expression in ECPC-4, we analyzed the transcriptional factors NFkB and IkB. These transcription factors were expressed in ECPC-4, the phosphorylated-IkB appeared at 10 min and IkB disappeared at 15 min after addition of LPS. Furthermore NFkB gene silencing inhibited the expression of iNOS by LPS.
Conclusions: These results suggested that LPS activated iNOS in the choroid plexus via autocrine induction of IL-1β and TNF-α, and NFkB was a key factor in iNOS transcriptional process. The NFkB gene silencing possibly regulate the iNOS expression in choroid plexus on the brain inflammation process. It may hypothesize that choroid plexus epithelial cells has an important role as the inflammatory sensor in the CNS.

MO03-24

INDUCTION IN NG2 EXPRESSION IN HIPPOCAMPUS OF LIPOPOLYSACCHARIDE-TREATED RAT

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The NG2 expressing glia cells (NG2 cells) were first identified as oligodendrocyte progenitor cells (OPC). The proteoglycan NG2 cells have more than 5–10% of all glia in the developing and adult brain. Several studies have demonstrated an increasing in number and a changing in cellular morphology of NG2 cells in adjacent brain damage area. However, recent information of NG2 cell's functions in the brain still remains unclear. Therefore, the main purpose of this study was to investigate the functional role of NG2 cells in inflammatory responses in adult rat brain. We found that NG2 protein levels in rat hippocampus was high at the postnatal day 8 (P8) and gradually decreased to low levels at the adult (8 weeks). The inflammatory process in adult Wistar rat was induced by lipopolysaccharide (LPS). LPS administration significantly induced increase in COX-2 and NG2 protein levels in hippocampus when compared with normal saline-treated rat. These findings demonstrate an activation of NG2 expressing cells in inflammatory reaction in adult rat brain.

MO03-25

BUTANOL FRACTION OF WHITE ROSE PETAL EXTRACT REDUCES ISCHEMIC BRAIN INJURY IN RATS

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We have demonstrated antiallergic, antibacterial and antioxidative activities of white rose petal extracts (WRPE) and its butanol and hexane fractions. In the present study, we examined the neuroprotective effects of WRPE-butanol fraction (WRPE-BF) in a middle cerebral artery occlusion (MCAO) model. Seven week-old male rats were orally administered with 10 or 32 mg/kg of WRPE-BF for 2 weeks, and subjected to MCAO, followed by reperfusion in 2 h. Twenty-four hours later, behavioral abnormalities as well as brain injuries were evaluated. MCAO induced behavioral dysfunctions on rota-rod performance and locomotor activity, which were markedly attenuated by pretreatment with WRPE-BF in a dose-dependent manner. WRPE-BF not only decreased infarction area from 47.5% to 7.3% in TTC staining, but also reduced astrogliosis as measured by glial fibrillary acidic protein (GFAP) immunoreactivity. In addition, WRPE-BF decreased nitric oxide and malondialdehyde levels in striatum and subventricular zone. Therefore, it is proposed that WRPE-BF exert neuroprotective effects by eliminating radical formation and ensuing lipid peroxidation in ischemia-reperfusion brain injury, and that WRPE-BF could be a candidate for the improvement of ischemic stroke.

MO03-26

IMPROVING EFFECTS OF SILK PEPTIDES ON THE COGNITIVE FUNCTION OF RATS WITH MEMORY DEFICIT INDUCED BY CHOLINOTOXIN AF64A

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In order to develop silk peptide (SP) preparation possessing cognition-enhancing effect, several candidates were screened through *in vitro* assays, and its effectiveness was investigated in rats. Incubation of brain acetylcholinesterase with SP-P (a degradation product of silk proteins with Aspergillus protease Protease P Amano 6G) did not inhibit the enzyme activity. The expression of choline acetyltransferase (ChAT) mRNA of neural stem cells expressing ChAT gene (F3.ChAT) was increased to 1.68 and 2.15 folds by 24-h treatment with 10 and 100 μg/mL of SP-P, respectively. Intracerebroventricular injection with a cholinotoxin AF64A [3 nmoles (690 ng)/rat] impaired learning and memory function in passive avoidance and Morris water-maze performances 4 weeks post-injection. AF64A decreased acetylcholine concentration in cerebrospinal fluid by 35–40%, and increased activated hippocampal astrocytes to 2.1 folds control level as measured by gilal fibrillary acidic protein (GFAP) immunostaining. Daily oral treatment with SP-P (50 or 300 mg/kg) for 5 weeks from 1 week prior to AF64A injection exerted recovering activities on acetylcholine depletion and brain injury (GFAP reactivity) as well as cognitive deficit induced by AF64A. The results indicate that SP-P restores cognitive function of cholinotoxin-challenged rats by increasing the release of acetylcholine, in addition to neuroprotective activity.

MO03-27

EFFECT OF ACUTE PARACETAMOL TREATMENT ON THE CSD INDUCED THE CGRP AND SP EXPRESSION IN THE TRIGEMINAL GANGLION

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Paracetamol is one of the most popular analgesic drugs used as the pain killer in several pathologic conditions including headache. Despite the popularity of this drug, its mechanism of action is still debated. In this study, we investigated the effect of paracetamol treatment on the alteration of the trigeminovascular nociceptive system in the cortical spreading depression (CSD) animal model. The expression of the calcitonin gene related peptide (CGRP) and substance P (SP) in the trigeminal ganglion were studied. The effect of this drug on the expression of Fos in trigeminal nucleus caudalis (TNC) and the ultrastructural changes of cerebral vessels were also studied. Rats were separated into four groups: control, CSD, paracetamol-treated, and paracetamol-treated with CSD. CSD was induced by solid KCl (3 mg) while NaCl was used in the non-CSD induced groups. In the animals with paracetamol treatment, paracetamol (200 mg/kg bw) was i.p. injected into the rat before the induction of CSD while NSS was given to the non-paracetamol treated groups. After induction of CSD for 2 h, the TG and the cerebral cortex were removed for CGRP and SP immunohistochemical and ultrastructural studies. The results have demonstrated that the expression of Fos in the TNC as well as the expression of CGRP and SP in the TG were higher in the CSD group. Interestingly, these changes were significantly lower ($p < 0.01$) with the paracetamol treatment. In addition to these effects, pretreatment with paracetamol could reduce the ultrastructural alterations of the cerebral vessels induced by CSD. We suggest that the ability of the paracetamol in the decrease of the CGRP and SP expression in the TG may at least in part involved in the anti-nociceptive effect of this drug in headache.

MO04 Molecular Mechanism of Parkinson's Disease

MO04-01

A STUDY ON CYBRID MODEL OF PARKINSON'S DISEASE FROM INDIAN POPULATION

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Existence of mitochondrial dysfunction is reported in the blood and brain of parkinsonian patients. Cybrids are cytoplasmic hybrids created by the fusion of anucleate cells with rho0 (ρ0) cell line that has been deprived of its mitochondrial DNA. Present study describes production of ρ0 cells from SH-SY5Y cells and then uses these cells for the creation of normal and parkinsonian cybrids. Platelets from patients and age- and gender-matched controls were used to create cybrids by fusion of these platelets with the ρ0 cells. Further study involves their differentiation, characterization and comparison between control and Parkinson's disease (PD) with specific consideration into the mitochondrial function or dysfunction in relation to the expression of certain nuclear and mitochondria encoded mitochondrial subunits. The expression in the proteins and their up- or down-regulation were extensively studied with the help of immunoblot and 2-dimensional polyacrylamide gel electrophoresis (2-D PAGE). PD cybrids showed significant decline in the mitochondrial electron transport chain protein subunit expression (ND4, ND5 and ND6) with respect to the control cybrids. Twenty one mitochondrial proteins were differentially (up- or down-regulated) expressed as identified by 2-D PAGE followed by MALDI-TOF-TOF analysis. The platelets isolated from PD patient blood samples exhibited significantly lower complex I and IV activities, as compared to control population. This, taken along with decreased mitochondrial complex-I subunit expression in the PD cybrids suggests the newly generated cell lines as a reliable cellular model of PD, and is an invaluable tool for mitochondrial research on this disease, and has been achieved for the first time in this part of the world. It is expected that the cybrids created in the laboratory would enable elucidation of mitochondrial machinery underlying PD pathology.

MO04-02

METHYLPHENIDATE REGULATES DOPAMINE OVERFLOW VIA ALPHA-SYNUCLEIN SPECIFIC PRE-SYNAPTIC MECHANISM

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Alpha-synuclein (α-syn) deficient mice display altered compartmentalization of pre-synaptic dopamine storage and facilitated dopamine release following burst stimulation. Methylphenidate (MPD), a preferred drug for treatment of attention-deficit hyperactivity disorder (ADHD), also affects dopamine storage and increases dopamine release. However, mechanisms underlying the paradoxical calming effect of MPD in ADHD patients have remained elusive. We hypothesized that MPD and α-syn share same molecular targets. We studied the effect of MPD on stimulated and basal extracellular dopamine levels in the dorsal striatum in wild type and two mouse lines lacking α-syn by *in vivo* voltammetry and *in vivo* microdialysis, respectively. MPD-induced increase in stimulated dopamine overflow was attenuated in mice lacking α-syn. During burst stimulation, MPD (1 mg/kg) enhanced facilitation of dopamine release in the wild type mice but decreased it in mice lacking α-syn to the levels found in the untreated wild type mice. A comparison of equipotent doses (in terms of increasing the extracellular half-life of DA) of MPD (5 mg/kg) and a specific dopamine reuptake inhibitor GBR12909 (10 mg/kg) revealed that MPD augments DA overflow not only by inhibiting reuptake but also by increasing DA overflow. MPD (5 mg/kg) preserved while GBR12909 eliminated the facilitation of dopamine overflow in response to repetitive burst stimulation. No difference was observed between three lines in MPD-induced increase in the basal extracellular DA levels. We conclude that MPD and α-syn share the same pathways regulating dopamine overflow, which involve redistribution of vesicles in dopamine storage pools.

MO04-03

EFFECT OF TYROSINE HYDROXYLASE ON α-SYNUCLEIN AGGREGATION

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Parkinson's disease (PD) is a common neurodegenerative disorder affecting 1% of the population over 60. Major pathological features of PD include the loss of the dopaminergic neurons in the substantia nigra and the development of intracytoplasmic inclusions known as Lewy bodies. α-synuclein (α-Syn) is a major component of the Lewy bodies. α-Syn is normally a soluble protein but is found in a fibrillar form in the Lewy bodies. It is therefore thought that the aggregation of α-Syn is a key component in the development of PD. α-Syn is thought to go through a protofibril stage before being converted to the fibrillar form. The protofibrils are aggregates which are made up of multimers of α-Syn that are resistant to separation by sodium dodecyl sulphate and boiling (SDS-resistant). As tyrosine hydroxylase (TH) can directly bind to α-Syn we examined the effects of TH on α-Syn aggregation. TH induced the formation of an SDS resistant α-Syn multimer of 90–100 kDa. This complex was only found in aggregated α-Syn and not soluble α-Syn. Incubation of α-Syn with a series of control proteins or different forms of iron did not generate the 90–100 kDa α-Syn complex indicating that it was a specific effect of TH. When dopamine-bound TH was incubated with α-Syn there was a sixfold increase in the amount of the SDS resistant α-Syn complex compared to that when TH without bound dopamine was incubated with α-Syn. Incubation of α-Syn with dopamine alone did not generate the SDS resistant

α-Syn complex. This data suggests that the aggregation of α-Syn may be influenced by the presence of TH and therefore the aggregation process in TH containing neurons may be different from that in other neurons.

MO04-04

ASSESSMENT OF ALPHA-SYNUCLEIN SECRETION IN MOUSE AND HUMAN BRAIN PARENCHYMA

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Genetic, biochemical, and animal model studies strongly suggest a central role for alpha-synuclein in the pathogenesis of Parkinson's disease. Alpha-synuclein lacks a signal peptide sequence and has thus been considered a cytosolic protein. Recent data has suggested that the protein may be released from cells via a non-classical secretory pathway that is mediated, at least in part, by exosomes. As such, it may therefore exert paracrine effects in the extracellular environment. However, proof that alpha-synuclein is actually secreted into the brain extracellular space *in vivo* has not been obtained. We developed a novel highly sensitive ELISA in conjugation with an *in vivo* microdialysis technique to measure alpha-synuclein in brain interstitial fluid. We show for the first time that alpha-synuclein is readily detected in the interstitial fluid of both alpha-synuclein transgenic mice and human patients with traumatic brain injury. Our data suggest that alpha-synuclein is physiologically secreted by neurons *in vivo*. This interstitial fluid pool of the protein may have a role in the propagation of alpha-synuclein pathology and progression of Parkinson's disease.

MO04-05

DEVELOPING NEW DRUGS FOR PARKINSON'S DISEASE

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Aims: A disease-modifying treatment for Parkinson's is required to prevent or slow nigral degeneration. PBT434 was developed from a library of novel, redox-silencing, orally-bioavailable, brain-penetrating compounds screened for their ability to preserve neuronal viability and motor function in wild type and A53T alpha-synuclein transgenic mice challenged with nigral toxins.
Methods: To determine whether this class of drug might be effective when SN degeneration was already advanced, the drug was administered when cell death cascade is established by either 6-OHDA or MPTP. Efficacy was assessed by stereological counts of nigral cells, tyrosine hydroxylase expression and nigrostriatal terminal density and rotational behaviour in response to amphetamine challenge (6-OHDA) or Pole test (MPTP).
Results: Treatment with PBT434 resulted in significant preservation of tyrosine hydroxylase positive SN neurons and significantly improved motor function in 6-OHDA and MPTP-treated wild-type mice and MPTP-treated A53T animals compared with lesioned, untreated animals. PBT434 prevented the MPTP mediated elevation in alpha-synuclein and nigral iron and significantly increased levels of the redox sensitive chaperone PARK 7 (DJ-1) and PARK 1 (alpha synuclein).
Discussion: The ability of PBT434 to preserve nigral viability in both MPTP and 6-OHDA models after the neurotoxic cascade and its effects upon major disease-associated biomarkers make it an authentic candidate as a disease-modifying drug. Because PBT434 does not interact with elements of the dopamine anabolic or clearance pathways it offers a differentiated therapeutic strategy that may treat a broad spectrum of patients, from early onset to those more advanced disease, including those on L-Dopa medication.

MO04-06

DJ-1 OXIDIZED AT C106 INHIBITS P53-ACTIVATED DUSP1 EXPRESSION BY SEQUESTERING P53 FROM THE PROMOTER

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DJ-1 is an oncogene and causative gene for familial Parkinsons disease. Although oxidative status of DJ-1 at cysteine at 106 (C106) is thought to affect all of the activities of DJ-1 and excess oxidation leads to onset of various diseases, the precise molecular mechanisms underlying the effects of oxidation of DJ-1 on protein-protein interaction of DJ-1 remain unclear. In this study, we found oxidative stress-dependent enhancement of DJ-1-p53 complex formation. DJ-1 bound to the DNA-binding region of p53, and C106 of DJ-1 was essential for its binding. When the expression levels of p53-target genes in mouse (DJ-1 (+/+)) primary cells treated with H_2O_2 were examined, the expression of the dual-specific threonine and tyrosine phosphatase (DUSP1) gene was first induced at a peak of 30 min and that of the p21 gene was observed at 2 h after H_2O_2 addition. DUSP1 and p21 promoters harbor non-consensus and consensus p53-recoginition sequences, respectively. Furthermore, expression level and promoter activity of the DUSP1 gene, but not those of the p21 gene, were further increased in H_2O_2-treated (DJ-1 (-/-)) primary cells and decreased in wild-type DJ-1- but not C106S DJ-1-transfected H1299 cells. Chromatin immunoprecipitation assays showed that the DJ-1-p53 complex stably bound to the p21 promoter but not the DUSP1 promoter and that wild-type DJ-1 but not C106S DJ-1 sequestered p53 from the DUSP1 promoter. These results indicate that DJ-1 inhibits the expression of a p53-target gene(s) that contains a non-consensus p53-binding region in an oxidation of C106-dependent manner.

MO04-08

ROLE OF DJ-1 AS AN ANTI-OXIDATIVE STRESS SENSOR

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DJ-1, a Familial Parkinson's disease related-protein, plays roles in transcriptional regulation, protease, regulation of mitochondrial complex I and oxidative stress function. Parkinson's disease (PD) is characterized by progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta and effects 1–2% of people over the age of 60 years. Oxidative stress contributes to the cascade leading to dopaminergic neuron degeneration in PD.

DJ-1 has three cysteines at residues 46, 53 and 106 amino acid from the N-terminal. In particular, superfluous oxidation of cysteine at amino acid 106 (C106) of DJ-1 renders DJ-1 inactive, and such oxidized DJ-1 has been observed in patients with the sporadic form of PD. The underlying mechanism of DJ-1 oxidation process, however, remains unclear.

Therefore, we first examined the effect of nitric oxide on DJ-1 oxidization. As a result, an oxidized form of DJ-1 was increased by a NO donor. C106 of DJ-1 was shown to be the most sensitive cysteine residue toward NO-mediated oxidation.

Furthermore, anti-oxidative stress function of DJ-1 also remains unclear. We studied the effect of Peroxiredoxin (Prdx) family proteins, the antioxidant enzymes, on anti-oxidative stress function of DJ-1. We found that DJ-1 is associated with Prdx4 and Prdx6, and that this interaction leads to reduction of DJ-1.

MO04-09

MODULATION OF THE DOPAMINE TRANSPORTER BY BETA-SYNUCLEIN AND GAMMA-SYNUCLEIN

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The synuclein proteins, including α-synuclein (α-Syn), β-synuclein (β-Syn), and γ-synuclein (γ-Syn), have been associated primarily with a role in neuropathology. The normal, physiological function of the synucleins, however, has remained elusive. Recent work has established a role for synucleins in modulating neurotransmission through various pre-synaptic mechanisms, including regulated trafficking of the monoamine transporters. It has been shown previously that α-Syn modulates trafficking of the transporters of dopamine (DAT), norepinephrine (NET), and serotonin (SERT). Also, β-Syn modulates NET, while γ-Syn modulates both NET and SERT, and may do so by a mechanism distinct from the cytoskeleton-dependent trafficking employed by α-Syn. Modulation of DAT by β-Syn and γ-Syn, however, has not been analyzed. We show here that β-Syn and γ-Syn are also capable of modulating DAT. We found that, like α-Syn, when either β-Syn or γ-Syn is co-expressed with DAT at high ratios of co-transfection, dopamine (DA) uptake is reduced. Reduced uptake is not associated with a decrease in total expression of DAT, suggesting that the decrease is due instead to altered distribution of DAT. Fractionation of co-transfected cells illustrates the distribution of DAT upon co-expression with β-Syn and γ-Syn. Confocal microscopy of co-transfected cells indicated that α-Syn, β-Syn, and γ-Syn all co-localize with DAT. Intensity correlation analysis shows that each pair is co-distributed in a region-specific manner, with the strongest co-localization occurring in a peri-nuclear compartment. Live-cell imaging experiments demonstrate the trafficking of DAT in the presence or absence of each synuclein. Co-immunoprecipitation assays confirmed an interaction between α-Syn and DAT, but a similar interaction between DAT and either β-Syn or γ-Syn could not be detected. Taken together, our results indicate both β-Syn and γ-Syn are capable of modulating DAT trafficking, and suggest that this modulation may occur by a mechanism distinct from α-Syn modulation of DAT.

MO04-10

SUPPRESSION OF NURR1 EXPRESSION BY SYNUCLEIN UNDERLIES THE SELECTIVE VULNERABILITY OF DOPAMINERGIC NEURONS IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is one of the most common neurodegenerative disorders, characterized by a relatively preferential loss of midbrain dopaminergic (mDA) neurons. Up to present no mechanism-based treatment is available and this could mainly be attributed to our limited knowledge on the events leading to the relatively selective degeneration of mDA neurons. Genes linked to PD allowed the generation of animal models that have been quite useful in dissecting the pathogenesis of PD. A number of genetic and pathological studies point out a prominent role of α-syn in PD; however, no current α-syn transgenic mice present substantial loss of mDA. Therefore, we decided to model the PD-related A53T α-synuclein (α-syn) in transgenic mice by selectively driving its expression in the mDA neurons. These mice developed profound motor disabilities as well as robust and progressive mDA neurodegeneration, resembling key clinical and neuropathological phenotypes of PD. In an attempt to appreciate the molecular events leading to α-syn-dependent phenotypic outcome, we found a progressive reduction of Nurr1 protein expression in the nucleus of a subset of mDA neurons expressing A53T α-syn, which correlated with decreased expression of Nurr1-controlled DA marker proteins. Nurr1 is a key transcription factor for the function and survival of mDA neurons and mutations of its gene were related to PD. The decrease in the Nurr1 levels was a crucial determinant of mDA neuronal loss since inhibition of its proteasome-dependent degradation ameliorated the degeneration of DA neurons. Together, our data suggest that α-syn-mediated suppression of Nurr1 levels may serve as a critical pathogenic mechanism of the selective vulnerability of mDA in PD, and regulation of Nurr1 expression may become a key therapeutic strategy for the disease.

MO04-11

THE ROLE OF SPHINGOSINE KINASE(S) IN EXPRESSION, SECRETION, AND TOXICITY OF ALPHA-SYNUCLEIN. IMPLICATION IN PARKINSON'S DISEASE

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Our previous data indicated that oxidative stress induced secretion of alpha-synuclein (ASN) from brain nerve endings

(synaptosomes). The presynaptic protein ASN is now accepted to be responsible for pathogenesis of Parkinson's disease (PD) and other synucleinopathies. However, the molecular mechanisms of ASN toxicity are not fully understood.

The aim of this study was to investigate the involvement of sphingosine kinases (SKs) in expression, secretion and toxicity of ASN. Moreover, the effect of extracellular ASN on the expression and activity of SKs in hippocampal neuronal HT22 and PC12 cells was evaluated. Our study carried out on synaptosomes isolated from brain cortex with hippocampus indicated that inhibition of SKs evokes ASN secretion into extracellular space and enhanced its liberation evoked by oxidative stress. In addition, inhibition of phospholipase D that participates in sphingolipids turnover leads to ASN secretion from brain synaptosomes. Oligomers of ASN (10 μM) that could be liberated into extracellular space by alteration of sphingolipid metabolism lead to changes in the expression of the proapoptotic protein from Bcl 2 family and induce apoptosis of HT22 and PC12 cells. Our results presented the pro survival role of SKs in these cells. Inhibition of SKs leads to apoptotic cell death and suppressed the NGF-induced differentiation of PC12 cells into dopaminergic neurons. However, inhibitor of SKs had no effect on the ASN mRNA level. The role of SKs in sequence of molecular events involved in death signaling evoked by extracellular ASN in HT22 and PC12 cells will be widely discussed. Summarizing, the alteration of sphingolipids metabolism by inhibition of SKs could be significantly involved in ASN translocation and toxicity.

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MO04-12

IDENTIFICATION AND ANALYSIS OF INTERACTION PARTNERS OF DLRRK2 IN DROSOPHILA MELANOGASTER

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Parkinson disease (PD) is the second most common neurodegenerative disease among the elderly population. Clinical characteristics are resting tremor, akinesia and rigidity. Pathological hallmarks are loss of dopaminergic neurons in the substantia nigra and presence of Lewy bodies. Most cases of PD are sporadic but several genes have been linked to inherited forms of PD. Among those, mutations in Leucine-rich repeat kinase 2 (LRRK2) appear to be most frequent. Some identified mutations in LRRK2 lead to elevated activity of its inherited kinase domain, thus suggesting a gain-of-function mechanism. Using genetic tools applicable in Drosophila, we are able to show, that the Drosophila homologue LRRK2 (dLrrk) is involved in the extracellular signal regulated protein kinase (ERK) pathway. We have identified several interaction partners of dLrrk and further characterization of them might lead to a better understanding about LRRK2-induced PD. In addition, we show that human LRRK2 phosphorylates MEK1 (upstream of ERK) *in vitro* and provide functional implication of LRRK2-induced ERK activation in cultured neuronal cells. Our results were corroborated by finding of elevated ERK activity found in dopaminergic neurons of PD brains.

Taken together, the results from our fly experiments strongly propose a link between LRRK2-mediated ERK activation and PD.

MO04-13

TARGETING THE CMA PATHWAY IN NEURONAL CELLS MITIGATES ALPHA-SYNUCLEIN INDUCED TOXICITY

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The presynaptic protein alpha-synuclein (AS) plays a crucial role in the pathogenesis of Parkinson's Disease (PD). However, how this leads to nigral degeneration, remains elusive. Alpha-synuclein protein levels are considered as a major determinant of its neurotoxic potential. Nevertheless, the manner of AS degradation in neurons remains controversial. Our previous data suggest that a major pathway for AS clearance in neuronal cells is Chaperone-Mediated Autophagy (CMA). We have also shown that CMA inhibition via down-regulation of it's rate-limiting step, the receptor LAMP-2A, led to accumulation of aberrant AS species in neuronal cells (Vogiatzi et al., 2008). Furthermore, we have recently shown that the neurotoxic effects of aberrant AS in neuronal cell cultures are in part mediated by CMA inhibition (Xilouri et al., 2009). In the present study we have investigated whether enhancement of CMA activity by increasing lysosomal LAMP-2A abundance could facilitate the clearance of endogenous or overexpressed AS in neurons, and how this might be associated with the restoration of lysosomal function and prevention of neuronal death. For this purpose we have generated stable human SH-SY5Y neuroblastoma cell lines over-expressing LAMP-2A. Our data show that SH-SY5Y cells overexpressing LAMP-2A upregulate the CMA pathway and they also display an overall improvement of total lysosomal function. Turnover of endogenous AS is enhanced in the cell lines overexpressing LAMP-2A and most importantly these lines are protected against adenoviral-mediated AS-induced neurodegeneration. Interestingly, this neuroprotection is selective, as cells that overexpress LAMP-2A are not protected against MPP^+ or camptothecin-induced toxicity. Our study suggests a close relationship between LAMP-2A levels and AS clearance and toxicity in neurons, supporting the hypothesis of boosting LAMP-2A expression as a potential therapeutic target for the treatment of PD or other related synucleionapathies.

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MO05 Neurological Dysfunction

MO05-01

EYE-TO-FOOT COORDINATION IN STANDING CERVICAL DYSTONIA PATIENTS

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Idiopathic spasmodic torticollis (ST), the most common form of focal dystonia, is characterized by abnormal head posture, with the chin deviating frequently towards the shoulder on one side. ST is considered to represent a basal ganglia disorder affecting both sensory and motor systems. Previous studies have dealt with various aspects of gaze displacement, head movement and eye-head coordination when sitting. The present measurements include movements of the trunk and feet during more or less natural voluntary pivot turns. Ten ST patients and 10 healthy adults, (mean age 58.3 ± 11 and 52 ± 2.6 years) volunteered for the study. Participants were required to stand in the centre of a circular array of lights (LEDs) in darkness. They fixated their gaze on and align their bodies with a centrally located LED. After a delay of 10s the central LED extinguished cueing the participant to rotate his whole body in order to align it with a second LED that lit up in one of seven eccentric locations (45, 90 and 135 degrees either right or left of centre as well as at 180 degrees). After an additional time interval of 15 s the eccentric LED was turned off cueing the subject to return back to the initial position. Head, upper body, and feet horizontal movements were recorded using a motion analysis system, while horizontal eye in head rotations were recorded using electro-oculography. Latencies of all segments were prolonged in patients. However, prediction reduced the latencies mainly for the foot and trunk such that the segments moved more or less en bloc, just as in controls. Head-on-trunk and trunk-in-space peak velocity were reduced in patients both contra- and ipsilaterally to the torticollis. Our measurements of this multisegmental movement, common in everyday life, reveal that cervical dystonia is not simply a disorder of head posture; it represents a generalised movement disorder with prolonged initiation times and reduced peak velocity.

MO05-03

STUDY OF NMDA RECEPTOR NR2A SUBUNIT AND PSD-95 EXPRESSION AFTER THE ADMINISTRATION OF THE CONVULSANT 3-MERCAPTOPROPIONIC ACID

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NMDA receptors play an important role in synaptic plasticity and participate in the initiation and/or propagation of epileptic discharges. NMDARs are heteromeric complexes of different subunits and most of the NMDARs function as tetramer assemblies of two glycine-binding NR1 and two glutamate-binding NR2 subunits. The complex of proteins NR1/NR2A interacts with many of the synapse-associated proteins, including the protein postsynaptic density 95 (PSD-95). These interactions may be playing a crucial role in epilepsy. The objective of this work is to study the effect of the administration of the convulsant drug 3-mercaptopropionic acid (MP), as an experimental epileptic model, and the adenosine analogue cyclopentyladenosine (CPA) on NR2A and PSD-95 expression. Methods: Wistar rats (250-300g) were daily injected with MP 45 mg/kg i.p. or CPA (2 mg/kg) or CPA 30 min previous MP (CPA + MP) during 4 days. Control rats were injected with saline. Western blot assays on the whole hippocampal tissue showed a significant increase in NR2A and PSD-95 expression (47–53%) with respect to control after 4 days of MP administration. CPA previous MP recovered the normal values and after CPA administration a tendency to reduce NR2A and PSD-95 expression was observed. Immunoassays of NR2A and PSD-95 showed hippocampal pyramidal layer staining. These results suggest an excitotoxic effect due to MP-induced seizures and a protective effect of cyclopentyladenosine.

MO05-04

PKCε REGULATES THE NUCLEOCYTOPLASMIC SHUTTLING OF NEUROFIBROMIN IN POST-MITOTIC NEURONS

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Neurofibromatosis 1 (NF1) is an autosomal dominant, progressive disorder and its main clinical features include learning difficulties and development of benign and malignant tumors of the nervous system; yet, the current knowledge on the function of the NF1 gene product, the RasGAP neurofibromin, remains very limited. Neurofibromin also contains a functional NLS and we have previously reported its PKC phosphorylation-dependent subcellular shuttling in neuroblastoma cells. In chick embryo neurons, *in vivo* and in culture, this PKC substrate is highly detected in the nucleus and we have begun to address the mechanism of it's nucleocytoplasmic shuttling in this post-mitotic environment. For this, we used a dephosphorylation assay and a phospho-sensitive antibody (JNC 2009;109:573–83) to detect neurofibromin's relative abundance in subcellular fractions. As PKCε is the primary isoform in these neurons, we treated the cultures with the PKCε-selective peptide inhibitor (εV1-2) and activator (ψεRACK) and found that PKCε-induced phosphorylation increased the abundance, but not the synthesis of the protein, both in the nucleus and the cytoplasm, in a time dependent manner. More importantly, we found that PKCε-phosphorylated neurofibromin was insensible to CRM1-dependent export and thus retained in the nucleus. These effects were further enhanced in the presence of phosphatase inhibitors like calyculin A. Further investigations showed that the PKCε-dependent stabilization was proteasome-independent. Specifically, we observed significant increase of nuclear neurofibromin levels, upon treatment with proteasome inhibitors, like MG-132, which, however, rescued only the pool of neurofibromin that was not phosphorylated at least on Ser2808, a PKC specific site. Our results suggest that a pool of neurofibromin is proteasomally degraded inside the nucleus of post-mitotic neurons, and that neurofibromin shuttles between the nucleus and the cytoplasm in a dynamic and PKCε-regulated manner for roles possibly related to cargo transport (PENED 03ED778).

MO05-05

FREQUENCY OF THE FORGETFULNESS AMONG THE CELLULAR PHONE USERS: A RISK ASSESSMENT

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There is widespread public concern about the potential adverse health effects of cellular phones in general. The human brain and the way in which it stores and retrieves information is a subject of debate and speculation. There are many possible causes of memory loss or forgetfulness; some factors may be like tiredness, concentration problem, pregnancy, general anxiety, normal aging and more serious causes are Alzheimer's disease, Parkinson's disease, depression, emotional problems, chronic alcoholism etc. But, Can the cellular phone irradiation be a cause of forgetfulness? This study was particularly designed to investigate the possibilities of association of the common symptom 'forgetfulness' among the cellular phone users. The close proximity of the cellular phone to the head causes likely 40–60% energy emitted by the device to be absorbed in the brain. We conducted a survey among the 188 cellular phone users in Lucknow city of India to assess the possibilities of forgetfulness. All subjects (77.7% male and 22.3% female) were enquired about general profile through a well designed questionnaire (age, sex, income, education etc.), calls detail and general health (disease, never smoker or smoker). The subjects belonged to 14–62 years age range (mean age ± SD; 29.1 ± 9.5). Study revealed that 12.7% (14) subjects were reported their association to forgetfulness symptom. The frequency of forgetfulness among high user (HU>500 h use in life) and low user (LU<500 h use in life) was found 2.1% and 10.6% respectively. In general it was found that the users, who held cellphone above the 4–5 years, were supplementary associated to the symptom of forgetfulness. The present study is of significance and need for more laboratory findings on cellular and molecular level to better understand how cellular phone irradiation may alter Central Nervous System functioning. We acknowledge to UPCST, Lucknow, India for financial support.

MO05-06

AGING INCREASES ANGIOGENESIS-RELATED GENES IN RAT STRIATUM

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Angiogenesis has been reported to be impaired in old age. There are controversial studies regarding angiogenesis-related genes in striatum, a basal ganglion of the brain that is particularly affected during aging. Vascular endothelial growth factor (VEGF) is known as the major inducer of angiogenesis and plays a key role in new vessel growth. This work is addressed to clarify this current controversy, investigating the expression of two angiogenesis-related genes, VEGF and adrenomedullin, as well as the number of blood vessels in the striatum of adult and aged rats. The study was performed on adult (4–5 months old) and aged (24–25 months old) Wistar rats. The expression of VEGF and adrenomedullin was determined by RT-PCR (TaqMan gene expression assays). For vessel labelling, lectin expression and location were analyzed using both immunohistochemical and image processing techniques (fractal dimension). The results showed that both VEGF and adrenomedullin mRNA expression rose in aged rats in comparison with the adult ones. On the other hand, the quantification of blood vessels in the striatum of adult and aged rats showed no significant differences between groups. The results demonstrate that the angiogenesis process, analyzed by quantification of the number of blood vessels in the rat striatum, was not affected in old individuals vs. the adult ones. However, aging boosted angiogenesis-related genes (VEGF and adrenomedullin) in this basal ganglion of the brain, suggesting that molecular mechanisms at transcriptional level could affect the angiogenesis process in the striatum of aged rats.

MO05-07

NITRIC OXIDE PATHWAY IS ALTERED DURING AGING IN RAT STRIATUM

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The striatum is a basal ganglion of the brain that is particularly involved in neurodegenerative processes, most of them being frequently detected in old age. Nitric oxide (NO), synthesized by the hemoproteins NO synthases (NOS), has been widely involved in the oxidative injury that takes place during aging. In order to elucidate the role of the NO pathway in aging, we aim to analyze the expression and activity of NOS isoforms, as well as NO production in the striatum of adult and aged rats. The study was performed on adult (4–5 months old) and aged (24–25 months old) Wistar rats. In situ NOS activity (NADPH-diaphorase), as well as NOS isoforms (neuronal, endothelial, and inducible NOS) expression (RT-PCR) were analyzed in the striatum of adult and aged rats. Also, NO production was indirectly quantified by measuring nitrate/nitrite and S-nitrose compounds (NOx). The results showed that eNOS, iNOS, and nNOS mRNA expression increase in the aged group vs. the adult group, although this increase was statistically significant only for iNOS, and nNOS. NADPH-d activity was detected in some striatal neurons in both age-groups. The intensity of the staining in the sections from the old group show no slightly changes. NOx levels significantly decreased in aged rats in comparison with the adult group. These results showed interesting data, reflecting that the NO pathway is altered during aging in the rat striatum. In fact, although NOS isoforms expression increased during aging in this basal ganglion of the brain, NO decreased, and in situ NOS activity was not affected by aging. It seems to be molecular mechanisms, acting in the functioning of the NO/NOS system in the striatum of aged rats.

MO05-08

PROLONGED DECREASE IN THE EXPRESSION OF CONNEXINS IN THE COCHLEAR SPIRAL LIGAMENT FIBROCYTES FOLLOWING INTENSE NOISE EXPOSURE

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It is well known that noise-induced hearing loss results mainly from enhancement of oxidative stress and mitochondrial damage. In

particular, oxidative stress is thought to produce hair cell death in organ of Corti and dysfunction of lateral wall structure. Accumulating evidence indicates that gap junction pathway in cochlear spiral ligament fibrocytes (SLFs) plays a critical role in hearing system. The mutation in the gap junction beta 2 (GJB2)-encoding gap junction protein connexin26 (Cx26) and GJB6 encoding gap junction protein connexin30 (Cx30) are related to hereditary non-syndromic deafness through lack of gap junction pathway for the recycling of K^+ in the cochlear endolymph. In this study, we evaluated the expression of Cx26 and Cx30 during hearing loss induced by intense noise exposure. Adult male Std-ddY mice were exposed to 8 kHz octave band noise of 110 dB SPL for 1 h. The noise exposure produced a dramatic threshold shift at frequencies of 4, 12, and 20 kHz. 4-Hydoroxy tempo (tempol, ROS scavenger) was significantly reduced the threshold shift on day 2 to 7 post-exposure. Noise exposure decreased the level of Cx26 in the SLFs at least 2 h to 7 days post-exposure. In addition to Cx26, Cx30 level in the SLFs was decreased at day 7 post-exposure. Noise-induced reduction in Cx26 expression was completely abolished by pre-treatment with tempol. Taken together, our data suggest that intense noise exposure produced prolonged decrease in the expression of Cx26 and Cx30 in the cochlear SLFs. Furthermore, noise-induced hearing loss is at least a part due to dysfunction of gap junction in the SLFs through oxidative stress-induced down-regulation of Cxs.

MO05-09

EXOGENOUS PYRUVATE SUPPRESSES EPILEPTOGENESIS

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Many types of epilepsy of varied etiologies involve disruptions of brain energy homeostasis. Brain energy demands are extremely high and metabolic stress or failure is a key feature and a direct contributor to epileptogenesis. The aim of the present study was to explore whether administration of exogenous energy substrates may downgrade epileptogenesis after establishment of status epilepticus. Three groups of adult Wistar rats were studied: control, epileptic and epileptic with addition of pyruvate to the diet after status development (135 mg/day/rat). Status epilepticus was induced by the intraperitoneal pilocarpine injection. The EEG and biochemical parameters of hippocampus were analyzed after 3 months of the status onset. Experiments showed that addition of pyruvate to the diet significantly reduced the EEG pathology manifestation: no animals with unmistakable seizures or sharp waves were observed in this group. Moreover, in this group, a photostimulation (8 Hz) revealed similar changes in hippocampal EEG as in control rats while in epileptic rats the response to photostimulation disappeared. Comparative analysis of hippocampal homogenates from control and epileptic rats by NMR revealed a large decrease in the N-acetylaspartate concentration (NAA, marker of neuronal disfunction in epilepsy). This fact is in a good agreement with the data obtained by MRT in humans (Vermathen et al., 2003) and in rats (Tokumitsu et al., 1997). However, in rats fed on the diet with addition of pyruvate, NAA concentration was within the physiological range.

Our results suggest that metabolic crisis may be one of the major reasons for neuronal hyperexcitability and seizure development. Compensation of metabolic deficiency by administration of exogenous energy substrates offers a new approach to treat epilepsy.

MO05-10

A MOUSE MODEL OF CARDIAC DYSFUNCTION WITH DECREASED CHOLINERGIC NEUROTRANSMISSION SHOWS TRANSCRIPTIONAL ALTERATIONS IN THE HEART

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Cardiomyopathies are very common throughout the world and can lead to cardiac dysfunction and heart failure. In many cardiovascular diseases, decreased parasympathetic and increased sympathetic tone is seen prior to compensatory cardiac remodeling. Previous work from our laboratory indicated that genetically-modified mice with reduced cholinergic tone present heart dysfunction and substantial remodeling of cardiomyocytes. In this study, we used mice with reduced expression of the vesicular acetylcholine transporter (VAChT), and consequently reduced cholinergic tone, to understand the role of cholinergic neurotransmission in cardiac dysfunction. In order to analyze whether a long-term decrease in parasympathetic tone can lead to alterations in myocardial gene expression, microarray analyses were performed in hearts and cardiomyocytes from VAChT mutant mice. The gene ontology analysis revealed alterations in pathways related to several biological processes in cardiac tissue and myocytes. A list of genes whose expression is altered in both was generated and their expression was re-analyzed through qPCR. Real-time PCR data confirmed transcriptional alterations in many genes, including serine protease inhibitor A3N (Serpina3n), an ECM remodeling protein, in both the hearts and myocytes of mutant mice. Interestingly, gene expression changes found in cholinergic-deficient mice could not be detected in mice chronically treated with isoproterenol, a β-adrenergic agonist which induces cardiac remodeling through its sympathomimetic effects. This suggests that the gene expression changes observed in the VAChT mutants are related to decreased cholinergic neurotransmission and not simply the result of imbalance between sympathetic and parasympathetic tone.

MO05-11

ALTERATIONS IN LOCAL THYROID HORMONE METABOLISM AND BEHAVIOR IN THE SENESCENCE-ACCELERATED SAMP8 MICE

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The senescence-accelerated mouse (SAM) strains which consist of senescence-prone (SAMP) and senescence-resistant (SAMR)

strains have been established through selective inbreeding of the AKR/J strain based on phenotypic variations of accelerated aging. Among them, SAMP8 strain shows age-related deficits in learning and memory with AD-like characteristics. Since thyroid disorders have been linked to various psychiatric and neuropsychological disorders including learning deficits, impaired attention, anxiety and depression, we examined whether brain thyroid hormone (TH) metabolism is involved in the pathological aging of SAMP8 mice. Compared with the senescence-resistant SAMR1 mice at different ages (1, 3, 5, 8, & 10 months [M]), SAMP8 mice showed progressive deficits in learning and memory in the passive avoidance test starting at 5M of age as well as hyperactivity at 1, 3, 5, 8 M and lower anxiety at 3 and 5 M in the open-field test. Plasma levels of thyroxine as well as thyroid stimulating hormone were comparable in both strains at all time points, indicating that the overall thyroid status was not altered in SAMP8. However, the expression of thyroid hormone metabolizing enzymes in the hippocampus showed significant differences between the two strains. In SAMP8, deiodinase 2 which converts T4 into active T3 was down-regulated at 1, 3, 5, and 8 M, while T3-degrading enzyme deiodinase 3 tended to be up-regulated. Expression of the two known TH-dependent genes was significantly down-regulated in SAMP8; *hairless* at 1, 3 & 5 M, and *myelin basic protein* (*mbp*) at 1, 3, 5, 8 & 10 M. MBP protein was further confirmed to be decreased in SAMP8. The results thus suggest a decrease in active T3 leading to hypomyelination in the SAMP8 hippocampus during maturation. Alterations in local TH metabolism may thus underlie behavioral abnormalities as well as the pathological aging of SAMP8.

MO05-12

EFFECT OF *Centella asiatica* ON NEURONAL ACTIVATION IN THE SUPRAOPTIC NUCLEI OF ADULT AND AGED MALE RATS

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Psychological problems found in aging, such as stress, anxiety and aggressive, might be related to oxytocin (OXT) and arginine vasopressin (AVP) functions. Neurohypophysial concentrations and plasma basal levels of OXT and AVP, number of AVP and OXT neurons, and mean areas of AVP, but not OXT, neurons in the supraoptic nucleus (SON) were decreased in aging. Since *Centella asiatica* (*C. asiatica*) has anti-stress, anxiolytic and anti-aggressive activities. We, therefore, studied the effect of *C. asiatica* on the SON neuronal activation in adult and aged rats. Adult male Wistar rats (8 weeks old) received DDD water (1 ml/kg, p.o., $n = 5$) and 1 g/ml/kg *C. asiatica* ($n = 5$). Aged male Wistar rats (8 months old) received DDD water (1 ml/kg, p.o., $n = 6$), 1 g/ml/kg and 2 g/ml/kg *C. asiatica* (p.o., $n = 7$ each). One hundred and thirty min later, the rat brains were fixed, removed and sectioned. Neuronal activity in the SON was determined by Fos immunohistochemistry. *C. asiatica* could induce Fos-immunoreactive (IR) neurons in the SON in both adult and aged rats. In adult rats, significant increase in Fos expression was found in 1 g/kg/ml *C. asiatica* treated rats compared to control (76.4 ± 36.77 vs. 0 Fos-IR neurons/section, $p < 0.05$). In aged rats, Fos expression in 1 and 2 g/kg/ml *C. asiatica* treated rats was not significantly different from that in control rats but tended to increase in a dose dependent manner. No significant difference between adult control rats and aged control rats, but a significant difference was observed between adult and aged rats treated with 1 g/kg/ml *C. asiatica* (76.4 ± 36.77 vs. 12.67 ± 6.46 Fos-IR neurons/section, $p < 0.05$). Anti-stress, anxiolytic and anti-aggressive effects of *C. asiatica* might be related to oxytocin and/or vasopressin within the SON. *C. asiatica* may be used as a promising anti-stress, anti-aggressive and anxiolytic agent for both healthy adults and aging in the future.

MO05-14

INDUCTION OF FOS EXPRESSION IN THE RAT HYPOTHALAMIC AREAS CONTROLLING FOOD INTAKE BY *CENTELLA ASIATICA*

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Centella asiatica (*C. asiatica*) is a medicinal plant that contains several active compounds such as flavonoids (rutin and quercetin) and triterpenoids (betulinic and asiatic acids) that can prevent obesity by reducing food intake and body weight. Therefore, this study was focused on the effect of *C. asiatica* on Fos expression, a marker for neuronal activation, in the paraventricular (PVN), ventromedial hypothalamus (VMH) and arcuate nuclei (ARC) which are neuronal nuclei in the hypothalamus that involve a regulation of food intake and energy homeostasis. Adult male Wistar rats were orally administered *C. asiatica* extract (1 g/kg/ml, $n = 5$) or DDD water (1 ml/kg, $n = 6$). Ninety minutes after each treatment, brains were removed and Fos expression was assessed in the PVN, VMH and ARC using immunohistochemistry. No Fos-immunoreactive (Fos-IR) neuron was observed in these areas in control rats, while Fos- IR neurons were observed in the PVN and the ARC, but not in the VMH, in rats treated with *C. asiatica*. In comparison to control, *C. asiatica* induced significantly higher in the number of Fos-IR neurons in the ARC (19.35 ± 9.07 Fos-IR neurons/section, $p < 0.05$), no significant difference was found in the PVN. Surprisingly, ARC neurons were activated by *C. asiatica* in lateral and dorsal parts of this nucleus. The ARC contains two groups of neurons: neuropeptide Y and agouti-related peptide (NPY/AGRP) neurons, and pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript (POMC/CART) neurons. The orexigenic NPY/AGRP neurons are more concentrated in the medial ARC, while anorexigenic POMC/CART neurons are more concentrated in the lateral ARC. Hence, *C. asiatica* might reduce food intake and body weight by activating the lateral and dorsal ARC neurons. *C. asiatica* could be a new alternative agent for prevention of obesity. Further studies are required to clarify which neurons (NPY/AGRP or POMC/CART neurons) are activated by *C. asiatica*.

MO05-15

CHARACTERIZATION OF A LAMININ RECEPTOR PROTEIN AS A JAPANESE ENCEPHALITIS VIRUS PUTATIVE RECEPTOR ON MICROGLIAL CELLS

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Japanese encephalitis virus (JEV) a mosquito-borne flavivirus is a major cause of viral encephalitis in Asia. While the principle target cells for JEV in the central nervous system are believed to be neurons, microglia are activated in response to JEV infection and JEV antigens have been detected in microglial cells which may act as a long lasting virus reservoir. Viral attachment to the host cells is the first step of the viral entry process and is a critical mediator of tissue tropism. This study sought to identify molecules associated with JEV entry to microglial cells. Virus overlay protein binding assay (VOPBA) and liquid chromatography-mass spectrometry (LC/MS/MS) identified the 43 kDa laminin receptor precursor protein as a potential JEV binding protein, which was subsequently investigated for its role in JEV entry to mouse microglial BV-2 cells together with other possible candidate receptor molecules including Hsp70, Hsp90, GRP78 and CD4, CD14. In antibody mediated inhibition of infection experiments, both anti-laminin receptor and anti-CD4 antibodies significantly reduced virus entry (20-40% inhibition) while no inhibition of entry was observed in the presence of anti-Hsp70, 90 or GRP78 antibodies. Significant inhibition of virus entry (approximately 70%) was observed in the presence of lipopolysaccharide implicating a role for CD14 in JEV entry. These results suggest that multiple receptor proteins may mediate the entry of JEV to microglial cells.

MO05-17

THE CLINICAL EFFICACY OF NERVE GROWTH FACTOR (ANEWWAY) COMBINED NDT IN THE TREATMENT OF CEREBRAL PALSY

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Objective: To explore the Clinical effect of Nerve growth factor (Anewway) with Neurodevelopmental treatment (NDT) on rehabilitation of Cerebral Function in the children of cerebral palsy (CP). To provide a new way to study and theoretical basis for the clinical application of early intervention of Anewway and NDT for children with CP in order to promote their nerve repair.
Methods: Eight-Sixty of CP were randomly divided into a treatment group (46 cases) and a control group(40 cases), the treatment group with NDT and muscle injection of Anewway for 3 periods. The control group were treated only with NDT. Their clinical therapeutic effects and recoveries of brain lesion detected by MRI were investigated. The curative effect before and after Anewway and NDT for CP patients were quantitatively assessed by (GMFM)-88 items.
Result: The total effective rate was 88.1% in the treatment group better than 66.8%, of the control group.there being the significant difference($p < 0.01$). The total scores of GMFM-88 items in the treatment group was higher than the control group ($p < 0.01$).
Conclusions: Application of Anewway can benefit obviously the treatment of CP. Anewway and NDT can promote compensation of central nervous system. function in the children of cerebral palsy.

MO06 Cholinergic Transmission

MO06-01

MATERNAL DEPRIVATION IN RATS INDUCES MEMORY DEFICITS, MODIFICATION OF THE CHOLINERGIC SYSTEM AND PCREB AND PERK1/2 SYNTHESIS

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Here we investigated whether the cognitive deficits induced by maternal deprivation might be related to disruption of the cholinergic system and protein synthesis correlated with mnemonic process. The mothers were separated from their pups for 3 h per day from postnatal day 1 to 10. The dams were moved to a different cage and the pups maintained in the original home cage and transferred to a different room kept at 32°C. when adults, maternal-deprived (DEP) and non-deprived (N-DEP) male rats were subjected to some experiments: (i) The behavioral tests showed that DEP rats trained in inhibitory avoidance (IA) and Morris water maze (MWM) have cognitive deficits in both the tasks. (ii) DEP rats did not increase the phosphorylation of ERK and CREB after the training session in IA. Similarly, DEP rats that did not able to retain the reversed learning in the MWM showed an increasing in the protein phosphorylation measurement only 2 h after training. (iii) Pharmacological experiments using an acetylcholinesterase inhibitor shown that oral administration of galantamine (1 mg/kg) 30 min before training was able to reverse the cognitive deficit only in the IA of DEP. However, the galantamine (2 mg/kg) administered twice (180 and 30 min) before reversal learning in the MWM reversed the cognitive deficits of the DEP rats in this task. (iv) Using cholinergic agonists (nicotine 3.3 μg) or muscarine 10 mM) with 0.5 μl/size administered intra CA1 30 min before the session of training in IA showed that only the muscarine reversed the cognitive deficit of deprived rats, but in MWM, both nicotine and muscarine administered 30 min before the reversed learning were able to improve the memory in DEP rats. (v) Environmental enrichment after weaning shows efficient to reverse the cognitive deficit of DEP rats in the IA and MWM. So these memory impairments in DEP rats can be mediated by modification of the cholinergic and protein synthesis and reversed using cholinergic agonists or prevented by EE.

MO06-02

PRESYNAPTIC NICOTINIC AUTORECEPTORS OUTLYING RELEASE SITES FACILITATE CHOLINERGIC NEUROTRANSMISSION IN THE TORPEDO ELECTRIC ORGAN

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Previously, we have put in evidence the existence of α3β2-containing neuronal nicotinic receptors (nAChRs) mediating facilitation of ACh release from stimulated rat motor nerve terminals, which may operate as a presynaptic amplifier to increase the safety factor for neuromuscular (NMJ) transmission (Faria 2003). In this study, we used Torpedo marmorata electric organ as a NMJ homologous system to demonstrate real-time nAChR autofacilitation and assess the tridimensional relationship between ACh release sites and presynaptic nAChR that facilitate cholinergic neurotransmission. Pure Torpedo marmorata electric organ nerve terminals (synaptosomes) were isolated and ACh release evaluated directly by chemiluminescence. The nAChR agonist, 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP, 0.1 μM) raised the amount of ACh release (~30%,) induced by depolarizing agents, like veratridine (100 μM) or KCl (40 mM). The α3β2 nAChR antagonist dihydro-beta-erytroidine (DH-β-E, 3 μM) significantly attenuated DMPP facilitation indicating that a pre-synaptic α3/4β2-containing nAChR might be involved. Concomitantly, Torpedo marmorata electric organ stacks of electrocytes (prisms) were used as real-time detectors of synchronized ACh release by measuring compound evoked electroplaque potentials (EPP). The nAChR antagonists, DH-β-E and hexamethonium (HE, 100 and 1000 μM) reverted endogenous ACh release facilitation with a rank potency order of DH-β-E>>HE. We took advantage of prisms submillisecond resolution to evaluate the spatial relationship between nAChR and ACh release sites by incubating either the fast Ca^{2+}-chelator, BAPTA-AM (50 μM) or the ~400 times slower Ca^{2+}-chelator, EGTA-AM (50 μM). EPP amplitude of prisms incubated with EGTA rose 37 ± 14% while BAPTA decreased 20 ± 11%, indicating that ACh release sites are tightly coupled to voltage operated calcium channels. Conversely, EGTA and BAPTA decreased DMPP-induced EPP amplitude facilitation to 8 ± 1% and 9 ± 1%, respectively down from a 21 ± 2% facilitation obtained in the absence of chelators, implying that pre-synaptic nAChR and ACh release sites are not co-localized. This work was supported by FCT (FEDER funding, UMIB-215/94 and FSE-POPH-QREN)

Reference:

1. Faria et al., (2003). Synapse, **49**: 77–82

MO06-03

NICOTINE INDUCES THE RELEASE OF ENDOGENOUS BIOGENIC AMINES IN DROSOPHILA BRAIN VIA ACTIVATION OF α-BUNGAROTOXIN-SENSITIVE NACHRS

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Invertebrates, as vertebrates, respond to nicotine exposure displaying a set of behaviors including increased locomotion, stereotypy and behavioral sensitization. It is known that nicotine actions in vertebrates depend on the activation of nicotinic acetylcholine receptors (nAChRs), which regulate the release of Biogenic Amines (BAs). Here, we evaluated whether activation of nAChRs regulates the release of endogenous BAs in Drosophila brain. We also assessed the effect of chronic nicotine treatment on BA release and locomotor behavior.

Flies were treated with different concentrations of nicotine (up to 2 mg/mL). In the third and sixth day of treatment, locomotor activity was tested using negative geotaxis and hang assays. On the other hand, release of BAs was evaluated by chronoamperometry. The brains of flies were dissected out and placed in a recording chamber under constant superfusion (3 ml/min). Electrochemical detection of BAs outflow was done using a Nafion-coated carbon electrode connected to a computer-controlled chronoamperometric system (IVEC-10, Medical System Corp).

Fast nicotine application induces a transient, dose-dependent increase in the release of BAs (EC50 = 1.04 ± 0.204 mM). The nicotine-induced release of BAs is partially and reversibly blocked by the vertebrate homomeric nAChR blocker alpha-bungarotoxin (10 nM, 78 ± 12% inhibition of BAs release). On the other hand, results obtained in nicotine-treated flies show a decreased locomotor activity, which is accompanied by impairment in their 'hanging' ability. This is consistent with chronoamperometry results that show de-sensitization of the nicotine effect on BAs release (EC50 = 4.607 ± 1.143 mM).

Our results show that activation of alpha-bungarotoxin-sensitive nAChRs induces the release of BAs from fly brains, as it has been shown in vertebrate systems. Moreover, chronic nicotine treatment induces behavioral effects that are consistent with the desensitization of nAChRs in fly brain. Thus, our results support the hypothesis that, as in vertebrate brains, nAChRs regulate the release of neurotransmitters that modulate locomotor activity in Drosophila.

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MO06-04

STRIATAL CONDITIONAL VACHT KNOCKOUT MOUSE LINE REVEALS NOVEL ROLES OF ACETYLCHOLINE IN LOCOMOTION DOPAMINE-MEDIATED RESPONSE

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The central cholinergic system plays an important role regulating motor functions and behaviours, but the specific contribution of acetylcholine for behavioural functions remains poorly understood. Striatal cholinergic neurons express both VAChT and VGLUT3, therefore they are likely to co-release ACh and glutamate. Previous experiments have not been able to separate the contributions of these two transporters, as they used ablation of these neurons to probe for their role in behavioural functions. To address the specific role of acetylcholine in striatal functions we generated a striatal-selective VAChT-knockout mouse line using the Cre/lox system. Analysis of VAChT expression at the protein and mRNA levels shows deletion of VAChT in the striatum, but not in the hippocampus in VAChT-KO mice. In agreement with these data, acetylcholine release was abolished in the striatum, but not in the hippocampus. Interestingly, we found no alterations on object recognition memory, sensory gating and motor learning in striatal VAChT-KO mice, indicating that striatal ACh does not play a role in these behaviours. Because acetylcholine and dopamine usually play opposite roles in the striatum, we investigated if elimination of ACh release impacted on dopaminergic function and dopamine-dependent behaviours. Striatal VAChT-KO mice present an increase in mRNA levels of dopamine receptors D1R and D2R, but also on nicotinic alpha7 receptors (nAChα7R) and muscarinic receptor 4 (M4R), whereas M1R and M2R were unchanged. Despite these changes in receptor expression we did not detect any alteration in spontaneous locomotion in VAChT-KO mice. However, when these mice were treated with D1R or D2R agonists they showed increased response to both drugs. Contrary to previous experiments we did not find a role for ACh in spontaneous locomotor activity. Instead, elimination of ACh release seems to impact the expression of dopamine receptors. Striatal VAChT KO mice will be important to evaluate the specific roles of ACh in behaviour.

MO06-05

INTERACTIONS OF HUMAN β-AMYLOID AND MUSCARINIC TRANSMISSION IN YOUNG ADULT PPSWE/PS1DE9 MICE

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There is now general agreement that soluble oligomers of β-amyloid (Aβ) fragments might be the primary noxious entities driving pathogenesis of Alzheimer's disease. Transgenic APPswe/PS1dE9 mice are an appropriate model for studying *in vivo* effects of progressively increasing concentrations of human Aβ fragments on brain neuronal functions. Despite already increased concentrations of cerebral cortex soluble Aβ1-40 and Aβ1-42 in young female (7–10 weeks) transgenic animals we did not find any changes in the synthesis and release of either newly synthesized or previously stored acetylcholine from cortical slices, and in the density or activation of cerebral cortex muscarinic receptors. In young adult (5–7 months) transgenic mice, however, we found a decrease in depolarization-stimulated release of newly synthesized (but not previously stored) acetylcholine and rightward shift of carbachol potency and efficacy to activate GTP-γ35S binding in cerebral cortical membranes. The density of muscarinic receptors and VAChT protein was significantly reduced in transgenic mice in both cases by about 10%. These changes evidence both pre- and post-synaptic disorders that developed in transgenic mice in parallel with increasing concentrations of soluble Aβ fragments. Gene (mRNA) expression of individual receptor subtypes as well as ChAT and VAChT mRNA were not changed. These findings indicate that these early changes in cortical cholinergic synapses are not due to expression of respective genes but rather due to events at the posttranscriptional level. Relatively small decreases in muscarinic receptor protein concentration compared to a large functional deterioration of muscarinic transmission (decrease in both potency and efficacy of agonist-stimulation of GTP-γ35S binding) prompts usefulness of studies of molecular mechanisms of signal tranduction by muscarinic receptors. Supported by project AV0Z50110509 and grants IAA500110703, MSMT CR LC554, and EU project LipiDiDiet GA No211696.

MO06-06

WATER-SOLUBLE DOMAIN OF HUMAN LYNX1: NMR STRUCTURE, ACTION ON ACETYLCHOLINE RECEPTORS AND COMPARISON WITH THREE-FINGER NEUROTOXINS

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The identification of proteins expressed in the central nervous system that share the three-finger structure with snake α-neurotoxins

provoked much interest in the role of such molecules in brain functions. Prototoxin lynx1, the first identified member of this family, is membrane-tethered by GPI anchor which considerably complicates *in vitro* studies. We were able to develop an effective system for bacterial expression of water-soluble domain of human lynx1 lacking a GPI anchor (ws-lynx1). We report the NMR spatial structure of ws-lynx1 and its concentration-dependent activity on nicotinic acetylcholine receptors (nAChR). Exposure of Xenopus oocytes expressing the human α7 nAChRs to 1 μM ws-lynx1 enhanced the response to acetylcholine but no effect was detected on α4β2 or α3β2 nAChRs. Increasing the ws-lynx1 concentration to 10 μM caused a modest inhibition of these three nAChR subtypes and, similarly to lynx1, decreased the response to high concentrations of acetylcholine. At 5–30 μM ws-lynx1 competed with [^{125}I]-α-bungarotoxin for binding to the acetylcholine-binding proteins and to *Torpedo* nAChR. The weak positive allosteric interaction between ws-lynx1 and [^{3}H]-N-methylscopolamine was observed in pseudocompetition experiments at human M_3 muscarinic acetylcholine receptor (mAChR). The affinity of ws-lynx1 was relatively low (~ 3 μM), but this points to a possible existence of non-nAChR molecular targets for lynx1. The interactions with mAChR were previously described for some non-conventional three-finger snake neurotoxins. Computer modeling using the NMR structure of ws-lynx1 revealed potential interaction with nAChRs and possible differences in the binding modes of ws-lynx1 and snake neurotoxins.

MO06-07

INTERSTITIAL CELLS OF CAJAL CONTROL CHOLINERGIC NEUROTRANSMISSION IN THE TRIPARTITE MYENTERIC SYNAPSE BY RELEASING ADENOSINE

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Coordination of motor patterns in the gastrointestinal (GI) tract requires the interplay between neuronal firing and contraction of smooth muscle fibers, which activity is modulated by myenteric pacemaker cells known as Interstitial Cells of Cajal (ICC-IM) – tripartite myenteric synapse. Recently, we demonstrated that muscarinic M3 receptors facilitate acetylcholine (ACh) release from stimulated myenteric neurons through the release of endogenous adenosine (ADO) leading to activation of A2A receptors (Vieira 2009). Using confocal microscopy, we observed that ICC-IMs are highly immunoreactivity against M3 receptors, whereas A2A receptors are localized mainly in VAChT-positive cholinergic nerve terminals. The muscarinic agonist, oxotremorine (Oxo, 300 μM), increased [3H]-ACh and ADO release from stimulated longitudinal muscle-myenteric plexus (LM-MP) of the rat ileum. Blockade of M3 receptors with J104129 (6 nM) prevented Oxo (300 μM)-induced facilitation of [3H]-ACh and ADO release and partially attenuated LM-MP contractions elicited by Oxo (0.003–300 μM). Blockade of nerve action potentials and smooth muscle contractions with tetrodoxin (1 μM) and nifedipine (1 μM), respectively, was devoid of effect on ADO outflow facilitation caused by Oxo (300 μM). Mibefradil (3 μM), an inhibitor of T-type Ca^{2+} channels located predominantly in ICC-IM, decreased the Oxo-induced facilitation of ADO release by 97 ± 5% (n = 3). Mibefradil (3 μM) attenuated (~60%) spontaneous myographical recordings and the maximal tension caused Oxo (0.003–300 μM) in the LM-MP; Mibefradil (3 μM) also decreased Oxo (300 μM)-induced facilitation of [3H]-ACh release by a similar amount (22 ± 4%, n = 4) to that observed with J104129 (6 nM) or ZM241385 (50 nM, an A2A receptor antagonist). Data suggest that stimulation of M3 receptors mediate a positive feedback mechanism on evoked ACh release by increasing ADO outflow from ICC-IM leading to the activation of facilitatory A2A receptors on myenteric neurons. Supported by FCT (FEDER funding, PTDC/CVT/74462/2006).

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MO06-08

EFFECT OF NICOTINIC ACETYLCHOLINE RECEPTOR DESENSITIZATION ON NICOTINE SIGNALING IN PC12H CELLS

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Nicotine activates nicotinic acetylcholine receptors (nAChRs) in central nervous systems and affects neuronal functions in brains. A number of recent studies have shown that not only nAChR activation but also desensitization contribute to nicotine-induced behavioral effects. Because tobacco smoking may result in nAChR desensitization at low concentrations of nicotine, it is important to investigate cellular mechanisms of the desensitization and the subsequent effects on nicotine-inducing intracellular signaling. In the present study, we have investigated the effect of nAChR desensitization on phosphorylation of extracellular signal-regulated protein kinase (ERK) in PC12h cells. Nicotine at 0.1 mM transiently induced ERK phosphorylation. PC12h cells were pretreated with lower concetrations (0.1~20 μM) of nicotine for 2 or 24 h and subsequently stimulated with 0.1 mM of nicotine for 5 min. Pretreatment of nicotine reduced the nicotine-induced phosphorylation of ERK in a dose-dependent manner. Particularly pretreatment of nicotine at 20 μM cmpletely prevented the nicotine-induced ERK phosphorylation. Nicotine withdrawal after nicotine pretreatment recovered the nicotine-induced ERK phosphorylation. These results suggest that low concentrations of nicotine antagonize nAChR through nAChR desensitization in PC12 h cells.

MO06-09

RETROGRADE SIGNALLING BY ADENOSINE IS IMPAIRED IN MYASTHENIA GRAVIS: CROSS-TALK WITH NICOTINIC α3β2 AND MUSCARINIC M1 RECEPTORS

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Adenosine, via neuronal A2A receptors, controls neuromuscular transmission through a sophisticated interplay with cholinergic autoreceptors, which are either excitatory, like nicotinic α3β2 and muscarinic M1 receptors, or inhibitory, like the M2 receptor. Retrograde signalling mediated by adenosine released from skeletal muscle fibres is significantly impaired in α-bungarotoxin-induced Myasthenia gravis (TIMG) rats, thus causing profound changes in the control of neuromuscular transmission particularly during high frequency nerve stimulation (50 Hz-bursts) (Noronha-Matos 2011).

Most attempts to improve muscle weakness in myasthenic patients involve blockade of acetylcholine breakdown with cholinesterase inhibitors. The therapeutic benefit of cholinesterase inhibitors (e.g. neostigmine) may be partially mediated by the activation of excitatory cholinergic autoreceptors. This prompted us to revaluate the neuromodulatory role of adenosine and its interaction with excitatory nicotinic α3β2 and muscarinic M1 autoreceptors in TIMG rats. Neostigmine (0.5–1 μM) transiently (30s) increased the strength of diaphragm contractions in control and TIMG animals, an effect that was partiall y attenuated by blocking α3β2-containing nicotinic and muscarinic M1 autoreceptors with DH-β-E (3 μM) and MTx-7 (3 nM), respectively. Inhibition of [3H]-ACh release by DH-β-E (3 μM) was more evident in TIMG animals (- 25 ± 6%, n = 9) than in controls (- 9 ± 4%, n = 5). Likewise, MTx-7 (1 nM), inhibited by 47 ± 6% (n = 9) the release of [3H]-ACh evoked by 50 Hz-bursts in TIMG rats, but it was without effect in control animals. There is remarkable parallelism between tonic enhancement of transmitter exocytosis via nicotinic α3β2 and muscarinic M1 and the reduction of adenosine A2A activation (revealed by ZM241385-induced inhibition) in myasthenics. Data indicate that reduction of the retrograde adenosine signalling from myasthenic muscles via A2A receptors might partially compensate neuromuscular transmission failure through disinhibiting ACh exocytosis via nicotinic α3β2 and muscarinic M1 autoreceptors. Work supported by FCT (FEDER funding, PTDC/SAU-FCF/108462/2008 and UMIB-215/94) and by Univ. Porto / Santander Totta.

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MO06-10

DIFFERENCES IN BINDING AND REGULATION OF MUSCARINIC ACETYLCHOLINE RECEPTORS AFTER ACUTE EXPOSURE TO XANOMELINE

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Xanomeline is a functionally selective agonist (in terms of potency and efficacy) at M_1/M_4 muscarinic receptors but displays the same binding affinity for all muscarinic receptor subtypes. Thus, subtype differences in potency and efficacy of xanomeline cannot be explained by differences in affinity. A unique feature of xanomeline is its ability to bind to all muscarinic receptor subtypes in a wash-resistant (WR) manner. Measurement of binding of the radiolabeled antagonists N-methylscopolamine and quinuclidinyl benzilate after exposure of CHO cells expressing individual subtypes of muscarinic receptors to xanomeline for 1, 3 or 10 min followed by extensive washing revealed differences in kinetics of formation of xanomeline WR binding. Formation of xanomeline WR binding was the fastest at M_5 receptors where already after 1-min xanomeline WR binding reached 80% of receptor occupancy. Formation of xanomeline WR binding was the slowest at M_2 receptors where it took xanomeline 10 min to reach 20% occupancy. Subsequent 1-h washing did not change xanomeline WR binding except for M1 receptors where xanomeline binding decreased by 25%. Xanomeline WR binding also displayed subtype variations in affecting receptor regulation. Subtype differences in kinetics of xanomeline WR binding and receptor regulation may constitute the basis for subtype differences in potency and efficacy of xanomeline.

MO06-11

CENTRAL MUSCARINIC RECEPTORS ARE MODULATED BY NEUROTENSIN AN EFFECT WHICH DOES NOT INVOLVE THE HIGH-AFFINITY NTS1 RECEPTOR

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Neurotensin (NT) is a tridecapeptide distributed in central and peripheral nervous systems, which can behave as a neurotransmitter or neuromodulator at central and peripheral levels. We have tested the potential effect of this peptide on quinuclidinyl benzilate ([3H]-QNB) binding to muscarinic receptor in rat CNS membranes. Previously we observed that NT decreased up to 50–70% ligand binding at 1×10^{-7} $M^{-1} \times 10^{-5}$ M concentration in cerebral cortex, cerebellum and striatum. In order to test the involvement of high-affinity NT receptor (NTS1) in NT inhibitory effect, assays were carried out in the presence of 1×10^{-6} M NT and/or SR 48692 (Sanofi-Aventis, U.S., Inc.), a specific antagonist for this receptor, which was dissolved in dimethylsulfoxide (DMSO) 10% v/v. This inhibition was not observed with the DMSO control group. As controls, membranes incubated with DMSO and/or NT 1×10^{-6} M plus DMSO were processed. It was found that NT+DMSO decreased [3H]-QNB binding to cerebral cortex, cerebellum and hippocampal (49%, 32% and 53%), respectively. Membrane preincubation with 1×10^{-6} M SR 48692 failed to alter NT effect on SR 48692 binding at 1×10^{-6} M concentration decreased binding by 50% only in cerebral cortex, suggesting a possible direct effect of the antagonist on muscarinic receptors in this area. NT exerted a biphasic effect behaving as a stimulator in the presence of 1×10^{-12} M–1×10^{-10} M concentration but as an inhibitor at 1×10^{-8} M–1×10^{-5} M concentration, in hippocampal membranes, according to the concentration employed. NT enhanced 40%–50% of the ligand binding at 10^{-12}–10^{-10} M concentration, without significant differences between both conditions. Peptide diminished binding (15–25%) at 10^{-8} M and 10^{-7} M concentration and roughly by 75% at 10^{-6}–10^{-5} M concentration. To quantify the inhibitory ability of NT for every area, inhibition constants for NT versus [3H]-QNB were determined. It was therefore concluded that the high-affinity NT receptor is hardly involved in ligand binding inhibition to muscarinic receptor by NT.

MO06-12

COMPARISON OF RADIOACTIVE, BIOTINYLATED AND FLUORESCENTLY-LABELED LONG-CHAIN α-NEUROTOXINS EFFECTIVENESS IN α7 NACHR DETECTION

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Homopentameric α7 nicotinic acetylcholine receptors (nAChRs) are widespread in nervous system, where they can either modulate transmitter release or participate in signal transduction depending on their synaptic location. Their malfunctioning is associated with

number of psychiatric and neurodegenerative disorders. Thus, reliable α7 nAChRs detection and quantification are of great importance for such diseases diagnostics and investigation. The present work was focused on comparison of radioactive, biotinylated and fluorescently-labeled long-chain α-neurotoxins (antagonists of α7 nAChR) effectiveness in several α7 nAChR detection approaches including binding assays, histochemistry and Western blotting. Within the modifications iodination minimally impaired native toxins characteristics; consequently, radioactive derivatives had the highest affinity to α7 nAChR and in binding assay allowed to detect less receptor amount than biotinylated and fluorescent ones. Moreover only radioactive toxins gave absolute values of binding parameters that allowed quantifying α7 nAChR. Besides the protein detection task, using either radioactive or fluorescently labeled α-neurotoxins in competitive binding assay we could calculate inhibition parameters and estimate affinity and selectivity of other ligands. In particular, this method allowed selection of an inhibitor combination for fluorescent derivatives to discriminate between muscle and neuronal types of nAChRs. Further the examined fluorescently-labeled reporter and the combination of non-labeled blockers was shown to be the most effective in histochemical localization of α7 nAChRs, while biotinylated α-neurotoxins were more inferior because of higher background, possibly due to secondary detection reagents. Interestingly, in Western blotting all the tested derivatives were effective in detection of muscle-type nAChR of Torpedo californica electric organ (1–5 nmol/mg of protein), while the level of α7 nAChRs in brain and in α7 transfected cell membranes was insufficient (4 fmol–1 pmol/mg of protein).

MO06-13

KINETIC ANALYSIS OF ESTER HYDROLYSIS WITH SOME PYRIDINIUM OXIMES TO COMBAT CHOLINESTERASE INHIBITION CAUSED BY CHEMICAL WARFARE AG

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Acetylcholinesterase (AChE) hydrolyzes neurotransmitter acetylcholine and controls synaptic transmission at central and peripheral cholinergic synapses. Exposure to organophosphorus poisons leads to inhibition of AchE *in vivo*, resulting in acute toxicity caused by a cholinergic crisis. Novel approaches to overcome the toxic effects of organophosphorus pesticides (OP) and chemical warfare agents are needed due to their documented use in warfare and Terrorism. During the past few decades investigators in this field have realized the need for a comprehensive effort that can provide in-depth knowledge on various aspects of oximes not only as reactivators of acetylcholinesterase but also as micellar catalysts for detoxification of OP and nerve agents. Owing to this, present investigation deals with kinetic studies on the interactions between different OP stimulants (carboxylate, phosphate and sulfonate esters) and novel mono/bis-pyridinium oximes and oxime based functionalized surfactants. Most of the kinetic runs were performed in cationic micellar medium. The reactivation potency of oximes as antidotes towards acetylcholinesterase inbited by organophosphates and nerve agents is discussed and comparisons are made with some commercially available reactivators. Effect of position and presence of oxime group on the pyridinium ring, number of pyridinium rings and linkers have been analyzed. Bis-pyridinium oximes proved to be better reactivators than mono-pyridinium oximes and oxime at position-2 was the most efficient. Physicochemical properties of some novel oxime based functionalized surfactants have also been explored using conductometric and kinetic measurements and their role in decontamination of toxic warfare agents and pesticides have been considered. The results proved that oxime based functionalized surfactants are far more effective and versatile in their mode of action against different esters.

MO07 Synaptic Transmission

MO07-01

PHARMACOLOGICAL CHARACTERISTICS OF PROTEIN SYNTHESIS INHIBITORS BY RADIOACTIVE LEUCINE INCORPORATION IN RAT HIPPOCAMPAL SLICES

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Protein synthesis inhibitors (PSIs) constitute a major tool to validate the hypothesis of protein synthesis-dependent phase of synaptic plasticity and memory consolidation. However, several reports have showed inconsistent findings about the effect of these drugs on behavioral learning and synaptic plasticity. Testing the potencies of these drugs is hence crucial for validating such negative findings and in planning future studies. It is also necessary to examine the dose dependence, onset dynamics and reversibility, and possible effects on basal proteins. Here we used the labeled leucine as marker for the newly synthesized proteins. The fraction of leucine incorporation, following 50 min of pre-incubation, was compared between two groups of slices: a PSI-treated and a control group. Both anisomycin and cycloheximide revealed a dose-dependent but time-independent manner of inhibition reaching over 92% at concentrations well below those used in previous experiments which revealed effects on synaptic plasticity and learning. Surprisingly, washout of a 'reversible' inhibitor, anisomycin was not followed by rapid reversibility of the action of the drug, the case that differs with cycloheximide. Interestingly, emetine revealed a time-dependent inhibition of protein synthesis, where levels above 80% needed drug pre-incubation for as long as 90 min. Since the duration of labeling relates to the half-life of the proteins, short-time labeling as used in this study will result in radioactivity incorporation into short-lived proteins and proteins that are synthesized in large quantities. We therefore studied the availability of newly synthesized proteins at 8–10 h following leucine incorporation. The results revealed virtually the same protein content as in slices retrieved for analysis immediately following the labeling period, indicating that the main pool of the newly synthesized proteins is of intracellular long-lived pool. This likely reflects a stable metabolic state of our prepared slices. These findings challenge current idea on the role of de novo protein synthesis in synaptic plasticity as well as brain changes underlying several neurological and psychiatric disorders.

MO07-02

INTERMITTENT FASTING DIETARY RESTRICTION AND BRAIN PLASTICITY: ROLE OF SYNAPTIC PROTEINS

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Dietary restriction (DR) is known to have beneficial effects on the brain including enhanced learning and memory, neurogenesis, increased synaptic plasticity and resistance to oxidative and metabolic insults. Recent studies on the effects of DR in rodents and primates have shown that short-term regimens can bring about comparable beneficial changes seen in animals subjected to life long DR. Although, the underlying mechanism(s) remain unknown, but the need for reduction in caloric intake to achieve these benefits has been assumed. In the current study we investigated whether synaptic proteins such as synaptophysin, calcineurin and CaM kinase play a mechanistic role in plastic morphological changes in different brain region of female rats on IF-DR (alternate day feeding regimen). Further to demonstrate the effect of IF-DR on brain plasticity, the expression of glial cell marker, GFAP and synaptic proteins was studied by immunohistochemistry, western blotting and reverse transcriptase-PCR in different brain regions. These results show increased expression of synaptophysin, GFAP and CaM kinase, whereas the expression of calcineurin was decreased in DR animals as compared to Ad libitum fed control rats. Our results suggest that IF-DR regimen may have beneficial effects by facilitating the dynamic plastic changes and structural remodeling of glial and neuronal cells in the different brain regions.

MO07-03

A PHOTOACTIVATABLE ENKEPHALIN NEUROPEPTIDE FOR SPATIOTEMPORALLY PRECISE STUDIES OF OPIOID SIGNALING

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Neuropeptides activate G-protein coupled receptors (GPCRs) to acutely modulate cellular excitability and synaptic transmission and thus cause complex changes in neural network function. Despite their importance, direct investigations into the spatio-temporal dynamics of neuropeptide signaling in the mammalian central nervous system are scarce. To enable rapid and spatially delimited delivery of an opioid neuropeptide in neural tissue, we have developed a photoactivatable Leucine-Enkephalin (L-Enk). This molecule contains a carboxy-nitrobenzyl (CNB)-modified Tyrosine (Y) residue and is thus called CYLE. We demonstrate that CYLE is functionally inactive and that it releases L-Enk when exposed to UV light. Whole-cell recordings from acute slices of rat locus coeruleus (LC) reveal that CYLE enables robust, graded delivery of L-Enk with ~100 μm spatial precision and kinetics that approach the limits of G-protein mediated signaling. We highlight the advantages of spatially-delimited and temporally-precise photorelease in determining the kinetics, spatial extent and ionic components of the opioid response in LC.

MO07-04

NAVβ4 IS RECRUITED TO THE AXON INITIAL SEGMENT THROUGH INTERACTION WITH Na^+ CHANNEL α SUBUNITS

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Action potentials are initiated at the axon initial segment (AIS). The molecular architecture of the AIS, characterized by high-densities of voltage-gated Na^+ and K^+ channels and supporting scaffold proteins, is similar among cell types; firing patterns, however, vary between neuron classes. Differences in voltage-gated Na^+ channel regulation could contribute to their divergent physiologies. The specific composition the multimeric Na^+ channel

complex, consisting of one pore-forming α subunit and two modulatory β subunits, dramatically influences channel activity as each β subunit differentially affects α subunit physiology. Sodium channel auxiliary subunit beta-4 (Navβ4) favors an open-channel conformation at depolarized potentials by destabilizing the inactivation state. Recent evidence indicates that Navβ4 may facilitate the generation of resurgent Na^+ current. Navβ4 expression is limited to a specific subset of cells within the nervous system including Purkinje neurons and is correlated with high-frequency firing capacity. To determine the subcellular localization of Navβ4, we generated antibodies targeting Navβ4-specific epitopes. Navβ4 is enriched at central and peripheral nodes of Ranvier and at the AIS of cerebellar Purkinje neurons and spinal motor neurons. To determine the domain of Navβ4 required for AIS-targeting, we expressed GFP-tagged terminal truncation mutants in cultured hippocampal neurons. Like full-length Navβ4, Navβ4ΔC, but not Navβ4ΔN, localized to the AIS. Furthermore, we found that C28 is required for Navβ4 AIS localization. To determine if Navβ4 is recruited to the AIS through interaction with Navα subunits, we silenced Navα expression in developing neurons. In the absence of Navα, Navβ4 was not enriched at the AIS. To determine the functional role of Navβ4, we are generating mice lacking Navβ4 (Scn4b-/-) expression. Knockout animals will be subjected to a range of physiological and behavioral tests. Modulation of Navα subunits through interaction with Navβ4 may provide a mechanism to sustain high-frequency firing at the AIS. Our results suggest that the cell-type specific molecular composition of the AIS may contribute to the variability in firing patterns observed between neuron classes.

MO07-05

REGULATION OF HUMAN D-AMINO ACID OXIDASE AND OF ITS INTERACTOR PLG72

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D-serine, an endogenous allosteric modulator of NMDA receptors (NMDAr), is synthesized by serine racemase and, at least in astrocytes, it is mainly degraded by the flavoprotein D-amino acid oxidase (hDAAO). A relationship between D-serine signalling deregulation, NMDAr dysfunction and neuropsychiatric diseases (like schizophrenia) is widely assumed. Interestingly, SNPs in the genes encoding for hDAAO and SR were correlated to schizophrenia susceptibility. Moreover, an association between this pathology and SNPs in the G72 gene was also reported: it encodes for pLG72, a specific interactors of hDAAO, present in primates only (1). *In vitro* pLG72 acts as an inactivator of hDAAO, and D-serine cellular concentration depends on the amount of active enzyme. We proposed a model to explain the relationships between hDAAO-pLG72-D-serine and schizophrenia susceptibility: an anomalous hypoexpression of pLG72 yields to an increase of hDAAO activity, then to a decrease of D-serine released at the synapse and finally to the hypoactivation of NMDAr (2). By using U87 human glioblastoma cells transiently or stably expressing pLG72 and/or hDAAO we demonstrated that the newly synthesized hDAAO is catalytically active and is transiently located in cytosol. Cytosolic hDAAO can interact with pLG72, which we propose to be exposed on the external membrane of mitochondria, before being targeted to peroxisomes. hDAAO is a stable 'long-lived protein': the largest part of the flavoprotein is degraded by the lysosomal system while a minor amount is polyubiquitinated and targeted to UPS. On the contrary, pLG72 is characterized by rapid turnover (t/2 ~ 25 min) and is essentially degraded through the proteasomal system. In conclusion, these studies aim to clarify the mechanism(s) of regulation of hDAAO activity in glial cells and its effect of D-serine cellular concentration.

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MO07-06

CHOLESTEROL-DEPENDENT INTERACTIONS OF SYNAPTOPHYSIN

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Synaptophysin, despite its high abundance on synaptic vesicles and years of study, has yet to be ascribed a function. Of the few characteristics which have been elucidated is a possible interaction with cholesterol, a critical lipidic component of vesicle membranes and a key contributor to synaptic activity. In our current work we aim to characterize the interaction of synaptophysin and cholesterol and using established biochemical techniques identify cholesterol-dependent interactions to advance our understanding of the role of synaptophysin in neuronal cell biology. To this end we have identified conserved motifs in the structure of synaptophysin which may regulate its association with cholesterol-rich microdomains in the vesicular membrane. Using a combination of biochemistry and live cell imaging we will identify the contributions these motifs make to the incorporation of synaptophysin into cholesterol-rich microdomains and the effect this has on the trafficking and subcellular localization synaptophysin.

MO07-07

NMDA RECEPTOR STIMULATION REDUCES STRESS-INDUCED INCREASE IN NOREPINEPHRINE OUTPUT IN THE RAT PREFRONTAL CORTEX

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Stress can precipitate episodes of anxiety and depression; two mental disorders characterized by poor concentration, reduced attention, altered memory, reduced socialization and altered state of arousal while episodes of depression are often preceded by stressful conditions. A number of experimental evidences suggest that norepinephrine and glutamate play an important role both in the stress response and in psychiatric disorders such as depression and schizophrenia.

The aim of this study was to evaluate whether the same stress protocol (footshock) at different intensities (from 0.2 to 0.8 mA) with different paradigms (mild – 0.2 mA for 8 min every 500 msec/s – or with the paradigm used to induce Learned Helplessness) is able to induce differential effects on extracellular concentration of norepinephrine in the prefrontal cortex of freely moving rats, using the microdialysis technique. We also studied the role of NMDA glutamate receptors in modulating the response of noradrenergic neurons to stressful stimuli. According with our previous results, exposure of rats to the mild stress protocol induced a significant increase in norepinephrine output (+100% of basal values) that was

maximal during stress exposure and returned to basal values within 20 min. Exposure to the Learned Helplessness paradigm induced a dramatic increase in cortical norepinephrine extracellular concentrations (+500% o basal values) that was maximal during stress exposure but persisted for at least 60 min after footshock termination. Local perfusion with NMDA (100 μM) 40 min before stress exposure, was able to significantly reduce the sensitivity of cortical noradrenergic neurons to stress. In fact, after NMDA perfusion the increase in norepinephrine output induced by the mild paradigm was only 48% of basal values, while that induced by the Learned Helplessness paradigm was similar to that observed with the mild stress in control rats (+100%).

These evidences suggest that the reduction of glutamatergic excitatory transmission in key limbic areas and neuronal circuits involved in anxiety responses might represent an important mechanism involved in the action of anxiolytic agents.

MO07-08

PHARMACOLOGICAL CHARACTERIZATION OF GLUTAMATE NA$^+$-INDEPENDENT TRANSPORT IN RETINAL CELLS CULTURE: IMPLICATIONS IN THE GLUTATHIONE ANTIOXIDANT MOLECULE

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L-Glutamate is the primary excitatory neurotransmitter in the mammalian central nervous system (CNS). Mechanisms for the removal of glutamate are vital for maintaining normal function of retina. In the present study, using retinal cells cultures obtained from chick embryos, we characterize, pharmacologically, the presence of two glutamate transporter mechanisms, Na^+-dependent and Na^+-independent uptake systems. Na^+-independent uptake system seems to present characteristics related to system xCG- (cystine-glutamate exchanger), that in the current work demonstrated highlighted contribution to the glutamate transport in retina, which is not observed in other tissues. Our results showed that glutamate shares xCG- system with another amino acid, L-cysteine, suggesting the possible involvement of this component in processes related to the release of the glutathione antioxidant molecule. Furthermore, cysteine uptake by Na^+-independent transport appears to be more evident in glial cell cultures than in neuronal cell cultures. So, Na^+-independent transport system seems to have other functions besides amino acid transport, demonstrating a physiological role in modulating cell redox status.

MO07-09

SYNAPTIC VESICLE RECYCLING IN SYNAPTOPHYSIN KNOCKOUT MICE

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Synaptophysin (SYP) is an integral membrane protein of unknown function that comprises 10.2% of total synaptic vesicle protein. Despite its abundance, knockout of the protein in mice produces a very mild phenotype. Knockout mice display impairments in learning and memory (3), while double knockout of SYP and the related protein synaptogyrin results in a defect in LTP (2). Cells that lack both SYP and SYPII have decreased levels of vesicles, that is more pronounced with increased synaptic activity (4), suggesting a potential role for these proteins in synaptic vesicle recycling. SYP binds to dynamin in a Ca^{2+}- dependent manner (1), further suggesting a role for SYP in the regulation of endocytotic mechanisms. However, despite much research the actual functional role of synaptophysin is yet to be determined. We have examined the effect of knockout of SYP on the recycling of synaptic vesicles by monitoring the trafficking of different synaptic vesicle proteins in both wild-type and knockout mouse cortical cultures. We have found that knockout of SYP results in alterations in the recycling dynamics of specific synaptic vesicle proteins that is rescued by transfecting SYP back into our culture system. This result suggests that SYP plays a role in the recycling of specific synaptic vesicle cargo from the plasma membrane to form synaptic vesicles.

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MO07-10

CORTICOSTERONE FACILITATES THE SYNCHRONIZED CALCIUM OSCILLATION IN HIPPOCAMPAL NEURONS

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Corticosteroid hormones are released high amount after stress. The hormones bind to the two specific receptors namely mineralocorticoid receptors and glucocorticoid receptors, and the signal mediated by these receptors affect the brain function for stress in hippocampus. Corticosterone (CORT) has the effect to the excitatory neural activity in hippocampus CA1 area. In this study, we investigated the CORT activates the spontaneous synchronous calcium oscillation in primary hippocampal neurons of mouse embryos. After 8–12 cultured days, the hippocampal neurons were loaded to fura-2/AM and measured the neuronal activity by calcium imaging method conditioned by removed extracellular Mg^{2+} of balanced salt solution. The results showed that spontaneous calcium oscillation induced a clear large increase in the CORT treatment (200 nM, 2 h) group cells compared with the control group cells. The frequency of calcium oscillations was decreased in CORT treatment group and the amplitude was increased in CORT treatment group. As this calcium synchronized oscillatory activity was blocked by TTX (1 μM), DNQX (5 μM), and MK-801(10 μM), it is suggested that this activation was synaptically driven accompanied with voltage-dependent oscillation. The mRNA expression of NMDA receptors (NR2A and NR2B) decreased at 20 min after CORT treatment, whereas the protein expression of these receptors was increased. On the other hand, the expression of both the mRNA and the protein of AMPA receptors (GluR1, GluR2) did not change. The result suggests that the synchronized calcium oscillation by CORT was promoted by the activation of translation of NMDA receptors. The facilitated synaptic oscillation activity by CORT may regulate the neural network in hippocampus for stress by the activation of glutamatergic synapse.

MO07-11

FACILITATION OF DISTINCT INHIBITORY SYNAPTIC INPUTS BY ANOXIA IN CRANIAL MOTOR NEURONS OF THE RAT

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by the selective loss of motor neurons in the brainstem and spinal cord. Clinical studies have indicated that there is a distinct region-dependent difference in the vulnerability of motor neurons. For example, the motor neurons in the facial and hypoglossal nuclei (VII and XII, respectively) are more susceptible to neuronal death than those in the oculomotor nucleus (III). To understand the mechanism underlying the differential susceptibility to cell death of the neurons in different motor nuclei, we compared the effects of chemical anoxia on the membrane currents and postsynaptic currents in different motor nuclei because we have already demonstrated that anoxia-triggered release facilitation of glycine results in potentiation of NMDA-induced currents through activation of glycine-binding sites in XII (Kono 2007). The membrane currents were recorded from neurons in III, VII and XII in brain slices of juvenile Wistar rats by using whole-cell recording in the presence of tetrodotoxin. In these three nuclei, NaCN induced a continuous inward current accompanied by a significant increase in the spontaneous action potential-independent synaptic inputs. Whereas the NaCN-induced increase in the synaptic input frequency in XII and VII was abolished by strychnine but not by picrotoxin, it was unaffected by strychnine but was abolished by picrotoxin in III. Blocking ionotropic glutamate receptors in any of the three motor nuclei did not affect the NaCN-induced release facilitation. These results suggest that anoxia selectively facilitates glycine release in the ALS-vulnerable motor neurons. The region-dependent differences in the neurotransmitters involved in the anoxia-triggered release facilitation might provide a basis for the selective vulnerability of motor neurons in the neurodegeneration associated with ALS.

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MO07-12

SUPPOSED ROLE OF MEMBRANE VESICLES IN PURINERGIC SIGNALING

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Cell derived membrane vesicles (MVs) are submicron structures (50–1000 nm) surrounded by phospholipid bilayer, released by both prokaryotic and eukaryotic cells. These MVs are considered as important mediators of intercellular communication. They represent efficient delivery platforms targeting complex molecular information to professional antigen presenting cells, and a population of them (exosomes) has been shown to modulate the activity of cellular signaling pathways with release of cytosolic proteins. Critical roles of these naturally formulated units of information have been described in blood coagulation and tumor invasiveness. MVs are not only potential biomarkers and possible pathogenetic factors in numerous diseases, but they also represent extrahepatic drug metabolism systems. Our goal was to investigate whether MVs can play a role in purinergic signaling. Besides the use of BV2 microglia cell line as model system sensitive to changes in the concentration of extracellular ATP, we worked out a fast and simple protocol for the preparation of MVs from tissue culture supernatants and from human synovial fluid of rheumatoid arthritis patients. Expression of NTPDase1 (an ectonucleotidase) and purinergic receptors in the MVs of different origin were investigated by quantitative real time RT-PCR, immunocytochemistry and enzyme cytochemistry at electron microscopic level. Our results indicate the involvement of MVs in purinergic signalling, and may support their potential use as diagnostic tools.

MO07-13

CATALYTIC ACTIVITY OF α -NUCLEOPHILES FOR ESTER HYDROLYSIS IN CATIONIC MICELLAR MEDIA

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Organophosphorus pesticides and nerve agents are highly toxic to humans and other living organisms, primarily because of their interaction with enzyme acetylcholinesterase that disrupts the neurological functions, which forms the basis of their activity as pesticides and chemical warfare agents. Many elegant studies have made significant advance towards understanding the mechanism of detoxification of chemical warfare agents. For the last few years, our group at Raipur has been working on the kinetic and mechanistic aspects of the toxic and nontoxic carboxylate and phosphate esters by various oximate and hydroxamate ions. Here we discuss the role of different α-nucleophiles and some acetylcholinesterase reactivators as potential hydrolytic catalysts for the cleavage of carbon, phosphorus and sulphur based esters spectrophotometrically. For comparison, the effects of various cationic surfactants viz. cetydiethylethanolammonium bromide (CDEABr), cetyltrimethylammonium bromide (CTAB) and cetylpyridinium bromide (CPB) have also been investigated. All the reactions were conducted under pseudo-first-order conditions with the neucleophiles in excess. The values of first-order-rate constant (kobs.) increase with increasing the pH of reaction media. It was observed that oximate ion bind more significantly to the cationic surfactants compare to other hydroxamate ions. The multifunctional neucleophilicity of α-neucleophiles fulfills the requirements of the reactive catalyst for the esterolytic cleavage of the carboxylate and phosphate esters. Outcome of the present investigation may encourage the use of nucleophilic reagents for detoxification of toxic pesticides and nerve agents.

MO07-14

MATRIX METALLOPROTEINASE 9 (MMP-9) EXPRESSION IN NEURONS IS REGULATED VIA SERUM RESPONSE FACTOR/C-FOS PATHWAY

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MMP-9 is and endopeptidase playing important role in physiological and pathological neuronal plasticity. Although multiple factors regulating MMP-9 expression have been described in different cell types, the molecular mechanism directly controlling its transcription in neurons remains poorly understood. As neurotrophins are key regulators of neuronal plasticity, we aimed at investigating, whether brain-derived neurotrophic factor (BDNF) can modulate MMP-9 expression in neurons. qRT-PCR analysis revealed strong upregulation of MMP-9 mRNA levels after stimulation of rat primary cortical neurons with BDNF. Additionally, increased protein expression and elevated MMP-9 enzymatic activity was observed using western blot and gelatin zymography. To investigate mechanism of MMP-9 promoter regulation, we used luciferase gene reporter assay system in which luciferase gene is controlled by MMP-9 promoter fragment (-1369/+35). Treatment of neurons with BDNF led to MMP-9 promoter activation, that was dependent on ERK1/2 actvity, as demonstrated using selective inhibitor or overexpressing constitutively active MKK1. As in MMP-9 promoter there are two AP-1 binding sites, we investigated whether AP-1 contributes to the BDNF-mediated MMP-9 transcription in neurons. MMP-9 reporter construct was induced upon overexpression of different AP-1 dimers in neurons, the most potent being those containing c-Fos. Moreover, we observed strongly reduced BDNF-induced activation of the MMP-9 reporter construct if proximal, but not distal, AP-1 binding site was mutated. Furthermore knocking-down c-Fos expression in neurons by shRNA decreased MMP-9 gene activation in response to BDNF. As c-fos gene is a known target of serum response factor, we tested whether SRF can contribute to MMP-9 transcription. Inhibition of SRF by the overexpression of dominant-negative mutant of SRF or using shRNA targeting SRF, abolished BDNF-induced activation of MMP-9 promoter. Our data indicate that MMP-9 expression in neurons can be induced by BDNF. The signal propagation could involve ERK1/2 pathway and SRF-mediated transcription of c-fos gene resulting in activation of MMP-9 promoter.

MO07-15

LOCAL TRANSLATION OF MMP-9 IS REGULATED BY POLYADENYLATION

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Matrix metalloproteinase 9 (MMP-9), gelatinase B that regulates pericellular environment through the cleavage of protein components of the extracellular matrix, plays a role in synaptic plasticity. The activity of MMP-9 increases shortly, 5–10 min, after stimulation of rat neuronal cultures with either glutamate or bicuclline and is reduced by polyadenylation and translation inhibitors. We observed the presence of MMP-9 protein and corresponding mRNA in synaptoneurosomes, the synaptic fraction isolated from rat hippocampus. Using MS2 mRNA labeling system we were able to track MMP-9 mRNA molecules transported in granules along the dendrites. In order to prove directly that MMP-9 mRNA is capable to undergo local translation in synaptoneurosomal compartment the polyribosomal fraction was isolated from resting or stimulated synaptoneurosomes and analysed by qRT-PCR. We observed that MMP-9 mRNA shifts to the polyribosomal fractions, upon glutamate or DHPG (mGluR5 agonist) stimulation. To further study the molecular mechanism of MMP-9 local translation we performed PAT assay on mRNA from synaptoneurosomes. Our data indicate that the level of polyadenyleted MMP-9 mRNA increases after glutamate or DHPG stimulation. We have also used cordycepin, a polyadenylation inhibitor in order to observe its effect on MMP-9 polyadenylation after neuronal stimulation.

MO07-16

BLOCKADE OF SEIZURE-LIKE EVENTS BY SK-CHANNEL ENHANCERS IN RAT HIPPOCAMPAL SLICES

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sK-channels are thought to be involved in regulation of neuronal excitability, upon activation results in hyperpolarisation of membrane causing in the reduction of excitability, thus it might have effect in suppressing seizures. In the present study we have evaluated the action of sK-channel enhancers by inducing seizure-like events (SLEs) in acute hippocampal slices of rat using 4-aminopyridine and low magnesium (zero Mg^{2+} ACSF). Electrophysiological field potential recording was performed in an interface setup. CyPPA (50 μM and 100 μM) and SKa-31 (100 μM and 150 μM) were tested after stabilization of SLEs. CyPPA at dose of 50 μM didn't block SLEs, rather delayed the interval between SLEs, whereas at the dose of 100μM SLE were completely blocked. Similarly SKa-31 didn't suppress 4-AP induced SLEs at dose of 100 μM but suppressed SLEs at dose of 150 μM. Both drugs failed to block SLEs induced by low magnesium. Based on these findings we suggest that sK-enhancer plays a role in reducing membrane hyperexcitability and thus may have potential to be an anticonvulsant agent.

MO07-17

CHARACTERIZATION OF NECTIN-3 AS A NEW MOLECULAR TARGET FOR MMP-9 METALLOPROTEINASE

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Nectin-3 is Ca^{2+}-independent immunoglobulin-like cell adhesion molecule that is involved in the organization of various types of intercellular junctions, including synapses in the central nervous system. Proteolytic shedding of Nectin-3 has been reported, however, the nature of the regulation of Nectin-3 cleavage in neuronal cells is poorly understood. We report herein that NMDA receptor activation results in robust Nectin-3 ectodomain shedding in the hippocampal neuronal cultures. The NMDA-driven cleavage was abolished when neurons were treated with the NMDA receptor

antagonists, APV and MK801. We have also investigated if MMP-9, matrix metalloproteinase implicated in neuronal plasticity, was mediating Nectin-3 cleavage. Towards this, we pretreated hippocampal neurons with MMP-9 inhibitor I and found the cleavage of Nectin-3 to be prevented. We also show that pretreatment of nifedipine and CNQX partially decreased NMDA-induced Nectin-3 shedding. Furthermore, no Nectin-3 cleavage was observed when the neurons were stimulated with NMDA in the presence of EGTA, the calcium chelator. In contrast, ionomycin, calcium ionophore, evoked robust shedding of Nectin-3 and this effect was blocked by inhibitor I of MMP-9. Our results suggest that ectodomain shedding of Nectin-3 is Ca^{2+}-regulated event and MMP-9 can potentially be responsible for these cleavage.

MO07-19

HISTOLOGICAL PROFILE OF SYNAPTIC MATRIX METALLOPROTEINASE ACTIVITY IN THE MOUSE HIPPOCAMPUS

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Structural plasticity of synapses is thought to be an important process for learning and memory. Recent analyses have demonstrated that the efficiency of synaptic functions depends on the shape and size of synaptic structures. In such studies, rapid remodeling of synaptic connections is postulated to be dependent on the activity of extracellular matrix proteins and/or cell adhesion molecules. We hypothesized that molecules which are cleaved by proteinases at the synapse of the hippocampus during animals' learning behavior might induce dynamic changes in synaptic morphology, and hence affect long-term potentiation in the Schaffer-collateral pathway. To test this hypothesis, we examined the local activity of matrix metalloproteinases (MMPs) at the hippocampal synapse, using high-resolution fluorescent *in situ* zymography in thin sections of polyester wax-embedded mouse brain tissue. Successful staining patterns were obtained, with excellent preservation of fine structural detail. Gelatinolytic activity of MMPs, determined using dye-quenched gelatin as a substrate, was observed as a fine fluorescent punctate profile indicative of active synaptic MMPs. Intraperitoneal injection of kainic acid, which induces neural hyperexcitation, caused an increased number and intensity of fluorescent punctate structures in the CA1 area of the hippocampus. These changes in fluorescence indicate that MMP activity was increased. We also observed neural activity-dependent changes in MMP activity, which may reflect dynamic molecular events in synaptic plasticity.

MO07-20

PHOSPHORYLATION OF $GABA_B$ RECEPTORS IS REQUIRED FOR LONG-TERM SPATIAL MEMORY

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$GABA_B$ receptors are heterodimeric G-protein coupled receptors composed of R1 and R2 subunits that mediate slow synaptic inhibition in the brain by activating inwardly rectifying K^+ channels (GIRKs) and inhibiting Ca^{2+} channels. Postsynaptic $GABA_B$ receptors are found predominantly on dendritic spines adjacent to excitatory synapses. We have previously reported that $GABA_B$ receptors are intimately associated with 5'AMP-dependent protein kinase (AMPK) and directly phosphorylate serine 783 (S783) in the R2 subunit to enhance $GABA_B$ receptor activation of GIRKs. In parallel, we have demonstrated that prolonged activation of NMDA receptors leads to endocytosis and subsequent lysosomal degradation of $GABA_B$ receptors. This mechanism is critically dependent on the dephosphorylation of S783 within the $GABA_BR2$ subunit. Thus NMDA receptor-dependent dephosphorylation of S783 acts as a molecular switch to decrease the abundance of $GABA_B$ receptors at the neuronal plasma membrane.

To further examine the significance of this mechanism we have generated a $GABA_BR2$ subunit serine-to-alanine mutant knock-in mouse (S783A). S783A mice form functional $GABA_B$ receptors; however, S783A mice are resistant to NMDA-dependent degradation and enhanced in $GABA_B$ receptor-mediated currents. Multiple behavioral experiments suggest that this S783A mutation causes specific deficits in long-term spatial memory. One of the key functions of the $GABA_B$ receptor is to inhibit the production of cAMP, a second messenger known to play an important role in long-term memory. We have shown that cAMP production and PKA activity are significantly decreased and that phosphorylation of CREB, an important protein for memory formation, is reduced in S783A mice. These results suggest that phosphorylation of $GABA_B$ receptors and downstream signaling of $GABA_B$ receptors play a critical role in long-term spatial memory formation.

MO07-21

SYNTHESIS AND BINDING CHARACTERISTICS OF A NEUROTENSIN-LIKE PEPTIDE: ^{3}H-NEUROMEDIN N

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Neurotensin (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu) (NT) and its analogue neuromedin N (Lys-Ile-Pro-Tyr-Ile-Leu) (NN) are synthesized by a common precursor (pro-NT/NN) in mammalian brain and intestine. Most of the biological effects mediated by NT and NN (analgesia, hypothermia) result from the interaction of these peptides with G-protein coupled receptors. Aims of the study were: synthesis, radiolabeling and binding characteristics of ^{3}H-neuromedin N and G-protein activation of the hexapeptide. The specific radioactivity of ^{3}H-neuromedin N was 16 C_i/mmol. In homologue displacement studies IC_{50} was 454 nM in rat brain and IC_{50} was 425 nM in rat spinal cord. In saturation binding experiments the equilibrium dissociation constant, K_d was 264.8 ± 30.18 nM, while the maximal number of binding sites (B_{max}) was 3.8 ± 0.2 pmol/mg protein in rat brain membranes. In kinetic experiments steady-state level of specific binding was achieved in 10–12 min in rat brain membranes. In sodium sensitivity experiments IC_{50} was 150.6 mM. In [^{35}S]GTPγS binding experiments maximal stimulation (E_{max}) was 112.3 ± 1.4% in rat brain and 112.9 ± 2.4% in rat spinal cord. Potency, EC_{50} was 0.7 nM in rat brain and 0.79 nM in rat spinal cord. NN showed moderate agonist activities in stimulating regulatory G-proteins. Stimulatory effect of NN could be maximally inhibited using the potent NTS2 receptor antagonist levocabastine but not by the opioid

receptor specific antagonist naloxone. In homologue displacement studies NN displaced ^{3}H-neuromedin N with a weak affinity. Specific binding of ^{3}H-neuromedin N was saturable, interacting with a single set of homogenous binding sites. The association of the ligand-receptor complex occurred rapidly. Na^{+} ions had a very weak effect on the binding of ^{3}H-neuromedin N. It seems that ^{3}H-neuromedin N in rat brain membranes labels NTS2 receptors. This is based on the following: low affinity, levocabastine selectivity and less sensitivity to Na^{+} ions.

MO07-23

THE AMYLOID PRECURSOR PROTEIN FAMILY IS PRESENT AT THE PRESYNAPTIC ACTIVE ZONE

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There is a wealth of literature describing the role of amyloid precursor protein (APP) in Alzheimer disease albeit our knowledge about the physiological function of APPs remains rudimentary. APP belongs to the amyloid precursor protein family, which in mammals includes the amyloid precursor-like protein 1 (APLP1) and amyloid precursor-like protein 2 (APLP2). All three members contain highly conserved regions in particular within the ectodomain and the intracellular tail. All three proteins are similarly cleaved by the α-, β-, γ-secretase, although APLP1 and APLP2 lack the expression of the Aβ region. APP and APLP2 mRNAs are ubiquitously expressed in a variety of developing and adult mouse tissues, in contrast, the APLP1 mRNA is restricted to the nervous system. Employing subcellular fractionation of synaptosomes derived from rodent brain and using a monoclonal antibody directed against the integral vesicle membrane protein SV2 we recently immunopurified synaptic vesicles and a presynaptic compartment containing the active zone with synaptic vesicles docked to the presynaptic plasma membrane. The immunoisolated fractions were analyzed by electron microscopy and individual protein bands separated by SDS-PAGE, 2-dimensional BAC/SDS-PAGE and double SDS-PAGE were subjected to mass spectrometry and Western blotting.

Upon subcellular fractionation of synaptosomes derived from mouse brain all three members of the APP family were found to migrate as a broad peak in the denser fractions of the sucrose gradient revealing an overlap with the migration behavior of the docked synaptic vesicle fractions. Whereas all APP family members were absent from the immunoisolated synaptic vesicle fraction, they were present in the immunoisolated presynaptic active zone fraction. Our results add novel information to the proteome of the presynaptic active zone and imply a functional role of amyloid precursor proteins at the active zone.

MO07-24

INHALED ANESTHETIC EFFECTS ON NEUROTRANSMITTER RELEASE IN RAT CNS

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Inhaled anesthetics inhibit Na^{+} channel-dependent 4-aminopyridine (4AP)-evoked release of glutamate at greater potencies than that of GABA. Although glutamate and GABA are the major excitatory and inhibitory neurotransmitters in the CNS, a direct comparison of the sensitivities of the release of non-amino acid transmitters to inhaled anesthetics has not been explored. We tested the hypothesis that inhaled anesthetics primarily affect the release of neurotransmitters via presynaptic Na^{+} channel block by comparing the effects of the model inhaled anesthetic isoflurane on 4AP- and elevated K^{+} (Na^{+} channel independent)-evoked transmitter release from neurochemically distinct nerve terminals. Isolated nerve terminals from adult male Sprague-Dawley rat brain regions were prelabeled with [^{3}H]glutamate, [^{14}C]GABA, [^{3}H]norepinephrine, [^{14}C]dopamine or [^{3}H]choline. Release was evoked by either 0.1 mM 4AP or 15 mM KCl from superfused synaptosomes at 37°C in the presence of calcium and various concentrations of isoflurane. Fractional release above baseline in the presence of isoflurane were fitted to sigmoidal curves and differences between IC_{50} values were determined by F-test. Isoflurane inhibited 4AP-evoked glutamate release (IC_{50} = 0.37 ± 0.03 mM: $p < 0.05$) more potently than GABA (0.52 ± 0.03 mM), norepinephrine (0.48 ± 0.03 mM), dopamine (0.48 ± 0.03 mM) or acetylcholine (0.48 ± 0.02 mM). Release evoked by elevated K^{+} was not inhibited by clinical concentrations of isoflurane (0.2–0.7 mM), except for dopamine (IC_{50} = 0.59 ± 0.03 mM), which was not inhibited as potently as dopamine release evoked by 4AP ($p < 0.05$). Na^{+} channel-dependent transmitter release was more sensitive to isoflurane inhibition than was Na^{+} channel-independent release for all five transmitters tested, consistent with Na^{+} channel block as the major mechanism determining anesthetic sensitivity to release inhibition. Isoflurane potencies to inhibit Na^{+} channel-dependent release of GABA, norepinephrine, dopamine and acetylcholine release were comparable, and less than that to inhibit glutamate release, consistent with nerve terminal differences in the properties of Na^{+} channels on glutamatergic nerve terminals. These findings suggest that presynaptic Na^{+} channel subtypes and/or Na^{+} channel coupling to transmitter exocytosis underlies the sensitivity of transmitter release to inhibition by inhaled anesthetics.

MO08 Signal Transduction

MO08-01

NOTCH SIGNALLING PATHWAY INVOLVEMENT IN THE REMYELINATION PROCESS

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Previous studies demonstrate that a single intracraneal injection of apotransferrin (aTf) in 3-day-old rats increases myelin constituents. These promyelinating effects of aTf were shown in cuprizone-demyelinated animals (Adamo et al., 2006) and lysolecithin (LPC)-induced focal demyelination in the corpus callosum (CC). Notch is a family of receptors involved in the proliferation and maturation of oligodendrocytes (OL) during myelination. We evaluated the effect of aTf and the involvement of the Notch pathway in remyelination in a non-immune toxic model of focal demyelination in the CC.

Adult rats were stereotaxically injected 2% LPC into the CC; 7 days later they received a single dose of aTf or saline solution (SS). A subset received an intraventricular injection of the γ-secretase inhibitor (DAPT) before the aTf injection.

Animals were sacrificed at different times after injection depending on experiments. Subventricular zone (SVZ) and CC were dissected for Western blot and Real time PCR. Oligodendrocyte precursor cell (OPC) proliferation was evaluated by incorporation of BrdU. We studied the activation of the Notch pathway by measuring its intracellular domain (NICD) and the expression of downstream genes by Real time PCR. We observed an increase in NICD and Notch ligand Jagged levels in SVZ of demyelinated rats as compared to controls. Concomitantly, we observed an increase in the expression of Hes genes. aTf induced an increase in the Notch ligand F3/contactin after 24 h, both in controls and demyelinated animals. According to the activation of the Notch pathway by F3/contactin ligand, Real time results showed an increase in MAG gene expression 24 h after aTf injection in LPC animals. Moreover, aTf induced an increase in APC+ OL population, as well as a decrease in OL precursors NG2+ in SVZ and CC. We observed a higher incorporation of BrdU in demyelinated rats compared to controls but aTf induced a decrease in cell proliferation. DAPT injection blocked aTf effects on remyelination. Results hint at the participation of the Notch signalling pathway in remyelination and in the promyelinating effects of aTf.

MO08-02

LOCALISATION OF THE LOW AFFINITY CATECHOLAMINE BINDING SITE IN TYROSINE HYDROXYLASE

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Tyrosine Hydroxylase (TH) performs the first and rate limiting step in the synthesis of catecholamines, which feedback to regulate the enzyme by irreversibly binding to the active site and inhibiting TH activity. During neuronal or chromaffin cell activation, phosphorylation of Ser40 relieves this inhibition by allowing the dissociation of catecholamine, thereby increasing the rate of catecholamine synthesis. We have recently documented the existence of a second type of catecholamine binding which has a lower affinity for the enzyme, is dissociable, is not abolished by phosphorylation and inhibits TH by competing with the essential cofactor, tetrahydrobiopterin. Here, we have localised this novel inhibitory site to the active site of TH by substituting a number of active site residues. E332D and Y371F increased the IC50 of dopamine for the low affinity site 10-fold and 70-fold respectively in phosphorylated TH, indicating dramatic reductions in affinity. Much smaller effects were seen in the non-phosphorylated form, with 2–4 fold increases in IC50 measured for E332D and Y371F, and also for L294A and F300Y. This suggests that the active site structure including the low affinity site changes upon phosphorylation of the N-terminus. The residues described here were also involved in the high affinity site; this included a loss of competition with tetrahydrobiopterin for E332D, A297L and Y371F, and a decreased ability to inhibit catalysis (V_{max}) for A297L and Y371F. The common roles of E332 and Y371 in low and high affinity catecholamine binding indicate that these sites are co-localised in the active site. However, it has been previously shown that the two types of catecholamine binding occur simultaneously, each measuring a stoichiometry of approximately 1mol catecholamine/mol TH dimer. Since the TH quaternary structure is known to be a dimer of dimers, a possible model of catecholamine inhibition may therefore involve one high and one low affinity site per dimer, where both types of binding can act to regulate TH activity in their respective monomers.

MO08-03

INDUCIBILITY OF HEAT SHOCK PROTEINS BY CELASTROL AND TEMPERATURE ELEVATION VARIES WITH DEVELOPMENTAL AGE OF CORTICAL CULTURES

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Neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS), have been termed 'protein misfolding disorders' that are characterized by the neural accumulation of protein aggregates. Upregulation of heat shock proteins (Hsps) could alleviate neurodegeneration by modulating protein misfolding in affected neural cells. Hsps are 'protein repair agents' that provide a line of defense against misfolded, aggregation-prone proteins. Primary cortical cultures have been widely employed as a model system for studies that include the investigation of neuroprotective mechanisms. Here we investigate heat shock proteins along the time course of developmental age of rat primary cortical cultures. We noted that Hsp27 and Hsp32, but not Hsp70, demonstrated basal expression in the absence of inducing agents, with Hsp32 arising at an earlier culture age compared to Hsp27. Celastrol induced a robust induction of Hsp27 and Hsp32 but this was achieved only at culture ages that showed basal expression. Induction of Hsp70 by celastrol was observed solely in cultures of advanced age. Temperature elevation was a less effective inducer of Hsp27 and Hsp32 at all culture ages compared to celastrol and failed to induce Hsp70 at any stage. These results demonstrate that inducibility of potentially neuroprotective Hsps

varies with the developmental age of primary cortical cultures and correlates with basal Hsp expression levels. In addition, temperature elevation, the classical inducer of the heat shock response, proved to be a less effective inducer of Hsps compared to celastrol which shows promise as a potential pharmacological agent to counteract protein misfolding, a central feature of neurodegenerative diseases.

MO08-04

CONFORMATIONAL CHANGES IN EXTRACELLULAR LOOP 2 ASSOCIATED WITH SIGNAL TRANSDUCTION IN THE GLYCINE RECEPTOR

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The Cys-loop family of ligand gated ion channels (LGICs) includes the nicotinic acetylcholine receptor (nAChR), serotonin type 3 receptor (5-HT3R), γ-aminobutyric acid type A receptors ($GABA_AR$) and the glycine receptor (GlyR). These receptors are pentamers with each subunit containing four transmembrane domains (M1-M4) (Betz 1990) and a large extracellular domain (ECD). The ligand binding site is located in the ECD and is spatially distant from the transmembrane domain M2, which lines the pore (Unwin, 1993; Corringer et. al., 2000). Several studies have proposed that loops 2 and 7 of the ECD might be involved in coupling ligand binding to receptor activation. In the present study, seven residues of loop 2 in the ECD of the human GlyR believed to be critical to the signal transduction process were investigated by assessing the availability of substituted cysteines for covalent modification. Mutants were produced using site-directed mutagenesis, expressed in HEK293 cells and assessed using whole-cell patch clamping. One of the mutants showed no glycine-evoked currents. Concentration-response curves for three of the mutants showed small increases in EC50, compared to WT, while the remaining three mutant receptors exhibited a 29-fold increase in the EC50 for glycine. Thus, introducing mutations in the ECD loop 2 disrupts the transduction mechanism that couples ligand binding and channel opening. By using two derivates of methane thiosulfonate (MTSET and MTSES) we also demonstrate that loop 2 undergoes a conformational change upon ligand binding. This demonstrates the important role played by the ECD loop 2 of the GlyR in signal transduction, which is likely to be applicable to other members of the Cys-loop family.

MO08-05

TYPE 1 EQUILIBRATIVE NUCLEOSIDE TRANSPORTER REGULATES ETHANOL DRINKING THROUGH ACCUMBAL NMDA RECEPTOR SIGNALING

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Mice lacking type 1 equilibrative nucleoside transporter exhibit increased ethanol-preferring behavior compared to wild-type littermates. This phenotype of ENT1 null mice appears to be correlated with increased glutamate levels in the nucleus accumbens (NAc). However, little is known about the downstream consequences of increased glutamate signaling in the NAc. To investigate the significance of the deletion of ENT1 and its effect on glutamate signaling in the NAc, we employed microdialysis and iTRAQ proteomics. We validated altered proteins using Western blot analysis. We then examined the pharmacological effects of the inhibition of the N-Methyl-D-Aspartate (NMDA) glutamate receptor and protein kinase Cγ (PKCγ) on alcohol drinking in wild-type mice. In addition, we investigated *in vivo* cAMP response element binding (CREB) activity using CRE-lacZ mice in an ENT1 null background. We identified that NMDA glutamate receptor-mediated down-regulation of intracellular PKCγ-neurogranin (Ng)-Ca^{2+}-calmodulin dependent protein kinase type II (CaMKII) signaling is correlated with reduced CREB activity in ENT1 null mice. Inhibition of PKCγ promotes ethanol drinking in wild-type mice to levels similar to those of ENT1 null mice. In contrast, an NMDA glutamate receptor antagonist reduces ethanol drinking of ENT1 null mice. These findings demonstrate that the genetic deletion or pharmacological inhibition of ENT1 regulates NMDA glutamate receptor-mediated signaling in the NAc which provides a molecular basis that underlies the ethanol-preferring behavior of ENT1 null mice.

MO08-06

ON THE INTERPLAY BETWEEN THE NORADRENERGIC SYSTEM AND CB1 RECEPTORS DURING THE CONSOLIDATION OF OBJECT RECOGNITION MEMORY IN RATS

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Noradrenaline modulates attention, novelty detection and arousal and, so, it could play a key role in the consolidation of declarative memories. CB1 cannabinoid receptors are abundant in medial temporal lobe structures, including the hippocampus, where they modulate the release of noradrenaline. Therefore, and since activation of hippocampal CB1 receptors interfere with the processing of declarative memories, we analyzed the possible interplay between the noradrenergic and the endocannabinoid systems during object recognition (OR) memory consolidation in rats.

Infusion of the $GABA_A$ receptor agonist muscimol to the locus coeruleus, the main source of noradrenergic fibers to the hippocampus, hindered OR memory formation and the training-induced increases in hipocampal mature BDNF (brain-derived neurotrophic factor) levels. Both of these effects were reversed when either noradrenaline or a membrane-permeable analog of cAMP (8-Br-cAMP) were injected in the dorsal CA1 region. These findings demonstrate the importance of noradrenergic signaling for the consolidation of information about novel objects.

We also found that, when injected into the CA1 region of the dorsal hippocampus immediately after OR training, the CB1 receptor selective agonist ACEA hindered recognition memory and diminished the learning induced increases in mature BDNF levels, phosphorylation of TrKB (the cellular receptor for BDNF)

and phosphorylation of tyrosine hydroxylase at the serine 40 residue. These results suggest that the amnesia caused by activation of CB1 receptors is due to a reduced functionality of the noradrenergic system. Indeed, we found that co-infusion of noradrenaline reversed the amnesic effect caused by intra-CA1 infusion of ACEA and restored the learning-induced increase in hipocampal BDNF and pTrKB levels hampered after treatment with ACEA.

MO08-07

INHIBITION OF PROTEIN SYNTHESIS INCREASES NITRIC OXIDE PRODUCTION AND ACTIVATION OF DOWNSTREAM SIGNALING PATHWAYS IN AVIAN RETINA

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The synthesis of nitric oxide (NO) is limited by the intracellular availability of the substrate L-arginine (L-Arg). Our previous work has shown that stimulation of NMDA receptors promotes an increase of intracellular L-Arg which supports an increase of NO production as observed by measuring intracellular and extracellular L-[^{3}H] citrulline accumulation by chick retinal cells in culture. This effect appeared to be mediated by phosphorylation of the translation factor eEF2, thereby decreasing the rate of protein synthesis. Indeed, free intracellular L-[^{3}H] arginine was six times higher in cultures treated with the protein synthesis inhibitor cycloheximide (CHX) than in control cultures, and the intracellular and extracellular L-[^{3}H] citrulline levels increased two or three-fold in CHX-treated cultures, respectively. Here we show the same levels of stimulation by directly measuring NO levels. Anisomycin (ANISO, another inhibitor of protein synthesis) and the NO donor S-nitroso-N-acetylpenicillamine (SNAP) showed the same stimulation profile when compared with CHX. Both CHX and ANISO effects were blocked with the NO synthase inhibitor L-N^G-Nitroarginine Methyl Ester (L-NAME). We also show that treatment with SNAP or L-Arg is able to enhance AKT and ERK phosphorylation approximately three times above control. Interestingly, CHX and ANISO similarly increased AKT and ERK phosporylation, but with different time courses (maximum in five minutes for ERK and thirty minutes for AKT). Those effects were blocked by L-NAME, KT-5823 (a cGMP protein kinase inhibitor) and 1H-[1,2,4] Oxadiazole [4,3-a]quinoxalin-1-one (ODQ, a guanylate cyclase inhibitor), indicating that protein synthesis inhibition can trigger NO downstream signaling pathways after increasing the intracellular availability of the substrate L-Arg. The results suggest that some of the effects of protein synthesis inhibitors could be mediated by NO and the activation of NO-dependent intracellular pathways.

MO08-08

N- AND C-TERMINAL SIGNAL DOMAINS ARE ESSENTIAL FOR TARGETING THE NEURONAL PROTEIN BM88/CEND1 TO THE MITOCHONDRIAL OUTER MEMBRANE

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Although targeting of proteins to the inner mitochondrial membrane has been studied in great detail, less is known on the biogenesis of the mitochondrial outer membrane (MOM). Many MOM proteins are also found in the endoplasmic reticulum (ER) and are anchored to the lipid bilayer by a short hydrophobic domain close to the C-terminus, with the N-terminal domain exposed to the cytosol. The neuronal pro-differentiation protein BM88/CEND1 has similar topological features and is distributed in several intracellular organelles, most notably the MOM and ER. BM88/CEND1 contains a hydrophobic transmembrane region followed by a short tail of three positively charged residues (RKK) on its C-terminus both of which represent potential MOM-targeting signals. In silico analysis revealed two ER-like retention motifs, ESRG and LARK on the N- and C-terminal region, respectively. To evaluate the significance of these motifs for proper targeting, two mutants were generated: one in which ESRG was deleted and a second in which RKK was replaced by AAA. Two additional mutants were produced, one with deletion of the first 19 amino acids and another with the double mutation (ΔESRG, RKK->AAA). All four mutants and the wild type protein were fused with EGFP at their carboxy-terminals. Co-localization studies were performed using organelle-specific markers, confocal microscopy and IMARIS software image analysis in transfected mouse neuroblastoma cells. EGFP-mutants showed significant reduction to MOM targeting by 6.5-fold, 10-fold, 4-fold and 7-fold for ΔESRG, RKK->AAA, (ΔESRG, RKK->AAA) and Δ19aa, respectively as compared to the non-mutant protein fused or not with EGFP. Notably, no significant changes were observed between wild-type and mutants in ER-localization. Fractionation analysis confirmed the differential mitochondrial distribution of mutant and non-mutant proteins. These results demonstrate that, in addition to the essential C-terminus RKK signal, amino-terminal sequences are also critical specifically for MOM, but not ER, targeting of the neuronal protein BM88/CEND1. Supported by FP7 REGPOT Project 264083 Neurosign.

MO08-09

DEVELOPMENTAL REGULATION OF PROTEIN O-GLCNACYLATION, O-GLCNAC TRANSFERASE, AND O-GLCNACASE IN MAMMALIAN BRAIN — A BIOCHEMICAL STUDY

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Protein O-GlcNAcylation is a dynamic, regulatory posttranslational modification of protein by β-N-acetylglucosamine (GlcNAc), which is transferred enzymatically from UDP-GlcNAc donor to the hydroxyl group of serine or threonine residues of proteins. O-GlcNAcylation is catalyzed by O-GlcNAc transferase (OGT), and the O-GlcNAc groups of proteins can be removed with the

catalysis of O-GlcNAcase (OGA). Numerous neuronal proteins, including those involved in neuronal communication, signal transduction, transcriptional regulation and cytoskeletal proteins, are modified by O-GlcNAc, suggesting that it regulates many brain functions. Both OGT and OGA are highly expressed in the brain. Here, we investigated the levels of global O-GlcNAcylation, OGT and OGA in rat brains during development from embryonic day 15 to the age of 24 months by Western blots. We found that the global O-GlcNAcylation was very high during embryonic stages, and it remained relatively stable after 5 days of age. Two isoforms of the nucleocytoplasmic OGT were differentially regulated during development. The 110-kDa isoform was highly expressed during early development and then its level declined gradually after the age of 15 days, whereas the 78-kDa isoform was seen only after 15 days old. The level of the cytosolic OGA increased after birth (birth to 15 days) and then kept stable during the rest of life, but the nuclear variant of OGA decreased dramatically during embryonic development and was hardly detectable after birth. These results demonstrate the detail developmental regulation of O-GlcNAcylation, ODT and OGA in the rat brain.

MO08-10

ANATOMICAL LOCALIZATION OF LYSOPHOSPHATIDIC ACID (LPA) RECEPTOR IN RODENT AND HUMAN BRAIN

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The lysophosphatidic acid (LPA) is a lipid molecule with multiple biological functions that may be modified in neurodegenerative diseases, such as Alzheimer's disease. The LPA has a relevant role in the CNS signaling during the embryo development and at least two LPA receptors, LPA1 and LPA2, are expressed in the developing brain. However the precise anatomical distribution of the LPA receptors in the brain is still unknown. Therefore, the present study, measures the density of the Gi/o proteins activity mediated by LPA receptors studying their anatomical distribution by using the [35S] GTPgammaS autoradiography assay. We compensated the lack of specific antagonists for the LPA receptor checking the selectivity of the results with those obtained for the maLPA1-null mice. The autoradiographic distribution of functional LPA receptors in human and rodent brain showed the highest densities located in white matter regions. The LPA stimulates the binding of [35S] GTPgammaS in the internal capsule of both, human (656% ± 539) and rodent brains (1002% ± 653). Furthermore, the white matter located at the circumvolutions of the human frontal cortex showed a percentage of stimulation of the [35S]GTPgammaS binding mediated by LPA of 861% ± 797. On the other hand, in the corpus callosum of rodent brain, a stimulation of 132% ± 32 was observed. The white matter that is surrounded by the cerebellar cortex also had a high stimulation (274% ± 128). Other regions analyzed such as amygdala, striatum or hippocampus showed minor densities of functional LPA receptors. The reported localization of LPA receptors in human and rodents will facilitate the study of their specific alteration in neurological diseases.

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MO08-11

AMPK REGULATES BDNF-INDUCED ACTIVATION OF MTOR SIGNALING AND ENHANCEMENT OF TRANSLATION IN CORTICAL NEURONS

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AMPK (AMP-activated protein kinase) is known as a cellular 'energy sensor', since its activity is sensitively regulated by the cellular energy state. An increase in the AMP: ATP ratio activates AMPK. In contrast to the situation in fibroblasts where amino acids sufficiency was essential for growth factor-induced activation of mTOR(mammalian target of rapamycin) signaling, glucose-deprivation (but not amino acid-deprivation) canceled the BDNF(brain-derived neurotrophic factor)-induced mTOR signaling activation in neurons. Thus we examined the roles of AMPK in mTOR signaling and succeeding translation that were activated by BDNF, insulin and serum in cultured rat cortical neurons. Glucose deprivation changed the phosphorylation level of translation regulators such as p70S6Kinase and eEF2 (eukaryotic elongation factor 2), to decrease translation efficiency. As an end point of biological response, glucose deprivation markedly suppressed protein synthesis and the re-addition of glucose restored it. The effect of growth factors, BDNF and Insulin, on the activation of translation factors and novel protein synthesis in the presence or absence of glucose were examined. Although they activated translation in neurons in the presence of glucose, BDNF and insulin had no effects on protein synthesis, nor on the activation of translation regulators under the glucose-deprived condition. Phosphorylation of p70S6K was inhibited specifically at Thr389, a substrate residue of mTOR, but not on MAPK targets Thr421/Ser424. The result suggests that glucose deprivation used in this study causes a rather specific activation of AMPK without causing general ATP-deficiency. In fact, similar results on both p70S6K phosphorylation and protein synthesis were obtained by the treatment of AMPK activators, metformin and AICAR in neurons. Furthermore, overexpression of constitutivelyactive AMPK (AMPKα1. 1-312) decreases p70S6K phosphorylation and protein synthesis. These results indicate that AMPK dominantly regulates mTOR signaling downstream of BDNF.

MO08-12

PHOSPHORYLATION OF CDK5 AT TYR15 IS INHIBITED BY P35 CDK5 ACTIVATOR AND DOES NOT CONTRIBUTE TO THE ACTIVATION OF CDK5

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Cdk5 is a member of cyclin-dependent kinase family. While other Cdks play a role mainly in cell cycle progression of proliferating cells, Cdk5 play a role in various neuronal activities such as neuronal migration, synaptic activity and neuron death. Cdk5 is activated by binding to p35 or p39. In contrast to Cdk1 and Cdk2, which are inhibited by phosphorylation at Tyr15, Cdk5 is reported to be activated by phosphorylation at Tyr15 with Src family tyrosine kinases (SFK) or receptor tyrosine kinases (RTK). However, it is not known how cycling Cdks and neuronal Cdk5 are

oppositely regulated by their Tyr15 phosphorylation. In this study, we have reinvestigated the effect of Tyr15 phosphorylation of Cdk5 on its activation using the COS-7 cell overexpression system and cultured neurons. When Cdk5-p35 was coexpressed with constitutive active Fyn, however, phosphorylation of Cdk5 at Tyr15 was not detected. Its phosphorylation was obesrved only when Cdk5 alone was coexpressed with active Fyn. Increasing p35 expression decreased Tyr15 phosphorylation gradually. These results indicate that Cdk5 is phosphorylated at Tyr15 by Fyn only when it is a monomeric free form, and the binding of p35 inhibits the Tyr15 phosphorylation. Further, we found by cell fractionation and immunoprecipitation experiments that phosphorylated Cdk5 did not bind to p35. These results suggest that Cdk5 is not activated by phosphorylation at Tyr15. If so, how is Cdk5-p35 activated by SFK or RTK? When Cdk5-p35 was coexpressed with Fyn, the total activity of Cdk5-p35 increased along with increased p35, suggesting that, extracellular signals, which activate SFK or RTK, would increase the Cdk5-p35 activity by stabilizing p35.

MO08-13

STRUCTURE AND DOMAIN MOTION OF 5′-NUCLEOTIDASE

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In vertebrates, ecto-5'-nucleotidase (CD73, 5NT) hydrolyzes extracellular AMP to adenosine as part of extracellular purinergic signaling pathways. The structure of a related nucleotidase from *E. coli* has been characterized in two conformations (open and closed), which differ in the relative orientation of the two domains by a rotation of up to 96° (1). The domain movement can be described as a rotation of the C-terminal domain around an axis, which passes through the center of the C-terminal domain. The resulting domain movement is unique in that the cleft between the domains does not open up, but the residues of the domain interface slide along the interface. The conformational change is necessary for the catalytic action of the enzyme, presumably to allow for substrate binding and product release (2, 3).

In more recent work, we have determined X-ray structures of the dimeric human ecto-5NT, which is specific for AMP. The bacterial enzymes hydrolyze ATP, ADP and AMP as well as other nucleotides. Furthermore, the domain motion of E. coli 5NT is studied by NMR, EPR and FRET spectroscopy. Due to the independent motion of the two domains, the NMR spectra show relatively sharp peaks for a 58 kDa protein. In order to study the domain orientation in solution via residual dipolar couplings, the two domains were expressed independently and the resonances are assigned via HNCACB and HNCOCACB spectra.

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MO08-14

POSSIBLE DIFFERENT ROLES OF TWO PHOSPHORYLATION SITES ON $GABA_B$ RECEPTORS R2 SUBUNIT

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$GABA_B$ receptors are members of the G protein-coupled receptor (GPCR) superfamily and mediate slow and prolonged synaptic inhibition in the brain. Unlike other members of the GPCR superfamily, functional $GABA_B$ receptors are heterodimers formed by 2 subunits identified as $GABA_BR1$(R1) and $GABA_BR2$ (R2). R2 is highly phosphorylated at serine 892 (S892) by protein kinase A. In addition to protein kinase A, 5'-AMP-activated protein kinase (AMPK) phosphorylates another residue serine 783 (S783). Phosphorylation of both sites suppresses desensitization of $GABA_B$ receptors. To date, however, it is difficult to distinguish the importance and timing of the phosphorylation in these sites. Therefore, we tried to elucidate functional significance and regulation of phosphorylation in these sites. Of mouse discrete brain structures including the olfactory bulb, cerebellum, medulla-pons, hypothalamus, striatum, midbrain, hippocampus, and cerebral cortex, the hippocampus and cerebral cortex had the highest expression of R2 with phosphorylation of both sites (p892 and p783). Relative phosphorylation of p892 to total R2 was almost even among these brain regions, whereas that of p783 was uneven among these brain regions. In the acute hippocampal slices, the level of p892 was decreased during the incubation with the artificial cerebrospinal fluid (aCSF) with being blocked by okadaic acid. In addition, the level of p892 was decreased by tetrodotoxin in cultures of the hippocampal neurons. In the acute hippocampal slices, the level of p783 was decreased immediately during the incubation with aCSF, coincident with the decrease in the AMPK activity. Phosphorylation status of S783 was easily disappeared during the preparative procedures of immunoprecipitation *in vitro*, suggesting that endogenous serine/threonine phosphatases dephosphorylate the p783. However, exogenously added phosphatases were ineffective in dephosphorylating the p783. These results suggest that in the central nervous system, S892 is highly phosphorylated during neuronal excitation process, with being dephosphorylated by okadaic acid-sensitive phosphatases. Phosphorylation of S783 may be promptly regulated by AMPK and/or undefined phosphatase in response to cellular signals.

MO08-15

IDENTIFICATION OF REGULATORY PHOSPHORYLATION SITES IN THE C-TERMINAL DOMAIN OF NEUROFIBROMIN

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Neurofibromin, the protein product of the Neurofibromatosis I (Nf1) tumor suppressor gene (TSG), is a negative regulator of plasma membrane-bound Ras GTPases through its RasGAP domain; its function as a TSG is, however, expected to additionally depend on its ability to shuttle to the nucleus via its Nuclear Localization Signal-containing C-terminal domain (CTD). Towards understanding the mechanism of action of this TSG we have recently established, using a phospho-sensitive NF1 antibody that

PKC phosphorylation of neurofibromin on its CTD correlates with its translocation from the nucleus to the cytosol in differentiated SH-SY5Y cells after long-term exposure to PKC agonists. To further investigate the properties of neurofibromin-CTD we undertook a systematic mutagenesis study of Ser residues. Wild type CTD retained its nuclear localization, while PKC activation increased its extranuclear localization and, more importantly, its expression levels. Mutation of Ser2808 to Ala (phospho-ablating) revealed that this is a primary site for PKC, as the recognition by the phospho-sensitive antibody was abolished. Moreover, mutation of this serine to Asp (phosphomimetic) led to a subcellular distribution similar to phosphorylated WT-CTD and to similar increases of expression. To establish this apparent PKC phosphorylation-dependent stabilization of the CTD, and feasibly of full length neurofibromin, we introduced an additional mutation of a proximal Ser (2813) to Ala which resulted in proteasome-dependent degradation. Our data indicate that PKC phosphorylation of specific serines in neurofibromin may regulate its stability and subcellular distribution, and provide a mechanism for the nucleocytoplasmic shuttling of this TSG.

MO08-16

RELEASE OF ATP FROM MÜLLER CELLS OF THE EMBRYONIC CHICK RETINA IN CULTURE

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In this study, we investigated the effect of glutamate receptors activation, KCl-induced depolarization and intracellular calcium rise on the vesicular release of ATP from Müller cells of the embryonic chick retina in culture. We estimated ATP release by examining quinacrine staining and by measuring the extracellular levels of ATP in enriched Müller glia cultures.

Quinacrine staining revealed fluorescent vesicles in the soma of Müller cells that could not be observed when cultures were pre-treated with 1 μM bafilomycin A1 or 2 μM Evans blue. Whilst no difference in quinacrine staining was observed when cells were kept for 10 min in Hanks' solution, treatment with 50 mM KCl, 1 mM glutamate or 0.1 mM kainate resulted in reduced intracellular levels of quinacrine fluorescence at the same period of time. KCl-induced quinacrine staining reduction was blocked by BAPTA-AM (30 μM). Treatment of cells with ionomycin (5 μM) induced a reduction in quinacrine intracellular levels after 10 min, an effect that was blocked by 1 mM EGTA. Either 50 mM KCl or 1 mM glutamate was able to increase the levels of ATP in the extracellular medium by 77% and 89.5%, respectively, after a 5 min incubation of the cells. Glutamate-induced rise in extracellular ATP could be mimicked by 100 μM kainate (81.5%) but not by 100 μM NMDA in medium without $MgCl_2$ but with 2 mM glycine. However, glutamate-induced increase in extracellular ATP levels were blocked by 50 μM DNQX, MK-801 or 200 μM AP5, suggesting the involvement of both NMDA and non-NMDA receptors. Kainate-induced ATP release was also inhibited by DNQX and MK-801. Extracellular ATP accumulation induced by glutamate was also blocked by incubation of the cells with 30 μM BAPTA-AM or 1 μM bafilomycin A1. These results suggest that cultured glial cells from chick embryonic retina present ATP-containing vesicles that can be released in a calcium-dependent way by activation of glutamate receptors and KCl depolarization. Supported by: CNPq, FAPERJ, PROPPi-UFF.

MO08-17

CREB1 AND CREB BINDING PROTEIN IN STRIATAL MEDIUM SPINY NEURONS REGULATE BEHAVIOURAL RESPONSES TO PSYCHOSTIMULANTS

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The transcription factor cAMP responsive element binding protein 1 (CREB1) has a complex influence on behavioural responses to drugs of abuse that varies depending on the brain region in which it is expressed. In response to drug exposure CREB1 is phosphorylated in the striatum, a structure that is critically involved in reward related learning. The aim of the present study was to investigate the role of striatal CREB1 and its coactivator CREB binding protein (CBP) in behavioural responses to psychostimulants. Using the 'cre/lox' recombination system we generated mice with a postnatal deletion of CREB1 or CREB binding protein (CBP) directed to medium spiny neurons of the striatum. qRT-PCR and immunohistochemistry were used to confirm the deletion, and mice were assessed with respect to their locomotor response to acute cocaine (20 mg/kg), cocaine sensitization (10 mg/kg), amphetamine induced stereotypies (10 mg/kg) and ethanol-induced hypnosis (3.5 g/kg). Here we show that CREB1 mutant mice have increased sensitivity to psychostimulants, an effect that does not generalise to ethanol-induced hypnosis. Furthermore, in the absence of CREB1 there is rapid postnatal upregulation of the related transcription factor CREM, indicating possible redundancy amongst this family of transcription factors. Finally striatal deletion of CBP, a critical coactivator for the CREB1/CREM signalling pathway, also results in increased sensitivity to psychostimulants. These data suggest that striatal CREB1 regulates sensitivity to psychostimulants, and that CREM, acting via CBP, is able to partially compensate in the absence of CREB1 signalling.

MO08-18

IDENTIFYING PDZ PROTEINS INVOLVED IN SCAFFOLDING THE SEROTONIN/ CRFR1 RECEPTORS COMPLEX

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Our group has shown that the activation of CRFR1 receptors causes an increase in 5-HT_2 signalling due to an increase of these receptors at the cell surface, a mechanism we named heterologous sensitization. This synchronized serotonin receptors recruitment to the cell surface is dependent on CRFR1 activation and consequent internalization followed by Rab 4-dependent rapid recycling. Our studies also showed that the molecular engagement between these receptors relies on the association of PDZ proteins since PDZ binding motif removal from both receptors abolishes the observed effect. More importantly, this molecular mechanism has been shown to be physiologically relevant *in vivo* since mice stimulated with CRF peptide and then challenged with a serotonin agonist showed increased serotonin-mediated anxiety behavior. We are now concentrating on identifying the PDZ protein(s) that is (are) crucial for the

scaffolding of these receptors complex by using siRNAs to knock-down potential candidates and potentially inhibit this heterologous sensitization of serotonin receptors. Since we observed the heterologous sensitization of 5-$HT_{2A/C}$ receptors in HEK cells and cortex slices, we started screening for PDZ proteins that are expressed in both. We started with 16 different PDZ proteins that are related to regulation of GPCR trafficking and signalling and designed primers to conduct RT- PCR. We identified a few PDZ proteins (among them SAP97 and SAP102) which are expressed in both HEK cells and cortex. We are now starting to test different siRNA delivery methods to knock down the expression of each of these proteins in HEK cells. We will then be able to assess their role in the heterologous sensitization of 5-$HT_{2A/C}$ receptors using physiological assays such as IP_3 formation. The heterologous sensitization mechanism presents a way to integrate different signalling pathways that are important for normal and pathophysiological states, opening a new line of research on the molecular pharmacology field and translational science.

MO08-19

INFLUENCE OF CHANGES IN MEMBRANE CHOLESTEROL CONTENT ON MUSCARINIC M1, M2, AND M3 RECEPTOR SUBTYPE SIGNALING

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We investigated the influence of membrane cholesterol content on Gq/11 G-protein activation induced by activation of preferential M1/3 or non-preferential M2 muscarinic receptors. To this end we used Chinese hamster ovary (CHO) cells expressing these receptor subtypes individually. We used free or cholesterol-saturated methyl-beta-cyclodextrin to deplete or increase membrane cholesterol content, respectively. Cholesterol depletion significantly reduced efficacy of carbachol in stimulating IP accumulation through both preferential M1/3 and non-preferential M2 subtypes of muscarinic receptors. The decrease in efficacy of carbachol was accompanied by a decrease in potency at the M1 subtype but, in contrast, by an increase in potency for M2 and M3 subtype-mediated responses. Cholesterol enrichment significantly reduced the efficacy of carbachol in stimulating IP accumulation at M1 receptors but had no influence at M2 and M3 receptors, and did not change the potency of carbachol at any studied receptor subtypes. Cholesterol-modifying treatments had no effect on the concentration of Gq/11 G-protein alpha subunits. Cholesterol supplementation decreased 3H-NMS binding in intact cells to a similar extent at M1 and M3 subtypes (by about 35%) but had no significant effect at M2 subtype. Increased membrane cholesterol had no influence on receptor affinity at any subtype. Cholesterol depletion significantly increased 3H-NMS binding by about 40%, 70%, and 20% at M1, M2, and M3 subtypes, respectively. The increases in 3H-NMS binding were accompanied by the significant 2.10-fold and 1.62-fold decrease in affinity at M2 and M3 subtypes, respectively while the affinity at M1 subtype was not changed. These results demonstrate differential sensitivity of muscarinic receptor signaling to changes in membrane cholesterol content. The deviations in signal transduction observed at the M1 receptor subtype by both membrane cholesterol enrichment and depletion may have relevance to the role of impaired M1 signaling in Alzheimer's disease progression.

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MO08-20

SUSTAINED PHOSPHORYLATION OF TYROSINE HYDROXYLASE AT SERINE 40 *IN VIVO*

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Tyrosine hydroxylase (TH) is the rate-limiting enzyme in catecholamine synthesis. Its activity is know to be controlled acutely (minutes) by phosphorylation and chronically (days) by protein synthesis. Using bovine adrenal chromaffin cells our laboratory found that nicotine and PACAP, the major neurotransmitters released on activation of splanchnic nerves, induced sustained activation of TH (via Ser40 phosphorylation) for 24 h, which was completely independent of TH protein synthesis. Glucoprivation elicited by 2-deoxyglucose (2DG) which is a metabolic stressor known to activate the adrenal chromaffin cells by splanchic nerve activation did not show sustained TH phosphorylation. TH protein levels were significantly increased (2.3 fold, $p < 0.001$) but Ser40 phosphorylation levels at 24 h were not increased. However another stressor, neonatal injection of lipopolysaccharide (LPS) did cause a sustained increase in Ser40 phosphorylation levels at 4 h and 24 h following injection ($F(1,21) = 6.19$, $p < 0.05$). In this case TH protein levels were not significantly altered. This is the first study to our knowledge to identify sustained Ser40 phosphorylation *in vivo*. Sustained Ser40 phosphorylation has the ability to cause sustained TH activation and sustained catecholamine synthesis without the need for TH protein synthesis. It represents a novel phase between acute and chronic maintenance of catecholamine synthesis.

MO08-21

KAPPA OPIOID RECEPTOR SIGNALING IS DIFFERENTIALLY MODULATED BY THE REGULATORS OF G PROTEIN SIGNALING RGS4 AND RGS2 PROTEINS

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Regulators of G protein Signaling (RGS) comprise a large multifunctional protein family that accelerate GTP hydrolysis of Gα subunits, thus modulating G protein coupled receptor (GPCR) signaling. RGS proteins also act as effector antagonists and serve as "platforms" where protein complexes can be formed (1). We have previously demonstrated that RGS4 directly interacts with mu (μ-OR) and delta (δ-OR) opioid receptors to regulate their signaling (2, 3). To deduce whether selectivity in coupling between members of RGS proteins and opioid receptors exist we tested the ability of members of B/R4-RGS family to interact with kappa opioid receptor (κ-OR). Pulldown experiments using GST fusion peptide encompassing the carboxyl terminal tail of kappa opioid receptor (κ-OR) indicated that RGS2 and RGS4 interact within the C-terminal region of κ-OR. Using a truncated version of RGS4 that lacks the N-terminus (ΔNRGS4) we showed that this domain is responsible for RGS4 interaction with κ-OR. Co-immunoprecipitation studies indicated that RGS2 and RGS4 associate with κ-OR constitutively and retain upon agonist stimulation of the kappa receptor. RGS2 and RGS4 display a differential regulatory effect in

κ-OR signaling as assessed by a series of functional assays ranging from cAMP accumulation to ERK1,2 phosphorylation and internalization measurements. Collectively, our results suggest that although κ-OR directly interacts with RGS2 and RGS4 each of these proteins affect signaling in a distinct manner.

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MO08-22

EFFECTS OF SHORT- AND LONG-TERM *IN VIVO* ADMINISTRATION OF MORPHINE ON MAO ACTIVITY AND CAMP SYNTHESIS IN THE RAT MYOCARDIUM

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Short term application of morphine has been shown to induce protection in cardiac myocytes (Schultz and Gross, 2001), isolated working heart (Shi et al., 2003), as well as *in vivo* (Ludwig et al., 2003). However, the mechanism(s) and duration of cardioprotection are not known. Here we investigated whether and for how long *in vivo* administration of a relatively high dose of morphine (10 mg/kg per day, i.m.) influences β-adrenoceptor signaling and the activity of monoaminoxidase (MAO) that is an important source of oxygene radicals in rat heart ventricles. Adult rats were treated with morphine for 10 or 28 days and then used either immediately or after another 7 days without treatment for measurements. Activity of MAO was determined in a crude mitochondrial fraction and cAMP synthesis *in vitro* in heart ventricle slices. We found that 28-day treatment significantly diminishes MAO activity and this effect is blunted after additional 7 days without drug. Interestingly, comparable inhibition of MAO activity to that after 28-day treatment is present after 10-day treatment followed by 7 days without drug. In heart slices, 10-day treatment followed by 7 days without drug has no effect on basal and forskolin-stimulated cAMP synthesis but reduced the response to isoprenaline. Conversely, 28-day treatment followed by 7 days without drug inhibited basal cAMP synthesis but had no effect on isoprenaline- as well as forskolin- stimulated cAMP synthesis. Our data demonstrate similar regulation of MAO activity and isoprenaline-stimulated cAMP synthesis induced by shorter (10 days) and longer (28 days) morphine treatment followed by 7 days without drug while basal cAMP is reduced only after longer treatment. The reductions in cAMP synthesis are not due to changes in adenylylcyclase characteristics because forskolin-stimulated activity remained unchaged by the respective treatments. Supported by AV0Z50110509, IAA501110901.

MO08-23

SIGNAL TRANSDUCTION CHANGES INDUCED BY SPONTANEOUS GLUR-DELTA2 GENE KNOCK-OUT IN LURCHER MICE

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Lurcher mice represent a natural model of olivocerebellar degeneration. Heterozygotes (+/Lc) carry a mutation in the glutamate receptor delta-2 subunit gene resulting in complete postnatal cerebellar Purkinje cells apoptosis causing a secondary degeneration of granule cells and inferior olive neurons leading to a lurching gait and worsened spatial learning. This study was to investigate signal transmission changes in brain parts involved in learning and locomotive functions (striatum, hippocampus, cerebellum). Compared to the wild type, higher amounts of NMDA, D1-like and D2-like receptor binding sites were found in Lurcher mice male hippocampus. Both D1-like and D2-like receptor binding sites were similar in the male striatum and lower in the cerebellum. On the D1 to D5 receptor mRNA (D1R-D5R mRNA) level, higher amounts of D1R-mRNA and D2-mRNA were seen in hippocampus, and D2R-mRNA in male cerebella. No D4R-mRNA was detected in striatum and no difference in other receptor subtype mRNAs was found out in males. In females, no D4R mRNA was seen in striatum and cerebellum and overall, no difference in all mRNA amount was observed. Upon specific D-receptor agonist stimulation, an increase in intracellular hippocampal cAMP level was observed in wild-type males after a specific D1- and D5- agonist R-(+)-SKF-38393 whereas no change occurred in Lurcher. D2 and D3 agonist stimulation lead to similar decrease in cAMP both in wild-type and Lurcher. No difference was seen upon D4 receptor agonist. Presented study shows multiple changes in NMDA and dopamine receptor signalling including both receptor binding sites and gene expression as well, which ultimately results in different intracellular response.

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MO08-24

DEVELOPMENTAL REGULATION OF SPONTANEOUS NETWORK ACTIVITY IN MOUSE CORTICAL SLICES

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The cerebral cortex is intrinsically active. During quiescent brain states (e.g. non-REM sleep and anesthesia) cortical networks develop spontaneous rhythmic activity in the form of slow oscillations (SO). The cellular correlates of the SOs are sustained epochs of depolarization and increased likelihood of action potential firing (Up states), interspersed with epochs of hyperpolarization and decreased activity (Down states). Since Up/Down states develop spontaneously, in the absence of sensory inputs, and also *in vitro*, in brain slices, they are considered the default activity of the cortex, and thus an intrinsic network property that can serve as an endophenotype of cortical circuit function.

Despite their significance, the effect of development and ageing on cortical Up/Down states is unknown. Moreover,

previous research on SO activity has been conducted in different species, ages or brain areas, leading to contradictory findings and complicating functional interpretations. Here we investigate the effects of development across different cortical regions, by monitoring spontaneous activity *in vitro* with simultaneous intracellular and field potential recordings. Cortical Up states are examined in animals ranging from the first postnatal week, to adult and aged animals (24 months), thus covering the entire lifespan. Two cortical regions with distinct function and cytoarchitecture are monitored: (i) the primary whisker somatosensory cortex, or barrel cortex, and (ii) the primary motor cortex. Simultaneous recordings at different layers of the same cortical column, or in different columns are obtained to assess network and cellular activity correlations. Initial results reveal systematic differences in network dynamics as a function of age and cortical region, reflecting developmental changes in the cortical circuitry. Besides providing important information on intrinsic activity as an endophenotype of cortical function we believe this work will form a useful background upon which to compare and characterize a number of mouse models of neurological and psychiatric diseases.

MO08-25

EPAC-BASED CAMP SENSOR FOR STUDY OF MELANOCORTIN RECEPTORS AND THEIR LIGANDS

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Melanocortin 1 receptor (MC1R) is a G protein-coupled hormone receptor, which is involved in regulation of mammalian skin and hair colour. MC1R binds pituitary peptide hormones, melanocortins: adrenocorticotropic hormone (ACTH) and different forms of melanocyte-stimulating hormone (MSH). These receptors are positively coupled to Gs proteins resulting in activation of adenylate cyclase and accumulation of cAMP. We have used this property of MC1R to generate an assay system for characterization of biological activity of different ligands of this receptor. We have used a version of Epac-camp sensor protein, which was originally constructed in M. Lohse's laboratory (Würzburg, Germany) (1) and contains a cAMP-binding protein between a FRET pair of CFP and YFP. Upon cAMP binding to the sensor-protein FRET between CFP and YFP is decreased resulting in increase and decrease of fluorescence intensity in both channels, respectively. This enables to analyze the spatial and temporal aspects of cAMP-signaling in different living cells and this approach is also applicable for online high throughput screening. Herewith we have used mouse melanoma cell line B16F10 that endogenously expresses MC1R. Among different strategies studied to express the sensor-protein, the most reliable results were obtained using BacMam viral transfection system, which was hence used in all further experiments. The system obtained revealed expected concentration - response curves for all melanocortin agonists studied with potencies and efficacies in agreement with their properties measured in other assay systems. Using fluorescence anisotropy/intensity measurement assay for characterization of ligand binding affinities (2), in parallel, generates a set of homogenous assays, which allows to determine affinities, potencies and efficacies of pharmacological compounds and is applicable for high throughput screening of new drugs.

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MO08-26

FUNCTIONAL PROPERTIES OF A TRUNCATED α-CAMKII MUTANT

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The functional roles of the important regulatory enzyme calcium-calmodulin stimulated protein kinase II (CaMKII) are controlled by phosphorylation and targeting through binding to specific proteins (1). Protein binding interactions occur mainly through regions of CaMKII distant from the phosphorylation sites that modify their binding (2). Identification of regions of the CaMKII surface whose binding is modified by phosphorylation offers the opportunity for the development of drugs that inhibit functions of CaMKII that are specific to cell type or signalling pathway. To identify such binding sites and develop molecules that can be used as inhibitors of such binding, we have made a kinase dead mutant of αCaMKII by deleting 43 amino acids from the N-terminus and 96 amino acids from the C-terminus. Unlike the full length CaMKII, this truncated mutant does not assemble into a dodecamer but behaves as a molecule of 80kDa (as determined by size exclusion chromatography) indicating that it has retained the dimeric structure found in the full length subunit when it is part of the holoenzyme. The truncated mutant also displays identical protein binding characteristics to the full length native protein in an overlay binding assay (2) and can competitively inhibit the binding of native CaMKII in a pull down assay. These results indicate that all the protein binding sites in non-phosphorylated αCaMKII are located between amino acids 44 and 382. We are examining the effects of phosphomimic mutations on the protein binding properties of this truncated mutant.

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MO08-27

ON THE MODULATION OF D-SERINE CELLULAR CONCENTRATION BY DAAO

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In brain the flavoenzyme D-amino acid oxidase (DAAO) catabolizes D-serine, an endogenous allosteric modulator of NMDA receptors. We demonstrated that the cellular concentration of D-serine depends on the expression of active DAAO and on the presence of the inactivating interactor pLG72 (Sacchi 2008). Genetic evidence indicates that both pLG72 and DAAO are related to schizophrenia susceptibility. We proposed a model to explain the DAAO/pLG72/D-ser association with schizophrenia: an anomalous hypoexpression of pLG72 yields to an increase of DAAO activity,

to a decrease of D-ser released at the synapse and to the consequent hypoactivation of NMDA receptors (Sacchi 2008).

This intriguing mechanism of D-ser regulation does not match with the subcellular localizations proposed for DAAO (peroxisomes) and pLG72 (mitochondria). Here, by using U87 human glioblastoma cells expressing EYFP-DAAO and/or pLG72-ECFP fusion proteins, we provide evidence that newly synthesized DAAO is transiently located in the cytosol where it likely interacts with pLG72 (proposed to localize on the external membrane of mitochondria). Immunolocalization and FRET analyses strongly support this hypothesis. We also demonstrate that neosynthesized cytosolic DAAO is catalytically active, and therefore pLG72 binding -and ensuing DAAO inactivation- might play a protective role against D-ser depletion. Concerning the degradation pathways, the largest part of DAAO is degraded by the lysosomal/endosomal pathway (only a minor amount of DAAO is polyubiquitinated) while pLG72 is mainly targeted to the ubiquitin-proteasome system (UPS). Furthermore, in cotransfected cells pLG72 was shown to increase the degradation of DAAO.

In conclusion, since hypofunction of NMDA receptors has been implicated in the pathophysiology of schizophrenia, future pharmacological approaches may involve targeting enzymes that affect D-ser metabolism, and thus the mechanisms underlying DAAO regulation need to be fully elucidated.

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MO08-28

MEMBRANE-ACTIVE PEPTIDE AURELIN FROM JELLYFISH AURELIA AURITA COULD ACT AS A K^+ CHANNEL BLOCKER. NMR AND COMPUTER MODELING STUDY

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Cationic peptide aurelin (40 a.a., total charge +7) was purified from the mezoglea of a scyphoid jellyfish *Aurelia aurita*. The peptide is active against Gram-positive and Gram-negative bacteria. Structure of its molecular precursor, consisting of a canonical signal peptide, an anionic propiece and a mature cationic part, resembles the common structural features of animal defensins. At the same time, the distribution pattern of cysteine residues, forming six disulfide bridges, makes aurelin similar to K^+-channel blocking toxins of sea anemones (ShK and BgK). To investigate mechanism of aurelin action, the recombinant peptide and its ^{15}N-labeled analogue were overexpressed in Escherichia coli and purified. The spatial structure and backbone dynamics of aurelin in aqueous solution were determined using high-resolution NMR spectroscopy. The peptide encompasses two α-helices and one short 3_{10}-helix, and demonstrates pronounced structural homology with ShK and BgK. NMR spectroscopic analysis indicates that aurelin does not bind to zwitterionic lipid (POPC) vesicles, which mimic membrane of eukaryotic cells. By contrast, binding to anionic (POPC/POPG) vesicles, mimicking prokaryotic membranes, were observed even in high salt conditions. Structural NMR investigation conducted in the environment of membrane mimicking DPC micelles revealed that the peptide binds to the micelle surface by helical regions, and its spatial structure is only slightly changed upon binding. Most probably, antimicrobial activity of aurelin is mediated by electrostatic interactions with membranes of bacterial cells. At the same time, the computer docking of aurelin to homology-based model of Kv1.1 channel revealed potential interaction with the external vestibule of the channel, with the mode of binding similar to one observed for sea anemone toxins. Molecular dynamics simulations of aurelin suggested that the pore-blocking lysine residue of aurelin and one of the proximate hydrophobic residues may constitute the functional dyade by analogy with BgK and ShK.

MO08-29

DIRECT VISUALIZATION OF SINGLE RECEPTOR STRUCTURE/DYNAMICS WITH FAST-SCANNING ATOMIC FORCE MICROSCOPY

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Purinergic P2X4 receptors, expressed in microglia, are essential in neuropathic pain. Despite their functional importance, structural and functional relationships of P2X4 receptors are not fully understood. To address this, we employed atomic force microscopy (AFM) enables us to visualize receptors at subnanometer resolution. P2X4 receptors were overexpressed in 1321N1 astrocytoma cells and purified from their plasma membrane fractions. The P2X4 receptors were purified in forms of trimer, and they exhibited electrophysiological activities. In AFM observation, P2X4 receptors were adsorbed on a mica substrate and found that they exhibit circular shapes without subunit-like structures in control and trimeric structure when they were activated by ATP, an agonist for P2X4 receptors. In combination with time-lapse analysis using AFM and functional analysis revealed that ATP induced opening followed by further enlargement of ion channel pore in P2X4 receptors.

MO08-30

THE ROLE OF CAMKII TARGETING IN THE SENSITIVITY OF NEURONAL CELLS TO ISCHAEMIA

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Calcium/calmodulin stimulated protein kinase II (CaMKII) plays an important role in ischaemic neuronal cell death induced by transient middle cerebral artery occlusion (MCAo) (J Biol Chem 285:20675-82). Spontaneously hypertensive rats (SHR) are more sensitive to MCAo- and excitotoxicity-induced neuronal cell death compared to their parent Wistar Kyoto (WKY) strain. This enhanced sensitivity has been proposed to be due to increased expression of CaMKII leading to increased basal phosphorylation of AMPA receptors at S831-GluR1 (Stroke 38:3007-15). To test this proposal we have used western blotting to measure the expression and basal phosphorylation of CaMKII, the GluR1 subunit of the AMPA receptor and the NR2B subunit of the NMDA receptor in the striatum and cortex (relatively sensitive and resistant brain regions, respectively) from SHR (hypertensive, more sensitive) and WKY

and Sprague Dawley (SD) (normotensive, relatively resistant) rats ($n = 6$ per group). We have confirmed that the level of CaMKII expression and basal GluR1 phosphorylation at S831 is significantly higher in the striatum of SHR than WKY rats ($p < 0.01$). However, there was no significant difference in these variables between cortex and striatum in the same animal, nor between SHR and SD rats despite significant differences in sensitivity to MCAo-induced injury. There was no correlation between sensitivity to MCAo and the level of expression or basal phosphorylation of CaMKII, GluR1 or NR2B. However, phosphorylation of CaMKII at T253 or GluR1 at S831, but not NR2B at S1303, was significantly elevated in SD striatum, but not cortex, following stimulation with AMPA. Therefore, if differences in CaMKII mediated events are responsible for the differences in sensitivity to ischaemia between brain regions, the mechanism must involve alterations in ischaemia-induced CaMKII activation, probably due to altered CaMKII targeting, rather than differences in basal levels of CaMKII expression or activity.

MO08-31

AAV-MEDIATED BDNF OVEREXPRESSION DOES NOT DOWN-REGULATE TRKFL AND TRKTK RECEPTORS IN THE TRANSECTED SPINAL CORD OF THE RAT

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Brain-derived neurotrophic factor (BDNF) regulates its full-length TrkB (TrkBFL) receptor. A prolonged exposure of neurons to BDNF *in vitro* and BDNF infusions to the brain downregulate TrkBFL protein, reduce its phosphorylation and downstream signaling. We used AAV-mediated transfer of BDNF transgene to increase BDNF long-term delivery to isolated spinal cord transected at Th11-12 segments. It resulted in substantial improvement of treadmill locomotion, which weakened in time. The mechanism underlying this effect may arise from alterations in abundance/availability of TrkBFL and its truncated forms. To verify it, we compared levels of trkb/trkbTK transcripts (qPCR), TrkB/TrkB-P protein (Western blotting) and TrkBFL segmental distribution (fluorescence immunohistochemistry) in intact, spinal-PBS injected (spPBS) and spBDNF rats, 7 weeks after surgery. The level of trkb transcripts, which decreased in the scar and in L1-2 in spPBS rats, tended to increase in spBDNF rats (L1-2: $p < 0.07$). trkbtk levels, which were 10 times higher than those of trkB, showed the same tendency. The level of TrkBFL (140kDa) form decreased parallelly in spPBS rats; its deepening in spBDNF rats occured with concomitant increase of 40–60kDa forms. No group differences were found in L3-6 segments. Visualisation of rostro-caudal distribution of TrkBFL in combination with c-Myc labeling of transgene-derived BDNF, and image analysis revealed that: (i) caudally to the transection, TrkBFL was abundant in neurons and white matter oligodendroglia (ii) c-Myc (+) or (-) neurons demonstrated comparable intensity of TrkBFL labeling (iii) neuronal TrkBFL labeling was higher in segments featuring BDNF excess, than in those devoid of BDNF excess. In conclusion, BDNF overproduction in isolated spinal network does not downregulate TrkB transcripts. Cellular abundance and unchanged pattern of TrkBFL expression, decrease of 140 kDa TrkBFL and enrichment in low-molecular forms reveals importance of its turnover and post-transcriptional modifications possibly affecting BDNF signaling in regions with strong BDNF overproduction. Support-S007/Polish-German/2007/01 and EMBO(EZ) grant.

MO08-32

ATM IS A NOVEL REGULATOR OF NUCLEOLAR TRANSCRIPTION

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ATM (Ataxia-telangiectasia mutated protein kinase) mediates DNA double-stranded break (DSB) signaling. ATM deficiency results in the autosomal recessive disorder known as ataxia-telangiectasia (A-T). The cause of the neurodegeneration associated with this disorder remains unknown. Here we report the unexpected finding that, in neurons, ATM is a regulator of RNA-Polymerase-1 (Pol-1) -mediated transcription of nucleolar rRNA genes (rDNA). Low concentrations of the ATM-activating DSB inducer, etoposide, stimulated rDNA transcription in both cultured rat cortical neurons and cerebral cortices of postnatal day seven (P7) rats. The etoposide-mediated activation of rDNA transcription was reduced by ATM inhibition. Importantly, in the absence of etoposide, the specific ATM inhibitor, KU55933, reduced basal levels of nucleolar transcription and attenuated its induction in response to enhanced neuronal activity. Furthermore, $ATM^{-/-}$ mice display reduced Pol-1-mediated transcription in the cerebella compared to WT littermates. Finally, ATM is robustly present in neuronal nucleoli and several critical regulators of Pol-1 display potential ATM phosphorylation sites. Our results suggests that ATM is a major positive regulator of nucleolar transcription. Hence, defective ribosomal biogenesis may contribute to the A-T-associated neurodegeneration.

This work was supported by NIH grants NS047341 and RR015576 (M.H.)

MO08-33

THE ROLE OF SMALL RHOGTPASES IN CORTICAL GABAERGIC INTERNEURON DEVELOPMENT

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GABAergic interneurons play important roles in cortical function and provide the main source of inhibition to cortical microcircuits. Impaired interneuron function results in severe neurodevelopmental disorders such as schizophrenia, epilepsy and autism. Although recent studies have uncovered some of the molecular mechanisms underlying interneuron development, the intracellular components involved are still unknown. Rac proteins are RhoGTPases that integrate multiple extracellular signals and are required for many processes in diverse cell types, including cytoskeleton organization, vesicle trafficking, transcription, cell cycle progression, and apop-

tosis. We have addressed the specific role of Rac1 in interneuron progenitors originating in the medial ganglionic eminence, via Cre/loxP technology. We show that 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 CyclinD levels and Retinoblastoma protein phosphorylation. Additional studies are aimed at addressing the role of Rac3, the neuronal specific Rac. Our aim is to determine whether the phenotypes of double Rac1 and 3 mutants reflect qualitatively-distinct effects of these Rho GTPases or rather quantitative effects of their combined activities.

MO08-34

PROTEIN-PROTEIN INTERACTIONS BETWEEN BM88/CEND1, RANBPM AND MIRK/DYRK1B IN REGULATING CELL CYCLE PROGRESSION IN NEURAL CELLS

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BM88/CEND1 is a neuronal protein widely expressed in the mammalian nervous system. Its expression is low in neural stem cells while it is elevated in differentiated neurons. Previous studies have revealed a dual role for BM88/CEND1 in driving neural stem/progenitor cells towards cell cycle exit and neuronal differentiation. The dual function of BM88/CEND1 protein is accomplished by activation of the p53-p21-pRb signaling pathway via cyclin D1 down-regulation and cytoplasmic sequestration. Using a yeast-two hybrid system we have now identified and furthermore confirmed *in vitro* and *in vivo*, RanBPM protein, as a direct interacting partner for BM88/CEND1. RanBPM is a multi-domain intracellular protein that shuttles between the cytoplasm and the nucleus and has been found to act as a scaffold for signal transduction of several receptors, nuclear proteins, transcription factors and cytosolic kinases. Interestingly, RanBPM has also been implicated in the progression of neuronal precursors through M-phase while it has been identified as a Mirk/Dyrk1b kinase-binding protein. In turn, Mirk/Dyrk1b has been shown to destabilize cyclin D1 by phosphorylation at threonine 288, promoting its cytoplasmic translocation and subsequent degradation by the 26S proteasome. To investigate the potential tripartite interaction between BM88/CEND1, RanBPM and Mirk/Dyrk1b in regulating cell cycle progression and cyclin D1 levels, we performed co-transfection experiments in Neuro 2a cells. We show that the BM88/CEND1-dependent or Mirk/Dyrk1b-dependent down-regulation of cyclin D1 is reversed by RanBPM. Further we show for the first time that RanBPM inhibits the function of Mirk/Dyrk1b kinase by promoting its degradation through the proteasome and thus attenuates its capacity to destabilize cyclin D1. Triple co-transfection experiments with BM88/CEND1, Mirk/Dyrk1b and RanBPM are under way in conjunction with immunoblotting for cyclin D1 and FACS analysis to determine the 3-way interaction of these molecules and their role in limiting or facilitating cell cycle progression. Supported by FP7 REGPOT Project 264083 Neurosign - Development of a Center of Excellence in Neurosignalling

MO08-35

A FUNCTIONAL EXPRESSION PLATFORM FOR NEURONAL GPCRS AND LIGAND-GATED ION CHANNELS OF MAMMALIAN AND INVERTEBRATE ORIGIN

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In this presentation we will report on the development of a heterologous cell-based expression platform for neuronal receptors of mammalian and invertebrate origin. The neuronal receptors we have chosen as test cases are G-protein coupled receptors-GPCRs (such as the serotonin 4a and the μ- and δ-opioid receptors) and ligand-gated ion channels (serotonin 3a receptor and various mosquito odorant receptors), which are involved in important physiological processes, such as neurotransmission, analgesia and sense of smell. The expression system used was based on lepidopteran insect cells and plasmids containing genetic elements from the silkworm *Bombyx mori* and the *B.mori* nuclear polyhedrosis virus (BmNPV). The expression of the receptors was initially demonstrated by ligand-binding assays (serotonin 4a) or immunodetection (μ-opioid and mosquito odorant receptors). Most importantly, robust functional assays have been developed, which are based on genetically encoded optical probes allowing detection of ligand-dependent changes in intracellular cAMP accumulation (for GPCRs: serotonin 4a, μ- and δ-opioid receptors) and Ca^{2+} mobilization (for both GPCRs and ion channels: serotonin 3a and 4a, δ-opioid, mosquito odorant receptors) in real-time following addition of the specific ligands to cells expressing the receptor, the luminescent protein and the Gα16 (where appropriate). Taken together, the results show that all tested receptors, both GPCRs and ion channels, can be functionally expressed in this system. Consequently, the assays with the cAMP and Ca^{2+} biosensors could be used as reliable and straightforward tools for high-throughput screening and evaluation of putative specific neuronal receptor agonists and antagonists.

MO08-36

COMPARISON BETWEEN IONIC MATRIXES AND TYPICAL MATRIXES FOR THEDETECTION OF PHOSPHOLIPIDS IN HUMAN BRAIN EXTRACTS BY MALDI MS

Veloso, A.[1], González de San Román, E.[2], Fernández, R.[1], Manuel, I.[2], Ferrer, I.[3], Giralt, M. T.[2], Ochoa, B.[4], Rodríguez-Puertas, R.[2] and Fernández, J. A.[1]

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Analysis of lipids in biological samples is a difficult task, due to the difference in detection and abundance of the different lipid

classes. The problem is further complicated by the abundance of species with very small mass difference and even of isobaric species. While some lipid classes, like phosphatidylcholine are easily detectable, others like phosphatidyletanolamine, diacylglycerols or phosphatidylinositol, are hard to detect. In the last years, a new kind of MALDI matrices have appeared, which seem to ease detection of a larger number of lipid classes: they are called ionic matrices (IM), and they present good solubility for a variety of analytes, formation of homogeneous crystals with analytes, and high vacuum stability.

In this study the performance of two ionic matrices for the detection/identification of lipids, both in positive and negative detection, in human brain extracts are compared with the results obtained with the most commonly employed matrices: 2,5-Dihydroxybenzoic Acid (DHB), 2-Mercaptobenzothiazole (MBT) and 9-Aminoacridine (9-AA). As it will be demonstrated, election of the correct matrix substance results in a considerable increase of the number of species detected, which is a key issue for the study of influence of the lipid composition in neurodegenerative diseases such as Alzheimer disease.

MO08-37

EFFECT OF CATIONIC SURFACTANTS ON THE KINETICS OF ENZYME CATALYZED HYDROLYSIS OF CARBOXYLATE ESTERS IN MICELLAR MEDIA

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Enzymes are biocatalysts with high specificity, catalytic efficiency and bio-degradability and hence find major applications in industrial sectors and in medical. Herein, special attention has been given to explore the importance of serine protease enzymes viz. α-chymotrypsin and trypsin. The role of enzymes in self-organized medium for the hydrolysis of carboxylate esters has been investigated. The enzyme activity was tested in the presence of several cationic surfactants having different head groups maintaining the dodecyl hydrophobic residue and bromide counterion. The effect of chain length of cationic surfactants, which significantly enhances the α-CT activity for the hydrolysis of p-nitrophenyl acetate (PNPA) in aqueous medium was approached. The enzyme-surfactant interaction has been explored physicochemically using spectrofluorometric, conductometric and surface tensiometric measurements. This interaction increased the hydrophobicity of the enzyme-surfactant complex and the complex finally populated at the air/solution interface. Possible mechanism of micellar enzymology and the effect of organic cosolvents have been discussed. Under all the conditions employed, the reaction follows a Michaelis-Menten mechanism. The effect of surfactants on the apparent kinetic parameters of α-CT like Michaelis constant Km and the catalytic constant kcat has been determined. An increase in the head group size leads to increase in the interfacial area and the space between two head group is also enhanced. Due to enhanced interfacial region, the concentration of enzyme and substrate increased and showed higher activity. The enzyme surfactant concentrations were optimum with the surfactants having longer chain length (C16 > C14 > C12). Detailed knowledge of the effects of surfactants and the role of enzymes will greatly advance our understanding of the catalytic power of enzymes.

MO08-39

DEVELOPMENTAL REGULATION OF PROTEIN O-GLCNACYLATION, O-GLCNAC TRANSFERASE, AND O-GLCNACASE IN MAMMALIAN BRAIN — IMMU

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O-GlcNAcylation is a dynamic, regulatory posttranslational modification of protein by β-N-acetylglucosamine (GlcNAc), which is transferred enzymatically from UDP-GlcNAc donor to the hydroxyl group of serine or threonine residues of proteins. O-GlcNAcylation is catalyzed by O-GlcNAc transferase (OGT), and the O-GlcNAc groups attached to proteins can be removed with the catalysis of O-GlcNAcase (OGA). Numerous neuronal proteins, including transcription factors, synaptic and cytoskeletal proteins, are modified by O-GlcNAc, suggesting that it regulates many brain functions. Both OGT and OGA are highly expressed in the brain. Here, we investigated immunohistochemically the regional distributions of global O-GlcNAcylation, OGT and OGA in rat brains at ages of embryonic 19 days (E19d), postnatal 5 days, 6 months and 12 months. We found wide distributions of O-GlcNAcylated proteins, OGT and OGA at all ages examined, but they are regulated during development. At E19d, O-GlcNAcylated proteins, OGT and OGA had similar distributions with the highest staining in the cortical plate and subplate. More brain region-specific distributions were seen in rat brains after birth, but the distributions of O-GlcNAcylated proteins, OGT and OGA were similar at all ages examined. Higher immuno-staining was seen in the cerebral cortex and the pyramidal neurons of some sectors of the cornu ammonis of the hippocampus. These observations provide fundamental knowledge for understanding the regional regulation of brain functions by O-GlcNAcylation during development.

Tuesday Oral Sessions
Plenary Lecture 3

PL3

BREEDING AND BUILDING MOLECULES TO IMAGE CELLS, PROTEIN SYNTHESIS, ELECTRIC FIELDS, AND PERIPHERAL NERVE

Tsien, R.Y., Shu, X., Lev-Ram, V., Butko, M., Lin, J.Y., Miller, E.W., Whitney, M., Crisp, J.L., Steinbach, P.A., Deerinck, T., Ellisman, M.H. and Nguyen, Q.T.

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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 phototoxicity 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].

ESN Young Scientist Lecture 1

YSL2

ENERGY REQUIREMENTS OF GLUTAMATERGIC NEUROTRANSMISSION

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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:

1. Bak et al. (2006). *J. Cereb. Blood Flow Metab.* **26**: 1285–1297.
2. Bak et al. (2009). *J. Neurochem.* **109** (Suppl. 1): 87–93.
3. Sickmann et al. (2009). *J. Neurochem.* **109** (Suppl. 1): 80–86.
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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.

S10-04

MECHANISMS FOR MTOR REGULATION OF OLIGODENDROCYTE DIFFERENTIATION & MYELINATION/REMYELINATION

Wood, T. L.[1], Tyler, W. A.[1], Jain, M. R.[2], Cifelli, S. E.[1], Mahajan, K.[1], Li, Q.[2], Ku, L.[3], Feng, Y.[3] and Li, H.[2]

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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:

1. Tyler et al. (2009). *J. Neurosci*, 29: 6367.
2. Narayanan et al. (2009). *J. Neurosci*, 29: 6860.

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 preventing NMDAR-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, excitotoxicity, 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 α (SIRPα, 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 SIRPα. CD47 is also expressed predominantly throughout the brain, with the regions in which it is abundant overlapping extensively with those enriched in SIRPα. SIRPα 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 SIRPα 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 SIRPα and its formation of a complex with Shp2 in the brain. The FS stress-induced tyrosine phosphorylation of SIRPα 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 Kvβ2 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 trails 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.

Symposium 12
Cortical Interneuron Development and Function

S12-01

NEURONAL ACTIVITY IS REQUIRED FOR THE INTEGRATION OF SPECIFIC INTERNEURON SUBTYPES INTO THE CORTEX

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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.

S12-02

GENE NETWORKS IN CORTICAL INTERNEURON DEVELOPMENT

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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. Kcnc1, 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.

S12-03

RHOGTPASES FUNCTION IN CORTICAL INTERNEURON DEVELOPMENT

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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 Rac 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 CyclinD levels and

Retinoblastoma protein phosphorylation.

S12-04

THE EMBRYONIC ORIGIN OF GABAERGIC HUB NEURONS IN THE DEVELOPING HIPPOCAMPUS

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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:

1. Bonifazi et al. (2009). *Science*.

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 of 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 Ca^{2+} channels involved in retrograde BDNF release; expression of the $Ca_V1.3$ 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 Ca^{2+} 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:

1. Zucca et al. (2010). *J Neurosci*, **30**:6776–6781.

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 pro-apoptotic 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 parasaggital 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. Twennty-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 (Δ9-THC), increased GluR2-lacking AMPA receptors and permitted LTD only in the PPN-activated pathway. This was prevented by *in vivo* administration of the CB1 receptor antagonist AM251. Our findings demonstrate that cocaine has a more global effect upon AMPAR subunit composition in VTA DA neurons, whereas Δ9-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 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.

Symposium 14
Cytoskeletal Dynamics and Neuronal Polarity

S14-01

MICROTUBULE STABILIZATION INDUCES AXON REGENERATION

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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 ínjury.

S14-02

MICROTUBULE-ACTIN FILAMENT COUPLING IN AXON GUIDANCE

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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 focussed on the regulation of drebrin through phosphorylation in these different cellular contexts.

S14-03

MITOTIC MOTOR PROTEINS, MICROTUBULE TRANSPORT AND NEURONAL POLARITY

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In the axon, the microtubules (MTs) are nearly uniformly oriented with their plus ends distal to the cell body, while in the dendrite, MTs are non-uniformly oriented. We have proposed that these patterns are established and maintained by molecular motor proteins that transport MTs into these neurites with either the plus or the minus end of the MT leading. Our general hypothesis has been that cytoplasmic dynein is the principal workhorse for transporting MTs with their plus ends leading, and that the remainder of the work is performed by a small number of kinesins usually considered to be mitotic kinesins, namely kinesins 5, 6, and 12. The results of our recent studies on these motors indicate that, in fact, they do not fuel microtubule transport in the axon, but rather they somehow suppress it. We now think of these motors as 'brakes' on axonal microtubule transport. We will discuss the evidence for this conclusion, as well as potential mechanisms by which these motors might behave as brakes. A potentially exciting hypothesis is that these motors act, at least in part, at the level of the cell body to restrain the transport of MTs into the axon while simultaneously promoting the transport of MTs of the opposite orientation into the dendrites. We posit that it is by this mechanism that the neuron co-regulates the polarity orientation of MTs in the axon and the dendritic arbor.

S14-04

FROM SOMA TO SYNAPSE: SORTING OUT POLARIZED TRANSPORT IN NEURONS

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To establish and maintain their polarized morphology, neurons employ active transport driven by molecular motors to sort cargo between axons and dendrites. However, the basic traffic rules governing polarized transport on neuronal microtubule and actin arrays are unclear. To directly examine how cytoskeletal motors contribute to polarized transport, we established a trafficking assay in hippocampal neurons to selectively probe specific motor protein activity. This revealed that, unlike kinesins, microtubule minus-end directed dynein motors drive cargo selectively into dendrites, governed by their mixed microtubule array. Furthermore, several myosin family members were found capable of short-range cargo delivery into dendritic spines, the compartments where most excitatory synapses are located. These results demonstrate a powerful approach to study specific motor protein activity inside living cells and imply a key role for dynein in dendritic transport. We propose that dynein establishes the initial sorting of dendritic cargo while kinesins and myosins assist in subsequent delivery.

S14-05

CAMP SIGNALING IS INVOLVED IN THE DEVELOPMENT OF THE AXON

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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)

Symposium 15
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 Ca^{2+} influx through postsynaptic NMDA receptors. Astroglia can regulate activation of these receptors by releasing the NMDA receptor co-agonist D-serine, in a Ca^{2+}-dependent manner. However, Ca^{2+} 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 Ca^{2+} signalling in individual CA1 astrocytes by clamping their internal Ca^{2+} 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 Ca^{2+} -dependent activity of astrocytes. Activity in neighbouring astrocytes can have distinct effects on nearby synaptic connections, but each astrcoyte 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 Ca^{2+}-dependent exocytosis. However, the origin (cytosolic vs. vesicular) of the released gliotransmitter is still a matter of debate. The existence of Ca^{2+}-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. Immunoisolated 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 by using the SR KO mice as negative controls. In the SR KO mice, we found a reduced neurotoxicity induced by NMDA- and Aβ1-42 peptide injections into the forebrain. Furthermore, in the pentylenetetrazole (PTZ)-induced seizure model, we found that the duration of 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 the brain may lead to a novel strategy for neuroprotection against various excitotoxic diseases.

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 receptors (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.

Symposium 16
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 Ca^{2+}-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

Gasman, S., Ceridono, M., Ory, S., Momboisse, F., Calco, V., Haeberle, A.M., Bailly, Y. and Bader, M.F.

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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-β-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 view point 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.

Young Investigator Colloquium 1
Morphogenesis of the Central Nervous System

YIC01-01

COMBINATORIAL ACTIONS OF A PRONEURAL BHLH TRANSCRIPTION FACTOR WITH A ZINC FINGER REPRESSOR DURING CEREBRAL CORTICOGENESIS

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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 resulted 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.

YIC01-02

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, I12b, the Dlx2 proximal promoter and 3'UTR region *in vivo*. This interaction is mediated by E2F functional sites located in I12b 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 CIHR grant and a long-term faculty development grant to N. Ghanem from AUB.

YIC01-03

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 modulatory 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.

YIC01-04

REELIN IS REQUIRED FOR CLASS-SPECIFIC RETINOGENICULATE TARGETING

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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-linked proteins such as SOD1, TDP-43 and FUS are themselves secreted. However, the predominant pathway underlying secretion of ALS-linked 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 FRACTALKINE 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 temporospatial 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 plays 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 isoform 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-κB/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-)i]. Among different tools proposed for the monitoring of (Cl-)i, 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]i 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-]i, 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-)i in different compartments of cultured spinal and hippocampal neurons (7-8 DIV). For both neuron types, the highest [Cl-]i 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-)i was observed. These results demonstrate that Cl-Sensor enables monitoring non-invasively the (Cl-)i 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-)i 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 subcelullar 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.

Young Investigator Colloquium 4
Mechanisms and Consequences of Brain Injury

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 Nedd4-2 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.

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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 morbility. 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 intra-uterine 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 gaseoustransmitter 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 PATHOBIOLOGICAL 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, GFAPdelta, GFAPβ, GFAP7e, GFAPε, GFAP partial and GFAPκ. 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 glio-genesis.

Ontogenic profiling revealed the decrease in level of GFAP α (27% ± 2), GFAP β (18% ± 2), GFAP κ (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 κ (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 ε 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 κ /total GFAP decreased (47% ± 3) in control, but remained constant in treated. GFAP partial /total GFAP (65% ± 3) reduced while GFAP ε /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.

Tuesday Poster Sessions
TU01 Neurogenesis and Cell Differentiation

TU01-01

CARBON MONOXIDE IN NEURONAL DIFFERENTIATION – NOVEL APPROACHES FOR CELL THERAPIES APPLICATIONS

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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 neuronally 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 the final yield 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/PKCε/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 PKCε 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 03EΔ778/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 occurence 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

for either doublecortin (DCX), cell marker for immature neurons or glial fibrillary acid protein (GFAP) for labeling of astrocytes were used. However, the data from quantitative analysis of the numbers of proliferating cells are still under evaluation, our preliminary results showed, that fractionated irradiation has long-lasting effect on extent of neurogenesis in this radiosensitive region. Obtained results should have implications for clinical radiotherapy to avoid complications in therapeutic brain irradiation.

This work was supported by project 'Center of excellency for research in personalized therapy (CEVYPET)', code: 26220120053 and 'Identification of novel markers in the diagnostic panel of neurological diseases' co-financed from EU sources and European Regional Development Fund.

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. The neuropeptide Y (NPY), a key player of cognition, stimulates neurogenesis in the hippocampus and is neuroprotective. 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 nM 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 1nM 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.[1], 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 hipocampal 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 subgranule zone of hipocampal 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 hipocampal 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.[1], Guimarães, 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 Ca^{2+} 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 heterozigous,

reduction of STI1 did not affect muscle strength or memory in the grip force or in the novel object recognition test. Interestingly, STI1 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 STI1 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. STI1 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 STI1 mRNA levels are increased fourfold and protein levels are the same found in control mice. These results indicate that STI1 has a versatile role in signalling mechanisms with functions in early embryogenesis and regulation of STI1 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 poliubiquitination for its subsequent proteasomal degradation. This allows a fast degradation of the complex Cdk1/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 neurons. 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 tendency, 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 megachiroptera species and six microchiroptera species, which were not reported previously in literature. The animals were euthanized and transcardially perfused with saline followed by 4% paraformaldehyde 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 Triaenops 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, Triaenops persicus and Eidolon helvum, and the amygdala in both megabats. In conclusion, despite the suggestion that mega- and microchiroptera could have a paraphyletic 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

Do Nascimento, J.L.[1], Magalhães, R.C.[1], Hamoy, M.[1], Nascimento, M.V.[1], Silva, Y.Y.[1,3], Crespo-Lopez, M.E.[4], Silva, E.O.[2], Da Silva, M.N.[3] and Bastos, G.N.[1]

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The search for new compounds that enhance proliferation and differentiation of neural stem cells in the hippocampus represent a significant scientific challenge. Newborn neurons emerge from neural 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 hipocampal 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 subgranule zone of hipocampal 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), c-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 tranfected 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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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 neural 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 hindbtain, 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 Gal4 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 requirement. 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 four 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 individuals 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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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 I (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 OLIG2 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 saltatory 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.olig2) 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.olig2 (1 x 106 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 cyclider test, locomotor activity and rota-rod performance, and learning/memory function was tested on days 41-44 through passive avoidance performance. F3.olig2 recovered using rate of contralateral forelimb in cyclider 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·olig2 cells migrated to damaged areas; periventriclular 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.olig2 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, neuritogenesis, 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 (Prnp+/+) and PrPC-null (Prnp0/0) 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 Prnp0/0 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 Prnp+/+ neurosphere formation without further impairing the formation of Prnp0/0 neurospheres. Alternatively, neurosphere formation was enhanced by recombinant STI1 application in cells expressing PrPC, but not in cells from Prnp0/0 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 proliferation modulation under melatonin 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-deoxyuridine (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 melatonin 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 NECDIN 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. Coimmunoprecipitation 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. BrdU 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 though 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

control rats. These results suggest CFN treatment may exert deleterious neurotoxic effects on developing and adult rat brain by affecting neurogenesis through inhibition of wnt and Notch.

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. PrP-/- mice 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 PrP-/- mice grown in the absence or presence of NCAM-Fc, the chimeric soluble form of NCAM. We observed that NSCs derived from PrP-/- 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 PrP-/- mice do not respond to NCAM-induced neuronal differentiation. Moreover, upon NCAM-Fc addition in PrP-/- 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 KI67 (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, IFNgamma, and TNFalpha in SVZ extract. When neurospheres derived from rat cortical stem cells were treated with these cytokines, IFNgamma 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 KLF4 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, immunofluorescence 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 stem 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 hypothesize 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-c-myc 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 track 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 Prox1-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 astrogliogenesis. 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.

TU01-34

EFFECTS OF XENOESTROGEN ON HIPPOCAMPAL NEURAL STEM /PROGENITOR CELLS PROLIFERATION AND DIFFERENTIATION IN *IN VITRO*

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Generation of new neurons occurs continuously in sub-ventricular zone (SVZ) of lateral ventricles and dentate gyrus from neural stem cell (NSC) by process 'neurogenesis' in mammalian hippocampus through NSC proliferation, migration, differentiation and neuronal incorporation in to neuronal circuits. Recent studies revealed that BPA is a xenoestrogenic endocrine disrupting chemicals. It is monomer of polycarbonate plastics mainly used for production of consumer product like sunglasses, water and food containers, shatter resistant baby bottles and epoxy resin used for coating inside of almost all food and beverage cans and in dental sealants. We assessed the effects of BPA on rat neural stem/progenitor cells (NS/PCs) from hippocampus. We first established non-cytotoxic concentration in NSC for proliferation and differentiation studies and found 100 μm BPA is non-toxic. Studies revealed that BPA significantly decrease NSC proliferation compared to control NSC culture as assessed by Alamar Blue Cell proliferation assay and BrdU incorporation. We found significantly decreased size of neurosphere and number of neurosphere generated in presence of BPA as assessed by neurosphere growth kinetics. We observed a significantly decreased number of β-tubulin+/DAPI+ cells suggesting inhibitory effect of BPA on neuronal differentiation. We found that Lithium Chloride a mood stabilizer protects against BPA mediated deleterious effects on NSC. Results suggest that BPA affects neural stem/progenitor cell proliferation and differentiation *in vitro*.

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 trigerred 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 differentiating 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

undifferentation 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 are 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 immunostaining 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-1 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, Sox1 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.

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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 1–3 g/kg ascorbate i.p. 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).

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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 capsid 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 dimercaptosuccinate-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 dimercaptosuccinate-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-ANTHROYLOXY) 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 (SPMV) were isolated from fresh bovine cerebral cortex. Liposomes of total lipids (SPMVTL) and phospholipids (SPMVPL) extracted from the SPMV. The set of n-(9-anthroyloxy) stearic or palmitic 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 magnet 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 (flFeNP). Exposure of microglial cells to up to 15 μM iron as flFeNP for up to 3 hours did not result in an alteration of the distribution of intra- or extracellular 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 flFeNP. Incubation of microglia cells with flFeNP caused a time- and concentration-dependent accumulation of iron in the cells. After 4 hours of incubation with 15 μM iron supplied as flFeNP, 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 flFeNP. Absence of oxidative stress and co-localization of fluorescence and iron suggest that the accumulated flFeNP 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 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 [^{3}H] [D-Ala^2, MePhe^4, Gly-ol^5]-enkephalin ([^{3}H]-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 [^{3}H]-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-hydroxycrotonate, *gamma*-hydroxybutyrate, 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 conformation presumably similar to that of substantially fixed *trans*-4-hydroxycrotonate. 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-GlcNAcylation 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, ICQ = 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 PKFKB3 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)].

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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 LXR) 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 $14CO_2$. Carbon and nitrogen metabolism were further mapped employing [13C] glucose, [13C] glutamate, $15NH_{4+}$ and [15N]glutamate, and analyses of cell extracts and media were performed using mass spectrometry. Interestingly, the CO_2 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 glutamine after exposure to $15NH_{4+}$ 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 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 Neuroimmunology

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-arthritic 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-arthritic 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-1α (IL-1α) 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

were isolated at 48h post-TMT for qPCR analysis. Key molecules in the interleukin-1α pathway were induced by TMT exposure. Effects of IL-1α [150 pg/mL] were identified in the proliferation and differentiation of hippocampal neural progenitor cells (NPCs) *in vitro*. These data suggest a role for resident microglia and secreted IL-1α in regulation of NPC proliferation and differentiation for self-repair following chemical-induced hippocampal injury.

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TU03-04

ALZHEIMER'S DISEASE AND SEIZURES: INTERLEUKIN-18, KYNURENINE PATHWAY AND QUINOLINIC ACID PRODUCTION

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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. We exposed neuron-like differentiated human SH-SY5Y neuroblastomas and normal human astrocytes (NHA) to IL-18, interferon-gamma, other inflammatory cytokines or QA, and detected the expression changes of IDO and other 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 cell 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 DYSFUCTION

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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-α), 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-α, 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-ENVIROMENTAL 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 envorimental, immunological and genetic, as a tool to explain some fisiopathological mechanism ocurring in autism, all these factors contributing to dysregulation in autism spectrum disorder (ASD). Methods: We evaluated the influence of enviromental 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 regardin 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 stimated using a microanalitic assay. DNA damage was studied using both an electrophoretic method and 8 - hydroxyl 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 afectation and toxic effect of metal evidenced in this study as an element influencing pathogenesis of ASD itself. Conclusions:This study may confrm the previous hypothesis of autism pathology, the association between the severity of autism and body burden of toxic metals from the severity of learning impairtment analysis,and may conduce 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-β 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 secretome, 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 α-SYNUCLEIN ON CELLULAR HOMEOSTASIS - A FOCUS ON GLIAL CELLS

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α-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 α-synuclein still remains elusive, oligomeric intermediates of the protein are considered to be the toxic species. We have shown that α-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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TU03-11

INTERNALIZATION OF MISFOLDED TRUNCATED TAU INTO THE MICROGLIAL CELLS

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Tau protein represents a key player in the pathogenesis of Alzheimer's disease and related tauopathies. Tau protein is viewed as an intracellular cytoplasmic protein, however it is able to accumulate in the extracellular space usually as a consequence of neuronal death and may significantly contribute to the neurodegeneration. In the present study we report that misfolded truncated tau protein is a potent inflammatory mediator. We have found that truncated tau was internalized by reactive microglia. Two independent tau antibodies recognizing different tau epitopes DC25 and DC190 unequivocally revealed the presence of truncated tau inside of microglial cells. Western blot profile showed that internalized truncated tau was shifted to higher molecular weight pointing out its significant structural modification. Moreover, we showed that internalization of truncated tau by microglia led to the activation of the microglial inflammatory response characterized by the release of pro-inflammatory cytokines (IL-1beta, IL-6, TNF-alpha) from microglial cultures. On the basis of these results, we suggest that misfolded truncated protein tau represents viable target for immunotherapy of Alzheimer′s disease.

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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 phosphosphorylation 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 mature cultured 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 elevated hippocampal 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 (Aß) 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-1derived macrophages. NE

treatment reduced the Aβ-mediated cytotoxicity and production of the proinflammatory chemokine, monocytic chemotactic protein-1 (MCP-1/CCL2). However, NE treatment enhanced Aβ-mediated induction of IL-1β. Of note, the ability of NE to modulate the Aβ-induced 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, 2010-0022658).

TU04-04

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 vasoconstriction 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 ($Abeta_{1-42}$), total tau (T-tau) and tau phosphorylated at threonine 181 ($P\text{-}tau_{181}$) 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 ($Abeta_{1-42}$ 449 ± 209 vs. 460 ± 206 pg/mL; T-tau 697 ± 603 vs. 549 ± 298 pg/mL; $P\text{-}tau_{181}$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 $Abeta_{1-42}$ 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 $Abeta_{1-42}$/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 paractice.

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 Psychatrician. Serum metal levels are estimated by ICP-AES and ceruloplasmin by chemoluminisence method and Ferritin by ferro-oxidase technique. We report here differential increase of copper and iron levels serum samples in early and severe AD associated with increased level of ceruloplasmic 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.

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 regions and are similarly processed by the α-, β-, γ-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 modifier (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 transfect cells with a combination of SUMO-1, SUMO-2, UBC9 and SENP-1. Using propidium iodide and Hoechst staining, as well as measuring the released LDH, we assess the effect of increased/decreased SUMOylation on the protective effects of curcumin. 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 percentage 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 MICROTUBLE-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 Pin1 (Yotsumoto et al., J. Biol. Chem, 2009). Pin1 is a peptidyl-prolyl isomerase catalyzing the cis/trans isomerization of phospho-Ser/Thr-Pro sequences, stimulating dephosphorylation by protein phosphatase 2A (PP2A). We analyzed interaction between Pin1 and Tau phosphorylated by Cdk5-p25 using the GST-pulldown assay and Biacore. We firstly confirmed Ser202, Thr205, Ser235, and Ser404 as all major Cdk5 phosphorylation sites

using two-dimentional 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.

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 the IDE pathology, being manifested after the cognitive deficits have already been developed. Once developed, IDE and Aβ pathology may contribute to cognitive deficits progression.

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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 vulnereable 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.

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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. Threfore, 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 causes cell cycle re-entry in hippocampal slice cultures and subsequent neuronal cell death. Initially, amyloid-beta oligomers increases intracellular Ca^{2+} 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 pathwayand 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 findthat 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 O_2 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 triple-transgenic model of AD (3 × Tg-AD). These mice develop cognitive deficits and the typical neuropathology in a progressive and age-dependent manner.

Glutamate-induced NO production was similar along aging (3, 6 and 12 month old) in both, non-Tg and 3 × Tg-AD mice. However, the corresponding CBF changes in 3 × Tg-AD mice were attenuated with age, being significantly reduced in 12-month-old 3 × Tg-AD mice comparing to the matched non-Tg mice (32 ± 6 vs. 69 ± 7). Additionally, these mice presented CBF basal levels lower than the controls (96 ± 16 PU vs. 140 ± 21 PU).

Thus, neuronal-derived NO is the neurovascular diffusible coupler and the coupling is impaired in the later stages of AD at transducer vascular sites.

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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 Ca^{2+} 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 completed understood. In addition to Hcy implications in AD it has been largely recognized its role on the accumulation of cholesterol in

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. Furtermore, 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 neurotoxicant 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/α-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 Bcl-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, endoplasmin precursor, and calumenin, whereas α-tubulin, β-tubulin, vimentin, mitochondrial ATPase β 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/α-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 unilaterally 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

treatment groups. In another series of 6-OHDA lesioned rats the efficacy of MP without benserazide was tested using the same dose for five consecutive days, followed by two days washout, before testing another dose. Using this approach, we found effective doses at 12.5–20 mg L-DOPA/kg ip for MP (n = 17), whereas L-DOPA alone was not effective at these doses (n = 7). Chronic MP treatment induced a sustained motor improvement that took days to build up and lasted for at least 3–4 days after cessation of MP treatment. However, chronic MP administration at therapeutically effective doses caused also limbic AIMs in 8 out of 17 rats.

TU05-04

CATALASE AND SUPEROXIDE DISMUTASE PROTECT AGAINST MITOCHONDRIAL UNCOUPLING CAUSED BY 6-HYDROXYDOPAMINE

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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) derivate 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 ability 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 function. Furthermore, our results appear to support the idea of anion superoxide as the main ROS altering mitochondrial activity. [Supported by Grants SAF2007-66114 from the Ministerio de Ciencia e Innovación with the contribution of the European Regional Development Fund and 09CSA005298PR from the Xunta de Galicia. J.I.-G. was supported by a scholarship from the Fundación Obra Social La Caixa (Barcelona, Spain)].

TU05-05

DEGENERATION IN 12-22-YEAR OLD GRAFTS OF MESENCEPHALIC DOPAMINE NEURONS IN PATIENTS WITH PARKINSON'S DISEASE

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We demonstrate that grafted human fetal mesencephalic neurons can survive and extend axons for 22 years in the brain of a patient with Parkinson's disease (PD). In this patient, the overall survival and fiber outgrowth of the grafts were, however, relatively poor, which is consistent with the lack of significant clinical graft-induced benefit. We have compared the morphology of neurons in the 22-year-old grafts with those in two younger grafts (16- and 12-year-old), which were sequentially implanted in another PD patient. In the case with the 22-year-old transplant, a high proportion (up to 38%) of the grafted dopaminergic (pigment-granule containing) neurons do not express tyrosine hydroxylase and dopamine transporter and their perikarya appear atrophic. The proportion of pigmented neurons not expressing these markers is lower in the 12 to 16-year-old grafts. Furthermore, in the 22-year-old graft, 49% of the pigmented neurons display α-synuclein immunoreactivity in the cell body and 1.2% of them contain Lewy bodies. In conclusion, our results show that grafted dopaminergic neurons can survive for more than two decades. However, over time an increasing proportion of grafted neurons exhibit signs of degeneration.

TU05-06

MELATONIN SYNERGISTICALLY IMPROVES THERAPEUTIC OUTCOME OF L-DOPA IN EXPERIMENTAL PARKINSONISM IN MICE

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Parkinson's disease (PD) has an incidence of 550 and 260 per 100,000 respectively in USA and India in the age group of > 65, which is alarmingly increasing not only in these countries, but worldwide. PD results from loss of dopaminergic neurons of the nigrostriatal pathway, and the most preferred mode of treatment is dopamine replenishment by exogenously administered dopamine precursor, L-DOPA. Long-term treatment with L-DOPA results in tolerance and is further complicated by severe side effects, including the drug-induced dyskinesia. The present study was undertaken to find whether the endogenous neurohormone, melatonin could potentiate low dose effects of the dopamine precursor in MPTP-induced experimental Parkinsonism. MPTP administered twice (30 mg/kg, i.p.), 16 h apart produced more than 60% striatal dopamine depletion, which was not modified by pharmacological doses of melatonin (10, 20, 30 mg/kg; i.p.) 10 hours intervals, 6

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. Catalepsy 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-SY-5Y 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 nano molar 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 OLIG2 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.olig2) on myelin oligodendrocyte glycoprotein 35–55 peptide (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.olig2 (1×106 cells/mouse), and monitored neurobehavioral abnormalities up to 44 days. Transplantation of F3.olig2 cells greatly recovered the clinical scores of EAE. Around the brain lesions, much more F3.olig2 cells were detected by 44 days post-transplantation than parental F3 NSCs, and most of F3.olig2 cells differentiated into mature oligodendrocytes with thick myelin sheaths surrounding the axons. Moreover, F3.olig2 cells significantly attenuated autoimmune-mediated demyelination and axonal loss of host neurons. These results suggest that F3.olig2 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 astrocytosis, 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)

Mir17-92, miR338, miR219 may regulate human OL proliferation and differentiation. (iii) CSF proteome-mapping suggests perturbation of nodes-of-Ranvier in MS as an early event. (iv) The proteome maps presented here can be faithfully used as reference libraries for myelin health against which comparisons can be made to disease states.

TU06-04

THE HAEMATOLOGICAL PROFILE OF PATIENTS WITH MULTIPLE SCLEROSIS

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Red blood cells with impaired membrane fluidity, as has been reported in patients with multiple sclerosis are known to be targeted by phospholipase A2, an enzyme secreted by immune cells during inflammatory activation. The aim of the present study was to profile the red blood cells and immune cells in patients with multiple sclerosis. Venous blood from participants was collected into anti-coagulant EDTA tubes and Full blood counts were determined on a Beckman Coulter. C-reactive protein concentrations were determined on a Beckman auto-analyser. The heamotology profile was correlated with severity of neurological outcome as measured by the Kurtzke Expanded Disability Status Scale and Functional System Scores. There were no differences in white blood cell counts between patients with multiple sclerosis and control subjects. The haemoglobin was significantly decreased in patients (controls; median ± quartile range: 14.7 ± 1.60 g/dL; patients: 13.9 ± 1.40 g/dL; $p = 0.012$. Furthermore, both the red blood cell count and haematocrit was non-significantly decreased in patients. The immune cells showed no significant correlations with either the EDSS or CRP, except LUC % showed an inverse correlation with the brainstem FSS. The RBC count showed significant inverse correlations with both the EDSS and Bowel and Bladder FSS; $R = -0.41$; $p = 0.023$ and $R = -0.45$; $p = 0.011$ respectively. Results indicated that decreases in the RBC profile in patients with MS correlated with higher disability in patients with multiple sclerosis as measured by the EDSS. It is not clear from the results of this study whether decreases in the RBC profile in MS were due to unknown agents involved in the disease aetiology or from the resulting inflammatory responses.

TU06-05

ROLES OF ANNEXIN 2 IN THE SCIATIC NERVE OF LYSOLECITIN TREATED DEMYELINATION AND MYELIN MUTANT MICE

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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 paranodal region and Schmidt-Lanterman incisure (SLI) in normal appearing myelin in surrounding area of the demyelinationg region of lysolecithin-injected sciatic nerves. Reduction of AX2 by siRNA caused marked expansion of the demyelinationg 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.

U06-07

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 premyelinating 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 hypoglossal 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 apotransferrin (aTf)) prevented this de-differentiaton, 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 co-localization 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 knock-out (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 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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Myelinated 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 myelinated 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 lysophospholipase D catalyzing the production of the extracellular lipid mediator lysophosphatidic acid (LPA) from 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 CreER^{TM} and ROSA-loxP-stop-loxP-GAP43-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 bushy appearance under light microscopic observation with GFAP colocalization and extended endfeet onto capillaries under electron microscopic observation, both of which are the characteristics of astrocytes. In the cuprizone-induced demyelinating lesions, most of the GFP positive cells either kept Olig2 immunoreactivities or differentiate into mature oligodendrocytes with MBP expression in the corpus callosum, but few cells took astrocytic fates in contrast to the cryo-injured lesion. When environmental enrichment was applied to the double transgenic mice, the GFP positive cells proliferated, but scarcely differentiate into mature cell types (e.g., astrocytes, oligodendrocytes, and neurons) in the amygdala. These results suggest that Olig2 positive cells in the adult brain take brain-region specific fates in response to cellular environments (e.g., physiological or pathological microenvironments) and are thereby involved in remodeling and/or repair of brain functions. We are indebted to Dr. Hirohide Takebayashi at Department of Anatomy of Kumamoto University School of Medicine and Dr. Kazuhiro Ikenaka at National Institute of Physiological Sciences, Japan for the double transgenic mouse.

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-/-; plpTg(rTag-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 Ischemia and Oxidative Stress

TU07-01

REVERSIBLE INHIBITION OF H_2O_2 ELIMINATION BY CALCIUM IN BRAIN MITOCHONDRIA

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In the present work the Ca^{2+} -dependence of mitochondrial H_2O_2 elimination was investigated. Mitochondria isolated from guinea pig brain were energized by glutamate and malate and incubated with micromolar concentrations of Ca^{2+} in the presence of ADP preventing permeability transition pore formation. After the completion of Ca^{2+} uptake, mitochondria were challenged with H_2O_2 (5 μM), then at various time points residual H_2O_2 was determined using the Amplex red method and compared to that in mitochondria incubated with H_2O_2 without Ca^{2+} addition. Dose-dependent inhibition of H_2O_2 elimination by Ca^{2+} was detected, which was prevented by the Ca^{2+}-uptake inhibitor Ru 360. Stimulation of Ca^{2+} release from Ca^{2+} -loaded mitochondria by a combined addition of Ru 360 and Na^+ decreased the Ca^{2+}-evoked inhibition of H_2O_2 removal. Following Ca^{2+}-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 Ca^{2+} and H_2O_2 on the activity of aconitase were additive.

These results confirm that Ca^{2+} inhibits elimination of H_2O_2 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 Ca^{2+}-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%O_2/5%CO_2 in physiological medium containing different glucose concentrations (in mM): 0, 5.6 and 35 for 30 min. The consequences of hypoglycemia (0mM 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 0mM or 35mM 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 technique 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 previously 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-IRES-EGFP or Arg72p53-IRES-EGFP 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 dyferlinopathic 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 dysferliniopathic 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 of 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.

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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, nigrostratial 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 co-culture (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, CamKIIa 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 nitrated targets mediating cell death remain unknown. Out of 17 proteins that are major targets for peroxynitrite, nitrated 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 nitrated 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 nitrated 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 nitrated Hsp90 (ratio 10:1) for the binding to isolated mitochondria, suggesting an increased affinity of nitrated Hsp90 for a previous mitochondrial client or binding to a new client. The intracellular delivery of nitrated 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 nitrated 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 nNOS positive cells were observed in the cerebral cortex, putamen, caudate nucleus, substantia reticularis, olfactory bulb, nucleus caudatus, hippocampus and subcallosal cortex. No nNOS positive cells were observed in the cerebellum in any group. The mean nNOS 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 nNOS activity in ischemic brain injury.

TU07-11

HYPERHOMOCYSTEINEMIA AND ISCHEMIC PRECONDITIONING IN RAT BRAIN: RESPONSE OF SPCA CA^{2+} 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 Ca^{2+}-ATPase (SPCA1). Hyperhomocysteinemia was induced by administration of homocysteine (Hcy) (0,45 μmol/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 hyperhomocysteine 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 supression 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 Ca^{2+} 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 H_2O_2 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 H_2O_2 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 promoted 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 neurological disease. 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 consecuence, 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 cystein 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 24 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, dihydroetidium (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-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 gaglia 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.

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TU07-19

MEMORY ENHANCING EFFECTS OF SAFFRON IN ADULT & AGED MICE ARE CORRELATED WITH THE ANTIOXIDANT PROTECTION: *IN VITRO* & *IN VIVO* STUDIE

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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 Aß-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_2O_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 ANTIAPOPTOTIC FUNCTIONS OF MITOCHONDRIAL TARGETED SYNTHESIS OF GAMMA-GLUTAMYLCYSTEINE IN CORTICAL NEURONS

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Mitochondria are the main source of reactive oxigen 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 mito-GCL 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 pre-treatment 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 Ca^{2+} 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 ot 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.

TU08 Synaptic Plasticity

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 A_{2A} 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 A_{2A} receptors and metabotropic glutamate type 5 (mGlu5) receptors are co-localised both presynaptically and post-synaptically 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 A_{2A} receptors. The conditioned rewarding and locomotor activating properties of cocaine were evaluated using the conditioned place preference (CPP) paradigm. Adenosine A_{2A} 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 A_{2A} 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 A_{2A} 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]triazolol[2,3-a][1,3,5]triazin-5-yl-amino]ethyl)phenol ($[^{125}I]$ZM241385) binding to the A_{2A} receptor by MTEP.

TU08-03

SYNAPTIC ACTIVITY-INDUCED FLUCTUATIONS IN ASCORBIC ACID CONCENTRATION COULD DRIVE CHANGES IN THE AVAILABILITY OF SVTC2 AT 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 exposition 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). Phosphory%ADlation 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 kinaseinhibitor4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine (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 Ca^{2+} rather tan Na^{+} ions. Although recent studies identified the dominant negative NR3A and NR3B subunits, whether these subunits inhibit Ca^{2+} influx across functional NMDAR channels is not clarified so far. In this study, therefore, we investigated Ca^{2+} 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 Ca^{2+} 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 inhibited the increase by 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 Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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.

Berlin, Oregon-R and Canton-S demonstrated different ratio of C and D isoforms and similar total LIMK1 content. agnts3 had high activities of the both isoforms of LIMK1. Using our setup for automatic registration of courtship song parameters, we evaluated learning ability by calculating learning indices (LIs) as in the conditioned courtship suppression paradigm, based only on singing index. agnts3 and Berlin males demonstrated negative learning indices. LI in Oregon-R males was suppressed. To estimate a possible involvement of the agnts3 mutation in memory formation after 5-h massive training we analyzed distribution pCREB at adult thoracic NMJ fields are responsible for courtship song production. Using confocal microscopy, we found different distribution of pCREB in Canton-S and agnts3. Both in Canton-S and agnts3 pCREB was detected in thin nerve terminals but not at the synaptic boutons before learning. After learning pCREB level increased and bridges between axons were formed. In agnts3 pCREB was detected in nuclei of nervous and muscular cells before and after learning presumably, this distinctive localization of pCREB might be promoted by alteration in levels of LIMK1 D and C isoforms in agnts3due to disturbances of nuclear-cytoplasmic transport.

TU08-07

NOVEL 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. Kallikrein-related peptidase 8/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 littermates in an enriched environment consisting of a large transparent cage with paper bedding, a metallic running wheel and a plastic tunnel. Both neurospin 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 heterotetramerics made of at least two of the four proper subunits GluR1-4. AMPA receptors that are permeable to Ca^{2+} lack the GluR2 subunit.

The present study addressed the question of whether neonatal handling might have an effect on AMRARs, since it has been shown that the subunit composition and thus the Ca^{2+} 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 1(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 amydgala 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.

References:

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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 Ca^{2+}/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 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 complicatedly 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 $GABA_B$ 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 $GABA_B$ 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 isoform 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 isoform 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 controls glutamate stimulated release, calmodulin antagonist W-7 blocks release by 96% at concentrations above 100 μM, CamKII antaginst KN-62 reduces stimulated release (58% – 1 to 10 μM) as well as the phosphatase (PP) 1 and 2 antagonist okadaic acid (43% – 10 to 1000 μM) but not PP3 cypermethrin (10–1000 μM). The rigid analog of cAMP dibutyryl-cAMP was ineffective (1–600 μM) but the adenylate-cyclase activator forskolin tended to increase stimulated release (10 μM). While PKA inhibition by KT-5720 2 μM did not modify glutamate release, KT-5720 plus forskolin further increased stimulated release up to 85%. These results point towards a positive influence of adenylate cyclase activation in the glutamate release process while inhibition of calmodulin or CamKII have the opposite effect. The contrasting effects of dibutyryl-cAMP and forskolin might be explained assuming that forskolin increases cAMP in a specific subcellular compartment associated to glutamate release. Alternatively, Calcium channel phosphorylation states (or yet other ion channels) may directly influence release. Finally, the same drugs that affected 3H-L-glutamate release also affected 3H-L-aspartate in the same way arguing against a significant difference in the metabolic path involved in the releasable pool formation. Financial suport from FAPESP research grant 07/01066-4 to LRPT and PhD graduation grant 03/04408-2 to LM.

TU08-14

ADENOSINE/GLUTAMATE INTERACTIONS IN HIPPOCAMPUS INVOLVE PHOSPHORYLATION OF NMDAR AND ERK1/2 KINASES INDUCED BY MGLUR5R STIMULATION

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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 : a)significantly increased, in a dose dependent manner, the phosphorylation state of NR2B subunit (tyr-1472) of NMDA receptors compared to control levels, while CHPG had no effect on the phosphorylation level 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-administrated 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 hiippocampus. 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

Zisopoulou, S.[1,2], Asimaki, O.[1,2], Leontaritis, G.[1,2], Vasilaki, A.[1], Pitsikas, N.[1], Mangoura, D.[2] and Sakellaridis, N.[1]

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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 ψεRACK (both conjugated to a TAT carrier

peptide). 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 ψεRACK 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 ψεRACK and phorbol esters. Moreover, with similar analyses we found that ψεRACK 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 ψεRACK was co-administered. Taken together, these findings present the first direct evidence that PKCε activity is an essential molecular component of nonspatial recognition memory.

Wednesday Oral Sessions
Plenary Lecture 4

PL4

mRNA LOCALIZATION AND PROTEIN SYNTHESIS IN NEURONS

Schuman, E.M., Cajigas, I.C., Will, T., Tushev, G., Fuerst, N. and tom Dieck, S.

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An individual neuron in the brain possesses approximately 10,000 synapses, many of which are hundreds of microns away from the cell body, which can process independent streams of information. During synaptic transmission and plasticity, remodeling of the local proteome occurs via the regulated synthesis and degradation of new proteins. I will discuss previous and current studies aimed at understanding how local protein synthesis contributes to synaptic function and plasticity. Using deep-sequencing techniques and bioinformatic approaches, we can now describe the local transcriptome- a large population of previously undetected mRNAs that code for synaptically relevant proteins.

ESN Young Scientist Lecture 2

YSL3

THE NAD$^+$/NADH REDOX STATE OF ASTROCYTES: REGULATION AND FUNCTIONAL IMPLICATIONS

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NAD^+ and NADH constitute a redox pair crucially involved in cellular metabolism and function. However, while astrocytes are an important cell population contributing to brain metabolism and brain signaling, little is known about the control of the NAD^+/NADH redox state in these cells. Using biochemical and imaging approaches, we therefore analyzed the regulation of NAD^+ and NADH in astrocytes as well as the influence of the NAD^+/NADH redox state on Ca^{2+}-signals. While the NADH content of astrocytes was very sensitive to changes in energy metabolism, the NAD^+ content was surprisingly constant under these conditions but could, however, be modulated by NAD^+ synthesis and degradation. Furthermore, application of neurotransmitters like dopamine to astrocytes evoked a biphasic response of the NAD(P)H-fluorescence signal dependent of D1-receptors, protein kinase A and 5'-AMP-activated protein kinase signaling. Finally, the NAD^+/NADH redox state modulated astroglial Ca^{2+}-signals as shown both for spontaneous Ca^{2+}-signals and Ca^{2+}-waves. This interdependence of astroglial metabolism and Ca^{2+}-signals might contribute to fine tuned participation of astrocytes to neuronal activity and functional states of the brain.

Symposium 18
Intracellular Transport in Axons and Dendrites, and its Related Diseases

S18-01

A VESICULAR SNARE REQUIRED FOR SEMAPHORIN 3A AXONAL REPULSION

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Axonal growth cones are guided by attractive and repulsive molecules, particularly semaphorins. The role of vesicular trafficking in axonal guidance is still largely unknown. Here we show that the exocytic v-SNARE Synaptobrevin2 (Syb2) is required for Semaphorin3A-dependent repulsion, but not Semaphorin3C-dependent attraction. Syb2 knockout mice show defasciculation of Neuropilin 1-positive cortical axons. Syb2 associates with Neuropilin1 and PlexinA1, components of the Sema3A receptor, through Syb2's juxta and trans-membrane domain. Syb2 deficient neurons also fail to collapse and transport PlexinA1 to cell bodies upon Sema3A treatment. Sema3A inhibits the exocytosis of Syb2 and inactivation of SNAP-25, Syb2's target SNARE, blocks Sema3A repulsion. We conclude that Sema3A repulsion requires the regulation of Syb2 dependent exocytosis.

S18-02

BICAUDAL-D FAMILY PROTEINS: REGULATORS OF SECRETORY TRAFFICKING

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Active transport along polarized microtubules allows neurons to quickly and accurately transport a large variety of subcellular components to axons and dendrites. Cytoplasmic dynein together with the accessory complex dynactin, referred to as the dynein-dynactin complex, form the most important minus-end directed motor within neurons. The great number and variety of transport cargoes together with the paucity of minus-end directed motors necessitates an additional mode of regulation of the motor/cargo interaction. One way to regulate these interactions is through the use of adaptor proteins, which link cargo to motor proteins by interacting with both simultaneously.

A well-studied group of adaptor proteins is the evolutionarily conserved Bicaudal-D (BICD) family. Bicaudal-D was first described in Drosophila as an essential factor in oogenesis and embryogenesis and functions by controlling dynein-dynactin mediated RNA particle transport. In mammalian systems two BicD homologues are present, named BICD1 and BICD2, and have both been implicated in Rab6 secretory vesicle transport. The N-terminal part of BICD binds to the dynein-dynactin motor complex and is sufficient to induce microtubule dependent minus-end directed transport whereas the C-terminal part of BICD contains the cargo-binding domain and directly interacts with the small GTPase Rab6. Recently, we have identified two new members of the BICD family, BICD related protein 1 (BICDR-1) and BICD related protein 2 (BICDR-2). Similar to BICD, BICDR-1 binds to Rab6 as well as dynein-dynactin and is a key component of the molecular machinery that controls secretory vesicle transport in developing neurons. BICDR-1 interacts with kinesin motor Kif1C, the dynein/dynactin retrograde motor complex, regulates the localization of Rab6-positive secretory vesicles and is required for neural development in zebrafish. BICDR-1 expression is high during early neuronal development and strongly declines during neurite outgrowth. We propose an important role for BICDR-1 as temporal regulator of secretory trafficking during the early phase of neuronal differentiation.

S18-03

MOLECULAR MOTOR COORDINATION DURING BIDIRECTIONAL AXONAL TRANSPORT

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The microtubule motors kinesin and dynein drive bidirectional transport along the axon, but the mechanisms that coordinate the function of these oppositely-directed motors are not well understood. To investigate the mechanisms that coordinate kinesin and dynein motors for effective organelle transport, we used high-resolution live cell imaging to examine late endosome/lysosome dynamics in primary neurons. We compared these observations to the motility of purified Rab7-positive late endosomes/lysosomes *in vitro* in reconstitution experiments. Photobleaching and quantitative western blotting indicates that 1–5 dynein and 1–4 kinesin motors are sufficient to drive robust vesicle motility. These observations, in conjunction with mathematical modeling, suggest that a small number of tightly associated motors drive bidirectional vesicle transport along the axon, and that directional switching at short time scales is a consequence of the force-dependent dissociation kinetics of the motors in the absence of external regulation. While stochastic fluctuations in motor engagement mediate directional switching over short time scales, regulatory mechanisms likely control net flux on longer time scales to allow for targeted trafficking of vesicular cargoes. To better understand the regulatory mechanisms involved, we focused on JNK (c-jun N-terminal kinase) and the interacting scaffolding protein JIP1. Pharmacological inhibition of JNK induces a bidirectional inhibition in the axonal transport of both Rab7-positive and APP-positive organelles, with significant decreases in both speed of movement and percent motility. In contrast, activation of JNK enhances retrograde transport along the axon. Targeted knockdown of JIP1 also inhibits the motility of late endosomes/lysosomes and APP, suggesting that JIP1 is a mediator of JNK signaling in the axon. Biochemical studies demonstrate that JIP1 interacts with both anterograde and retrograde motor complexes, through interactions with both kinesin and dynactin. These interactions are mediated by distinct, non-overlapping domains of JIP1, suggesting that JIP1 serves as a bidirectional co-regulator of these motors. Together, these

data support a model in which both stochastic fluctuations in motor activity and active regulation by kinases, mediated via scaffolding proteins, contribute to the coordination of bidirectional transport along the axon. Supported by NIH grants GM48661.

S18-04

DISC1 ACTS AS A CARGO ADAPTER FOR NEURONAL TRANSPORT OF SPECIFIC PROTEINS AND MESSENGER RNAS

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Schizophrenia is a severe psychiatric disorder with lifelong disability. Although the causes of schizophrenia remain largely unknown, it has been widely reported that schizophrenia has high inheritance, indicating the existence of genetical risk factors (Owen et al., 2004; Craddock et al., 2005; Harrison and Weinberger, 2005). Disrupted-In-Schizophrenia 1 (DISC1) is a candidate gene for susceptibility to schizophrenia. Accumulating evidence suggests that DISC1 participates in neurodevelopment such neurogenesis, neuronal migration and axon/dendrite formation through the interaction with NUDEL/LIS1, FEZ1 and GSK3β. We also found that DISC1 accumulates at the tip of axons and regulates the axonal transport of NUDEL/LIS1/14-3-3 epsilon complex (Taya et al., 2007), Grb2 (Shinoda et al., 2007) and Girdin (Enomoto et al., 2009) through Kinesin-1 in rat hippocampal neurons. However, its modes of action remain largely unknown. Here, we comprehensively screened for DISC1-interacting proteins by proteomic analysis and identified many RNA-binding proteins including Hematopoietic zinc finger (HZF), which acts as a component of RNA-transporting granules and participates in the dendritic localization of inositol 1,4,5-trisphosphate receptor type 1 (IP3R1) mRNA. DISC1 co-localizes with HZF and RNA-transporting granules in hippocampal dendrites. DISC1 directly associates with IP3R1 mRNA and is co-transported into dendrites. Impairment of DISC1 function prohibits both the dendritic transport and BDNF-induced local translation of IP3R1 mRNA. Because Kinesin-1 also interacts with DISC1 and mediates the transport of DISC1 and IP3R1 mRNA along microtubules, we propose that DISC1 with HZF binds IP3R1 mRNA and thereby regulates its dendritic transport as a cargo adaptor for the local translation.

Symposium 19
Septin Research and Neuronal Disease

S19-01

ROLE OF THE SEPTIN IN NEURAL CELL MIGRATION DURING BRAIN DEVELOPMENT

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Septins are GTP/GDP-binding proteins interacting with cell membranes and the cytoskeleton, and thought to play pivotal roles in neuronal cell polarity, neurotransmitter release and development. Recent studies have revealed that abnormalities in the genetic expression and molecular function of septins are involved in neurological diseases such as Alzheimer's disease, Parkinson's disease and schizophrenia.

On the other hand, correct neuronal generation, migration and positioning during cortical development are essential for proper brain formation and functions, and abnormalities during corticogenesis may cause developmental disorders including mental retardation and epilepsy. In the study, we focused on the functions of septin family members, Sept14 and Sept4. By in utero electroporation, we found that the two septins, in a coordinated manner, play important roles in the brain development through regulation of neuronal morphology and migration. We then screened interacting molecules for Sept4 and Sept14 to investigate the molecular mechanism of their functions. By using a method combining affinity column chromatography with shotgun liquid chromatography tandem mass spectrometry (LC-MS/MS), we isolated ~100 candidates with various affinities to Sept4 and Sept14.

S19-02

EVIDENCE FOR THE INVOLVEMENT OF SEPTINS IN SCHIZOPHRENIA AND BIPOLAR DISORDER

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Schizophrenia is amongst the most debilitating of disorders and its core pathophysiology is poorly understood. Significant support exists for the presence of altered myelination, synaptic plasticity and GABergic function in the disorder. We have undertaken proteomic studies to identify novel mechanisms of disease pathogenesis in schizophrenia and in bipolar disorder. 2D gel eletrophoresis was used to characterise differential protein expression in the prefrontal cortex and hippocampus. In keeping with previous work we have found evidence of mitochondrial and cytoskeletal dysfunction. Remarkably, we have also found robust evidence implicating the septin family of proteins. First, in dorsolateral prefrontal cortex we observed upregulation of septin 5, 6 and 11 in both schizophrenia and in bipolar disorder. Septin 5 has roles in the presynaptic SNARE complex and cytoskeletal stability, functions which are implicated in schizophrenia. Subsequently, in hippocampus, using differential in-gel electrophoresis (DIGE) we observed prominent reductions in the expression of Septin11 in both disorders in the CA2/3 and also in CA4 subregions. We confirmed this reduction in Septin 11 by western blotting in CA4 in schizophrenia and in bipolar disorder. Septin11 has roles in myelination, dendrite spine morphology and GABAergic synaptic connectivity. Alterations of Septin11 are thus highly relevant to schizophrenia. The implications of Septin involvement in major psychiatric disorders will be discussed.

S19-03

A SEPTIN MULTIMER STABILIZES CENTRAL NERVOUS SYSTEM MYELIN

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Rapid nerve conduction in vertebrates is achieved by the insulation of neuronal axons with myelin, a multi-layered compacted membrane system extended from glial cells. We are interested in the molecules contributing to the structural organization of myelin. Based on the observation that mature myelin is actin-free we hypothesized that proteins of the septin family may constitute a cytoskeletal membrane cortex in myelin. By quantitative proteome analysis we identified SEPT8 as the most abundant septin of central nervous system myelin. Aiming at understanding the function of myelin septins *in vivo* we generated mice lacking SEPT8 either constitutively or conditionally in myelinating glia. Our results indicate that SEPT8 occurs as a heterooligomer together with SEPT2, -4, and -7 in normal myelin. Mice lacking the heterooligomer display pathogenic myelin outfoldings. We propose that this phenotype reflects the impaired function of oligodendrocytes to establish the lateral compartmentalization into functional domains of myelin required for long-term structural integrity.

S19-04

REGULATION OF COLLATERAL AXON BRANCHING BY SEPTIN GTPASES IN SENSORY NEURONS

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Development of the nervous system requires patterns of innervation, which are shaped by the collateral and terminal branching of axons. Collateral branches are formed from axonal shaft filopodia and require the coordinated action of the actin and microtubule cytoskeleton. Using chicken dorsal root ganglia as a model system, we found that septin 6 (SEPT6) localizes to actin-rich patches throughout the axon shaft. In contrast, septin 7 (SEPT7) localized to the base of axonal shaft filopodia. Time-lapse microscopy revealed that both SEPT6 and SEPT7 accumulate at sites of incipient filopodia. SEPT6 and SEPT7 depletion and over-expression resulted in significant decrease and increase in the number of >5 um-long

axon branches, respectively. To determine how SEPT6 and SEPT7 are involved in axon branch formation, we analyzed the dynamics of filopodia formation. Depletion or over-expression of SEPT6, but not SEPT7, affected the rate of filopodia formation. Imaging of actin dynamics showed that SEPT6 over-expression increases the transition of actin patches to filopodia, but has no effect on F-actin patch formation and life-span. Although SEPT7 had no role in the protrusive activity of F-actin patches, microtubule-presence in nascent filopodia depended on SEPT7. Absence of MAP1B from axonal sites of SEPT7-GFP accumulation indicated that SEPT7 may antagonize MAP1B for microtubule entry into actin-rich protrusions. Taken together, our data suggest a two-step mechanism for the development of axon branches. First, SEPT6 triggers the conversion of membrane F-actin patches to axonal filopodia, and second, SEPT7 facilitates the entry of axonal microtubules into nascent filopodia, and thus the formation of axon branches.

Symposium 20
The Amyloid Precursor Protein (APP) and Prion Protein Metabolomes: Interactions, Mechanisms and Neurodegeneration

S20-01

THE APP METABOLOME: NEW INSIGHTS AND NEW THERAPEUTIC TARGETS

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The importance of the amyloid β-peptide (Aβ) and its oligomers to Alzheimer pathology and disease progression is well established. However, understanding the physiology and functions of the amyloid precursor protein (APP) has been highly influenced by an amyloidocentric perspective of the protein despite the heterogeneity of its expression and its processing into multiple metabolites in different cellular compartments. Hence the overall complexity of the APP metabolic network, and its regulation, have rarely been addressed in their entirety. Metabolism of the amyloid precursor protein (APP) contributes to the pathogenesis of Alzheimer's disease. The consecutive processing of APP by β- and γ-secretases generates a soluble ectodomain (sAPPβ), the Aβ peptide and the APP intracellular domain (AICD). Current therapeutic strategies have focused on preventing Aβ formation or enhancing its clearance yet, in 20 years, they have failed to produce clinically effective drugs. The β-secretase pathway is predominantly restricted to endocytic compartments and is lipid-raft mediated. The APP intracellular domain, AICD, can regulate transcription of several neuronal genes, including glycogen synthase kinase-3β, aquaporin-1, and especially the Aβ-degrading enzyme, neprilysin (NEP). We have shown by chromatin immunoprecipitation studies that transcriptional upregulation involves direct binding of AICD to the NEP promoters competitively with the histone deacetylase HDAC1 in a neuronally specific manner. The alternative and predominant α-secretase pathway of APP metabolism generates a larger ectodomain (sAPPβ), which precludes the formation of Aβ but could allow formation of AICD. We have further explored the molecular mechanisms underlying the differential compartmentation of the α- and β-secretase mediated pathways of APP metabolism, their modulation by protein-protein interactions, and the molecular basis of the transcriptional regulation. A more complete understanding of the APP metabolic network and its interactions may provide new opportunities for therapeutic intervention in AD.

This work was supported by the UK Medical Research Council and Alzheimer's Research UK.

S20-02

IDENTIFICATION OF A NOVEL APP/AICD-BINDING PROTEIN THAT REGULATES APOPTOTIC CELL DEATH

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One of the most common neurodegenerative disorders is Alzheimer's disease (AD), which is characterized by excessive formation and accumulation of senile plaques in the brain. The major component of senile plaques are β-amyloid (Aβ) peptides derived from the β-amyloid precursor protein (APP) through sequential cleavages, first by β-secretase (BACE1) to produce a β-C-terminal fragment of APP (β-CTF or C99) and then by γ-secretase to generate Aβ. Aβ is highly toxic to neurons in susceptible brain regions and can trigger a cascade of pathogenic events including tau hyperphosphorylation, loss of synapses and eventually neuronal cell death. In addition to Aβ, γ-secretase cleavage of APP also generates the soluble APP intracellular domain (AICD) which has been found to have neurotoxic effects, enhance p53-mediated apoptosis, and regulate transcription of certain genes, such as p53, GSK-3 and EGFR, which are involved in cell survival/tumorigenesis. We have recently identified an AICD interacting mitochondrial solute carrier family protein (designated as appoptosin) that induces ROS release and intrinsic caspase-dependent apoptosis. The physiological function of appoptosin is to transport/exchange glycine/dALA across the mitochondrial membrane for heme synthesis. APP/AICD-appoptosin interaction modulates appoptosin-induced apoptosis. Levels of appoptosin are up-regulated in brain samples from AD and infarct patients and in rodent stroke models, as well as in neurons treated with Aβ oligomers. Down-regulation of appoptosin prevents the mitochondrial fragmentation and caspase activation caused by glutamate or Aβ insults in neurons. Our study identifies appoptosin as a crucial player in apoptosis and a novel proapoptotic protein involved in neuronal cell death, providing a possible new therapeutic target for neurodegenerative disorders and cancers.

S20-03

THE ROLE OF APP INTRACELLULAR DOMAIN IN ALZHEIMER'S DISEASE

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Amyloid precursor protein (APP) generates multiple smaller peptides including amyloid-β (Aβ) and APP intracellular domain

(AICD). Although Aβ is known to play a pivotal role in Alzheimer's disease (AD) pathology, there is growing evidence that amyloid-independent mechanisms also contribute to AD pathogenesis. AICD exerts significant biological effects by regulating intracellular signaling pathways and modulating gene expression.

We generated transgenic mice (AICD-Tg) that overexpress AICD in the forebrain and hippocampal neurons and showed that these mice recapitulate AD-pathologies, including memory deficits, in an age-dependent manner. AICD activates inflammatory pathways and neuroinflammation is the earliest pathological feature observed in these animals. Treatment of AICD-Tg mice with non-steroidal anti-inflammatory drugs (NSAIDs) blocks development of many pathological features.

These results indicate that inflammation plays a crucial role in AD-pathologies in AICD-Tg mice. We suggest a model in which Chronic Low-grade Neuroinflammatory Environment (CLONE), triggered by factors such as AICD, Aβ, aging, stroke etc, plays a causative role in the pathogenesis of AD. Chronic neuroinflammation inhibits synaptic transmission and causes neuronal cell death. Our results provide a basis for the observations that NSAID treatment provides significant protection against developing AD when initiated prior to the appearance of the symptoms. We suggest that the anti-inflammatory drugs lose their effectiveness against AD once the self-perpetuating cycle of inflammation sets in. We propose that anti-inflammatory treatment should be an integral component of the therapeutic strategy against AD.

S20-04

AICD CONTROLS P53 AND REGULATES βAPP PROCESSING

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Aβ production results from the sequential attack of βAPP by β- and γ-secretase. The latter enzyme is composed of at least four distinct subunits, namely presenilin1 (PS1) or PS2, nicastrin, Aph-1 and Pen-2. Besides Aβ, an intracellular domain named AICD could act as a transcription factor and regulates, besides others, the transactivation of the p53 promoter. All members of the complex regulate or are regulated by p53. Here we substantiate these data by delineating a molecular cross-talk between several members of the complex such as presenilin 1 and Pen-2 and by examining directly the influence of p53 in cellular and *in vitro* γ-secretase assays.

Symposium 21
Molecular, Cellular, and Circuit Mechanisms Underlying Brain Dopamine Functions

S21-01

BASIC MECHANISMS AND REGULATION OF NEUROTRANSMITTER RELEASE BY MIDBRAIN DOPAMINE NEURONS

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Regulation of the pre- and postsynaptic actions of dopamine is at the heart of a number of therapeutic strategies for the treatment of diseases such as Parkinson's and schizophrenia. However, little is currently known regarding the basic mechanisms that mediate and regulate dopamine release in the brain. At the axon terminal level, the D2 autoreceptor is known to tightly regulate axonal dopamine release through a negative feedback mechanism that is presently ill-defined. At the somatodendritic level, dopamine is known to be released from the dendrites and cell body of dopamine neurons, but the molecular mechanism involved has not been properly characterized. This presentation will provide a brief overview of my laboratory's work on these two questions. I will first describe the results of cyclic voltammetry experiments using selective potassium channel neurotoxins suggesting that the ability of the D2 receptor to inhibit dopamine release is dependent on the function of voltage-dependent potassium channels of the Kv1 subtype. In the second part of the presentation, I will present the results of experiments performed with a new *in vitro* primary culture system allowing us to detect dopamine release from the somatodendritic compartment of dopamine neurons. Using a siRNA strategy, we tested the hypothesis that the differential calcium-dependence of somatodendritic dopamine release is due to selective expression of different synaptotagmin isoforms in the axon terminal and somatodendritic compartment of these neurons. We provide evidence showing that while synaptotagmin 1 is required for axonal dopamine release, synaptotagmins 4 and 7 are particularly important for somatodendritic dopamine release. Work funded by the Canadian Institutes of Health Research.

S21-02

PHYSIOLOGICAL PROPERTIES AND ADULT NEUROGENESIS OF DOPAMINERGIC NEURONS IN THE MOUSE OLFACTORY BULB

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The olfactory bulb (OB) of mammals contains a large population of dopaminergic (DA) interneurons within the glomerular layer (GL) which have been shown to modulate several aspects of olfactory information processing. To study the functional properties of DA neurons, we used a transgenic mouse strain harbouring an eGFP reporter construct under the promoter of tyrosine hydroxylase, allowing the identification of dopaminergic neurons (TH-GFP cells) in living preparations. The most prominent feature of these cells was the autorhythmicity, that we show is supported by the interplay of the a persistent Na^+ current and of a T-type Ca^{2+} current. In these cells we have identified six main voltage-dependent conductances, that we have completely kinetically characterized, developing a numerical model of TH-GFP cells, capable of reproducing accurately the properties of living cells.

A significant fraction of the bulbar DA interneurons is added in adulthood. In the OB, DA neurons are restricted to the GL, but in TH-GFP transgenic mice we also detected the presence of GFP+ cells in the mitral and external plexiform layers. We show that these are adult-generated neurons committed to become DA but not yet entirely differentiated. Accordingly, TH-GFP+ cells outside the GL exhibit functional properties (appearance of pacemaker currents, synaptic connection with the olfactory nerve, intracellular chloride concentration, and other) marking a gradient of maturity toward the dopaminergic phenotype along the mitral-glomerular axis. Finally, we propose that the establishment of a synaptic contact with the olfactory nerve is the key event allowing these cells to complete their differentiation toward the DA phenotype and to reach their final destination.

S21-03

MOLECULAR MECHANISMS REGULATING DOPAMINE SIGNALING

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Dopamine (DA) is a major neuromodulator of the central nervous system, where it regulates very diverse physiological functions ranging from the control of locomotion to hormone synthesis and release. Consequently, dysfunctions of the dopaminergic system underlie major neurological and psychiatric human disorders, such as Parkinson's disease and schizophrenia. DA elicits its control through the binding to membrane receptors, which belong to the family of seven transmembrane domain G-protein coupled receptors. Our research focuses on dopamine D2 receptors, one of the leading actors of the dopaminergic system. Importantly, D2 receptors are the major target of antipsychotics, this feature together with the major role of D2 receptors in regulating dopamine synthesis and release, makes this receptor a strong candidate gene involved in the etiology of schizophrenia. D2 receptors *in vivo* have multiple roles. In fact these receptors are present presynaptically on dopaminergic, cortical and thalamic neurons as well as on interneurons. The presynaptic localization of D2 has been shown to modulate release not only of dopamine, but also of other neurotransmitter such as GABA, acetylcholine or glutamate. At the same time, D2 receptors have also major postsynaptic functions. In addition, two isoforms of dopamine D2 receptors are present in the brain, D2L and D2S, both isoforms are generated from the same gene by a mechanism of alternative splicing. Thus, addressing the

function of D2 receptors *in vivo* is very complex. We have approached this study by generating genetically engineered mice in which the expression of the dopamine D2 receptors is either abolished or modified. The biochemical, molecular and behavioral analyses of these mice are clarifying the physiological role of these proteins in specific D2-mediated functions. In particular, we have been able to demonstrate that the two isoforms, D2L and D2S, have different functions *in vivo*. D2L appears to have mainly post-synaptic activities while D2S has preponderant presynaptic/hetero-synaptic release-modulating functions. Ongoing studies are aimed at identifying the involvement of pre- versus post-synaptic D2 mediated effects on animal physiology.

S21-04

BEHAVIORAL AND PHYSIOLOGICAL ROLES OF STRIATAL PROJECTION PATHWAYS IN INSTRUMENTAL LEARNING

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Instrumental conditioning is a critical adaptive mechanism by which animals learn an association between sensory cues and motor responses leading to reinforcement. The dorsal striatum in the basal ganglia circuit plays an important role in learning processes contributing to instrumental motor actions. Extensive studies indicate that lesion or pharmacological blockade of the dorsal striatum and its related structures impairs acquisition and performance of different paradigms for goal-directed and habitual actions. For instance, excitotoxic lesion of striatal neurons disturbs choice accuracy of learned motor response and reduces their responding rate in conditional discrimination learning. The dorsal striatum receives excitatory inputs from many cortical areas and the thalamic nuclei and projects to the output nuclei through two major pathways composed of the direct and indirect pathways. The striatonigral spiny neurons in the direct pathway provide monosynaptic inhibition to the output nuclei, whereas the striatopallidal spiny neurons in the indirect pathway inhibit the globus pallidus. The balance between opposing inputs from the two pathways is considered to be implicated in motor control though the regulation of basal ganglia output activity. However, the mechanism remains unclear how specific neural pathways in the basal ganglia circuit contribute to the learning processes of instrumental actions. In the present study, we aimed to address the behavioral roles of the striatonigral and striatopallidal neurons in the performance of conditional discrimination task. The striatonigral neurons containing dopamine D1 receptor and the striatopallidal neurons containing dopamine D2 receptor were selectively eliminated from the brain in transgenic mice or rats by using immunotoxin cell targeting, and behavioral consequence of neuronal elimination was assessed by using several types of operant conditioning tasks. Our results suggest that the stiratonigral and striatopallidal pathways act to cooperatively regulate the accuracy and response time of learned motor actions in the performance of conditional discrimination.

Symposium 22
On the Mechanisms of Memory Facilitation and Erasure

S22-01

ON THE RECONSOLIDATION OF FEAR EXTINCTION

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The non-reinforced expression of long-term memory may lead to two opposite protein-synthesis-dependent processes: extinction and reconsolidation. Extinction weakens consolidated memories whereas reconsolidation allows incorporation of new information into them. Knowledge about these two processes has accumulated in recent years, but their possible interaction has seldom been evaluated. In this talk I will present recent results showing that extinction memory is susceptible to a retrieval-induced process similar to reconsolidation in the hippocampus. I will also discuss the implications of these results at the theoretical and clinical levels.

S22-02

FACILITATING (AND INDUCING) MEMORY FOR FEAR EXTINCTION

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The resurgence of interest in the neural mechanisms of extinction is based, in part, on the promise of facilitating extinction of conditioned fear. This is because extinction is thought to underlie cognitive treatment approaches for anxiety disorders such as PTSD and OCD. It is becoming apparent that fear extinction involves a broad network of structures, including the basolateral amygdala, prefrontal cortex, hippocampus and even midbrain areas. The infralimbic PFC (IL) can inhibit fear expression via projections to GABAergic interneurons in the amygdala, while the prelimbic (PL) PFC drives fear through projections to excitatory area of the amygdala. BDNF has been implicated in synaptic plasticity and NMDA-dependent learning. We observed that infusion of BDNF into the IL (but not PL) induced long lasting extinction memory, even in the absence of extinction training. Like extinction, this memory was NMDA-dependent and could be reinstated with unsignaled shocks. Rats unable to retrieve extinction showed decreased BDNF in ventral hippocampal inputs to IL, suggesting that impaired hippocampal control of IL could contribute to extinction failure. Another approach we are using is to modulate PL and IL with deep brain stimulation (DBS) of prefrontal axons passing through the striatum. Depending on the specific site, 3 hours of DBS given concurrently with extinction training was able to strengthen or weaken extinction memory. Thus, modulation of fear extinction may underlie the beneficial effects of striatal DBS for intractable OCD.

S22-03

CREB AND MEMORY

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Competition between neurons governs the refinement of neural networks during development and may be important for selecting which neurons participate in a given memory in the adult brain. To examine neuronal competition and selection during memory formation we manipulated the function of the transcription factor CREB in subsets of neurons. Here we show that changes in relative CREB function bias the probability that individual lateral amygdala neurons are recruited into a fear memory trace. Our results suggest a competitive model underlying memory formation in which eligible neurons are selected to participate in a memory trace as a function of their relative CREB activity at the time of training.

S22-04

USING HISTONE DEACETYLASE INHIBITORS TO ELIMINATE FEAR AND DRUG SEEKING: FACILITATION OF EXTINCTION OR ERASURE OF MEMORY?

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Recent work on the molecular mechanisms of learning has examined the processes that underlie extinction, which occurs when an organism learns that a previously established relation between two events has been severed. Many studies have demonstrated that extinction suppresses a previously formed memory, but that this suppression is often reversed through the passage of time or through reminder treatments. A challenge for pharmacological approaches to extinction is therefore to determine molecular processes to target that may result in a persistent form of extinction that does not reverse with time or when reminders of the original experience are encountered. One potential target for these manipulations is the control of gene expression by pharmacological modulation of chromatin, the protein complex that packages genomic DNA. Relaxing chromatin structure by administering a histone deacetylase (HDAC) inhibitor can promote gene expression by facilitating interactions between transcription factors and DNA. In this talk, I will review results of experiments that examine the effects of HDAC inhibitors on initial learning and extinction in two commonly used preparations, fear conditioning and cocaine-induced place preferences. Our results show that HDAC inhibitors facilitate extinction and weaken reinstatement, reconditioning, and spontaneous recovery. These findings are consistent with both a memory facilitation account (i.e., promoting the new extinction memory) and a memory erasure account (i.e., erasing the original memory). I will discuss implication of these theoretical accounts for molecular mechanisms underlying memory and will suggest that modulating chromatin modification during extinction may be a useful tool for behavioral approaches to disorders that involve failures in extinction.

S22-05

INHIBITION OF THE PROTEASOME AFFECTS LEARNING AND MEMORY

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Temporal phases of memory are defined according to memory sensitivity for psychological or pharmacological interventions, including the well documented effect of protein synthesis inhibitors. Long term memories of sensory information are presumed to be stored at list in part in the relevant cortical area. However, the molecular machinery underlying both consolidation and maintenance of sensory information in the cortex is poorly understood. We tested the effect of proteasome inhibitors locally infused to the rat gustatory cortex on different phases of positive and negative forms of taste learning and memory. We found that proteasome inhibitors improve memory when they were introduced before taste learning. Co-application of proteasome inhibitors with protein synthesis inhibitors during learning rescued the protein synthesis dependent memory impairment. Moreover, in contrast to the enhancing effect of proteasome inhibitors on learning, inhibition of the proteasome impairs memory long after it was acquired. These results demonstrate that protein turnover is the major factor in the molecular consolidation process in the cortex and suggest that long term storage of memories across cortical networks is affected by rates of protein degradation.

Symposium 23
The Prion Protein: Beyond Prion Diseases

S23-01

STI1-PRP^C AS A POTENTIAL THERAPEUTIC TARGET IN NEURODEGENERATIVE DISORDERS

Prado, M.A.[1], Beraldo, F.H.[1], Caetano, F.A.[1], Guimarães, A.L.[1], Mohammad, A.[1], Ostapchenko, V.[1], Hajj, G.N.[2], Prado, V.F.[1] and Martins, V.R.[2]

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Understanding the biology behind prion protein (PrP^C) functions is critical to dissect mechanisms of pathological insults in prion and other neurodegenerative disorders. We have proposed that PrP^C acts as a signaling scaffold in neurons and glia, allowing for distinct ligands to signal and modify neuronal function. Distinct partners of PrP^C have been uncovered in the last few years and some of them can bind PrP^C to trigger neuronal signaling. STI1, a protein secreted from astrocytes, binds PrP^C in neurons triggering an increase in intracellular calcium via alpha7 nicotinic acetylcholine receptors. This response is the upstream signal for an intracellular cascade that protects cells from death and helps them to differentiate. To understand the roles that STI1 play *in vivo* we generated genetically modified mice with a disruption in the STI1 gene. Heterozygous STI1 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. This is remarkable because STI1 is not required for survival in yeast or *C. elegans*. More recently, PrP^C has also been implicated in Alzheimer's disease, although its precise role is still controversial. We find that Aβ oligomers modify PrP^C trafficking and activate PrP^C dependent signalling in neuronal cells. In order to investigate if STI1 levels could be altered in neurodegenerative diseases, we used a transgenic Alzheimer's mouse model which develops plaques and cognitive dysfunction. STI1 protein levels were reduced by 50% in 6 month-old transgenic mice. Importantly, reduction in STI1 levels increased Aβ oligomer-mediated toxicity. Our data suggest that the STI1/PrP^C complex may play important roles in protecting neuronal cells from toxic activities of Aβ oligomers. PrioNet-Canada, CIHR, FAPESP-Brazil

S23-02

NEUROTOXIC AND NEUROPROTECTIVE ACTIVITIES OF THE PRION PROTEIN

Harris, D.A.[1], Solomon, I.H.[1], Turnbaugh, J.A.[1], Massignan, T.[1], Westergard, L.[2], Unterberger, U.[1], Huettner, J.E.[2] and Biasini, E.[1]

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There is evidence that alterations in the normal physiological activity of PrPC contribute to prion-induced neurotoxicity. This mechanism has been difficult to investigate, however, because the normal function of PrPC has remained obscure, and there are no assays available to measure it. Transgenic mice expressing PrP deleted for a highly conserved block of residues (105–125) in the central region display a neonatal lethal phenotype characterized by massive death of cerebellar granule neurons via a novel, non-apoptotic mechanism that is independent of Bax and caspases (Li et al. (2007). EMBO J, **26**:548–558; Li et al. (2007). J Neurosci, **27**: 852–859. This neurodegenerative phenotype is reversed in a dose-dependent fashion by co-expression of wild-type PrP. We have found that cells expressing deletions or disease-associated point mutations in the conserved, central region of PrP exhibit spontaneous ionic currents and hypersensitivity to certain classes of cationic drugs (Massignan et al. (2010). J Biol Chem, **285**:7752–7765; Solomon et al. (2010). J Biol Chem, **285**: 26719–26726, Using cell culture assays, as well as expression in transgenic mice, we have found that the toxic activity of these mutant PrP molecules requires localization to the plasma membrane and depends on the presence of a polybasic amino acid segment at the N-terminus (residues 23–31). The sequence domains identified in our study are also critical for PrPSc formation, suggesting that common structural features may govern both the functional activity of PrPC and its conversion to PrPSc.

S23-03

THE PRION PROTEIN BEYOND NEURODEGENERATION

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The prion protein (PrPC) is a highly conserved GPI-anchored cell surface protein, expressed mainly in the nervous and immune systems. Although abnormal conformers of PrPC are associated with neurodegenerative disorders, the cellular content of the normal protein affects not only neural activity and integrity, but also innate and acquired immunity, and possibly other organs and systems. Based on mounting evidence that PrPC has pleiotropic signaling properties, we proposed the hypothesis that the prion protein is a dynamic cell surface platform, or scaffold, for the assembly of signaling modules, based on which selective interactions with many ligands and transmembrane signaling pathways translate into wide-range consequences upon both physiology and behavior. Our recent work focused on structural properties associated with interaction of PrPC with its ligands, as well as on general physiological properties of the protein. Our data showed that interaction of PrPC with its ligand hop/STI1 entails reciprocal remodeling that may be involved in the propagation of signals mediated by PrPC. Outside of the nervous system, our previous work suggested that PrPC modulates phagocytosis by macrophages and inflammatory responses. Neutrophils play critical roles in both acute and chronic inflammation, and are implicated in a cross-linked circuit of inflammation, sensitivity to stress, and disease. Our recent work shows that both peripheral inflammation and behavioral stress modulate the content of PrPC at the plasma membrane of neutrophils, with consequences for peroxide-dependent cytotoxicity towards vascular endothelial cells. These data may be relevant for clinically observed associations of stress and anxiety with either the severity or the progression

of various diseases, inclusive of, but not restricted to neurodegeneration. Our studies add to the understanding of how both allosteric properties of the prion protein and systemic control of its expression and function modulate cellular physiology and pathology.

S23-04

PRION PROTEIN IN AMYLOID-BETA AND ZINC METABOLISM

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Alzheimer's disease is characterised by the accumulation in the brain of amyloid-beta peptides derived from the amyloid precursor protein (APP). The cellular prion protein (PrPC) inhibits the action of the beta-secretase BACE1 towards APP in cellular models and the levels of endogenous amyloid-beta are significantly increased in the brain of PrPC null mice. BACE1 co-immunoprecipitates with PrPC from cells, mouse brain and human brain, and PrPC is decreased in the hippocampus in sporadic Alzheimer's disease. Immunofluorescence microscopy and FACS analysis reveals that PrPC decreases the amount of BACE1 at the cell surface and in endosomes, and retains it in the Golgi. By site-directed mutagenesis PrPC was found to interact directly with Pro29 in the pro-domain of the immature Golgi-localised form of BACE1. Zinc is released into the synaptic cleft upon exocytotic stimuli. Using zinc specific fluorescent dyes, PrPC was found to enhance the uptake of zinc into neuronal cells. This PrPC-mediated zinc influx was dependent on the octapeptide repeats in PrPC but did not require the endocytosis of the protein. The PrPC-mediated zinc uptake was blocked by selective antagonists of AMPA receptors and PrPC interacted with both GluA1 and GluA2 subunits. Zinc-sensitive tyrosine phosphatase activity was decreased in cells expressing PrPC and increased in the brains of PrPC null mice. This PrPC-mediated zinc uptake was ablated in cells expressing a range of familial prion disease-associated mutants of PrPC and in prion-infected cells. Thus, PrPC appears to have roles in amyloid-beta and zinc metabolism, and disruption of these functions may contribute to the neurotoxicity observed in Alzheimer's disease.

Symposium 24
Molecular Mechanisms of Opiate Actions

S24-01

OPIOID RECEPTOR IMAGING AND LIGAND BIASED RESPONSES *IN VIVO*

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The combination of fluorescent genetically-encoded proteins with mouse engineering provides a fascinating means to study dynamic biological processes in mammals. We used enhanced-GFP (eGFP) to achieve functional imaging of a G protein-coupled receptor (GPCR) *in vivo*. We created mice where the delta opioid receptor (DOR) is replaced by a functional DOR-eGFP fusion (Scherrer PNAS 2006). Confocal imaging revealed detailed receptor neuroanatomy throughout the nervous system. Real-time imaging in primary neurons allowed dynamic visualization of drug-induced receptor trafficking. In DOR-eGFP animals, drug treatment using a classical delta agonist triggered receptor endocytosis throughout the nervous system. Mice with internalized receptors were insensitive to subsequent agonist administration, providing first evidence that receptor sequestration desensitizes the behavioral response and limits drug efficacy *in vivo*. We further addressed the physiological consequences of receptor internalization and compared effects of two agonists with equipotent analgesic efficacy but distinct internalizing properties the hypothesis in a model of inflammatory pain. The high-, but not the low-internalizing drug produced behavioral desensitization, establishing that ligand-biased trafficking impacts on receptor function *in vivo*. This effect was transient, and surface receptor expression as well as delta agonist-induced analgesia were both restored after 24 hours (Pradhan PLoS ONE 2009). We finally investigated the consequences of chronic treatment with the two drugs. The high-internalizing compound produced receptor down-regulation, leading to generalized tolerance to all *in vivo* effects of delta agonists. In contrast, the low-internalizing compound did not modify receptor expression and G protein coupling, and tolerance developed specifically to analgesic effects, but not locomotor or anxiolytic effects of delta agonists. Ligand-biased receptor trafficking *in vivo*, therefore, leads to distinct forms of tolerance (Pradhan J Neurosci 2010). Our findings have both fundamental and therapeutic implications for slow-recycling/degrading GPCRs. Direct receptor visualization in mice is a novel and unique approach to receptor biology and drug design.

S24-02

OPIOID-EVOKED ADPATATION OF SYNAPTIC TRANSMISSION THE VENTRAL TEGMENTAL AREA

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Opioids, such as morphine target the ventral tegmental area (VTA) affecting the function of the mesocorticolimbic system. In the acute situation, they cause an increase of dopamine (DA) levels through disinibition of projection neurons of the VTA. Morphine binds to μ-opioid receptors that are selectively expressed on GABA neurons in the VTA, which leads to hyperpolarization and a decrease of the release probability. Beyond the actual presence of the drug in the brain, adaptive changes can be observed already after a first morphine dose. Within hours glutamate receptors in synapses of excitatory afferents onto DA neurons are redistributed. Calcium permeable AMPA receptors appear and NMDA function decreases, which leads to an overall potentiation at physiological potentials and inverts the rule for further activity dependent synaptic plasticity. Drug-evoked plasticity can also be observed with other addictive drugs and after optogenetic stimulation of DA neuron activity that mimics the increased firing rates observed with morphine.

After several injection of morphine additional adaptations are observed. In DA neurons the efficiency with which Gio coupled receptors, such as $GABA_B$ receptors activate potassium currents of the GIRK family increases. This effect involved a downregulation of RGS2, which dynamically regulates $GABA_B$ to GIRK coupling. As a consequence slow inhibitory transmission within the VTA may become more efficient, which may represent a mechanism that counterbalances the potentiated excitatory transmission.

In summary opioids leave traces in synaptic transmission of the VTA that profoundly alter network properties and may thus contribute to the behavioral changes associated with drug exposure.

S24-03

REGULATION OF MU OPIOID RECEPTOR FUNCTION BY RGS9-2

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The signaling modulator RGS9-2 plays a potent role in dopaminergic and opioidergic transmission in the striatum via actions as a GTPase accelerating protein or as effector antagonist for the G protein alpha subunit. Evidence so far points to RGS9-2 as a potent modulator of antiparkinsonian, antipsychotic, psychostimulant and opiate drug actions (reviewed in Traynor et al., Trends in Pharm Sci 2008). In this study, we use genetically modified mice to further understand the role of RGS9-2 in addiction, analgesia and depression like behaviors associated with chronic pain or with long term exposure to opiates. Our data suggest that increased activity of RGS9-2 in the nucleus accumbens (NAc) following stereotaxic infection with an AAV-RGS9-2 construct blocks the rewarding and locomotor sensitizing actions of morphine and leads to a milder opiate withdrawal syndrome. Our behavioral studies also suggest that manipulation of RGS9-2 levels in the NAc reduce the analgesic actions of opioids, whereas increased RGS9-2 activity in this brain region accelerates the development of analgesic tolerance. We next examined the way RGS9-2 affects the actions of agents used to alleviate chronic pain symptoms. Using a neuropathic pain model we show that mice lacking the Rgs9 gene develop tolerance to the antiallodynic actions of morphine much later than their wild type controls, and that they are more sensitive to the antiallodynic actions of tricyclic antidepressants. This phenotype is related to RGS9-2 actions in the NAc as it can be rescued by local overexpression of

the protein. Finally, using immunoprecipitation assays we examined changes in RGS9-2 complexes in the striatum associated with acute and chronic actions of opiates. Our findings provide new insights into the cellular mechanisms of opiate drug actions in the NAc and suggest that interventions in the formation of RGS9-2 complexes may be used to improve treatment efficiency.

S24-04

GENOMIC NETWORK ACTIVATED BY OPIOIDS

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Opioids produce molecular and cellular alterations in the brain reward system that are essential to the development of addiction. We applied whole-genome microarray profiling to evaluate detailed time-courses (1, 2, 4 and 8h after injection) of transcriptome alterations within striatum/accumbens following acute opioid (morphine, heroine) administration in C57BL/6J mice. The study elucidated the modules of opioid-induced genes and their regulatory elements which may be involved in mechanisms of early stages of opioid addiction development. Drugs of abuse have different mechanisms of action but produce similar behavioural and functional effects. To reveal genes regulated by both opioids and other classes of drugs of abuse, we analysed the transcriptional networks activated by acute administration of cocaine, methamphetamine, nicotine and ethanol. We identified 42 drug-responsive genes that were segregated into two main transcriptional modules. The first group consisted of activity-dependent transcripts which were induced by opioids and psychostimulants. The second group, which was in part controlled by the release of steroid hormones, was strongly activated by opioids and ethanol. We demonstrated that knockdown of the selected opioid-responsive genes Sgk1 and Tsc22d3 resulted in alterations in dendritic spines in mice, possibly reflecting their role in neuronal plastic changes. We further analysed the transcriptional effect of prolonged chronic heroin treatment and protracted withdrawal, and compared it with effects of methamphetamine. Chronic heroin and methamphetamine treatment activated common genes (Pdyn, Cartpt, Inmt, Fam40b) which are enriched in the nucleus accumbens. The study showed that despite distinct pharmacological profile of heroin and methamphetamine they have similar long-term molecular effects. Our study identified modules of drug-induced genes that share functional relationships. These genes may play a critical role in the opioid addiction.

Symposium 25
Sticking to the Plan: The Role of Extracellular Matrix In Modulating Nervous System Function and Repair

S25-01

HELPING TO START AND STOP MYELINATION: CSK HAS DUAL REGULATORY FUNCTIONS DURING OLIGODENDROCYTE DEVELOPMENT

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The timing and location of oligodendrocyte differentiation is tightly controlled but the molecular mechanisms that underlie this control are poorly understood. Many intrinsic regulators of myelination have been identified, but less is known regarding how oligodendrocytes and their progenitors respond to extrinsic signals in their environment during development and during repair. The Src Family Kinases (SFKs) are important downstream effectors for extracellular matrix (ECM) and growth factors, and thus many of the extrinsic factors thought to contribute to oligodendrocyte development likely require the contribution of SFKs. The SFK Fyn is essential for normal CNS myelination, yet little is known about how Fyn or other SFKs are regulated in developing oligodendrocytes. We recently observed that, in conjunction with delayed oligodendrocyte differentiation, mice that lack the ECM protein laminin have dysregulated SFK phosphorylation as well as elevated expression of C-terminal Src Kinase (Csk), a putative negative regulator for oligodendroglial SFKs. We now report that Csk acts as a molecular switch for SFK activity in oligodendroglia, with, interestingly, distinct roles in early versus late oligodendroglial development. Early in oligodendroglial development Csk was critical for the appropriate onset of OPC differentiation. Here, Csk suppressed OPC proliferation such that Csk depletion in OPCs led to proliferation under conditions that normally promote cell cycle exit. Hyperproliferation of Csk-deficient OPCs resulted in delayed oligodendrocyte maturation. In adult mice, however, Csk deletion caused hypermyelination, with analysis of myelin ultrastructure revealing increased numbers of myelin wraps. This suggests that, during myelination, Csk normally promotes the termination of myelin wrapping. We propose that Csk is a novel regulator of oligodendroglial development with two distinct roles: generating appropriate numbers of oligodendrocytes at the onset of myelination, and terminating wrapping at the close of myelination. Preliminary studies furthermore suggest that Csk loss leads to enhanced remyelination following myelin damage.

S25-02

FIBRONECTIN: FRIEND OR FOE IN REMYELINATION?

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Remyelination following central nervous system demyelination is crucial for the prevention of neurodegeneration. However, in some demyelinating diseases, such as multiple sclerosis (MS), remyelination ultimately fails. This failure of remyelination is likely mediated by a variety of factors, including changes in the extracellular signaling environment. The extracellular matrix molecule fibronectin, which is nearly absent in healthy adult central nervous system, accumulates following demyelination. In experimental toxin-induced lesions undergoing efficient remyelination, fibronectin expression was transient and declined as remyelination proceeded. Fibronectin levels increased within demyelinated regions by both leakage from the blood circulation and production by reactive astrocytes. In chronically demyelinated MS lesions, however, fibronectin expression persisted, and is associated with inflammation-mediated fibronectin aggregation. Interestingly, astrocytes isolated from MS patients showed enhanced fibronectin aggregation. These aggregates inhibited myelin-like membrane formation, likely by perturbing (secondary) process outgrowth, myelin-membrane directed vesicular transport and membrane microdomain formation. Taken together, upon central nervous system demyelination, transient expression of fibronectin is associated with remyelination, whereas the pathological fibronectin aggregates present in MS lesions may contribute to remyelination failure. Therefore, strategies to promote remyelination in both demyelinating and neurodegenerative diseases might benefit from eliminating or preventing fibronectin aggregation.

S25-03

TWO PINCHS, TWO FUNCTIONS IN PNS DEVELOPMENT

Relvas, J.B.[1,2],Gonçalves A.F.[1], Pereira, J.A.[1], Dias, N.G.[2], Normen, C.[1], Ricci, R.[1], Nave, K.A.[3], Fassler, R.[4] and Suter, U.[1]

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There is substantial evidence that the control Schwann cell differentiation derives, at least in part, from instructive cues originating within the extracellular environment, of which growth factors and proteins of the extracellular matrix (ECM) are essential components. Recently, we have shown essential functions for integrin-linked-kinase (ILK), a member of the IPP complex of proteins acting in both integrin and growth factor transduction pathways, in the radial sorting and remyelination of axons. During my talk, I will focus on another member of the IPP complex, the

LIM domain adapter protein, particularly interesting new cysteine-histidine-rich protein (PINCH). There are two known PINCH genes PINCH1 and PINCH2, which have been previously reported to play both distinct and partially compensatory roles in the homeostasis of different cell types. Using Schwann cell specific gene ablation in mice, we show that PINCH1 controls radial sorting of axons by negatively regulating rho/ROCK activation, and that PINCH2 signaling is essential for the developmental transition from axon-dependent (paracrine) to axon-independent (autocrine) Schwann cell survival by a mechanism involving the regulation of IGF1 expression directly at promoter level.

S25-04

A ROLE FOR FIBRONECTIN IN DRIVING CEREBRAL ANGIOGENESIS

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The extracellular matrix (ECM) is an important influence on angiogenesis and vascular remodeling. We have shown that angiogenic vessels in the developing central nervous system (CNS) express high levels of fibronectin and the fibronectin receptor α5β1 integrin. In keeping with an angiogenic role for fibronectin in other systems, this implies that fibronectin may provide an important angiogenic drive in the CNS. To investigate whether this mechanism also applies to the adult CNS, we examined these events in a mouse model of cerebral hypoxia, in which mice are exposed to 8% O2. Over a 2-week period, this results in a robust increase (50%) in vessel density in the brains of these mice. Immunohistochemistry and western blot revealed that hypoxia strongly induced fibronectin and brain endothelial cell (BEC) expression of the fibronectin receptors, α5β1 and αvβ3 integrins. To directly test whether these BEC integrins are required for cerebral angiogenesis, the hypoxic response was examined in transgenic mice deficient in either the α5 or β3 integrins. This revealed that while the αvβ3 integrin is not essential for the angiogenic response, the α5β1 integrin plays an important role in driving BEC proliferation. In current experiments, we are generating α5/β3/double-KO mice to examine whether the hypoxic-angiogenic response is "flat-lined" in the absence of both BEC fibronectin receptors, or whether other redundancy or compensation exists in this response.

Workshop 5
Engineered Receptors as Molecular Switches in the Study of Physiology, Behaviour, and Neurological Disorders

W05-01

USING ENGINEERED RECEPTORS TO DISSECT THE CONTRIBUTION OF STRIATOPALLIDAL AND STRIATONIGRAL NEURONS IN BRAIN REWARD MECHANISMS

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The striatum is a key site for many of the behavioral and neurobiological adaptations thought to underlie the development of addiction. However, the "direct" (striatonigral) and "indirect" (striatopallidal) medium spiny neurons, respectively, have not been previously targeted in rat behavioral models. In order to address this, we developed HSV viral vectors that use the preprodynorphin and preproenkephalin promoters to target transgene expression to the direct or indirect pathway, respectively. We then utilized some novel engineered "DREADD" receptors developed by Bryan Roth's laboratory that can be selectively activated by a synthetic and otherwise inert ligand, clozapine-N-oxide (CNO). The hM4D receptor couples to Gi and inhibits excitability whereas the GsD receptor couples to Gs and excites neurons.

These novel, phenotype-specific vectors were highly selective for the direct or indirect pathways. The hM4D receptor transiently inhibited targeted striatal neurons only when rats were treated with CNO; this was confirmed electrophysiologically. Transient silencing of striatopallidal neurons in rat dorsal striatum facilitated the development of locomotor sensitization to amphetamine whereas silencing striatonigral neurons attenuated sensitization. We found that transiently decreasing activity of striatonigral neurons disrupted the acquisition of lever pressing for a sugar reward and impaired the acquisition of a decision making task for small versus large magnitude natural rewards. However, inhibiting activity of the striatopallidal pathway had no effect on the motor performance of this decision-making task.

These are the first examples of using selective manipulation of direct and indirect pathways in rats performing complex, motivated behaviors. The DREADD receptors altered time- and experience-dependent plasticity but did not disrupt overt behavior during CNO treatment, suggesting that they may represent a model for treatment interventions that manipulate disease states without interfering with normal behavior. In addition, these viral vectors, by targeting the direct and indirect pathway selectively, are useful tools for dissecting the roles of these pathways in striatal-dependent behaviors in rats or mice.

W05-02

CONTROL OF GI/O SIGNALING AND NEURONAL ACTIVITY BY LIGHT TO MODULATE SPINAL CORD AND MOTOR FUNCTION

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The tractability of light activated receptors makes them attractive tools by which to study the brain. They allow for the non-invasive and specific control of neuronal signaling and could allow for the study of receptor pathways that occur faster than the rate of diffusion. Furthermore, with the aid of implantable light devices light activated receptors have the potential for use in live animals and later in humans to control and cure GPCR pathways involved in diseases. We demonstrate here the use of vertebrate rhodopsin to control ion channel modulation, spinal cord, cerebellar and serotonergic signaling via activation of the pertussis toxin sensitive Gi/o pathway by light.

W05-03

ASTROCYTE SIGNALING IN BEHAVIOR

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It has been exceedingly difficult to investigate the role of astrocyte signaling in physiology due, in part, to our inability to selectively activate astrocytes *in vivo*. We have developed a transgenic line of mice that expresses a unique Gq-linked G-protein coupled receptor (GPCR) in astrocytes that responds to a ligand that crosses the blood brain barrier. This receptor, known as Gq-DREADD, responds to a ligand that does not activate any known GPCR other than Gq-DREADD. The specific astrocyte localization of this receptor has been demonstrated using calcium imaging and immunocytochemistry. Administration of CNO, the ligand that activates Gq-DREADD, leads to a striking phenotype in mice expressing Gq-DREADD but not in wild type mice. The phenotype includes changes in the acoustic startle response, rotorod performance and center time activity. In addition, activation of this receptor in astrocytes leads to marked saliva formation and changes in blood pressure and heart rate suggesting activation of the autonomic nervous system. Overall, these findings suggest that astrocytes may be playing a role in a number of previously unsuspected behaviors.

W05-04

5-HT4-RASSL AND NEURODEGENERATIVE DISEASES

Claeysen, S.[1,2,3], Gaven, F.[1,2,3], Pellissier, L.P.[1,2,3], Kremer, E.J.[4], Dumuis, A.[T,2,3] and Bockaert, J.[1,2,3]

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Molecular devices have been developed to modulate the cellular signalling pathways and membrane potentiels. These tools range from optogenetic proteins (channelrhodopsins, light-driven pumps, photoactivated cyclases and synthetic photoreceptors) to engineered G protein-coupled receptors, insensitive to their endogenous ligands (RASSLs: Receptor Activated Solely by Synthetic Ligand and DREADDs).

We generated one of the first RASSLs: the 5-HT4-RASSL from the 5-HT4 serotonin Gs-coupled receptor introducing a single mutation. 5-HT4-RASSL became totally insensitive to serotonin but still responds to synthetic ligands having affinities in the range of nanomolar concentrations, exhibiting full efficacy and able to cross the blood brain barrier. A viral gene delivery strategy has been developed to achieve efficient intra-neuronal transfer of the 5-HT4-RASSL. A recombinant canine adenovirus (CAV-2) has been engineered to preferentially transduce neurons.

Excessive NMDA receptor activation has been implicated in pathophysiology of chronic neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. We examined cAMP neuroprotective potentialities on an excitotoxic model of dopaminergic cells in primary cultures. Intracellular cAMP accumulation was controlled by the 5 HT4-RASSL. BIMU8, a selective 5 HT4R agonist, still totally active on 5-HT4-RASSL, prevented dopaminergic neurons from death. This effect was strengthened by IBMX, a phosphodiesterase inhibitor. Moreover, the intrinsic basal activity of RASSL, which correlated with an increase of cAMP level, was also neuroprotective.

In Alzheimer's disease, β-amyloid peptide (Aβ) formation results from the amyloidogenic degradation of APP by β- and γ-secretases. The non-amyloidogenic proteolysis of APP within the Aβ by α-secretases releases the extracellular fragment of APP (sAPPα), which is neurotrophic. Recently, we showed that the 5-HT4-RASSL can be used to stimulate the non-amyloidogenic pathway in neurons.

Workshop 6
Role of the α7 Nicotinic Acetylcholine Receptor (α7nachr) in Brain Function

W06-01

α7NACHRS, THEIR LIGANDS AND ASSOCIATED PROTEINS, OFFER APPROACHES TO DRUG THERAPY FOR HUMAN NEURODEGENERATIVE DISORDERS

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Nicotinic acetylcholine receptors (nAChRs) are targets for drugs under development which aim to ameliorate the symptoms of Alzheimer's disease. This is based on earlier work showing that nicotinic receptor agonists are pro-cognitive and the activation of nAChRs appears to be neuroprotective against the adverse, toxic actions of β-amyloid peptide, which is part of the signature pathology of Alzheimer's disease (AD). Since human α7nAChRs levels are altered in AD, allosteric modulators of these receptors are potentially of therapeutic value in maintaining cholinergic signalling. Unlike agonists, which rapidly desensitize α7nAChRs, allosteric modulators can maintain and fine-tune cholinergic signaling. Advances in comparative genomics have highlighted a rich diversity of α7-like nAChR isoforms in organisms from invertebrates to man. Tapping into this rich vein of molecular and functional diversity can be instructive in understanding and interpreting comparative receptor pharmacology. Combining studies on evolutionarily remote orthologues of human α7nAChRs with those on wild type and site-directed α7nAChR mutants adds to our understanding of allosteric drug actions. Many of the Type I and Type II positive allosteric modulators (PAMs), effective on human α7nAChRs, are ineffective on C. elegans ACR-16, which, like its human counterpart, also forms a fast-desensitizing, homomeric nAChR when heterologously expressed in Xenopus oocytes. Site-directed mutagenesis studies on human α7nAChRs, ACR-16 and related cys-loop ligand-gated ion channels point to the importance of residues in the transmembrane region in the actions of PAMs. Thus, in the immediate future, human α7nAChRs are likely to remain important targets for new candidate AD drugs. There may be challenges resulting from the diverse roles of a α7nAChRs and hence their presence on several cell types within and outside the nervous system. This is generating interest on the exploration of α7nAChR-interacting proteins as potential drug targets for the future.

W06-02

CURRENT EFFORTS TOWARDS UNDERSTANDING STRUCTURE-FUNCTION RELATIONSHIP OF HUMAN ALPHA7 NACHR

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In-depth knowledge of nicotinic acetylcholine receptor (nAChR) structure, and of the interaction between neuronal nAChRs and their ligands, is necessary for understanding synaptic action and designing nAChR subtype-specific drugs for major neurological diseases. The alpha7 homopentameric nAChR (α7nAChR) plays a major pathophysiological role. The crystal structures of a) homologous water-soluble molluscan ACh-binding proteins (AChBP), b) the extracellular domain (ECD) of muscle α1nAChR subunit, and c) prokaryotic precursors of the Cys-loop receptor superfamily have been determined. Nevertheless, diffraction quality crystals of intact nAChRs or of neuronal nAChR ECDs have not yet been produced. We aim at the expression of major human nAChR domains, with emphasis on α7nAChR, for their structure elucidation and understanding their interactions with cholinergic ligands. Specifically, we are studying their ECDs (~210 amino-acids) and their truncated forms which lack only their cytoplasmic domains. α7ECD expressed in yeast was in the form of microaggregates; its water-solubility was improved by substituting its Cys128-Cys142 loop with the hydrophilic AChBP Cys-loop and by introducing six additional single-point mutations. This mutant was expressed in an oligomeric form and at high yield. To reduce its still considerable heterogeneity, stringent tag-based fractionation led to the isolation of an apparently pentameric form, as deduced by gel filtration, DLS and cryo-EM studies. Crystallization trials resulted in microcrystals, needing further optimization. In addition to the ECDs, truncated transmembrane nAChRs, including the α7nAChR, in which the flexible cytoplasmic part (probably the major factor hindering crystallization) was deleted, were expressed in insect and mammalian cells. These expressions resulted in high affinity ligand-binding membrane molecules. Yet, detergent solubilization was inefficient; nevertheless, the solubilized molecules retained the ligand-binding properties and exhibited a molecular weight corresponding to the pentameric nAChR. Crystallization efforts will be initiated after optimization of their solubilization.

W06-03

Aβ NEUROTOXICITY AND THE ROLE OF THE α7 NICOTINIC ACETYLCHOLINE RECEPTOR IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common form of dementia in the elderly. AD is characterized by the presence of amyloid plaques which are formed from deposits of β-amyloid protein (Aβ). Accumulation of oligomeric Aβ in the brain contributes to neuronal dysfunction and ultimately leads to neurodegeneration. Cholinergic abnormalities are also commonly observed in AD and it has been suggested that these abnormalities contribute to the cognitive dysfunction. For this reason, acetylcholinesterase inhibitors are used to boost cognition in AD patients. Both muscarinic and nicotinic receptor agonists have also been proposed for the treatment of AD. The α7 nicotinic receptor is of interest in AD for several reasons. First, the α7 receptor may play an important role in synaptic

plasticity, cognition and memory. Second, there are several reports suggesting that Aβ may bind to the α7 receptor and either stimulate or inhibit activity of the receptor. Our own studies have shown that α7 receptor antagonists can inhibit an Aβ-mediated increase in acetylcholinesterase activity in neurons. We have examined the nature of the Aβ-α7 receptor interaction using a variety of different systems (tissue binding assays, Xenopus oocyte expression, biochemical analysis). Our studies show that Aβ does not bind directly to the α7 receptor. Instead, Aβ interacts predominantly with lipid membranes and may stimulate calcium entry by perturbing several ion channels in the neuronal cell membrane. Nevertheless, as Aβ stimulates calcium influx into neurons and as the α7 nicotinic receptor can contribute to calcium influx and synaptic plasticity, there is some logic associated with the idea of using α7 nicotinic receptor drugs for the treatment of AD.

W06-04

ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTORS AS POTENTIAL TARGET FOR IMAGING TRAUMATIC BRAIN INJURY WITH POSITRON EMISSION TOMOGRAPHY

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The α7nAChR subtype is involved in the pathogenesis of neurodegenerative diseases. Furthermore, it has been hypothesized that cholinergic hypofunction, including diminished α7nAChR density, augments inflammatory signalling after traumatic brain injury (TBI) and thus may contribute to neurodegenerative processes. Therefore, we have investigated the α7nAChR density in experimental TBI using the controlled cortical impact rat model and the fluid-percussion pig model. Early widespread and significantly lowered α7nAChR densities (down to ∼ 50%) were found in both models by receptor autoradiography. Other cholinergic targets (α4β2nAChR, muscarinic AChR, acetylcholine esterase, vesicular acetylcholine transporter) were less influenced. Accordingly, α7nAChR is the preferred target to investigate cholinergic hypofunction after TBI with PET, the today most sensitive non-invasive molecular imaging method which uses highly affine and selective PET radioligands.

We have recently developed a 18F-labelled 1,4-diazabicyclo-[3.2.2]nonane derivative (NS10743) and investigated this radioligand regarding target specificity, pharmacodynamics, and pharmacokinetics *in vitro* and *in vivo*. *In vitro*, NS10743 and [18F] NS10743 showed high affinity and specificity towards human α7nAChR. The brain permeation of [18F] NS10743 in mice was fast and sufficiently high. Brain autoradiography and organ distribution showed target-specific accumulation of [18F] NS10743 in brain substructures and peripheral α7nAChR expressing organs. The radiotracer showed a high metabolic stability *in vivo* with a single polar radiometabolite, which did not cross the blood-brain-barrier.

Also in pigs [18F] NS10743 readily entered the brain, with the highest uptake in α7nAChR-expressing brain regions such as the colliculi, thalamus, temporal lobe, and hippocampus. Pretreatment and constant infusion with NS6740, a selective α7nAChR antagonist, significantly reduced the specific binding of [18F] NS10743 in receptor-dense regions (temporal lobe: −29%, midbrain: −35%) without significantly altering the specific binding in regions with low receptor density such as the cerebellum.

In conclusion, experimental TBI is accompanied by cholinergic hypofunction with α7nAChR as the most sensitive target. The alterations of α7nAChR are potentially measurable non-invasively with PET using [18F] NS10743 which is of importance for therapeutic drug monitoring.

Young Investigator Colloquium 5
Mechanisms of Synaptic Plasticity and Memory

YIC05-01

MOLECULAR SURFACE DYNAMIC AS A VARIABLE OF SHORT TERM PLASTICITY

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Neuronal synapses are composed of a large population of variable signaling proteins that are accumulated at these sub cellular compartments. The precision of synaptic communication is highly dependent on the local arrangement of these molecules, like transmitter receptors or adhesion molecules. Plastic changes at the synapse mostly caused by a rearrangement or a change in the concentration, density and number of receptors, ion-channels or adhesion proteins. The dynamic of these changes is crucial for the plastic capacity of a synapse. Interactions of trans membrane signaling molecules in the outer neuronal membrane with intracellular or extracellular signaling molecules tethers a certain population to the synaptic compartment. Conduction of single molecule tracking and FRAP experiments revealed, that many proteins at the synaptic membrane are not stable and therefore participate only in a certain time window to synaptic transmission. In particular mobile AMPA receptors contribute to the reliability of glutamatergic synaptic transmission by compensating the kinetic property of temporal desensitization by lateral exchange through surface diffusion. The degree of lateral mobility is restricted by many passive and active interactions inside and outside the membrane. Adhesion molecules like Neuroligins at the postsynaptic side and Neurorexins presynapticaly are identified to stabilize synaptic receptors and calcium channels. Both adhesion molecules are highly mobile. Artificial immobilization of AMPAR and presynaptic Neurexins strongly influence synaptic short term plasticity. Thus one can hypothesis that short term plasticity is partially determined by the degree of mobile signaling proteins in the synaptic compartment and influenced by the dwell time within the surface of the synaptic membrane.

YIC05-02

EVIDENCE THAT TOLL-LIKE RECEPTOR 3 SIGNALING INHIBITS MEMORY RETENTION AND CONSTRAINS ADULT HIPPOCAMPAL NEUROGENESIS

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Toll-like receptors (TLR) are innate immune receptors that have recently emerged as regulators of neuronal survival and developmental neuroplasticity. We investigated learning and memory in TLR3-deficient (TLR3-/-) mice using both hippocampus-dependent and -independent behavioral tests. Adult TLR3-/- mice exhibited enhanced hippocampus-dependent working memory in the Morris water maze, novel object recognition and contextual fear conditioning tasks. In contrast, TLR3-/- mice demonstrated impaired amygdala-related behavior and anxiety in the cued fear conditioning, open field and elevated plus maze tasks. Further, TLR3-/- mice exhibited increased hippocampal CA1 and dentate gyrus (DG) volumes, increased hippocampal neurogenesis and elevated levels of the AMPA receptor subunit GluR1 in the CA1 region of the hippocampus. In addition, levels of activated forms of the kinase ERK and the transcription factor CREB were elevated in the hippocampus of TLR3-deficient mice, suggesting that constitutive TLR3 signaling negatively regulates pathways known to play important roles in hippocampal plasticity. Our findings reveal novel roles for TLR3 as a suppressor of hippocampal cellular plasticity and memory retention.

YIC05-03

STRESS, AMYLOID β AND TAU: THE TRIANGLE OF OBLIVION

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Microtubule-associated protein tau (TAU) is postulated as a common crucial protein in mechanism of neurodegeneration in many chronic neurological diseases termed tauopathies, including Alzheimer's disease (AD) raising this protein as strong candidate for therapeutic intervention. While the mechanism(s) by which TAU mediates neurodegeneration are not completely understood, many studies have established that TAU protein dysfunction is central to AD neurodegenerative process. While the etiology of the disease is largely unknown, there is growing consensus that lifetime events such as environmental stressors may increase the risk for the disease. Specifically, stress and its primary manifestation, glucocorticoid (GC) secretion are causally implicated to AD but also strongly associated with memory and learning deficits, impaired cognitive performance as well as mood and affective disorders such as depression. Although cumulative evidence suggests a continuum between depression and AD, and stress is suggested to play a detrimental role in both diseases, considerably less attention has been given to the suggested role of stress as a connecting risk factor. We have been investigating the inter-relationship between these various pathogenic elements in transgenic and non-transgenic mice, with a particular focus on the mechanisms through which stress and TAU precipitates brain pathology. Our studies show that stress and GC trigger APP misprocessing towards the production of neurotoxic amyloid-β (Aβ) as well as TAU hyperphosphorylation and aggregation resulting in associated impairments of cognitive and emotional status. Furthermore, we show that the presence of TAU predisposes animals to stress/GC detrimental effects and that TAU protein is a essential regulator of synaptic plasticity mechanisms highlighting dendritic and synaptic tau as a key protein of neuronal dysfunction and synaptic degeneration. Conclusively, these studies suggest that tau plays a crucial role in the mechanism through which stress/GC exert their neurodegenerative effects upon the substrates of cognition and emotion.

YIC05-04

SPIKAR, SPINE RESIDENT TRANSCRIPTIONAL COACTIVATOR, IS A NOVEL DREBRIN BINDING PROTEIN AND REGULATES DENDRITIC SPINE NUMBER

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Dendritic spines are small protrusions emerging from the dendrites that receive most of excitatory inputs and contain many unique proteins. It is widely accepted that dendritic filopodia are precursors of dendritic spines, and filopodia formation can be considered to be an early step for spine formation. In many previous studies, various molecules have been identified as regulators of dendritic spine formation and morphogenesis. However, much less is known about molecular mechanism regulating filopodia formation. We have previously proposed that drebrin, a side-binding protein of actin filament, governs spine morphogenesis. The accumulation of PSD-95 and actin filaments at the spine is facilitated by the prior drebrin accumulation at the filopodia. Based on these evidences, we think that drebrin plays a significant role in early synaptic development and filopodia formation. However, the molecular machinery by which drebrin regulates spinogenesis is unclear. To find a novel molecule playing a role in the spinogenesis concerned with drebrin, we carried out a molecular screening of novel drebrin-binding protein. We performed a yeast two-hybrid screen and isolated a novel drebrin binding protein named as ''spikar''. In neuron, spikar was localized mainly in nuclei, dendrites and colocalized with drebrin in dendritic spines. Using RNA interference (RNAi) approaches, we found that spikar knockdown in cultured hippocampal neurons resulted in a significant decrease of dendritic spines and filopodia, and the effects of spikar depletion were different between developmental stages of neuron. Our data indicate that spikar plays a role in filopodium formation and spine maintenance. Gain of function experiment using extranuclear-spikar mutated its NLS showed that spikar is involved in filopodia formation during spine development. Furthermore, we revealed that spikar acts as a transcriptional coactivator of nuclear receptors. Thus, our data indicate that spikar is a multifunctional protein that is involved in filopodia formation in early step of spinogenesis and spine maintenance in matured stage of spinogenesis, and is a transcriptional coactivator in nucleus.

Young Investigator Colloquium 6
New Therapeutic Approaches for Neurodegenerative Disorders

YIC06-01

ALZHEIMER'S DISEASE AS A NEW FORM OF BRAIN SPECIFIC DIABETES INSTIGATED BY ABETA OLIGOMERS

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Alzheimer's disease (AD) has been linked to impaired brain insulin signaling, a novel type of brain diabetes. Serine phosphorylation of IRS-1 (IRS-1pSer), a central feature in peripheral insulin resistance, blocks insulin signaling. Here we show that AD brains present elevated IRS-1pSer636/639 levels, reminiscent of what is found in muscle and adipose tissue in type 2 diabetes. To determine the mechanism underlying pathological IRS-1pSer, we investigated the role of synaptotoxic Abeta oligomers, increasingly recognized as the proximal neurotoxins in AD pathogenesis and recently implicated in neuronal insulin resistance. Oligomers induced IRS-1pSer636 and inhibited physiological IRS-1pTyr465 in mature hippocampal neurons in culture. IRS-1pSer was blocked in neurons expressing a dominant negative form of c-Jun N-Terminal Kinase (JNK) and by the JNK inhibitor, SP600125, as well as by infliximab, a tumor necrosis factor-alpha (TNF-alpha) blocking antibody. Involvement of JNK and TNF-alpha in oligomer-induced neuronal IRS-1pSer parallels the pathway underlying peripheral insulin resistance. Consistent with aberrant activation of JNK, SP600125 blocked oligomer-induced disruption of axonal transport, a defect linked to JNK dysregulation in neurodegenerative diseases. Insulin and exendin-4, drugs used to treat diabetes, blocked oligomer-induced pathologies. By establishing a molecular link between dysregulated insulin signaling in AD and diabetes, results open new avenues for rapid implementation of therapeutics in AD.

YIC06-02

THE MECHANISM OF ACTION OF METAL-LIGAND COMPLEXES AS POTENTIAL THERAPEUTICS TO TREAT NEURODEGENERATIVE DISEASE

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Our research team has been developing metal-ligand complexes as potential therapeutics to treat neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's and amyotrophic lateral sclerosis. We have tested orally available, membrane- and blood-brain barrier-permeable complexes in multiple mouse models of neurodegenerative disease and have found the complexes to be highly effective in attenuating cognitive and locomotor deficits. In order to establish the potential for our metal-ligand complexes to proceed towards clinical trials, a priority of our current research is to define their molecular mechanism of action. Mechanistic work to date indicates that metal-ligand complexes are effective towards important disease-associated pathologies (e.g. preventing accumulation of neurotoxic amyloid-beta trimers and the phosphorylation of tau in Alzheimer's disease model mice) by hitting high profile molecular targets (e.g. inhibition of the kinase GSK3). Molecular pathways mapped to date include activation Akt- and ERK1/2-related cell signalling. The activation of these pathways is in part dependent on the inhibition of phosphatases such as calcineurin. Downstream effects that may contribute to the *in vivo* therapeutic efficacy of metal-ligand complexes include activation of the transcription factor CREB and the promotion of neurite extension. This talk will describe the therapeutic outcomes for metal-ligand complexes in mouse models of neurodegenerative diseases and will present data on their mechanism of action.

YIC06-03

CURCUMIN: A POTENTIAL THERAPEUTIC MOLECULE FOR PARKINSON'S DISEASE?

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Parkinson's disease (PD) is a neurodegenerative disease and a movement disorder characterized by loss of dopaminergic neurons in the substantia nigra (SN) causing dopamine depletion in the striatum. Neurodegeneration in PD occurs due to multiple pathways including oxidative/nitrosative stress, mitochondrial damage, protein aggregation etc. The current drugs for PD provide symptomatic relief and replenish striatal dopamine but their ability to prevent neurodegeneration is not validated in humans. Therefore, novel therapeutics that target multiple pathways and prevent neurodegeneration need to be explored. Turmeric (Curcuma longa) is a dietary spice used in Indian cuisine and traditional medicine. Curcumin (diferuloylmethane), the most active and non-toxic component of turmeric (i) is a polyphenol with antioxidant and anti-inflammatory properties (ii) can cross the blood brain barrier and (iii) has therapeutic potential in neurological disorders. We found that curcumin directly detoxified peroxynitrite (PN) and protected brain mitochondria against protein nitration, inhibition of mitochondrial complex I (CI), loss of mitochondrial membrane potential and mitochondrial swelling. Further, curcumin significantly protected mitochondria *in vivo* against nitrosative and oxidative stress by induced synthesis of cellular glutathione (GSH) in dopaminergic neurons. Chronic oral supplementation with turmeric protected against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mediated neurodegeneration in mouse SN. However, the bioavailability of curcumin is limited due to poor absorption, rapid metabolism and quick systemic elimination. We demonstrated that the di-glutamoyl

derivative of curcumin with improved bioavailability showed enhanced neuroprotection compared to curcumin. Based on our studies and others, we conclude that curcumin and its derivatives have therapeutic potential for adjunctive therapy along with dopamine replacement in PD.

YIC06-04

INTERFERON-BETA AND HMG-COA REDUCTASE INHIBITION IN MULTIPLE SCLEROSIS: A TRICKY COMBINATION?

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Recent studies support the notion that statins, widely-prescribed cholesterol-lowering agents, may target key elements in the immunological cascade leading to inflammation and tissue damage in the pathogenesis of multiple sclerosis (MS). Compelling experimental and observational clinical studies highlighted the possibility that statins may also exert immunomodulatory synergy with approved MS drugs, resulting in initiation of several randomized clinical trials testing statins in combination with interferon-b (IFN-b).

Some data, however, suggest that this particular combination may not be clinically beneficial. In this regard, a small North American trial indicated that atorvastatin administered in combination with IFN-b may increase disease activitiy in relapsing-remitting MS. Indeed, a potential loss of therapeutic efficacy was shown in cell culture experiments to be induced by blocking tyrosine phosphorylation of the STAT1 transcription factor (P-Tyr STAT1) by statins, which is essential for type I IFN (a/b) signalling. Both, similarities and differences of statins and IFN-b with regard to the immunomodulatory pattern of action were observed. While IFN-b 1b reduces and simvastatin increases the expression of the pro-inflammatory cytokines IFN-b and IL-12 in-vitro, patients on a combination therapy of IFN-b 1b and atorvastatin had significantly increased IL-12p70 levels. Likewise, in-vitro expression of the anti-inflammatory cytoline IL-10 is raised by IFN-b1b and decreased by simvastatin, and a trend for an increase of IL-10 serum levels was found in-vivo by the combination treatment. To this end, simvastatin may increase the proteolytic activity MMP-9, a protease facilitating the transmigration of leukocytes to the brain. Hence, many of the in-vitro findings either indicate potential interference of IFN-b and statins, or differential action on the immune system.

Eventually, the evident question whether usage of the combination requires caution, since the number of IFN-b treated MS patients receiving statins for lowering cholesterol is expected to grow, needs to be raised.

Wednesday Poster Sessions
WE01 Neuronal Polarity

WE01-01

THE ABSENCE OF TAG-1 RESULTS IN PERTURBATION OF OLFACTORY BULB ORGANIZATION AND FUNCTION

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During development,the main projecting neurons of the olfactory bulb(OB)(mitral,tufted cells) express the cell adhesion molecule TAG-1,an immunoglobulin superfamily member that plays an important role in neurite outgrowth,fasciculation,neuronal migration and axon guidance.The aim of the current project is to elucidate the role of TAG-1 in the development and organization of the olfactory system by using mice deficient for TAG-1(Tag-1KO) as a genetic tool.In adult and newborn TAG-1 deficient mice,a significantly decreased number of mitral cells in the olfactory bulb mitral cell layer(MCL) compared to control animals,has been observed.This defect in mitral cell number can be attributed either to apoptosis or to other deficits during the development of the olfactory bulb.TUNEL assay and caspase-3 immunohistochemistry at E13.5 to E18.5 did not show any changes in apoptotic cells between mutant and control animals.These data indicate that the reduction of mitral cells in adult mice is probably not an outcome of cell death.In order to discriminate the underlying mechanisms of this reduction we used Tbr-1 as a post-mitotic neuronal marker for mitral and tufted cells.There was no difference in post-mitotic Tbr-1+neurons pool,as detected at E14.5 Tag-1KO OB.This finding suggests that the deficit may occur during the migration and final positioning of projecting neurons towards their target-layers.For this purpose,we are performing tracing and immunohistochemical analysis at different developmental stages.Behavioral analysis on Tag-1 deficient animals indicates an olfactory memory deficit.We plan to subject the animals in extensive olfactory behavioral trials,to elucidate the importance of mitral cell layer organization in the olfactory system function.

WE01-02

THE ADHESION GPCR BAI1 CONTROLS MULTIPLE ASPECTS OF NEURONAL DEVELOPMENT

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Adhesion G-protein coupled receptors (A-GPCRs) are surface receptors consisting of a large extracellular region containing multiple cell adhesion domains linked to a GPCR domain by a GPCR cleavage site. The biology of A-GPCRs is obscure, despite increasing evidence that many impact neural development. Brain angiogenesis inhibitor 1 (BAI1) is an A-GPCR that regulates angiogenesis in the brain and acts as an engulfment receptor elsewhere. We sought to determine BAI1's function in neuronal development. Here, we show that BAI1 is expressed in hippocampal neurons and interacts with Tiam1, a Rac GTPase activator (GEF) that regulates dendritic spine and synapse formation. Knock down of BAI1 leads to immature spines and synapse loss. RNAi-resistant BAI1 rescues these defects, but not a BAI1 mutant that fails to bind to Tiam1. BAI1 also associates with PAR3, the structural scaffold of the PAR complex, which regulates the establishment of cellular polarity. PAR3 has previously been shown to regulate spine development by restricting Tiam1 to excitatory synapses, and we show that PAR3 interacts with BAI1 earlier in development than Tiam1. BAI1 may target PAR3 and Tiam1 to spines, since loss of BAI1 causes Tiam1 to accumulate in the soma. In addition to its effect on synapses, knockdown of BAI1 expression leads to the elaboration of dendritic arbors late in development (17 DIV), when wild-type neurons normally stop showing any net dendritic growth. This defect is rescued by wild type BAI1 and by the Tiam1 non-interacting mutant, but incompletely by a mutant that does not interact with ELMO1/DOCK180, another Rac GEF. Overexpression of BAI1 leads to shortening of the dendritic arbor starting at 17 DIV, while the mutant that does not interact with ELMO1/DOCK180 does not. These results indicate that BAI1 occupies a critical nexus between multiple Rac-dependent signaling pathways and the PAR complex. As BAI1 contains multiple extracellular ligand-binding domains, we propose that it integrates multiple signals to regulate both dendritic arbor formation and synaptogenesis.

WE01-03

PURKINJE CELL-DERIVED MATRICRYPTINS INDUCE THE FORMATION CLIMBING FIBER NERVE TERMINALS

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Matricryptins are enzymatically-released fragments of extracellular matrix (ECM) molecules that have distinct bio-activities compared with the full-length molecule from which they are generated. One large group of ECM molecules capable of generating matricryptins is the collagen superfamily. Outside of the nervous system collagen-derived matricryptins modulate cell adhesion, regulate cell proliferation and inhibit angiogenesis. In the peripheral nervous system, collagen-derived matricryptins regulate the formation and function of synapses. Here, we sought to understand whether similar synaptic roles exist for collagen-derived matricryptins in the mammalian brain. By screening for collagen gene expression in developing mouse brain, we identified one matricryptin-releasing collagen, collagen XVIII, whose expression was restricted to a single neuronal cell type in the cerebellar - Purkinje cells (PCs). We further discovered that endostatin, the collagen XVIII-derived matricryptin, was present in cerebellar synaptosomes - biochemical fractions enriched for synaptic proteins. To assess the necessity of this collagen-derived matricryptin in the formation of cerebellar synapses, we examined synaptic architecture in mutant mice lacking collagen XVIII (col18a1-/-). While most cerebellar synapses appeared unaffected by the loss of collagen XVIII, a significant decrease in the number of Climbing Fiber (CF) nerve terminals was observed in col18a1-/- mutant cerebella. To test whether endostatin was sufficient to induce the formation of these CF terminals, we generated dissociated cultures of inferior olivary (IO) neurons, which are the cell-type that generates

CFs. Application of endostatin onto cultured IO neurons produced a robust increase in the number of CF presynaptic terminals. Thus, PC-derived endostatin appears necessary and sufficient to induce CF nerve terminal formation. Outside of the nervous system endostatin signals through integrin receptors. Likewise, we discovered that the ability of endostatin to induce CF presynaptic differentiation *in vitro* required RGD-dependent integrin function. Expression analyses, immuno-precipitation experiments, and application of function-blocking antibodies all revealed that endostatin signals through integrin $\alpha 3\beta 1$ to induce CF-PC synapse formation. Together, our studies have identified a novel role for collagen-derived matricryptins and integrins in directing synaptogenesis in the mammalian brain.

WE01-04

THE FORMATION OF THE AXON INITIAL SEGMENT AND POLARITY IN DEVELOPING NEURONS

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Neurons are polarized into axonal and somatodendritic compartments. In addition there are subcellular polarized domains including the axon initial segment (AIS), nodes of Ranvier and the synapses. The AIS is enriched in voltage-gated sodium channels that initiate action potentials. Although the AIS is a critical structure in neurons, our knowledge of the AIS is still limited. So far no study has determined when the AIS is formed during neural development *in vivo*. The AIS is also required to maintain neural polarity, but whether the AIS is also required to establish neural polarity is unknown. Here, we describe the temporal relationship between initiation of axon-dendrite polarity and the formation of the AIS. We studied the formation of the AIS in developing neurons *in vivo* and *in vitro*. We used ankyrinG (ankG) as the AIS marker since it is the master organizer and it clusters at the AIS first among the known AIS proteins. For the *in vivo* study, we introduced GFP to cells at the ventricular zone of neocortex by in utero electroporating E14.5 mouse embryos. Then we followed the development of labeled cortical neurons by collecting brains at later time points. We found that neurons initiate ankG clustering after their axons are specified and after they migrate to their targets near the pial surface. In cultured hippocampal neurons, ankG clustering does not occur until stage 4, whereas axons are specified at stage 3. Thus we conclude that AIS assembly occurs after specification of axon-dendrite polarity. To determine if ankG clustering is required for establishment of neural polarity, we silenced ankG expression to disrupt AIS assembly by in utero electroporating ankG shRNA into neocortex of E16.5 rat embryos. Our results show that axons are still formed and relatively intact in cortical neurons without ankG. Thus, ankG clustering is not required to initiate axon-dendrite polarity *in vivo*.

WE01-05

DIFFERENTIAL PROPERTIES OF NEUROFIBROMIN GRDI AND II IN DEVELOPING NEURONS

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Neurofibromin, the product of the NF1 gene, is abundantly expressed in the CNS, and large deletions of the gene, presumably causing Ras hyperactivation, may lead to mental retardation. It functions as Ras-GAP through a central 360 amino-acid domain termed GRD, which exists as two variants type I and II. GRDII includes an additional exon (23a) and has significantly lesser GAP activity at least *in vitro*, while mice with depletion of 23a suffer from behavior deficits, all suggesting that exon 23a plays an important role in the function of neurofibromin. We have previously shown that PKC-dependent phosphorylation of neurofibromin-GRDII increases both its association with F-actin and its Ras-GAP activity. In a series of studies in the chick embryo telecenphalon, we found that at embryonic day 7, the onset of neuronal differentiation, there was an abrupt switch in the ratio of GRDI:GRDII transcript expression from 1:2 to 2:1; this switch slightly preceded a great gain in Ras transcription levels. We then transiently overexpressed GRDI-GFP or GRDII-GFP in primary neuronal cultures derived from E8 telenchephalon and assessed primary neurite length in pyramidal neurons. Both proteins induced an elaborate phenotype and conferred significant increases in the length of the major processes as well as in total neuritic length. Yet, immunofluorescence analysis revealed that GRDII, but not GRDI, showed significant increased colocalization with F-actin and with the neuron-specific, F-actin binding protein drebrin. Moreover, overexpression of GRDI mimicked the developmental switch in transcript expression, as it lowered the expression of endogenous GRDII transcripts in younger neurons. Our results demonstrate that GRD variants may play additional and specific roles in targeting neurofibromin within neuronal subcellular compartments where different pathways, such as the Ras/ERK or the Rac1/LIMK1/cofilin pathways, may be regulated.

WE01-06

SPATIOTEMPORAL REGULATION OF G-ACTIN LOCALIZATION IN GROWTH CONE GUIDANCE

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During axonal guidance, the motile growth cone senses spatiotemporally distributed extracellular signals and then translates into directional steering of the axon through a complex environment to the correct target cells. While the signaling mechanisms underlying distinct guidance molecules are different, the actin cytoskeleton is believed to be the major target of intricate intracellular signaling pathways that leads to specific motile behaviors of the growth cone. Our previous studies showed that directional growth cone responses are mediated partly by local protein synthesis of actin molecules and spatial severing/depolymerization of actin filaments. However, the involvement of globular actin monomers (G-actin) and its implication in actin assembly/disassembly during axonal guidance remain unclear. In this study, we provide evidence to show an unanticipated spatial pattern of G-actin in growth cones of the cultured Xenopus spinal neurons, which may play an important role in axonal guidance. First, using various specific probes for monomeric G-actin, we consistently detected a local enrichment of G-actin at the peripheral domain of the growth cone. Notably, the ratiometric analysis showed that the peripheral localization of G-actin was inversely related to that of filamentous actin (F-actin). In live cultured neurons, the differential distribution of G- and F-actin in growth cones was also observed by simultaneous dual-channel imaging of GFP-actin and RFP-Liveact. Importantly, we detected an asymmetric distribution of G-actin across the growth cone in

response to the local application of a guidance cue BMP7. Taken together, these results suggest a novel regulation of growth cone guidance by spatially restricting the availability of G-actin for actin polymerization induced by extracellular stimuli.

WE01-07

LOCAL APPLICATION OF NEUROTROPHINS SPECIFIES AXON THROUGH INOSITOL 1, 4, 5-TRISPHOSPHATE/ CA^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASES

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Neurons are highly polarized cells that possess axons and dendrites, both of which differentiate from common immature neurites in cultured hippocampal neurons. One of these immature neurites stochastically initiates rapid extension and becomes an axon, whereas the other immature neurites finally become dendrites. Various extracellular signals including neurotrophins, Wnts, IGF-1, TGF-β and Reelin have been implicated in axon specification among the immature neurites. However, the causal relationship between the extracellular and intracellular signals and axon specification remains elusive, because live imaging of the signals during axon specification is difficult in practice. We found here that neurotrophins including NT-3 and BDNF derived from the cultured neurons were required for axon specification in an autocrine fashion. To evaluate neurotrophins and their intracellular signals, we developed the local application method using micropipette, and found that stimulation of the selected neurite by NT-3 and BDNF induced neurite outgrowth and subsequent axon formation. A local application of Wnt5a, Wnt3a, IGF-1 and TGF-β, but not NGF and Reelin, also induced neurite outgrowth. NT-3 induced a rapid increase in Ca^{2+} in an inositol 1, 4, 5-trisphosphate (IP3)-dependent fashion, and activation of calmodulin-dependent protein kinase kinase (CaMKK), a Ca^{2+} effector, in the growth cone. Impairment of neurotrophin receptors and CaMKK prohibited NT-3-induced axon specification in cultured neurons and axon formation in cortical neurons *in vivo*. These results reveal a novel role for IP3/Ca^{2+} signaling in axon specification via CaMKK.

WE01-08

IMPACT, A DEVELOPMENTALLY REGULATED PROTEIN IN NEURONS, OPPOSES GCN2 IN MODULATING NEURITE OUTGROWTH

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Development of the nervous system requires the precise modulation of sprouting and elongation of neurites in a timely manner. The control of protein synthesis, specially the one localized to neuronal compartments such as growth cones and synapses, is one of the most important determinants of neuronal development and synaptic function. eIF2α phosphorylation is a key regulatory step for translation, resulting in inhibition of general protein synthesis but increasing translation of specific mRNAs, such as ATF4 mRNA. Recently, GCN2, one of the four mammalian eIF2α kinases, was characterized as a negative regulator of synaptic plasticity and memory. Here, we demonstrate that IMPACT, a protein preferentially expressed in the brain, prevents GCN2 activation and signaling, and is associated with increased levels of translation in neuronal cells. Furthermore, in neuronal processes, IMPACT was found in granules that were sensitive to translational arrest treatments and contained the ribosomal protein S6 (rpS6) and FMRP suggesting an association with the translational machinery. The transport of IMPACT to neuronal processes and its association with rpS6 were dependent on its C-terminal "Ancient Domain", of unknown function until now. Remarkably, we found that IMPACT expression increases drastically during neuronal differentiation along with the expression of neuronal differentiation markers. IMPACT increase is accompanied by a decrease in GCN2 phosphorylation at T898 that indicates that GCN2 becomes inactivated during neuronal cells differentiation. Consistent with a role during neuronal differentiation, we provide evidence that IMPACT promotes neuritogenesis and, conversely, that GCN2 is a potent inhibitor of spontaneous neuritogenesis. Accordingly, primary neurons from Gcn2-/- mice presented increased neuritogenesis. Together, these results suggest that the conserved GCN2-IMPACT module provides another step of translational control to the extremely regulated process of neuronal differentiation. Supported by: FAPESP

WE01-09

LAMININ-γ1 AND STI1 SYNERGISTICALLY MEDIATE PRP^C-DEPENDENT AXONAL GROWTH VIA CA^{2+} SIGNALING

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Prions are infectious pathogens associated to neurodegenerative diseases generated by the structural conversion of the cellular prion protein (PrP^C), a cell surface glycoprotein abundantly expressed in the nervous system. The cellular functions of PrP^C and its possible loss-of-function in prion diseases are under intensive investigation. PrP^C mediates neuritogenesis of hippocampal neurons through interaction with laminin-γ1 chain or stress inducible protein 1 (STI1). Herein, we investigated the involvement of PrP^C engagement with Ln-γ1 or STI1 in the axonogenesis of dorsal root ganglia neurons, assessing the role of calcium signaling. The Ln-γ1 peptide, corresponding to the laminin binding site to PrP^C, and STI1 were able to promote axonogenesis (axon sprouting and outgrowth) in wild-type neurons while no effect was observed in PrP^C-null neurons. Moreover, neuronal treatment with a combination of Ln-γ1 peptide and STI1 at suboptimal concentrations induced axonogenesis, suggesting a synergistic effect of these proteins upon interaction with PrP^C. The binding of Ln-γ1 peptide or STI1 to PrP^C causes an increase in intracellular Ca^{2+} levels by distinct mechanisms, whereas STI1 promotes extracellular Ca^{2+} influx, Ln-γ1 peptide recruits Ca^{2+} from intracellular stores. These results suggest that PrP^C acts as a pivotal scaffold molecule organizing multimolecular complexes able to promote diverse cellular signaling with synergistic activity in axonogenesis.

WE01-10

STABILIZATION OF MICROTUBULES BY POLYAMINES AND TRANSGLUTAMINASE: ITS ROLES IN BRAIN FUNCTION

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Neurons contain large numbers of microtubules that support intracellular transport, facilitate axon growth, and form a structural basis for neuronal morphology. While microtubules in non-neuronal cells are generally quite dynamic and can be depolymerized by cold, calcium or antimitotic drugs; many neuronal microtubules are unusually stable. The mechanisms for such stability are still unclear. In this study, a novel mechanism for axonal microtubule stabilization will be demonstrated based on four lines of evidence: First, neuronal tubulin, polymerized microtubules, and Taxol-stabilized microtubules can be polyaminated by transglutaminase. The polyamination sites were mapped using LC-MS-MS, and the identified sites are consistent with a role for this modification in stabilizing microtubules. Second, neuronal tubulin polyaminated by endogenous brain transglutaminase exhibits biochemical characteristics similar to neuronal stable microtubules in three significant ways. Third, there are strong spatial and temporal correlations between transglutaminase activity, TG2 (a predominant transglutaminase isoform in brain) immunoreactivity and microtubule stability in both cell and animal models. Fourth, inhibiting either polyamine synthesis or transglutaminase activity significantly decreases the amount of neuronal stable microtubules *in vivo*. Moreover, transglutaminase activity contributes to neurite outgrowth in the cell model and is correlated with postnatal axonal maturation in animal models.In sum, a novel posttranslational modification of neuronal tubulin has been identified, and this modification may contribute to microtubule stability as well as to neurite development, maturation and maintenance. Such a mechanism is critical for regulating neuronal cytoskeleton in development, regeneration and aging.

WE01-11

AATYK1 PHOSPHORYLATION BY CDK5 REGULATES AXON OUTGROWTH VIA RECYCLING ENDOSOME PATHWAY

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Membranes are supplied to extending neurites of neurons through recycling endosomes. Membrane trafficking to and from recycling endosomes is regulated by Rab11 small GTPase, but the regulatory mechanism remains elusive. We have recently shown that AATYK1 Ser/Thr kinase plays a role in the formation of Rab11A-positive pericentrosomal endocytic recycling compartment in CHO-K1 cells and that activity is regulated by Ser34 phosphorylation of AATYK1 with cyclin-dependent kinase 5 (Cdk5). Considering that both AATYK1 and Cdk5 are expressed highly in brains, it is important to identify the neuronal function of the Cdk5-AATYK1 pathway. Here, we investigated a role of AATYK1 and its phosphorylation by Cdk5 in neurite outgrowth using primary cortical neuron cultures. AATYK1 expression and Ser34 phosphorylation of AATYK1 were increased in cultured neurons at DIV3 at the time of axonal outgrowth. Downregulation of AATYK1 by RNAi promoted axonal outgrowth in primary cortical neurons, indicating the role of AATYK1 in axon outgrowth. Neither AATYK1-WT nor AATYK1-S34D affected outgrowth of axon, but the expression of AATYK1-S34A resulted in longer axon. Exogenously expressed AATYK1 co-localized with Rab11A-positive recycling endosomes at perinuclear region and neurites. AATYK1-S34A showed co-localization with Rab11A more than AATYK1-WT and AATYK1-S34D in neurites. AATYK1-WT showed co-localization with constitutively active Rab11A-Q70L more than dominant negative Rab11A-S25N in neurites. Axonal outgrowth induced by AATYK1-S34A was reversed by Rab11A-S25N. These results suggest that Cdk5-dependent phosphorylation of AATYK1 at Ser34 suppresses the axonal outgrowth via regulation of Rab11A activity.

WE01-12

LOCAL SYNTHESIS OF BETA-CATENIN DURING SYNAPSE FORMATION

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Proper synapse formation requires the expression of presynaptic β-catenin protein which is critical for establishing the synaptic vesicle reserve pool. From previous work we know that synapse-competent central axons contain β-catenin mRNA. We also know that β-catenin is locally translated in growth cones of hippocampal neurons. Here we tested the hypothesis that β-catenin is locally synthesized in axons during synapse formation. To experimentally address this hypothesis, we used a compartmentalized microfluidic platform to obtain an isolated field of axons, allowing us to add target mimics to the axonal compartment and induce presynaptic terminal formation. An advantage of this set-up is the ability to define the precise timing of presynaptic terminal development, which is not possible *in vivo*. Another advantage is the ability to selectively apply compounds, such as protein synthesis inhibitors, to axons. We show that functional presynaptic terminals form within 24h after the addition of the target mimics. We show that ribosomes and β-catenin mRNA localize to these differentiated presynaptic terminals within 24h– to our knowledge providing the first clear evidence of translational machinery in synapse-competent hippocampal axons. We demonstrate that localization of β-catenin protein at presynaptic terminals depends, in part, on axonal protein synthesis. Finally, we report that β-catenin is locally and specifically synthesized in hippocampal axons during presynaptic terminal formation.

WE01-13

BMP SIGNALING ENHANCEMENT BY ARKADIA2C IS REQUIRED FOR MOTOR NEURON AXON ELONGATION

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Little is known about extrinsic signals that regulate axon elongation particularly that of motor neurons (MN), which possess some of the longest axons in the body. The Transforming Growth Factor (TGF)-β superfamily includes two major classes of ligands: Nodal-like, which activate by receptor-phosphorylation, the Smad2/3 effectors, and Bone Morphogenetic Proteins (BMP), which activate Smad1/5/8. Arkadia/Rnf111, a RING-domain E3 ubiquitin ligase, specifically enhances Nodal-Smad2/3 signaling and is essential for head development in vertebrates. Arkadia mediates the degradation of the TGF-β negative regulators, Smad6/7 and SnoN/Ski, however, its activity depends on its specific interaction with activated Smad2/3. The existence of a similar factor with BMP signaling specificity remained unknown. Here we show that a close homologue of Arkadia, Arkadia2C/RNF165, is specifically expressed in the nervous system. Its loss in mice causes motor axon growth and innervation defects including the forelimb and the diaphragm. Arkadia2C functions by mediating the degradation of negative regulators and enhancing specifically BMP/Smad1/5/8 downstream transcriptional response. Reduction of BMP signaling in MN by genetic removal of alleles encoding signal transduction components in Arkadia2C heterozygous mice causes the emergence of forelimb innervation defects. This confirms the molecular function of Arkadia2C *in vivo*. Collectively, the data revealed a novel role of the BMP-Smad signaling pathway in MN axon elongation.

WE01-14

PAT1, A NOVEL KINESIN LIGHT CHAIN-LIKE PROTEIN: ITS INTERACTION WITH ZBP1 MEDIATING MRNA GRANULE LOCALIZATION IN NEURONS

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Subcelluar localization of messenger RNAs (mRNAs) gives precise temporal and spatial control over localized protein synthesis, which is important for axon guidance, filopodia and synapse growth, synapse function and neuronal asymmetry. It appears that motor-based transport of mRNA has become a predominant mechanism for the localization of mRNAs in animal cells. Currently, there are still difficulties in identifying the molecular linkers between localized transcripts and motor proteins in animal cells. By using of yeast 2-hybrid screen, we managed to identifiy PAT1 (Protein interacting with APP tail-1), a kinesin light chain-like protein. This protein interacts with both ZBP1 (zipcode-binding protein), and kinesin light chain (KLC), and could be a potential molecular linker. Cell immunofluorescence images showed co-localization of ZBP1 and PAT1 in primary hippcampal cultures. Co-immunoprecipitation and GST pull-down with recombinant proteins confirmed the interaction between ZBP1 and PAT1 *in vitro*. siRNA was designed to knock down PAT1 mRNA. As predicted, knocking down of PAT1 impairs dendritic β-actin mRNA granule transport, decreases β-actin mRNA granule density in dendrites, and further influences growth cone guidance and synaptogenesis. We postulate that PAT-mediated transport of RNA granules through ZBP1 and perhaps other mRNA-binding proteins serves to enhance stimulus-induced local protein synthesis in filopodial-spine growth, growth cone guidance, and synaptogenesis. BDNF signaling is known to induce rapid actin remodeling in growth cones and filopodia-spines, and may act to signal to the PAT1/ZBP1 complex to deliver beta-actin mRNA for rapid actin assembly dynamics.

WE02 Disorder-Related Bioenergetics

WE02-01

ALTERATION OF DOPAMINE AND ITS METABOLITE IN PLASMA OF HEROIN ABUSERS

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Heroin is known as a drug of abuse. It alters dopamine metabolism in neurons. In this study we examined the level of dopamine (DA) and its metabolite (3,4 dihydroxyphenylacetic acid; DOPAC) in plasma of heroin users (22–30 years of age) who used 600–1,200 mg/day of heroin for 3–4 years compared with a control group (24–33 years of age) by using high performance liquid chromatography connected with electrochemical detection (HPLC-ECD) analysis. The result showed that heroin users exhibited a significant decrease in plasma DA, but an increase in DOPAC levels ($p<0.05$). The present results showed the changes of dopamine turnover from plasma of heroin addicts, this may help to better understand the mechanism of heroin addiction.

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WE02-02

THE ANTIRETROVIRAL PROTEASE INHIBITORS INDINAVIR AND NELFINAVIR STIMULATE MRP1-MEDIATED GSH EXPORT FROM CULTURED BRAIN ASTROCYTES

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Combinations of antiretroviral drugs are successfully used as therapy to slow down the progression of AIDS and to prevent severe HIV-associated dementia. Since the tripeptide glutathione (GSH) is important for detoxification processes in the brain, we have investigated the consequences of a treatment with antiretroviral drugs on the GSH metabolism of brain cells, using astrocyte-rich primary cultures as model system. Exposure of these cultures to the protease inhibitors indinavir or nelfinavir resulted in a rapid time- and concentration-dependent depletion of cellular GSH and a matching increase in the extracellular GSH content. Half-maximal loss of cellular GSH was observed 6 h after application of around 5 μM nelfinavir or 10 μM indinavir. Although exposure of astrocytes to 30 μM indinavir for 6 h or 24 h almost completely deprived the cells of GSH, the viability of the cells was not compromised under these conditions. In contrast, treatment with 30 μM nelfinavir deprived the cells within 6 h only by around 50% of their GSH, but caused substantial cell damage after incubation periods longer than 6 h. The indinavir- or nelfinavir-stimulated GSH export from viable astrocytes was completely prevented by the application of MK571, an inhibitor of the multidrug resistance protein 1. These data demonstrate that the antiretroviral protease inhibitors indinavir and nelfinavir stimulate Mrp1-mediated GSH export from brain astrocytes. This alteration of astroglial GSH metabolism by antiretroviral protease inhibitors may be involved in the development of mild cognitive impairments in AIDS patients during a chronic treatment with combinations of antiretroviral drugs that include protease inhibitors.

WE02-03

DISRUPTION OF ASTROCYTIC GAP JUNCTIONAL COMMUNICATION BY DIABETES AND AMYLOID-BETA 1-40

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Astrocytes are extensively coupled by gap junctions to form large syncytia within which trafficking of metabolites and signaling molecules is important for normal brain function. To better understand the influence of disease states on astrocytes, gap junctional communication was evaluated in experimental diabetes and Alzheimer's disease. Dye transfer among coupled astrocytes cultured in high (25 mmol/L) glucose slowly fell to about half that of control cells grown in low (5.5 mmol/L) glucose, with an onset that lagged the increase in generation of reactive oxygen-nitrogen species (ROS-NOS) by several days. This deficit was not reversed by return to low glucose medium for two weeks, and was prevented but not rescued by treatments that reduce oxidative stress. In contrast, small molecules known to facilitate protein folding both prevented and rescued impaired dye transfer, even in the presence of high glucose. Growth in high glucose did not alter Cx26 level, but reduced Cx30 by 30%, and elevated Cx43 by 1.9-fold; impaired Lucifer yellow transfer was not due to lower connexin levels. Nitrosative stress was implicated as a causative factor because brief treatment of high-glucose cultures with dithiothreitol normalized dye transfer and exposure of low-glucose cultures to NO-donors quickly impaired dye transfer. Streptozotocin-diabetic rats also exhibited oxidative stress, reduced dye transfer, and lower Cx30 and Cx43 levels in the inferior colliculus. In experimental Alzheimer's disease, acute treatment of cultured astrocytes grown in low glucose with amyloid-beta1-40 caused a small, transient increase in ROS-NOS and rapid, prolonged inhibition of dye transfer; amyloid treatment further reduced the subnormal dye transfer in high-glucose cultures. However, dye transfer in slices from three brain regions of 9-14-month-old transgenic mice that contain human amyloid precursor protein with the Swedish and Indiana mutations was similar to that of age-matched controls. Thus, amyloid-beta has greater effects on gap junctional communication in cultured astrocytes compared to aged astrocytes *in vivo*, whereas diabetes may impair transcellular shuttling of molecules required for neuroenergetics and cell-cell signaling.

WE02-04

INCREASED BRAIN GLYCOGEN AFTER RECOVERY FROM ACUTE HYPOGLYCAEMIA SUGGESTS INVOLVEMENT IN HYPOGLYCAEMIA UNAWARENESS

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Diabetes mellitus is characterised by hyperglycaemia that is associated with the occurrence of well described microvascular complications that affect different organs, which development is dependent on the duration of the disease and glycaemia control. For many individuals with diabetes, episodes of severe hypoglycaemia are a major complication of glycaemia control. Moreover, recurrent hypoglycaemia impairs mechanisms of defence against hypoglycaemia. Thus, diabetes patients display a progressive decay in the physiological counter-regulatory response, resulting in hypoglycaemia unawareness, and therefore prolonged exposure to hypoglycaemia insults may become lethal. One mechanism through which the brain adapts to hypoglycaemia may involve glycogen metabolism and its buffering effect on brain glucose concentrations. Conscious freely moving rats were submitted to hypoglycaemia bellow 35 mg/dL for 90 minutes by insulin administration, followed by a recovery period of 24 hours either under normoglycaemia or hyperglycaemia achieved by glucose infusion. Rats were then sacrificed by microwave fixation and glycogen concentration was determined in different brain regions. Control rats underwent the same treatment without the preceding hypoglycaemia period. Hypoglycaemia depleted brain glycogen content in the brain. In the cortex, glycogen concentration was increased by 65 ± 27% or 114 ± 16% when recovery from hypoglycaemia was performed under hyper- or normoglycaemia. Similar glycogen supercompensation was observed in the hippocampus but not in the hypothalamus and striatum. Brain glycogen concentration did not increase after 24 hours under hyperglycaemia without a preceding hypoglycaemia insult. In conclusion, supporting brain metabolism during recurrent hypoglycaemia periods, glycogen may have a role in hypoglycaemia unawareness.

WE02-05

GHRELIN REGULATES ENERGY BALANCE THROUGH HYPOTHALAMIC RECEPTORS IN A RAT OBESITY MODEL

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Feeding behavior and energy balance is maintenained through integration of orexigenic and anorexigenic signals from the periphery in the hypothalamus, in particular in the arcuate nucleus. ARC neurons express anabolic Growth Hormone Secretagogue Receptor (GHSR), catabolic leptin (Ob-R) and Melanocortin 4 (MC4R) receptors, to detect ghrelin, leptin and α-MSH, respectively. Ghrelin is mainly produced in the stomach where, after acylation by O-acyltransferase (GOAT), may bind to GHSR. Acyl-ghrelin stimulates the gut-brain orexigenic axis to increase food intake and reduce fat mobilization, in contrast to leptin, which, circulating at levels proportional to body fat, promotes the synthesis of the appetite suppressant α-MSH. To further understand how the neuroendocrine ghrelin system regulates energy homeostasis through the gut-brain-axis, we employed a novel approach to reduce the endogenous ghrelin levels through the removal of the stomach fundus by sleeve gastrectomy, the procedure of choice for morbid obesity. We developed a rat obesity model, where animals were fed a high fat diet for 12 weeks and then underwent sleeve gastrectomy or sham operation. All animals were then fed normal diet, and, when sacrifized one or three months later, ghrelin and GOAT in the stomach, GHSR, Ob-R and MC4R expression levels in the hypothalamus were assessed by semi-quantitative RT-PCR and immunodetection. We found that sleeve gastrectomy significantly decreased ghrelin message in the stomach when compared to sham- and non-operated animals. GOAT patterns followed that of ghrelin suggesting co-regulation of their expression as well as acylation of ghrelin. In the hypothalamus we observed decreased GHSR and Ob-R expression both at the message and protein levels, possibly due to lower acyl-ghrelin levels. As expected, lower fat and thus lower leptin levels, led to significantly lower expression of MC4R, upon withdrawal from the fat diet for at least 3-months. Our results indicate that ghrelin participates in the control of energy balance through the regulation of its own receptor GHSR, Ob-R and MC4R expression in the hypothalamus of obese animals.

WE02-06

IMPAIRED INSULIN SIGNALING IN A PRIMATE MODEL OF ALZHEIMER'S DISEASE: LINK WITH TYPE-2 DIABETES

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Alzheimer's disease (AD) has been linked to defective brain insulin signaling, a proposed third type of diabetes (1, 2, 3). 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. In type 2 diabetes, tumor necrosis factor-α (TNF- α) signaling stimulates c-Jun N-Terminal Kinase (JNK). This results in serine phosphosphorylation of the insulin receptor substrate (IRS-1), blocking downstream signaling and triggering insulin resistance (4). The link between diabetes and Alzheimer's disease was found in the ability of Aβ oligomers, toxins that accumulate in Alzheimer brain and instigate synaptic damage, to activate the JNK/TNF-α pathway leading to phosphorylation of IRS-1pSer636. The aim of this study was to investigate whether Aβ oligomers injected into the cerebral ventricles of adult cynomolgus monkeys are capable of triggering mechanisms similar to those described for type 2 diabetes. Our findings indicate JNK activation and increased levels of IRS-1pSer636 in primate hippocampus. They reinforce the link between diabetes and AD. Furthermore, considering the dearth of animal model systems that truly recapitulates the main features of Alzheimer's disease, this new non-human

primate model offers a potential to provide insight into central aspects of AD that may be exclusively present in primates.

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WE02-07

ADMINISTRATION OF 17β-ESTRADIOL MODULATES THE AGE RELATED NEURONAL MARKERS IN NATURALLY MENOPAUSAL RATS FROM DIFFERENT AGE GROUPS

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Background: Aging in females and males is considered as the end of natural protection against age related diseases like osteoporosis, coronary heart disease, diabetes, Alzheimer's disease and Parkinson's disease. These changes increase during menopausal condition in females when the level of estradiol is decreased.

Objective: The objective of this study was to observe the changes in activities of monoamine oxidase, glucose transporter-4 levels, membrane fluidity, lipid peroxidation levels and lipofuscin accumulation occurring in brains of female rats of 3 months (young), 12 months (adult) and 24 months (old) age groups, and to see whether these changes are restored to normal levels after exogenous administration of estradiol.

Methods: The aged rats (12 and 24 months old) (n= 8 for each group) were given subcutaneous injection of 17β-estradiol (E2)(0.1μg/g body weight) daily for one month. After 30 days of hormone treatment experimental animals of all the groups were sacrificed and brains were isolated for further study.

Results: The results obtained in the present work revealed that normal aging was associated with significant increases in the activity of monoamine oxidase, lipid peroxidation levels and lipofuscin accumulation in the brains of aging female rats, and a decrease in glucose transporter-4 level and membrane fluidity. Our data showed that estradiol treatment significantly decreased monoamine oxidase activity, lipid peroxidation and lipofuscin accumulation in brain regions of aging rats, and a reversal of glucose transporter-4 levels and membrane fluidity was achieved. Administration of E2 brought these changes to near normalcy.

Conclusions: It can therefore be concluded that E2's beneficial effects seemed to arise from its antilipofuscin, antioxidant and antilipidperoxidative effects, implying an overall anti-aging action. The results of this study will be useful for pharmacological modification of the aging process and applying new strategies for control of age related disorders.

WE02-08

NEUROCHEMICAL ALTERATIONS IN THE HIPPOCAMPUS AND OLFACTORY BULBS OF RTG4510 TRANSGENIC MOUSE MODEL OF TAUOPATHY

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Tauopathies are characterized by aggregation of tau protein in the brain with a variety of neurodegenerative diseases including frontotemporal dementia and Alzheimer's disease. The several contributing molecular mechanisms that underlie tauopathies include oxidative stress and neuro-inflammation. However, the metabolic consequence of tauopathies on neurochemical levels has not been well described. Among several transgenic mouse models of tauopathy, rTg4510 is a recently developed double transgenic mouse model that expresses a repressible human tau variant and develops progressive age-related neurofibilary tangles, neuronal loss, and behavioral impairments at as early as 4–5 months of age (mos). This study aims to determine neurochemical alterations that reflect the ongoing disease progression and related mechanisms in rTg4510 mice. Neurochemical profiles were measured in the hippocampus and olfactory bulbs (OB) of nine rTg4510 and ten littermate wild-type (wt) mice longitudinally at 5, 9, and 12 mos using *in vivo* 1H MRS at 9.4T. In the hippocampus, taurine levels were significantly lower in rTg4510 mice than in wt mice ($p < 0.01$) at 5 mos, higher myo-inositol, glutamine, glycerophosphocholine, and GABA levels, while lower glutamate, glutathione, and taurine levels ($p < 0.01$) than those in wt mice at 9 mos. The distinctive neurochemical alteration patterns observed in rTg4510 mice at 9 mos were persistent and more pronounced at 12 mos. In addition, NAA levels started to be lower in rTg4510 mice compared with those in wt mice ($p < 0.05$) at 12 mos. Interestingly, neurochemical alterations in the OB appear to occur earlier than in the hippocampus, suggesting potential early pathologic development of tau pathology in the OB. Therefore, the neurochemical profile measured by MRS would provide an insight to the neurological effect in the development and progression of tauopathy.

WE02-09

1H MRS OF METABOLIC CHANGES AFTER PERMANENT CEREBRAL ISCHEMIA *IN VIVO*

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The course of ischemic episodes is very variable and makes prediction of the outcome of an individual patient early after ischemia difficult. Recently, 1H magnetic resonance spectroscopy (MRS) at early time points reliably estimated the severity of ischemia, identified transient ischemic attacks and thus would greatly facilitate clinical decisions. Such method should allow us monitoring the cascading damages after ischemia and thus reveal

insightful information for better understanding the underneath metabolic alterations after ischemia. Therefore, the aim of the present study was to study metabolic changes after permanent ischemia using 1H MRS.

ICR-CD1 mice were subjected to permanent (n = 18) endoluminal filament middle cerebral artery occlusion (pMCAO) with regional cerebral blood flow (CBF) < 20% of baseline CBF during ischemia by a laser-Doppler flowmetry. All MR studies were carried out in a horizontal 14.1T magnet. T2-weighted images were acquired to localize the volume of interest and evaluate the lesion size. Immediately after improvements of field homogeneities, localized 1H MRS was applied to obtain the neurochemical profile consisting more than 18 metabolites from the ipsilateral striatum (6–8microliter). Six animals (sham group) underwent nearly identical procedures without pMCAO.

Two metabolites were useful to determine whether reperfusion has occurred (glutamine increase) or not (GABA increase). Since N-acetyl aspartate + glutamate + taurine can be used to determine if a tissue is already irreversibly damaged or not after transient ischemia, we have also identified an exponential decrease of such metabolites and other involving metabolites after permanent ischemia and thus could be used to determine the onset time of ischemia.

In conclusion, 1H MRS can be used as a diagnostic tool to monitor reperfusion, identify reversibly and irreversibly damaged tissue and evaluate the time of ischemia onset. If these results can be translated to stroke patients, this technique would greatly improve the diagnosis and help with clinical decisions.

WE02-10

RISK OF ALZHEIMER'S DISEASE IN RELATION TO DIABETES: A POPULATION-BASED COHORT STUDY IN TAIWAN

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Objective: Using the National Health Insurance claim data, we prospectively investigated the age- and sex-specific incidence density and relative hazard of Alzheimer's disease (AD)(ICD-9: 331.0) in the diabetic and control groups.

Research Design and Methods: A total of 615,529 diabetic patients and 614,871 age- and sex-matched control subjects, selected from the ambulatory care claims and the updated registry for beneficiaries in 2000, respectively, were linked to the inpatient claims in years of 2000 to 2008 to identify the first episode of primary or secondary diagnoses of AD. Incidence density was calculated under the Poisson assumption. We estimated, using Cox proportional hazard regression models, the relative risks of AD in realtion to diabetes, selected co-morbidities, and certain sociodemographic characteristics.

Results: Over nearly 8 years of follow-up, a total of 4,615 diabetic patients developed AD, representing a cumulative incidence rate of 0.75% (vs. 0.63% in controls). The overall incidence desnity of AD for diabetic men and women was 0.82/1000 and 1.15/1000 person-years, respectively, which were also higher than those of control men (0.63/1000 person-years) and women (0.89/1000 person-years). Hazard ratio (HR) of AD was significantly increased in diabetic patients (1.45; 95% CI, 1.38–1.52), and the most increased HR was noted for diabetic women over 65 years old (1.52; 95% CI, 1.42–1.62), In addition to diabetes, older ages, higher insurance premium, and living in less-remote areas were also positively associated with AD. Moreover, certain co-morbidities including cerebrovascular disease, cardiovascular disease and hypertension were all significant predictors for increased risk of AD.

Conclusions: In Taiwan, diabetes may increase the risk of AD in both sexes and in all ages. Higher HR of AD was especially notable in older diabetic women.

WE02-11

IN VIVO PROTON MAGNETIC RESONANCE SPECTROSCOPY IN A TRANSGENIC RAT MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's Disease (AD) is a neurodegenerative disease characterized by extracellular amyloid plaques, intracellular neurofibrillary tangles, loss of neurons and synapses, and progressive cognitive dysfunction. The current study employed the McGill-R-Thy1-APP rat model, in which rats express the human amyloid precursor protein carrying the Swedish and Indiana mutations. Homozygous rats develop intraneuronal Aβ oligomers within 1 week, cognitive decline within 3 months and extracellular amyloid plaques by 6 months (Leon et al., 2010). The aims of the study were to investigate cerebral metabolic status noninvasively before and after the appearance of amyloid plaques and to longitudinally characterize metabolite concentrations as the pathology progresses. *In vivo* 1H MRS of the dorsal subiculum (DS) was performed in transgenic rats and age-matched controls at 3 and 9 months and in the frontal cortex at 9 months. At 3 months of age, the concentration of glutamate in DS was decreased while that of taurine was increased. This shows that altered glutamate homeostasis in AD is not merely a consequence of decreased neuronal viability/number and that intraneuronal accumulation of Aβ oligomers is sufficient to alter glutamate metabolism. Furthermore, it supports involvement of altered glutamate homeostasis in cognitive dysfunction in AD. By the age of 9 months the concentration of glutamate, GABA and NAA were decreased and that of myo-inositol and taurine were increased in DS, indicating decreased neuronal viability and metabolic alterations in both glutamatergic and GABAergic neurons at this stage. Furthermore, concentrations of glutamate and glutamine were decreased in the frontal cortex at 9 months, implicating altered neuronal - astrocytic metabolic interactions.

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WE02-13

OBESITY, DIABETES AND HYPERTENSION ASSOCIATED WITH ANTIPSYCHOTIC USE IN SCHIZOPHRENIA

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Objective: To ascertain the prevalence of diabetes, obesity and hypertension associated with antipsychotic use in remitted patients with schizophrenia.

Methods: This study included a cross sectional survey of diabetes, obesity and hypertension among all remitted patients diagnosed with schizophrenia/schizoaffective disorder (n = 130) on at least 6 months of antipsychotic treatment.

Results:A prevalence rate of 35.4% obesity, 1.5% hypertension and 3.8% (ADA) or 5.4% (WHO) prevalence of diabetes was observed. Conclusions: Long term antipsychotic use.

Conclusion: The use of antipsychotic drugs in the long run may be associated with a significantly greater risk of developing obesity with moderate influence on development of diabetes and minimal to none on hypertension.

WE02-14

REGULATION OF CARBOHYDRATE METABOLISM BY ZINC DURING ALUMINIUM INDUCED NEUROTOXICITY

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Aluminium has been correlated etiologically to several neurodegenerative diseases including Alzheimer's and Parkinson. The aim of this study was to elucidate the role of zinc (Zn) in mitigating the adverse effects inflicted by aluminium (Al) in cerebellum and cerebrum of rat. Male Sprague Dawley rats weighing 140–160 g were divided into four different groups viz: Normal control, Aluminium treated ($AlCl_3$ orally 100 mg/kg b.wt./day in drinking water), Zinc treated ($ZnSO_4$ in drinking water 227 mg/l/day) and combined aluminium and zinc treated. All the treatments were continued for a total duration of two months and their effects were studied on carbohydrate metabolizing enzymes viz: hexokinase, glucose-6-phosphatase (G6P), glucose-6-isomerase (G6I), lactate dehydrogenase (LDH), succinate dehydrogenase (SDH) and glycogen content. Additionally, expressions of the proteins which help in regulating carbohydrate energy metabolism were also studied. Al treatment resulted in increased activities of the G6P, G6I and LDH whereas the activities of hexokinase, SDH and glycogen content were found to be decreased. However, Zn supplementation to Al treated rats was able to significantly reduce the Al induced increased activities of G6P, G6I and LDH, but elevated the levels of hexokinase, SDH and glycogen contents. Further, Al treatment increased the protein expression of glycogen synthase kinase-3 (GSK3) but decreased the protein phosphatase (PP1) expression which was significantly reversed upon Zn administration. Therefore, Zn shall prove to be protective in regulating the carbohydrate metabolism following neurotoxic effects caused by Al.

WE02-15

METHYLMERCURY ALTERS SYNTHESIS, RELEASE, AND METABOLISM OF DOPAMINE IN PC12 CELLS

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Methylmercury (MeHg) is a potent, bioaccumulative neurotoxicant linked to severe neurological dysfunction in humans. One neuronal population susceptible to the neurotoxic effect of MeHg is the nigrostriatal dopamine (DA) pathway, which is known to degenerate in Parkinson disease. The mechanisms underlying MeHg targeting of DA neurons is not well understood. Therefore, the present investigation sought to characterize the concentration-response and time-course effects of MeHg on DA homeostasis in undifferentiated PC12 cells, which synthesize and secrete large amounts of this catecholamine. Cell viability after treatment with 1, 2, or 5 μM MeHg was assessed using Hoechst and propidium iodine staining. Significant cell death was only observed 60 and 120 minutes following treatment with 5 μM MeHg. The time-course and concentration-response effects of MeHg on DA release were determined by measuring the concentration of extracellular DA by HPLC-ED. Treatment with 2 or 5 μM MeHg significantly increased extracellular DA concentrations after 60 minutes compared to controls. Increased DA release was not associated with significant changes in stored DA, however a concentration-dependent decrease in intracellular concentrations of the DA metabolites DOPAC and HVA was observed at 60 minutes. The effects of MeHg on DA synthesis were assessed by quantifying amounts of total and phosphorylated tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis. Although total TH was not significantly altered after treatment MeHg, pTH Ser40 was significantly increased following treatment with 2 or 5 μM MeHg. These results indicate that increased DA release after treatment with 5 μM MeHg is due, in part, to an increase in cell death. However the increase in DA release after 2 μM MeHg is not due to cell death and involves altered DA homeostasis, including increased synthesis and decreased metabolism of DA.

WE02-16

IN VIVO INTRACEREBROVENTRICULAR ADMINISTRATION OF ORNITHINE AND HOMOCITRULLINE INDUCE OXIDATIVE STRESS IN CEREBRAL CORTEX OF RATS

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Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome is a genetic disorder biochemically characterized by tissue accumulation of ornithine, ammonia and homocitrulline. Affected patients present lethargy, ataxia, delayed development and severe mental retardation whose pathogenesis is poorly understood. The objective of the present study was to investigate the effects of intracerebroventricular administration of ornithine and

homocitrulline on parameters of oxidative stress in cerebral cortex of rats. A single injection of ornithine, homocitruline or saline was given to 30-day-old male Wistar rats. Animals were killed 30 or 120 minutes after the injection and cerebral cortex was isolated and used for the biochemical assays. Ornithine and homocitrulline induced lipid and protein oxidation (increased thiobarbituric acid-reactive substances values and carbonyl formation, respectively). Furthermore, homocitrulline also decreased glutathione levels and the activity of antioxidant enzymes catalase and glutathione peroxidase in cerebral cortex homogenates, indicating that homocitrulline provokes a reduction of brain antioxidant defenses. These results suggest an induction of oxidative stress caused by ornithine and homocitrulline *in vivo*. The mechanisms of this induction may be involved in the pathophysiology of the neurological dysfunction characteristic of HHH syndrome.

WE02-18

THE EFFECT OF ALZHEIMERS-RELATED TRANSGENE EXPRESSION ON METABOLITES IN THE MOUSE BRAIN: A 1H MRS STUDY *IN VIVO*

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Introduction: The transgenic mouse strain TASTPM (1), was investigated as a model of Alzheimer's disease. The aim of this study was to investigate neurometabolic changes occurring over time in the brains of TASTPM mice compared to their base strain (C57/BL6).

Methods: 1H MRS was performed *in vivo* on both strains of mice from 3 to 19 months of age, with data acquired at age intervals of 2 months (9 time points in total). A $3 \times 3 \times 3$ mm voxel was centred over the hippocampus and thalamus, and localized spectroscopy performed using the PRESS sequence with the following parameters: TR 2500 ms, TE 20 ms.

Results: Significant effects of genotype were observed for creatine (increase: $p < 0.005$), myo-inositol (increase: $p < 0.0001$) and N-acetyl aspartate (decrease: $p < 0.0001$). There were significant effects of age (decrease: $p < 0.001$) and genotype (decrease: $p < 0.0001$) on glutamate-glutamine (Glx) levels and on choline-containing (Cho) compounds (increase: $p < 0.0001$)

Discussion: The decrease observed in Glx levels could indicate an impairment in neuronal energy production in the TASTPM mice, a similar effect has been seen in human AD sufferers. The lower N-acetyl aspartate signal is an indicator of impaired neuronal function or neuronal death. The higher myo-inositol signal could be indicative of glial cell proliferation or microglial activation. Higher levels of Cho could be indicative of differences in phospholipid metabolism between the two strains. Overall there is good agreement between this study and previous *in vivo* studies on transgenic AD mice (2, 3) and data from human investigations. These similarities suggest that transgenic mouse models will be valuable in understanding metabolic derangement in Alzheimer's disease.

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WE03 Molecular Mechanism of Alzheimer's Disease

WE03-01

RELATIONSHIPS BETWEEN POLYMORPHISM OF ANTIOXIDANT GENE IN PATIENTS WITH ALZHEIMER'S DISEASE

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Ageing is a physiological process but it is connected with an increasing risk of origin and development of several diseases including neurodegenerative diseases. Frequency of neurodegenerative diseases increase significantly with the age. Alzheimer's disease (AD) is the most common cause of dementia. Dementia is a collective name for progressive degenerative brain syndromes which affect memory, thinking, behaviour and emotion. Protein oxidation and generation of protein aggregate may be cause of the loss of or decline in memory and other cognitive abilities and decreased ability of old organisms to resist physiological stresses and oxidative damage. The relationship between protein aggregation, oxidative damage and neurodegenerative diseases is unclear. Eenvironmental factors and genetic factors could be important agents in AD origin. Glutathione S-transferase (GST) is critical for protecting cells from reactive oxygen species. The catalase (CAT) neutralizes hydrogen peroxide, thus limiting the deleterious effects of ROS. The activity of antioxidant enzymes is genetically determined and correlates with the presence of polymorphisms in gene encoding these enzymes. We have investigated the association among GSTM1, GSTT1, GSTP1, CAT and XRCC polymorphism and AD. Patients with AD had a higher prevalence of the GSTM1 and GSTT1 null genotype than the control group. Patients were characterised by lower frequency of the GSTP1 Ile/Ile genotype. The CAT T/T homozygotes were more frequent in the patients than among the controls. The null genotype of GSTM1, GSTT1, Ile/Ile genotype of GSTP1 and T/T genotype of catalase were predominated in AD. Oxidative stress and changed antioxidant defense are included in the asthma pathology and therefore elimination of oxidative stress could be potentially an appropriate strategy for treatment of Alzheimer's disease. This work was supported by project "Identification of new markers in a diagnostic panel of neurological diseases" co-financed from EC sources and European Regional Development Fund.

WE03-02

DECIPHERING THE AMYLOID PRECURSOR PROTEIN NUCLEAR SIGNALLING PATHWAY

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The amyloid cascade hypothesis, in which the accumulation of the amyloid beta-peptide (Abeta) is proposed as the trigger for Alzheimer's disease (AD), has contributed much of the focus for research into AD therapeutics. Abeta is derived from the transmembrane amyloid precursor protein (APP). Most therapeutic strategies have focused on modifying the formation, aggregation or removal of Abeta. Yet, two decades on, available treatments are limited and palliative, rather than disease modifying. Current strategies focus on the amyloid cascade hypothesis, which has distracted from attempts to understand the normal physiology of APP, of which there are 3 isoforms, and of its metabolites. One of these is the APP intracellular domain (AICD) proposed as a transcriptional regulator. Using SH-SY5Y expressing APP, we have shown (Belyaev et al., 2010) that the neuronal isoform (APP695) preferentially produces functional AICD through a beta-secretase-dependent, lipid raft-mediated pathway. This, in turn, regulates transcription of the amyloid-degrading enzyme neprilysin. Using chromatin immunoprecipitation we reveal that AICD is associated with the neprilysin promoter in APP695 whereas HDAC1 enriches this region in APP751/770. Similar phenomena were observed in Neuro2A but not HEK293 cells. Structurally the isoforms differ in that APP751/770 contain a domain homologous to the Kunitz proteinase inhibitor whereas in APP695 exon 7 is spliced out. By identifying the binding patterns of APP751/770 our data explore the mechanism by which, unlike APP695, the KPI-containing isoforms are limited from sequestering with BACE1 and γ-secretase complexes in lipid raft domains and thus produce a non-function intracellular domain. Cellular detection of AICD is difficult due to its short half-life and rapid degradation. In order to resolve this issue, we have manipulated AICD production, optimized AICD detection and examined the effects on a range of putative AICD-regulated genes. We confirm that both treatment with the tyrosine kinase inhibitor Gleevec (20 μM, 24 hour) and the alkalizing agent ammonium chloride (10 mM, 24 hour) significantly increase AICD levels by 6-fold and 5-fold respectively. This corresponds with altered transcriptional regulation of AICD-dependent genes.

WE03-03

ROLE OF MEDIATOR SUBUNITS IN AMYLOID PRECURSOR PROTEIN INTRACELLULAR DOMAIN (AICD)-REGULATED GENE EXPRESSION

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APP (Amyloid Precursor Protein)-regulated gene expression is primarily considered to be mediated by the APP intracellular domain (AICD) as a result of the sequential cleavage of APP by α- or β-secretases followed by the action of γ-secretase. It is now established that AICD is translocated to the nucleus and participates in chromatin remodelling of its target genes altering the levels of their expression. There are only a few proposed AICD-regulated genes, one of which is neprilysin (NEP) that functions as the major amyloid-β degrading enzyme. Consequently, upregulation of NEP expression in human brain is a potential therapeutic strategy in Alzheimer's disease. Recently it has been demonstrated that a key

step in transcriptional activation by AICD is its interaction with the MED12 subunit of the Mediator complex regulating RNA polymerase II-dependent transcription (Xu et al., 2011). The MED12 subunit is mainly neuron specific, playing a role in neuronal development and some neurological diseases. Previously we have demonstrated that generation of AICD does not necessarily result in activation of NEP expression. We have now explored whether lack of AICD translocation to the nucleus and subsequent down-regulation of NEP expression is the result of a deficiency of MED12. Studies were performed using neuronal SH-SY5Y cells overexpressing the three different APP isoforms. Cells over-expressing APP695 isoform produce nuclear, transcriptionally active AICD and have high NEP levels while cells over-expressing APP751 and APP770 isoforms produce only cytoplasmic AICD and have lower levels of NEP expression. However, the levels of MED12 mRNA and protein expression were not significantly different in all tested cell lines. We conclude that while MED12 participates in AICD regulatory functions, the simple presence of MED12 is not a sufficient criterion for AICD translocation and regulation of gene activity.

This work was supported by the U.K. Medical Research Council and Alzheimer's Research UK.

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WE03-04

INVESTIGATION OF SIZE-DEPENDENT NEUROTOXIC EFFECTS OF BETA AMYLOID OLIGOMERS

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Miss-folded amyloid beta (Aβ) peptides are thought to be implicated in pathogenesis of Alzheimer's disease. Among various aggregate forms of Aβ, soluble oligomers are considered as the primary neurotoxic species though the mechanisms by which Aβ oligomers cause cell death remain largely unclear. We have recently demonstrated continuous transition from highly toxic to non-toxic species as the size of Aβ1-42 oligomer particles increased. Small Aß1-42 oligomers (dimers - pentamers) at around 1 μM concentration were found to cause rapid neuronal death in cultures of cortical neurons or cerebellar granule cells, whereas various non-neuronal cells were relatively resistant to Aβ1-42-induced cell death. Exposure of neurons to 500 nM Aβ1-42 oligomers caused neuronal necrosis over longer (3–7 days) period of incubation. Aβ1-42 monomers and fibrils had no effect on neuronal viability even after 7 days incubation. Bigger oligomeric particles of Aβ1-42 ($n > 14$) did not cause neuronal death directly, however their neurotoxicity increased in the presence of macrophage/microglial cells suggesting indirect action of Aβ1-42 oligomers. An assessment of cell densities revealed that both Aβ1-42 oligomers (small or large) similarly decreased the number of neuronal cells in mixed neuronal-astroglial cultures. While both Aβ1-42 oligomers caused a similar decrease in neuronal densities in the culture, only the small Aβ1-42 oligomers caused significant neuronal death. This suggests that Aβ1-42 oligomers may stimulate phagocytic activity of microglia resulting in disappearance of Aβ-oligomer affected neurons. In contrast, fibrils and monomers had no effect on density of neuronal cells in cultures. Altogether, our data suggest that Aβ1-42 oligomers may exert several neurotoxic effects depending on the oligomeric particle size. These effects include direct activation of cell death programme, activation of glial cells and stimulation of their phagocytic activity resulting in removal of Aβ-oligomer-affected neurons.

WE03-05

Aβ OLIGOMERS INDUCE GLUTAMATE RELEASE FROM HIPPOCAMPAL NEURONS

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Soluble oligomers of the amyloid-β peptide (AβOs) accumulate in Alzheimer's disease (AD) brain and have been implicated in mechanisms of pathogenesis. The neurotoxicity of AβOs appears to be, at least in part, due to dysregulation of glutamate signaling. Here, we show that AβOs promote extracellular accumulation of glutamate and D-serine, a co-agonist at glutamate receptors of the N-methyl-D-aspartate subtype (NMDARs), in hippocampal neuronal cultures. The increase in extracellular glutamate levels induced by AβOs was blocked by the sodium channel blocker tetrodotoxin (TTX), by the NMDAR blocker (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801) and by removal of Ca^{2+} from the extracellular medium, indicating dependence on excitatory neuronal activity. AβOs enhanced the release of pre-synaptic vesicles labeled by FM1-43 as well as spontaneous post-synaptic activity measured by whole-cell patch-clamp. Activation of inhibitory GABA A receptors by taurine blocked the increase in extracellular glutamate levels, suggesting that selective pharmacological inhibition of neuronal activity can counteract the impact of AβOs on glutamate dyshomeostasis. Results reveal a novel mechanism by which Aβ oligomers promote abnormal release of glutamate from hippocampal neurons, which may contribute to dysregulation of excitatory signaling in the brain.

WE03-06

AMYLOID-BETA ALTERS CONSTITUTIVE TRAFFICKING OF PRION PROTEIN

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The cellular prion protein (PrPC) has striking features to control cell signaling. Our group has proposed that PrPC functions as a dynamic platform for the assembly of signalling modules at the cell surface through its interaction with several ligands. Our previous results suggest that PrPC trafficking is an important mechanism for signalling. Recent data have implicated the PrPC as a putative receptor for Amyloid-β peptide (Aβ). Several evidences suggest that these peptides play a central role in Alzheimer's Disease pathogenesis. The physiological consequences to the PrPC-Aβ interaction are

still not fully understood, but it is likely that some of the effects of Aβ may be related to regulation of PrPC mediated signaling. We show that Aβ oligomers signal in cells in a PrPC dependent manner. Several different approaches indicated that treatment with Aβ oligomers, but not monomers, increased the localization of PrPC at the cell surface. We used confocal and TIRF microscopy to follow PrPC in living cells and we show that treatment with Aβ oligomers increase the localization of PrPC at the cell surface and to clusters and vesicles in very close proximity to the cell surface. Aβ oligomers inhibited the constitutive endocytosis of PrPC, whereas Aβ also seemed to recruit more PrPC to clusters at the cell surface. Our experiments show for the first time that Aβ oligomers affect PrPC trafficking, increasing its localization at the cell surface. Future experiments will examine how changes in trafficking regulate signaling properties of PrPC.

WE03-07

AN APOLIPOPROTEIN E4 FRAGMENT IS INVOLVED IN NEUROINFLAMMATORY RESPONSE

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Apolipoprotein E (apoE) plays a crucial role in lipid transport in the circulation and the brain and has three common isoforms (apoE2, apoE3, apoE4) in humans. ApoE4 isoform is a major risk factor for Alzheimer's disease (AD) and is more susceptible to proteolysis than other apoE isoforms. ApoE4 fragments have been found in brains of AD patients and it has been proposed that specific apoE4 fragments may be involved in discrete processes associated with the pathogenesis of AD. In the present study we have examined the effect of apoE4 fragments apoE4 [del(186-299)] and apoE4 [del(166-299)] on inflammation in human neuroblastoma SK-N-SH cells. Western blot and zymography analysis showed that treatment of cells with apoE4 [del(186-299)], but not full length apoE4 or the shorter apoE4 [del(166-299)] fragment, leads to increased extracellular levels of matrix metalloproteinase 9 (MMP9) and tissue inhibitor of metalloproteinase 1 (TIMP1) in SK-N-SH cells. Treatment of SK-N-SH cells with interleukin-1 beta (IL-1beta) also leads to increased extracellular levels of MMP9 and TIMP1. Real-time PCR showed that IL-1beta gene expression is increased in cells treated with apoE4 [del(186-299)], indicating that apoE4 [del(186-299)] increases MMP9 and TIMP1 levels by inducing IL-1beta gene expression in cells. Additionally, it was found that IL-10 gene expression is decreased in cells treated with apoE4 [del(186-299)]. Our findings indicate that a specific apoE4 proteolytic fragment (apoE4 [del(186-299)]) is involved in inflammatory response in neurons, an event that has been associated with the pathogenesis of AD.

WE03-08

THE SYNAPTIC PROTEOME IN ALZHEIMER'S DISEASE

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Synaptic dysfunction occurs early in Alzheimer's disease (AD) and is a target for treatment. It contributes to clinical signs of dementia through altered neuronal communication (1) and the degree of synaptic loss strongly correlates with cognitive impairment (2). To explore molecular mechanisms underlying synaptic degeneration and identifying abnormally expressed proteins we isolated synaptosomal fractions from human brain tissue obtained at autopsy from AD ($n = 6$) and normal control ($n = 6$) subjects and them compared using 2D-differential in gel electrophoresis. AD pathology is region-specific; human subjects vary in age, agonal state, and other factors. To mitigate the effects of these factors, two pathologically vulnerable areas (hippocampus and temporal cortex) were compared with two relatively spared areas (motor and occipital cortices). Protein spots exhibiting significant differences in expression (≥ 20% change, Newman-Keuls $p < 0.05$) were identified by either MALDI-TOF or ESI-QTOF mass spectrometry. A total of 28 different synaptic proteins exhibited more than 2-fold differences in expressions between AD and normal subjects. These proteins comprise various cellular components and activities, including energy metabolism, signal transduction, vesicle transport, cytoskeleton, and antioxidant functions. Several key synaptic proteins in human brain differ significantly between AD and normal control subjects, including septin-8, annexin A5, and G(o). Supported by US Alzheimer's Association, Proposal #GMS-6352.

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WE03-09

REGULATION OF EXPRESSION AND CELLULAR RELEASE OF ACETYLCHOLINESTERASE: IMPLICATIONS IN ALZHEIMER'S DISEASE

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The action of the neurotransmitter acetylcholine is terminated through hydrolysis by acetylcholinesterase (AChE), a membrane-bound enzyme, inhibitors of which are the main class of drugs currently used to treat Alzheimer's disease (AD). AChE has also been found to be enriched in the amyloid plaques present in AD. Some actions of AChE are mediated by a soluble, secreted form of the enzyme, although the mechanism of its release remains unclear. This work has compared the mechanism of release using cholinergic SN56 cells and neuroblastoma SH-SY5Y cells. Soluble AChE has been quantified in cell media by immunoblotting and enzyme activity following a variety of pharmacological treatments, in order to establish the nature of the sheddase activity. SN56 and SH-SY5Y cells were incubated with the metalloprotease inhibitor ilomastat and also with EDTA. Measurement of AChE activity in the cell media indicated an EDTA-sensitive but ilomastat-insensitive metalloprotease. The nature of the unknown sheddase is currently under investigation. The intracellular domain of amyloid precursor protein (AICD) is known to regulate the expression of certain neuronal genes, especially the amyloid-degrading enzyme, neprilysin. Given that a cholinergic deficit is an early event in Alzheimer pathogenesis, we have also explored the possibility that AICD may regulate AChE expression at the transcriptional level further linking their physiological and pathological actions. Both SH-SY5Y and SN56 cells have been used in these studies. Although significant changes in AChE activity and protein levels were detected in SH-SY5Y cells in which AICD levels were elevated, no changes in activity, protein or mRNA transcript levels were detected in the cholinesterase-rich SN56 cells, even after treatment with a gamma-secretase inhibitor. It is clear that AChE is linked to the pathology of

AD and greater understanding of the mechanisms underlying its cellular secretion and regulation of its expression may have potential future therapeutic benefit.

This work was supported by the U.K. BBSRC and MRC.

WE03-10

CALCIUM REGULATES THE INTERACTION BETWEEN AMYLOID PRECURSOR PROTEIN AND HOMER3

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Mutations in Amyloid Precursor Protein (APP) or proteins that regulate its metabolism and Ca^{2+} dysregulation are both linked to Alzheimer's Disease (AD) pathogenesis. However, the relationship between the regulation of Ca^{2+} homeostasis and the biology of APP and vice versa is not fully understood. Previous work in our laboratory has shown that APP interacts with Homer2 and Homer3 proteins and their expression inhibits APP processing towards Aβ. The focus of this study is to understand the significance of the APP/Homer interaction in Ca^{2+} homeostasis or how dysregulation of Ca^{2+} affects this interaction.

In order to investigate the effect of Ca^{2+} dysregulation on APP/Homer interaction, we caused alterations of Ca^{2+} homeostasis by incubating HEK293 cells with thapsigargin, an inhibitor of the SERCA pump of the ER, which leads to ER Ca^{2+} store depletion, either in the presence or in the absence of extracellular Ca^{2+}. We monitored APP/Homer3 interaction by co-IP at various time points. In the presence of extracellular Ca^{2+}, ER Ca^{2+} store depletion decreased APP/Homer3 interaction. In contrast, there was no effect on APP/Homer3 interaction when extracellular Ca^{2+} was chelated with EGTA suggesting that influx of extracellular calcium in response to ER Ca^{2+} store depletion disrupts APP/Homer interaction. We are currently investigating whether store-operated channels or other calcium channels mediate extracellular Ca^{2+} entry that disrupts the interaction between APP and Homer. This study could contribute in our understanding of the role of Ca^{2+} in AD development and provide the molecular basis for developing novel prognostic/diagnostic tests as well as novel therapeutics.

This work is supported by a grant from the Alzheimer's Association USA (IIRG-09-133340).

WE03-11

ROLE OF NEPRILYSIN IN NEURONAL PLASTICITY AND MEMORY IN RATS

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The metallopeptidase neprilysin (NEP) has received significant attention in relation to the pathogenesis of Alzheimer's disease (AD) due to its ability to degrade amyloid-β peptide. NEP activity and expression in the brain cortex (Cx) decline with age and are further reduced in AD brains. We have recently shown that neuronal NEP expression is up-regulated by the amyloid-precursor protein intracellular domain (AICD) and down-regulated by histone-deacetylase (HDAC) and that an HDAC inhibitor valproic acid (VA) can up-regulate its expression. Using a model of prenatal hypoxia in rats we have found that NEP protein levels and activity are reduced in the Cx of affected animals. These animals also demonstrated learning and memory deficits. Moreover, i.c. injections of NEP inhibitors phosphoramidon and thiorphan to normal adult rats led to a decrease in cortical NEP activity and short-term memory deficits. Administration of VA to adult animals subjected to prenatal hypoxia restored their memory and NEP activity. The changes in NEP activity observed in these paradigms correlated with the number of labile synaptopodin-positive dendritic spines in rat brain Cx which reflecting the changes in neuronal plasticity and adaptive response of the neuronal network. We found that prenatal hypoxia, normal ageing or i.c. injections of phosphoramidon led to a decrease in the number of neurones possessing neuronal spines while injections of VA to hypoxic rats increased their number. Hence, we suggest that NEP plays an important role in neuronal plasticity and memory and its decrease after prenatal hypoxia or in the process of normal ageing correlates with the number of neuronal spines and changes in cognitive functions which decline with age and, to a greater extent, in AD.

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WE03-12

PROTEOMICS ANALYSIS OF THE MUTANT APP E693DELTA TRANSGENIC MICE HIPPOCAMPUS AND CORTEX

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Objective: Amyloid precursor protein (APP) mutations cause famillial Alzheimer's disease. Recently, we have reported that APP E693Delta transgenic mice show synaptic alternation, abnormal tau phosphorylation, glial activation and neuronal loss in the absence of amyloid plaques. However, it remains unclear whether APP E693Delta mutation causes the qualitative and quantitative changes. Thus, we aim to analyze the comparative protein expression and phosphorylation in the hippocampus and cortex of the APP E693Delta transgenic mice and non-transgenic littermate.

Methods: Samples prepared from mouse hippocampus or cortex were applied to two-dimensional gel electrophoresis (2DE), and the gels were stained with SyproRuby and a phosphoprotein sensor, ProQ Diamond. Quantitative differences in protein among samples were selected by using Prodigy software, and each protein spots were identified by MS analysis.

Results: The 2DE profiles showed more than 500 spots in the hippocampus and 500 spots in the cortex respectively. The differentially expressed 77 proteins were selected by using software, and significantly changed 39 proteins were identified in APP E693Delta transgenic mice hippocampus and cortex. The down-regulated proteins include proteins involved in cytoskeltal proteins, energy-related enzymes and synaptic component, which were coactosin-like protein, actin related protein, phosphoglycerate mutase 1 and alpha-synuclein. Among the up-regulated proteins were those also involved in cytoskeltal proteins, metabolism,

energy-related enzymes and synaptic component, which were profilin-2, transketolase and vacuolar adenosine triphosphatase. In addition, 9 phospho-proteins changed in the hippocampus and cortex were identified. The up-regulative phosphorylated proteins, which were glyoxalase and vacuolar adenosine triphosphatase. Identification of such differentially expressed proteins provides new target for future studies that will allow assessment of their physiological roles and significance in AD pathology.

WE03-13

ALZHEIMER'S DISEASE AMYLOID BETA-PROTEIN MUTATIONS AND DELETIONS THAT DEFINE NEURONAL BINDING

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Amyloid β-protein (Aβ) accumulation in neurons has been demonstrated to precede its formation as amyloid plaques in the extracellular space in Alzheimer's Disease (AD) patients. Consequently, intraneuronal Aβ accumulation is thought to be a critical first step in the fatal cascade of events that leads to neurodegeneration in AD. Understanding the structural basis of neuronal binding and uptake of Aβ might lead to potential therapeutic targets that could block this binding and the subsequent neurodegeneration that leads to the pathogenesis of AD. Previously, we demonstrated that mutation of the two adjacent histidine residues of Aβ40 (H13,14G) resulted in a significant decrease in its binding to PC12 cells and mouse cortical/hippocampal neurons (Poduslo, et al. 2010. PLoS One, **5**: e8813),. We now demonstrate that the decreased neuronal binding follows the mutation order of H13G < H14G22 (E22 deletion) in Japanese pedigrees and showed AD-type dementia has enhanced oligomerization without both fibrilization and amyloid plaque formation but contains extensive intraneuronal Aβ (Tomiyama, et al. 2010. J Neuroscience, **30**: 4845). Our PC12 assay showed that deletion of glutamate-22 of Aβ resulted in a six-fold enhancement of neuronal binding. This enhanced binding explains the high level of intraneuronal Aβ seen in this pedigree. Blocking the binding of Aβ to neurons may provide a novel therapeutic approach for preventing neurodegeneration in AD.

WE03-14

AMYLOID BETA PEPTIDE INHIBITS SMALL GTPASES PRENYLATION: A NEW MECHANISM OF Aβ-INDUCED NEUROTOXICITY

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Introduction: Alzheimer's disease (AD), the most common form of dementia, is characterized by the accumulation of amyloid beta (Aβ) peptide in the brain. Cholesterol is a key player in AD. It regulates Aβ production and the isoform 4 of the cholesterol transport protein apolipoprotein E (apoE4) is the only risk factor consistently associated with non-familial AD. Epidemiological studies suggested that treating humans with cholesterol lowering medications might reduce the risk of developing AD or help treat it. Statins, the most prescribed medications in North America, inhibit the mevalonate/cholesterol synthesis pathway. Randomized control trial studies concluded that statins could not be recommended for prevention of AD. The outcome regarding treatment of AD has not been published yet. Our studies indicate that Aβ42 itself inhibits the mevalonate pathway. We aim to investigate the link between Aβ, the mevalonate pathway and neurotoxicity.

Methods: We used cultured primary sympathetic and forebrain neurons exposed to oligomeric Aβ42. Cholesterol synthesis was examined by metabolic labeling using 3H-acetate. Cholesterol and Aβ subcellular localization were identified by confocal microscopy. Hoechst staining was used to test apoptosis. Protein prenylation in neurons and brains of mice models of AD (TgCRND8) was examined by Triton X-114 extraction and GDI-capturing. SREBP-2 cleavage was investigated by immunoblot analysis.

Results: (1) Aβ significantly inhibits cholesterol synthesis via inhibition of SREBP-2 cleavage. (2) Aβ also inhibits prenylation of small GTPases in neurons and mice brains. (3) Aβ induces intracellular sequestration of cholesterol in late endosomes and plasma membrane. (4) Addition of the isoprenoid GGPP significantly reduces cholesterol sequestration and largely prevents Aβ-induced neurotoxicity.

Conclusions: we identified a new mechanism of Aβ-induced neurotoxicity via inhibition of small GTPases prenylation. Our work suggests that the combination of statins with high levels of Aβ in AD brain may have detrimental synergistic actions.

WE03-15

GENOME-WIDE SCREEN FOR MODIFIERS OF Aβ42-INDUCED NEURODEGENERATION IN DROSOPHILA

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Alzheimer's disease (AD) is a progressive neurodegenerative disease. It is characterized by two neuropathological hallmarks: neurofibrillary tangles and extracellular plaques composed of amyloid beta peptides (Aβ).

The Aβ peptides are generated by proteolytic cleavage of Amyloid Precursor Protein (APP). The endoproteolysis is done by β-site APP-cleaving enzyme (BACE) and γ-secretases. The cleavage products are Aβ peptides of 40 and 42 amino acids length. While both peptides are found in senile plaques, Aβ42 is considered as main amyloidogenic peptide. In addition, Aβ42 is the predominant form of APP peptides found in senile plaques.

To investigate the molecular mechanisms underlying Aβ42-induced neurodegeneration, we are using an established AD model, the fruit fly (*Drosophila melanogaster*). APP and γ-secretases and are conserved in Drosophila. However, β-secretase-like enzyme (dBACE) activity is low. It cleaves human APP but not at the β-site, lacking significant homology.

Therefore, the transgenic fly model for AD directly expresses human Aβ42 fused to a secretion signal for extracellular localization under control of UAS/GAL4 dual activation system.

The over expression of Aβ42 in the nervous system results in progressive structural and behavioural phenotypes such as locomotor deficits, reduced lifespan and age-dependent neurodegeneration.

Importantly, expression of Aβ42 limited to the fly eye causes a rough eye phenotype (REP) due to a degeneration of photoreceptors. The severity of the REP thus indicates Aβ42-induced toxicity. The REP provides a tool to identify enhancers and suppressors of Aβ42-induced neurodegeneration, as they alter the REP severity. Thus a genetic screen for modifiers of Aβ42-induced neurodegeneration, as observed in the REP, was performed through utilization of a well-established genome-wide RNAi library. Only RNAi lines

silencing fly genes with human homologues were included. The modifier screen yielded about 50 RNAi lines silencing genes involved in transcriptional regulation, protein degradation, several signalling pathways and vesicular transport. Found modifiers are analyzed by histochemical, biochemical and molecular biology methods with respect to their functional aspects in the pathomechanisms in Alzheimer's disease.

WE03-16

PROCESSING OF AMYLOID PRECURSOR PROTEIN IS AFFECTED BY VEGF IN PRIMARY NEURONAL, ASTROCYTIC AND VASCULAR ENDOTHELIAL CELLS

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A large number of Alzheimer patients demonstrate cerebrovascular pathology, as well as enhanced cortical VEGF expression in vicinity to β-amyloid (Aβ) plaques, suggesting a link of VEGF upregulation and formation of Aβ.

Primary neuronal, astrocytic, and vascular endothelial cells that over express the Swedish mutation of human amyloid precursor protein (APP), were exposed by VEGF, and the effect on APP metabolism was examined: APP cleavage products sAPPβ, and Aβ, released into the culture medium, were assessed by ELISA.

Exposure of neuronal cells by VEGF for 24 hours led to reduced sAPPβ release, accompanied by decreased β-secretase activity 12 hours after VEGF exposure. Incubation of neurons by the VEGF receptor inhibitor SU5416 for 24 hours resulted in increased release of sAPPβ, and strikingly enhanced secretion of Aβ into the culture medium, accompanied by a significant increase in β-secretase activity, compared to controls.

In astrocytes, VEGF reduced the secretion of Aβ and sAPPβ into the medium after 6h and 24h of exposure, respectively, whereas incubation by SU5416 for 6 hours and 24 hours resulted in decreased secretion of Aβ and in reduced sAPPβ release into the medium after 24 hours of exposure, compared to controls.

Six hours after VEGF exposure of EC significant lower levels of sAPPβ were observed in the medium, while incubations with SU5416 for 6 hours and 24 hours resulted in decreased secretion of Aβ into the medium, and in lower sAPPβ level after 6 hours, compared to controls.

The SU5416-induced effects on APP processing could not be suppressed by VEGF, suggesting that SU5416 affects pathways that are apparently independent of VEGF receptor signaling. The data strongly support our hypothesis that VEGF may play a role in the pathogenesis of Alzheimer′s disease by affecting APP processing.

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WE03-17

ALTERATIONS OF GLYCOGEN SYNTHASE KINASE-3β ACTIVITY BY ALZHEIMER'S AMYLOID BETA IS MEDIATED BY CYCLIN DEPENDENT KINASE 5

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Glycogen Synthase Kinase-3β (GSK-3β) is involved in several intracellular molecular events. The alterations of this enzyme play an important role in pathomechanism of Alzheimer's Disease (AD). The molecular mechanism responsible for the regulation of GSK-3β is not fully elucidated. Phosphorylation of GSK-3β on Tyr216 is probably necessary for its activity and its enhancement induces cells death. Inactivation of GSK-3β by its phosphorylation on Ser9 is important for cells survival. In this study the short time effects of Amyloid β peptide (Aβ1-42) oligomers on the alteration of GSK-3β were investigated in PC12 cell line. Moreover, this short term effect of extracellular Aβ was compared with long term action of endogenously liberated Aβ in PC12 stably transfected with human gene for Aβ precursor protein (APP) bearing Swedish mutation (APPsw).

Our data indicated that Aβ1-42 (1 μM) added into PC12 cells for 24 h enhanced GSK-3β phosphorylation on Ser9 what is responsible for lowering of its activity. The inhibitor of Cyclin Dependent Kinase 5 (CDK5) BML-259 efficiently prevented this phosphorylation, indicating that alteration of CDK5 activity is involved in mechanism of GSK-3β inactivation. In APPsw cells long term liberation of Aβ leads to lowering of GSK-3β phosphorylation on Ser9 and to enhancement of its activity and in consequence higher MAP tau Ser396 phosphorylation. The lower activity of CDK5 in APPsw transfected cells may be responsible for this alteration of GSK-3β phosphorylation.

Our data presented that CDK5 plays a crucial but opposite roles in modification of GSK-3β activity in PC12 cells during short and long term action of Aβ. Moreover, our data indicated that independently of time and of extracellular or endogenous pool of Aβ, the prosurvival/adaptive processes and proapoptotic events are activated, and the proportion between them decided on the pool of cells that survive during different stage of Aβ toxicity and neurodegeneration.

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WE03-18

SMAD PROTEINS CONTROL TRANSCRIPTION OF CDK4 - IMPLICATIONS FOR ALZHEIMER′S DISEASE

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Neuronal development and differentiation are regulated by Smad proteins, which are factors of the canonical TGF-beta signalling pathway, linking receptor activation to transcription processes. Previously, we reported Smad2 and Smad3 expressed in hippocampal neurones of the adult human brain, being constitutively phosphorylated and predominantly localised in the nucleus. Under neurodegenerative conditions such as Alzheimer′s disease the subcellular localisation of their phosphorylated forms is heavily

raising the question whether a nuclear Smad deficiency in neurones might contribute to neuronal dedifferentiation which is accompanied by increased expression of various cell cycle proteins in neurones of AD patients. Supporting our previous data we also confirmed the reduction of Smad4 levels in AD brain and thus examined the role of Smad protein deficiency for the expression of the G1-phase protein cyclin-dependent kinase 4 (cdk4). Using a siRNA approach and electromobility shift assays we analyzed cdk4 promoter targeting of Smad proteins and validated the relevance of binding studies by luciferase reporter assays. Neuritic growth retardation by reduced Smad levels, which are accompanied by elevated cdk4 demonstrates the role of Smad deficiency for neuronal dedifferentiation.

WE03-19

TWO ACTIVE DOMAINS OF ACE ARE ESSENTIAL FOR Aβ43-CONVERTING ACTIVITY

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Amyloid β-protein (Aβ) varies in length at its carboxyl terminus. The longer Aβ species, Aβ42 or Aβ43, is highly amyloidogenic and responsible for the neurotoxicity and the early amyloid plaque formation that lead to memory and cognitive defects in Alzheimer's disease (AD). In contrast, a shorter Aβ species, Aβ40, is easier to be maintained as a monomer, which has a neuroprotective effect. We have demonstrated that angiotensin-converting enzyme (ACE) converts Aβ42 to Aβ40 and this activity is specifically located in its N-terminal active domain. Here we found that ACE with two active domains converts Aβ43 to Aβ41, whereas ACE with either N- or C-terminal active domain did not convert Aβ43 to Aβ41. A mixture of N- and C-terminal active domain of ACE also failed to convert Aβ43 to Aβ41. This study suggests that a certain carboxyl dipeptidase activity may require ACE with two active domains. We also demonstrated that Aβ43 and Aβ42, but not Aβ40, deposit in diffuse amyloid plaques in APP transgenic (Tg2576) mouse brain. This Aβ43-to-Aβ41-converting activity of ACE may also be involved in the regulation of brain Aβ deposition.

WE04 Neurodegenerative Disease

WE04-01

A RODENT MODEL OF BLAST-INDUCED MILD TRAUMATIC BRAIN INJURY

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Proximity to an explosion exposes one to rapid changes in air pressure, which can cause a mild traumatic brain injury (mTBI). Clinical data from recent military conflicts suggest that blast-induced mTBI may induce long-lasting changes in behavior, but the mechanism underlying this form of brain injury has not been elucidated. Recently, calpain-mediated disruption of the axon initial segment (AIS) was reported as a new mechanism of neuronal injury, but it is unknown if the AIS and other excitable domains of axons are susceptible to damage following blast-induced mTBI. Using a novel blast tube, we have exposed rodents to controlled blast overpressures. Two weeks following blast exposure, animals underwent behavioral testing and were sacrificed for examination of the AIS using immunofluorescence. We report here preliminary results from rodents exposed to a blast overpressure of 690 kPa, which lasted approximately 5 ms. Cognitive function of the animals was assessed using a novel object recognition task. Control animals spent significantly more time with the novel object while animals exposed to a blast spent equal amounts of time with the familiar and novel objects, indicating a disruption of learning and/or memory. Using immunofluorescence to stain for the AIS-specific cytoskeletal protein βIV spectrin and a novel counting technique, we do not see disruption of the AIS in the cortex, however we found a significant decrease in the length of the AIS in the animals exposed to a blast. Additionally, there is a significant increase in staining for the injury markers BAPP and Iba-1 in the corpus callosum. Preliminary results indicate that exposure to the rapid changes in air pressure caused by an explosion induce changes in rodents at both a behavioral and molecular level.

WE04-02

FUNCTIONAL ANALYSIS OF MIRNAS RELATED TO NFL MRNA THAT HAVE ALTERED EXPRESSION IN AMYOTROPHIC LATERAL SCLEROSIS

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The motor neuron degeneration that is the core feature of Amyotrophic Lateral Sclerosis (ALS) is associated with the formation of neurofilament aggregates and a selective suppression of the steady state levels of the low molecular weight neurofilament (NFL) mRNA. The preferential localization of NFL mRNA to degradative granules (P-bodies) in ALS affected lumbar spinal motor neurons suggests that the suppression of NFL mRNA levels is related to an increase in RNA degradation. MicroRNAs (miRNAs) are small endogenous non-coding RNAs that participate in mRNA degradation through base pairing interactions in the mRNA 3' untranslated region (UTR). We have investigated the functional relevance of miRNAs expressed at differing levels in sALS and controls using reporter gene assays. HEK293T cells were cotransfected with 1 of 3 plasmids containing the human NFL mRNA 3'UTR linked to the firefly luciferase gene and miRNA predicted to interact with the 3'UTR. Three NFL 3'UTR constructs were screened, the first encoding the human NFL 3'UTR homologous to the murine NFL (3'UTR-S, 1-286) and the others corresponding to the predicted isoforms 3'UTR-M (1-1380) and 3'UTR-L (1-1838). Our results suggest a potential role for these differentially expressed miRNAs in altered NFL mRNA stability seen in ALS.

WE04-03

DEFINING THE NEUROPROTECTIVE ACTIVITY OF ENDOGENOUSLY EXPRESSED AMYLOID PRECURSOR PROTEIN IN TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) is a leading cause of morbidity and mortality affecting an estimated 10 million people annually. The amyloid precursor protein (APP) is upregulated following TBI. Our previous studies showed that exogenously administered APP ectodomain reduced apoptotic cell death and axonal injury and improved motor outcome following diffuse TBI in rats. However, it's unknown whether endogenous APP plays a similar beneficial role following TBI. To investigate this, APP knockout mice (APP-/-) were compared to APP wildtype mice following two types of TBI; a focal lesion induced by a controlled cortical impact injury and a diffuse lesion caused by a weight drop model.

Results: Following both types of injury APP–/– mice showed impaired spatial memory when compared to APP+/+ mice, with a significantly increased latency to locate a previously learned escape hole in the Barnes Maze on all days tested post-injury. After the diffuse injury APP–/– mice had a small but significant rotarod motor deficit when compared to APP+/+ mice, whereas APP–/– mice after focal injury demonstrated significantly more foot faults on the ledged beam than APP+/+ mice on days 2–5 post-injury. These deficits correlated with increased neuronal injury, with APP knockout mice having significantly more degenerating hippocampal neurons than APP wildtype mice, as detected with Flouro Jade staining at 24 hours following the focal injury. This corresponded with a decrease in the number of surviving hippocampal neurons in APP knockout mice at 7 days post-injury.

Conclusions: The improved outcome in APP wildtype mice as compared to APP–/– mice indicates that the upregulation of APP post-injury is a protective response.

WE04-04

SECONDARY ACCUMULATION OF GANGLIOSIDES IN SPHINGOLIPIDOSIS

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Sphingolipid metabolism is deeply deregulated in several pathologies. This seems to be responsible of neurodegeneration, in sphingolipidosis. Here, we focus the attention on secondary alterations of sphingolipid metabolism that have been sporadically reported in the literature, in some sphingolipidosis.

We present a detailed analysis of the lipid composition in different tissues from the acid sphingomyelinase-deficient mouse (ASMKO), the animal model for Niemann-Pick disease type A, characterized by the accumulation of sphingomyelin (SM). The animal model of NPD type A, was developed using gene targeting and embryo transfer techniques.

Results show, together with a general accumulation of SM, an unexpected tissue specific selection of the accumulated molecular species of SM, and of GM3 and GM2 gangliosides, that cannot be solely explained by the lack of sphingomyelinase. We observed the preferential accumulation of SM molecular species with shorter acyl chains in the nervous system, but not in extraneural tissues. The unbalance toward C18/C16-fatty acid containing SM species was detectable as early as SM accumulation started, and monosialoganglioside accumulation followed immediately afterwards. These changes in sphingolipid patterns should thus represent the effect of secondary biochemical pathways altered as a consequence of a non-related primary cause. The mechanism underlying these changes still remains to be elucidated and is probably the result of changes in the expression and/or activity of more than one single enzyme, and/or of anomalies in the traffic of the substrate/product concentrations in multiple cellular compartments. Several pieces of evidence suggest that altered sphingolipid metabolism results in a non-physiological plasma membrane composition and organization, leading to altered plasma membrane-originated signalling pathways that could be relevant to the onset of cellular damage and of tissue pathology.

WE04-05

RECENT ADVANCES IN UNDERSTANDING COMMON MECHANISMS OF NEURODEGENERATION

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Over the last 5 years, there have been considerable advances in our understanding of the pathogenic mechanisms occurring in neurodegenerative conditions, indicating that common pathways are involved in multiple disorders. A substantial finding has been the demonstration that TDP-43 is a major component of the polyubiquitinated aggregates found in motor neuron disease/ amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitin positive inclusions (FTLD-U). Importantly, the same mutation can give rise to either ALS or frontotemporal dementia (FTD) indicating that common mechanisms occurring in different regions of the CNS can underpin these contrasting phenotypes. The identification of new mutations associated with the familial forms of ALS (FALS), notably, in TAR DNA binding protein (TARDBP), Fused in sarcoma (FUS) and vesicle-associated membrane protein/ synaptobrevin-associated membrane protein B (VAPB) and FTLD-U, such as progranulin and valsosin containing protein (VCP) provide considerable insight into the mechanisms of neurodegeneration. TDP-43 is the major component of the polyubiquitinated cytoplasmic inclusions characteristic of ALS and VAPB, an ER protein involved in the unfolded protein response (UPR), is depleted in ALS. More recently, we have reported a novel mutation in the D-amino acid oxidase gene (R199W DAO) associated with classical adult onset FALS. Motor neuron cell lines expressing this mutation show high levels of ubiquitinated aggregates and increased apoptosis compared to cells expressing the wild-type protein. As levels of D-serine are known to accumulate in the spinal cord in cases of sporadic ALS and in the G93A SOD1 mouse model of ALS, an abnormality in DAO, which metabolises D amino acids may exacerbate this process. Overall, studies in FALS have contributed to the elucidation not only of the beneficial pathways involved in the degradation of misfolded proteins e.g. UPR, ubiquitin proteasomal system and autophagy but also the conditions that trigger motor neuron death.

WE04-06

RGNEF IS A RNA BINDING PROTEIN THAT FORMS AGGREGATES IN AMYOTROPHIC LATERAL SCLEROSIS AND ACTS AS NFL MRNA DESTABILIZING FACTOR

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Amyotrophic lateral sclerosis (ALS) is a progressive disorder characterized by degeneration of motor neurons resulting in paralysis and death 3–5 years after diagnosis in most patients. Although the cause of the disease remains elusive, protein aggregate formation in motor neurons is a neuropathological hallmark including neurofilamentous (NF) aggregates. Recent evidence supports the hypothesis that alterations in NF RNA metabolism in motor neurons can lead to the development of these aggregates. In mice, p190RhoGEF, a guanine nucleotide exchange factor, interacts with a destabilizing region of low molecular weight neurofilament (NFL) mRNA providing stability to the transcript and is involved in NF protein aggregation observed in a RNA-triggered transgenic model of motor neuron disease. We recently identified RGNEF, a human homologue of p190RhoGEF, as a protein that forms skeins and aggregates in motor neurons affected from ALS patients. Here, we demonstrate that RGNEF exhibits GEF activity and is an RNA binding protein that can destabilize all 3'UTR predicted isoforms of NFL mRNA. This provides further evidence that RNA metabolism pathways are integral to ALS pathology and is the first described link between ALS and a protein with aggregates formation that is also a central cell signalling pathway molecule.

WE04-07

CYTOTOXIC EFFECTS OF ZINC ON CHOLINERGIC SN56 NEUROBLASTOMA AND C6 ASTROCYTOMA CELLS

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Zinc excess in the synaptic cleft may be one of early pathologic signals triggering chronic neurodegenerative events. This cation may accumulate in this brain compartment during excitotoxic

stimulation of glutamatergic terminals. Therefore, in such conditions postsynaptic cholinergic neurons as well as adjacent astrocytes, may became overloaded with Zn. The aim of this work was to find relationships between Zn accumulation and integrity of cholinergic and astroglial cells. Exposition of cAMP/retinoic acid-differentiated (DC) and nondifferentiated cells (NC) cholinergic SN56 neuroblastoma and astroglial C6 cells to Zn yielded its fast concentration dependent accumulation. It caused instant inhibition of pyruvate dehydrogenase, aconitase, isocitrate dehydrogenase and ketoglutarate dehydrogenase activities. In neuronal cells, Zn resulted in decrease of [Ca] in mitochondria and its increase in the cytoplasmic compartment. It was accompanied by suppression of cytoplasmic [acetyl-CoA] and proportional decreases in acetylcholine content and release. Zn accumulation caused concentration-dependent death of both neuronal and astroglial cells. After 24 hours exposition of SN56 cells to 0.15 mM Zn their death rates were equal to 35 and 50% for NC and DC at intraneuronal cation levels equal to 4.03 and 5.52 nmol/mg protein, respectively. In same conditions, the death rates of astroglial NC and DC were close to 1-2% only, at intracellular Zn levels of 1.3 and 2.5 nmol/mg protein, respectively. Higher, about 0.25 mM Zn levels were required to evoke death rates of astroglial cells, similar to those seen in neuronal cells. In such conditions Zn levels in astroglia were about 5.0 and 27.0 nmol/mg protein, respectively. Such differential sensitivity of astroglial and neuronal cholinergic NC and DC to Zn may be due to respective differences in densities of ZnT1 transporters in their plasma membranes, resulting in higher Zn accumulation in the latter. Higher levels of Zn may cause deeper inhibition of acetyl-CoA synthesis and energy production leading to proportionally greater cell injury. Supported by MWiSW NN401029937 and W-109 GUMed projects.

WE04-08

NITRATED HSP90 INDUCES APOPTOSIS IN MOTOR NEURONS BY A FAS-DEPENDENT MECHANISM

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Production of peroxynitrite and tyrosine nitration are associated with several pathologies, including neurodegenerative diseases, central nervous system trauma and stroke. Peroxynitrite induces apoptosis by a mechanism involving nitration of tyrosine residues. The goal of this study was to identify targets of peroxynitrite nitration responsible for cell death. Out of 17 proteins that are major targets for peroxynitrite, nitration of a single tyrosine residue on a 2–4% of the pro-survival chaperone heat shock protein 90 (Hsp90) activated a toxic gain-of-function that induced motor neuron and PC12 cells death. Nitrated Hsp90 did not impair the proteasome activity. Caspase inhibition completely prevented motor neuron death upon intracellular delivery of nitrated Hsp90. After intracellular delivery of nitrated Hsp90 to motor neurons, incubation with the Fas receptor decoy Fas:FC completely prevented motor neuron death. These results reveal that the Fas-pathway is activated by nitrated Hsp90 leading to downstream activation of caspases. In motor neurons, the activation of Fas initiates two downstream pathways, (1) an apoptotic pathway that involves activation of caspase 8, release of cytochrome c from the mitochondria and activation of caspase 9, and (2) a pathway that leads to the activation of p38-MAPK, expression of neuronal nitric oxide synthase and production of peroxynitrite. Incubation with the NOS inhibitor L-NAME did not prevent motor neuron death stimulated by nitrated Hsp90, indicating that the second Fas-activated pathway is not involved in nitrated Hsp90-induced cell death. Our results demonstrate that nitrated Hsp90 induces apoptosis through the activation of the Fas receptor and downstream caspases.

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WE04-09

FUNCTIONAL CONSERVATION OF THE PATHOLOGICAL EFFECTS OF HUMAN MUTATIONS IN KIF5A IN A DROSOPHILA MODEL OF AUTOSOMAL-DOMINANT HEREDITARY SPASTIC PARAPLEGIA

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Impairments in intracellular transport are the hallmark of many neurological diseases including hereditary spastic paraplegia (HSP). HSP is a genetically heterogeneous neurodegenerative disorder causing spastic weakness of the lower extremities. On the cellular level the disease is characterized by distal axonopathy that affects the longest axons in the corticospinal tract. At present at least 45 HSP loci have been described. Mutations in atlastin and spastin (accounting for around 50% of all HSP cases) as well as mutations in 6 other identified SPG genes: (kif5a, nipa, spatacsin, spastizin, spartin and maspardin) have been implicated in disturbance of axonal transport and membrane trafficking.

The fact that spinal neurons with the longest axons are selectively affected might be due to their morphology. They have substantial dependence on efficient axonal transport of organelles, molecules and signals to and from nerve terminals. Disturbances in anterograde and retrograde transport might interfere with efficient synaptic function/maintenance and maintenance of the axon itself.

Perturbations of axonal transport emerge as a common pathological mechanism in many HSP cases.

To test this hypothesis and to further probe the pathological mechanisms we generated a Drosophila model for SPG10, an autosomal dominant form of HSP. SPG10 is caused by mutations in KIF5A gene, which codes for the heavy chain of the neuronal microtubule motor Kinesin-1. The Kinesin-1 family represents the major anterograde motor complex.

WE04-10

LONG TERM SUBTHRESHOLD EXPOSURE TO THE ORGANOPHOSPHATE CLORPYRIFOS INDUCES NEURODEGENERATION AND SENSORIMOTOR DEFICITS IN MICE

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Despite the well documented cholinergic symptoms that are associated with acute anticholinesterase organophosphate (OP) pesticide poisoning, limited information exists on the consequences of chronic subthreshold exposures to OPs. The present study examines the effects of chronic subthreshold chlorpyrifos (CPF) exposures on the central and peripheral nervous system of mice focusing on the accompanying neurological deficits. CPF was injected s.c. (5mgr/kgr) over the course of 45 consecutive days on adult mice (treated animals) and control animals received equivalent injections of vehicle (DMSO). Both treated and control animals were examined once a week with a comprehensive test battery to quantify their motor and sensory functions. The test battery was comprised of the following motor and sensory tests: landing footsplay, footprint analysis, grip strength, body suspension, hot plate and von Frey hair pinch test. Treated animals showed significant impairments in limb coordination, balance and motor responsiveness, as well as allodynia and hypersensitivity and abnormalities in nerve conduction rates. Histological analysis revealed signs of neurodegeneration and/or apoptosis in both central and peripheral nervous system. More specifically, intraneuronal eosinophilic spherical inclusions resembling Lewis bodies, degenerative dark neurons containing neurofilament tangles (NFTs) and dystrophic axons were found in brain, whereas apoptotic neurons (caspase 3 and fractin positive) were mainly detected in the ventral horns of spinal cord and in brain stem nuclei. Axonopathy was also evident in sciatic nerves. In addition, mild astrogliosis (as determined by GFAP immunohistochemistry) was detected in brain stem nuclei, striatum and thalamus. Finally, immunohistochemical detection of NMDAR1 revealed down-regulation of this receptor in treated animals indicating that the glutamatergic pathway was also affected.

The results of this study are in consistence with the notion that long term subthreshold exposures to anticholinesterase pesticides such as CPF, have the potential to induce neurodegeneration and sensorimotor deficits, which could be subsequent to changes in cholinergic and other pathways resulting from alterations in nerve conduction rates.

WE04-11

PROTEOMIC AND HISTOCHEMICAL ANALYSIS OF PROTEINS INVOLVED IN THE DYING-BACK-TYPE OF AXONAL DEGENERATION IN THE GAD MOUSE

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Local axonal degeneration is a common pathological feature of peripheral neuropathies and neurodegenerative disorders of the central nervous system, including Alzheimer's disease, Parkinson's disease, and stroke; however, the underlying molecular mechanism is not known. Here we analyzed the gracile axonal dystrophy (gad) mouse, which displays the dying-back-type of axonal degeneration in sensory neurons, to find the molecules involved in the mechanism of axonal degeneration. The gad mouse is analogous to a null mutant of ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), is a deubiquitinating enzyme expressed at high levels in neurons, as well as testis and ovary. In addition, we recently discovered UCH-L1 binds to and stabilize mono-ubiquitin in neurons, and the level of mono-ubiquitin was decreased in neurons, especially in axons of the sciatic nerve in gad mice. The low level of ubiquitin suggests that the target proteins of the ubiquitin proteasome system are not sufficiently ubiquitinated and thus degraded in the gad mouse; therefore, these proteins may be the key molecules involved in axonal degeneration. Our proteomic analysis demonstrated that age-dependent accumulation of several proteins, including glyceraldehyde-3-phosphate dehydrogenase (GAPDH), in gad mice compared with wild-type mice. In histochemical analysis, GAPDH were localized throughout axons in both gad and wild-type mice, but GAPDH accumulated in the axons of gad mice. Furthermore, sulfonated GAPDH, a sensor of oxidative stress that elicits cellular dysfunction, and 4-hydroxy-2-nonenal, a majormarker of oxidative stress, were detected in gad mice. Our findings suggest that GAPDH may participate in a process of the dying-back-type of axonal degeneration in gad mice and may provide valuable insight into the mechanisms of axonal degeneration.

WE04-12

BIOMARKERS OF NEURODEGENERATION IN CEREBROSPINAL FLUID OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Reactivation of cell cycle and proteolytic disbalance are among putative key mechanisms of neuronal cell death in neurodegenerative diseases. The aim of this study was to search for markers of neurodegeneration related to proteolytic systems (calpain, caspase-3, cathepsin B) and aberrant cell cycle (cdk-1) in cerebrospinal fluid (CSF) of patients with amyotrophic lateral sclerosis (ALS). CSF samples from 75 ALS patients and 40 controls were investigated. The expression of cdk-1 was elevated in ALS CSF (maximum at early stage of bulbar form ALS and rapidly progressing ALS); it negatively correlated with the duration of ALS ($r=-0.68$ $p<0.02$). Calpain-like activities were detected at pH 7.4 and pH 5.5. Calpain-like activity at acidic pH as well as CSF calpain inhibitory activity was significantly increased in ALS CSF as compared with the control group, calpain inhibitor being represented by a peptide with molecular mass lower than 10 kDa. Cathepsin B inhibitory activity was significantly increased in CSF of ALS patient. This activity inversely correlated with the time period before the disease generalization and positively correlated with the level of phosphorylated heavy chains of neurofilaments (pNF-H, reliable marker of neuronal cell death, significantly increased in ALS patients). From CSF biomarkers studied, significance of diagnostic value (AUC) decreased in a row pNF-H (0.91), calpain-1 inhibitory activity (0.82), calpain activity at low pH (0.82), cathepsin B inhibitory

activity (0.80), cdk-1 expression (0.68), LDH activity (0.65). The results suggest an involvement of specific proteolytic systems and of cell cycle reactivation in neuronal death in ALS patients.

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WE04-13

DOWN-REGULATION OF CYCLIN-DEPENDENT KINASE 5 ACTIVITY BY MOOD STABILIZER VALPROIC ACID

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Cyclin-dependent kinase 5 (Cdk5) is a neuron-specific Ser/Thr kinase, which is activated by regulatory subunit p35.Over-activation of Cdk5 induced by cleavage of p35 with calpain is implicated in neuron death of various neurodegenerative diseases. In contrast, Cdk5 knockdown makes neurons vulnerable to stresses. Thus, appropriate regulation of the Cdk5 activity is essential for neurons to survive. Cdk5 also regulates synaptic activity. Long term potentiation (LTP) is easily induced in mouse brains lacking Cdk5. Cdk5 may determine the threshold of neuronal excitation. Recent reports suggest the involvement of Cdk5 in mental disorders. We thought perturbation of the Cdk5 activity is related to mental conditions. To approach this question, we investigated effect of valproic acid (VPA) on the Cdk5 activity in cultured neurons. VPA is a drug of choice for the psychiatric treatment. VPA decreased protein and mRNA expression levels of p35 in cultured neurons in a dose-dependent manner. VPA is a well known inhibitor for histone deacetylase (HDAC). To see whether the effect of VPA is mediated via HDAC inhibition, we used valpromide (VPM), a VPA analogue without HDAC inhibition activity, and trichostatin A (TSA), another HDAC inhibitor. TSA, but not VPM, reduced p35 expression at both levels of protein and mRNA, indicating that VPA decreases p35 mRNA via HDAC inhibition. In addition, proteasomal inhibitors MG132 and epoxomicin also suppressed the VPA-induced p35 reduction, indicating that VPA induces p35 protein degradation by proteasome.VPA administration decreased the behavior activity of mice in open field. We compared the effect of VPA on the protein amount of p35 and behavior. Acute administration of VPA induced behavior abnormality at 5 min earlier than 3∼6 hours when p35 protein was decreased. We would like to discuss a possibility of Cdk5-p35 modulation as a possible VPA target in therapeutic treatment of mental disorder.

WE04-14

GENOME-WIDE SCREEN FOR MODIFIERS OF TDP-43 INDUCED NEURODEGENERATION

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Cytoplasmic accumulation of TAR DNA binding protein-43 (TDP-43) was discovered as a major component of abnormal protein aggregates found in patients suffering from Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLD). Alterations in TDP-43 function/location might represent a common link of FTLD and ALS, strongly suggesting shared mechanisms underlying neuron loss in these diseases. TDP-43 is multifunctional protein involved in transcription, splicing, and mRNA stabilization. It is primarily localized in the nucleus but shuttles between nuclear and cytoplasm. In the pathological state TDP-43 is depleted from the nucleus and found exclusively in cytoplasmic aggregates. In aggregates, TDP-43 is cleaved, phosphorylated and ubiquitinated. A direct role of TDP-43 in neurodegeneration is highlighted by the fact that neuronal expression of human TDP-43 in Drosophila causes age and dose dependent locomotion deficits and early lethality. Moreover, targeted expression of TDP-43 to the developing fly eye results in a rough eye phenotype (REP). We used the REP to perform unbiased screen for modifiers of TDP-43 induced neurotoxicity. Our primary screen grouped modifiers candidates broadly into four funtional groups, which are mRNA-related, vesicle transport, nuclear transport and autophagy/unfolded protein response. Our results implicate that TDP-43 plays an important role in these areas specifically in the context of TDP-43 induced neurotoxicity, thus enhancing our knowledge on disease mechanisms, inherent/pathological functions and potentially opening new avenues for therapeutical strategies to cure TDP-43 proteinopathies like ALS and FTLD.

WE04-15

APOER2 TRAFFICKING AND ITS SIGNALING INDUCED BY REELIN IN NIEMANN PICK TYPE C1 DISEASE MODELS

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Introduction: NPC1 is a lipid storage disorder caused by genetic mutations in npc1 gene that causes accumulation of cholesterol and sphingolipids in late endosome/lysosomes and is characterized by progressive neurodegeneration. Previous studies show that there are alterations in the trafficking and signaling of some membrane receptors in this disease. However this area of research remains largely unexplored. The Apolipoprotein E receptor 2 (ApoER2), is highly expressed in neurons and exhibits a significant association with cholesterol enriched membrane domains or lipid rafts in different cells lines and also in neurons. ApoER2 binds to reelin, triggering a signaling pathway that regulates neuronal migration and positioning in the developing brain and dendritic ramification. In the adult reelin participates in neuronal survival, synaptic plasticity, and neurogenesis. Since the segregation of ApoER2 into lipid rafts could have functional implications concerning trafficking, and/or signaling we evaluated whether these events are altered in different models of the disease.

Methods: As models we used neuronal hippocampal primary cultures treated with U18666A (a well-known class-2 amphiphile, extensively used to mimic NPC phenotype) and CHO cells/null for NPC1 stably transfected with ApoER2. We evaluated receptor internalization and recycling, receptor signaling (by phosphorylation levels of downstream targets of ApoER2/Reelin), and ramification by Sholl's method. The expression of apoER2 and reelin in cerebellum of wild type and NPC1 KO mice were determined by qPCR.

Results: In cellular conditions miming NPC there was a decrease in ApoER2 surface levels, explained by a more efficient internalization and less recycling. Neurons treated with U18666A, exhibited less dendritic ramification while the number of apoptotic cells was increased. Both effects were partial but significantly rescued when

exogenous Reelin was added. ApoER2/Reelin signaling was partially but significantly active in our cellular models indicating that it could act as a survival mechanism in NPC disease, however we found relevant differences in the expression of ApoER2 and its ligand reelin in the brains of the NPC KO mice.
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WE04-16

CALPAIN PARTICIPATION IN MANGANESE NEUROTOXICITY, *IN VIVO*

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Manganese (Mn) is an essential metal ion, but chronic exposure to this metal can induce toxic effects on the central nervous system, causing a neurodegenerative disease known as manganism, prevalent among miners. Some symptoms of manganism resemble those of Parkinson's disease, such as muscle stiffness, tremor, dystonia and hypokinesia. Mn accumulates in brain structures such as the striatum, globus pallidus and substantia nigra, and a correlation between its accumulation, neuronal damage and impaired motor function, has been suggested. The toxic effects of Mn are associated with altered mitochondrial metabolism, increased reactive oxygen species and energy deficiency. In addition, evidence shows that Mn can alter glutamate (Glu) levels and the expression of Glu transporters, suggesting an excitotoxic death mechanism. Excitotoxicity is associated with calcium influx through Glu receptors and with the activation of calpain, a cystein calcium-dependent protease. In the present study we show that the intra-striatal injection of 100 nmol Mn produces neuronal death, event partially mediated by calpain. Calpain activity was determined by western-blot analysis of the appearance of the 150/145 kDa fragment as a result of the cut of alpha-spectrin by the active protease, and by the fluorometric method. Mn affects neurons in the early hours, the calpain inhibitor MDL-28170 significantly reduced the cell damage (51 percent) and protease activity (62 percent, western-blot data). Calcium influx through NMDA glutamate receptors, does not appear to be responsible for the activation of calpain, inhibiting these receptors with MK-801 or memantine, does not prevent neuronal damage or protease activation. Results suggest that Mn might either activate calpain directly, or through the alteration of the intracellular calcium levels. These results suggest that additional mechanisms to excitotoxicity are involved in the neurotoxicity of Mn.

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WE04-17

THE ROLE OF CHAPERONE-MEDIATED AUTOPHAGY FOR THE SELECTIVE DEGRADATION OF MUTANT HUNTINGTIN PROTEIN

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Aberrant Huntingtin protein (Htt) degradation is implicated in Huntington's disease (HD) pathogenesis because Htt accumulates in nerve cells. The aim of study is to verify the association with HD and chaperone-mediated autophagy (CMA). The cell model of HD was produced by being infected adenovirus Htt. Htt with 18 polyQ was wt type and Htt with 100 polyQ was mutant type. The overexpression or knocking out of LAMP-2A, Hsc/p70 in cells was made to change the level of CMA. The protein levels of LAMP-2A, Hsc/p70 and Htt and the relationships of proteins were observed. LAMP-2A and Hsc/p70 could be well colocalized with Htt by microscope and IP. Western blot analysis certified the decreasing of LAMP-2A or Hsc/p70 resulted in the accumulation of Htt, vice versa. The mechanism was studied with intact lysosome. The present results suggest that CMA plays an important role in the degradation of Htt and protects cells expressing Htt from development of the disease.

WE04-18

A CELL-BASED ELISA ASSAY TO MONITOR GLT-1 INTERNALISATION AND DEGRADATION

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The predominant astrocyte glutamate transporter GLT-1 (EAAT2) is down-regulated in numerous neurodegenerative diseases. We wish to understand the intracellular mechanisms underlying GLT-1 loss from the cell-surface of astrocytes, and identify compounds which can block these processes. We previously developed a construct of GLT-1 with an epitope tag in the large extracellular loop. Using a cell-line (HEK293) stably expressing V5-GLT1 we developed a two-step cell-based ELISA assay to measure, in sequence, cell-surface and total cellular GLT-1. Cells grown in 96 well white plates are treated, fixed in 4% paraformaldehyde then subjected to two rounds of antibody binding, first using a mouse anti-V5 antibody then, following permeabilsation with Triton X-100, with a rabbit anti-V5 antibody. Chemiluminescent detection is used to measure cell-surface and total GLT-1 levels. The assay shows high sensitivity and specificity. Using this assay, we show that Phorbol Ester (PMA), 10 nM induces a 25% loss of V5-GLT1 from the cell surface within 30 minutes, with concomitant loss of total GLT-1. The assay system was used to screen a number of protease inhibitors for their ability to block PMA-induced GLT-1 internalisation and degradation and identify a potential role for cysteine proteases in regulating GLT-1 internalisation. Next we transfected primary mouse astrocytes with V5-GLT1 and showed that the cell based ELISA assay can measure down-regulation of GLT-1 following a range of metabolic and pro-oxidative stimuli including hydrogen peroxide (100 μM). We have developed a system to facilitate the identification of regulators of GLT-1 protein internalization and degradation in astrocytes *in vitro*.

WE04-19

INVOLVEMENT OF MACROAUTOPHAGY IN MULTIPLE SYSTEM ATROPHY AND GLIAL CYTOPLASMIC INCLUSION BODY FORMATION IN OLIGODENDROCYTES

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Glial cytoplasmic inclusions (GCIs), originating in oligodendrocytes, are the histological hallmark of multiple system atrophy (MSA), an adult onset neurodegenerative disease with symptoms of Parkinsonism. GCIs positively stain for α-synuclein, ubiquitin and a variety of heat shock proteins (HSP), specifically the small HSP αB-crystallin. The accumulation of aggregated proteins in MSA remains an enigma, defects in the protein degradation systems may be involved. α-Synuclein degradation occurs by both, the proteasome and the autophagic pathways within lysosomes. Macroautophagy (MA) involves the sequestration of cytoplasmic contents and large aggregates in double-membrane vesicles, the autophagosomes, which can be probed for by LC3-immunoreactivity. During autophagosome formation endogenous LC3 is processed to LC3-I, which is converted to LC3-II. Its amount correlates with the number of autophagosomes. Cells can respond to blockage of the proteasome by upregulation of MA. Our data demonstrate that in oligodendrocytes, derived from the brains of newborn rats, MA is efficiently upregulated by treatment with the proteasomal inhibitor MG-132. LC3-positive vesicular structures accumulate and an increase in LC3-II immunoreactivity is detectable by immunoblot procedure. Furthermore, we performed immunohistochemistry on 7 MSA cases which indicates that LC3 immunoreactivity is present in α-synuclein-positive GCIs. LC3 staining was either diffusely present in the GCIs or occurred as distinct vesicular patches. Hence, oligodendrocytes have the capacity to upregulate MA during proteasomal blockage which during acute phases may be involved in the removal of protein aggregates. The presence of LC3-positive vesicles in GCIs of all MSA cases investigated indicates that MA is upregulated during pathogenesis, however, not efficient in cargo removal. This might be due to a persistent downregulation of proteasomal activity and chronic upregulation of MA, which renders the cells unable to further activate MA in response to stress situations which occur during disease progression and aging.

WE04-20

ACIDOSIS-INDUCED INCREASE OF ZINC NEUROTOXICITY IN CHOLINERGIC SN56 CELLS

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Excessive increase of Zn concentration in synaptic cleft is thought to be an early excitotoxic event in long lasting neurodegenerative process. Levels of extracellular Zn^{2+} ions increase significantly during hypoxia/ischemia-induced acidosis. However, the mechanism of Zn neurotoxicity in acidotic conditions remains unknown. The decrease of the culture medium pH from 7.4 to 6.5 with 15 mM lactic acid caused no alterations in the count and viability of both differentiated (DC) and nondifferentiated (NC) SN56 cholinergic neuroblastoma cells. Also activities of key enzymes of energy and acetylcholine metabolism, as well as acetyl-CoA levels were not affected by acidosis. Exposition (24 hours) to Zn in concentrations up to 0.15 mM caused no structural and functional impairment of cells cultured in media of pH from 7.4 to 6.8. However, at pH 6.5 Zn caused 60% increase of nonviable cell fraction and 50% decrease of cell count irrespective on the degree of their differentiation. In acidotic conditions (pH 6.5) 0.15 mM Zn exerted in about 50% inhibition of pyruvate, ketoglutarate and isocitrate dehydrogenases as well as aconotase activities both in NC and DC. Also activity of choline acetyltransferase in DC was decreased by Zn for about 25%, remaining unaltered in NC. In accord with enzyme suppression whole cell acetyl-CoA, ACh levels were also decreased by 0.15 mM Zn at pH 6.5 for about 40%. The results indicate that acidosis augments Zn cholinotoxicity through the inhibition of PDH and key enzymes of the TCA cycle resulting in depletion of acetyl-CoA and to its decreased synthesis in cholinergic neuron mitochondria. It would explain suppression of acetylcholine transmitter functions and loss structural integrity of cholinergic neurons in acidotic brain. Supported by MNiSW NN401029937, IP2010035370 and GUMed founds St 57.

WE04-21

ROLE OF MITOCHONDRIAL DYSFUNCTION IN EXCITOTOXIC SPINAL MOTONEURON DEGENERATION *IN VIVO*

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Glutamate-mediated excitotoxicity has been proposed as a probable mechanism leading to motoneuron death in amyotrophic lateral sclerosis (ALS). Previously, our group developed an *in vivo* model of spinal motoneuron excitotoxic death by means of microdialysis perfusion of α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) in the lumbar region of the rat spinal cord. This treatment produces a permanent paralysis of the ipsilateral hindlimb and death of motoneurons by a Ca^{2+}-dependent mechanism (J Neurochem, 2004, **89**: 988; Neuropharmacology 2007, **52**: 1219). To study the possible participation of mitochondrial function deficiencies in this motoneuron degeneration, we have tested the neuroprotective effect of the energy metabolic substrates pyruvate, lactate, α-ketobutyrate, β-hydroxybutyrate and creatine, coperfused with AMPA. These treatments prevented the paralysis and motoneuron damage, and preserved motor function in the rotarod test, suggesting that mitochondrial energetic deficiencies are involved in motoneuron death. In addition, we studied oxygen consumption and transmembrane potential in mitochondria isolated from the ventral horn of the lumbar spinal cord of three groups of rats: treated with AMPA, AMPA + pyruvate, and Krebs–Ringer medium as controls. The AMPA-treated group showed decreased oxygen consumption, ADP-dependent respiratory control and transmembrane potential, and pyruvate prevented these functional deficits. Our results suggest that mitochondrial dysfunction plays a crucial role in spinal motoneuron degeneration induced by overactivation of AMPA receptors *in vivo*. These mechanisms could be involved in ALS motoneuron degeneration.

Supported by DGAPA, UNAM (IN215610) and CONACYT (60322 and 128229). L.D.S.-C. and S. G. -C. are recipients of a scholarship from CONACYT.

WE04-22

A GENOME-WIDE SCREEN FOR MODIFIERS OF POLYGLUTAMINE-INDUCED NEUROTOXICITY IN DROSOPHILA MELANOGASTER

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Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD) belongs to the group of polyglutamine (polyQ) neurodegenerative diseases and is the most prevalent autosomal dominant cerebellar ataxia worldwide. A highly variable polyglutamine tract is thought to confer toxicity upon the otherwise unrelated proteins causing polyQ diseases. Apart from the glutamine expansion, the physiological function and cellular context of these proteins and their interaction partners appear to be crucial for the specific pathogenesis and course of the disorders. In order to elucidate the molecular disease mechanisms triggered by trinucleotide repeats, we intended to identify genetic interactors enhancing or suppressing polyQ toxicity. Therefore, we targeted expression of a human Ataxin-3-derived polyQ transgene to the Drosophila compound eye. The resulting photoreceptor degeneration induced a rough eye phenotype (REP) in adult flies. Eye-specific silencing of specific genes (all fly genes with a human homolog, ca. 8000 genes) by RNAi was utilized to identify genetic interactors of the REP. Changes in the observed REP are likely to originate from the knockdown of the RNAi target. Thus, silenced candidate genes are capable of modifying polyQ-induced neurotoxicity. The gene products we discovered in this manner represent various biological pathways and molecular functions. We conducted secondary investigations with this set of candidate genes to gain more insight into the mode and quality of the interactions. Our results are likely to shed further light on the molecular pathogenesis of Machado-Joseph disease and the role of Ataxin-3 and its modulator proteins in this process.

WE04-23

THE ROLE OF P53 AND DRAM1 IN AUTOPHAGY ACTIVATION AND CELL DEATH INDUCED BY MITOCHONDRIA DYSFUNCTION

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In vivo administration of the mitochondrial inhibitor 3-nitropropionic acid (3-NP) produces striatal pathology mimicking Huntington disease (HD). However, the mechanisms of cell death induced by metabolic impairment are not fully understood. The present study investigated the role of the p53 target gene damage-regulated autophagy modulator 1 (DRAM1) in autophagy activation and cell death induced by 3-NP. DRAM1 was induced in rat striatum after stereotaxic injection of 3-NP. Induction of DRAM in striatum was blocked by the p53 inhibitor pifithrin-alpha (PFT). In A549 cells 3-NP treatment also induced expression of DRAM1 protein levels. Morphological and biochemical analyses demonstrated activation of autophagy in striatal cells as evidenced by increased formation of autophagosomes, the expression of active lysosomal cathepsin B and D, microtubule associate protein light chain 3 (LC3) and conversion of LC3-I to LC3-II. 3-NP also induced elevations in pro-apoptotic proteins Bax and PUMA. In A549 cells, knock-down DRAM1 reduced LC3-II and BAX protein levels. PFT treatment significantly inhibited 3-NP-induced striatal damage. Similarly, 3-NP-induced DNA fragmentation and striatal cell death were robustly attenuated by the autophagy inhibitor 3-methyladenine (3-MA) and bafilomycin A1 (Baf A1). In the present study, 3-NP caused cell death of A549 cells was significantly attenuated by knock-down of DRAM1, and autophagosome clearance was prevented as a result of a selective impairment of autolysosome acidification and cathepsin activation. These studies suggest that p53 and DRAM1 plays an important role in mitochondria dysfunction induced autophagy activation and cell death.

WE05 Psychiatric Disorders and Drug Abuse

WE05-01

PROTEOMIC PROFILING OF PLASMA AND PERIPHERAL BLOOD MONONUCLEAR CELLS OF FIRST ONSET SCHIZOPHRENIA PATIENTS

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Schizophrenia affects almost 1% of the population worldwide with devastating consequences for affected individuals and their families. Although there is strong evidence of the involvement of various neurobiological mechanisms in the pathogenesis of schizophrenia, it remains a disorder with an incompletely understood aetiology. In addition, schizophrenia's genetic complexity impedes the establishment of animal and cellular models specific to the core of the disease. The identification of biological markers at the onset of the disease is a fundamental step towards a better understanding of the pathogenesis of schizophrenia along with their potential for objective diagnostic and prognostic tests. In this study we performed a comparative proteomic analysis of peripheral blood mononuclear cells (PBMCs) from 15 drug-naive schizophrenia patients with first onset psychosis and 12 controls. We identified 45 differentially expressed proteins, 12 of which were downregulated and 33 upregulated in schizophrenia-derived samples. A group of the altered proteins have been strongly related to mental illness elsewhere, such as apolipoprotein A1, transthyretin, and syntaxin binding protein that reconfirmed previous proteomic studies in blood and CSF of first-onset psychosis patients. Most interestingly, many new putative disease-associated proteins were identified, including cytoskeleton-related proteins, molecular chaperones and signal transduction proteins. Some of these protein alterations may reflect the components of abnormal neurochemical pathways in schizophrenia.

WE05-02

BRAIN REGION-SPECIFIC GLUTATHIONE REDOX IMBALANCE AND INCREASED DNA OXIDATION IN AUTISM

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Autism is a severe neurodevelopmental disorder that causes impairment in language, cognition and social interaction. Accumulating evidence suggests that oxidative stress may provide a link between susceptibility genes and environmental stressors in the pathophysiology of autism. DNA is a major target for free radicals (reactive oxygen species: ROS)-induced damage. 8-hydroxy-2'-deoxyguanosine (8-OH-dG) is formed during oxidative DNA damage through the oxidation of guanosine bases in DNA. Glutathione (GSH) is the most important endogenous antioxidant in human tissues, which neutralizes ROS and participates in detoxification and elimination of environmental toxins. Glutathione in its reduced state (GSH) and oxidised disulfide form (GSSG) are the primary determinants of redox status in all human cells. A decrease in GSH-to-GSSG redox ratio is a marker of oxidative stress. In this study, we compared DNA oxidation and glutathione redox status in postmortem brain samples from the cerebellum and frontal, temporal, parietal and occipital cortices from autistic subjects and age-matched normal subjects. DNA oxidation, assessed by quantitation of 8-OH-dG, was significantly increased by two-fold in frontal cortex, temporal cortex, and cerebellum in individuals with autism as compared with control subjects. On the other hand, its levels in parietal and occipital cortex were similar between autism and control groups. Reduced GSH, increased GSSG, and a decrease in redox ratio of GSH/GSSG were observed in the cerebellum and temporal cortex in autism group compared with control group. Such changes were not observed in frontal, parietal and occipital cortices between autism and control groups. These results suggest brain region-specific glutathione redox imbalance in autism, and that oxidative stress differentially affects selective brain regions in autism. Increased oxidative damage coupled with reduced antioxidant status in specific brain regions may in part, contribute to the development of autism.

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WE05-03

BRAIN REGION-SPECIFIC CHANGES IN ACTIVITIES OF PROTEIN KINASE A, PROTEIN KINASE C AND MAP KINASES IN REGRESSIVE AUTISM

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Autism is a severe neurodevelopmental disorder that is characterized by impaired language, communication and social skills. In regressive autism, children first show sign of normal social and language development but eventually lose these skills and develop autistic behavior. The rate of regressive autism has varied from 15% to 62% of cases in different studies. Protein kinases are essential in G-protein coupled receptor-mediated signal transduction that is involved in neuronal functions, gene expression, memory, and cell differentiation. We studied the activities of protein kinase A (PKA), protein kinase C (PKC) and mitogen-activated protein kinases (MAP kinases) in the postmortem frozen brain regions, i.e., cerebellum, and cortices from frontal, temporal, parietal and occipital regions from autism with regressive autism, autistic subjects without clinical history of regression, and age-matched control subjects. The activities of PKA and PKC, and expression of PKA c-α (a catalytic subunit of PKA assessed by western blotting) were significantly decreased in the frontal cortex of individuals with regressive autism compared to developmentally normal subjects and autistic individuals without regression. Further studies in the frontal cortex showed that the levels of non-phosphorylated forms of MAP kinases (JNK, MEK 1 and P38) were not affected in individuals with regressive and non-regressive autism. However, the levels of

activated forms of these MAP kinases, i.e., their phosphorylated forms were increased in regressive autism. Such changes were not observed in temporal, parietal and occipital cortices and cerebellum in subjects with regressive autism. No significant difference in the activities of PKA and PKC or expression of PKA c-α was observed between non-regressed autism and control groups. These results suggest that regression in autism may in part, be caused by oxidative stress and altered PKA/PKC-mediated phosphorylation of proteins that are associated with cell signaling.

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WE05-04

CALPASTATIN REDUCES METHAMPHETAMINE-INDUCED INDUCTION IN C-JUN PHOSPHORYLATION, BAX AND CELL DEATH IN NEUROBLASTOMA SH-SY5Y CELLS

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Methamphetamine (MA) is one of the most commonly abused substances in today's society. Many studies have shown that the process of cell death induced by MA involves with the reception of death signals, an increase in pro-apoptotic proteins (Bax) and an activation of cysteine protease death pathway. The objective of this study is to investigate the neuroprotective effects of calpastatin against MA-induced toxicity in SH-SY5Y neuroblastoma cells by observing cell viability, phosphorylation of transcription factor, c-Jun (phospho-c-Jun) and levels of Bax and Bcl-2. We found that MA significantly decreased cell viability in SH-SY5Y cultured cells. Conversely, increase in phospho-c-Jun and Bax/Bcl-2 ratio was observed in MA-treated cells. Calpastatin reversed the toxic effect of MA by increasing cell viability, reducing phospho-c-Jun and Bax/Bcl-2 ratio in MA-treated cells. These results indicated that calpastatin has the capacity to reverse an activation of death process in MA-treated dopaminergic cell lines.

WE05-05

MAJOR DEPRESSIVE DISORDER: POLYMORPHISM OF GENES LINKED WITH SURVIVAL AND REPAIR OF NEURAL CELLS IN HUMAN PATIENTS

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Major depressive disorder (MDD) is caused by neurochemical imbalances in the regions of brain controlling mood, anxiety, cognition and fear. In genetics of psychiatric disorders, a lot of correlations is not yet solved. It is commonly anticipated that variants in many genes may contribute to the onset and mode of progression of MDD. Brain-derived neurotrophic factor (BDNF) is one of the critical regulators in neuronal growth, survival and diferenciation of neuronal cells. Genetic studies of BDNF Val66Met gene polymorphism in relationship with MDD led until now to nonconsistent results. The methylenetetrahydrofolate reductase (MTHFR) gene Ala677Val polymorphism reduces MTHFR activity, which is associated with elevation of blood plasma homocysteine. Reduced activity of MTHFR has been linked to schizophrenia, affective disorders, and depression. DNA repair gene XRCC1 (X-ray repair cross-complementing group 1) Arg399Gln and XPD (Xeroderma pigmentosum coplementation group D) Lys751Gln polymorphisms are positively associated with different forms of cancer, however little is known about their association with MDD. Methods: Selected polymorphisms were determined by PCR–RFLP method.

Results: The BDNF Val/Val genotype (72.2%) was more frequent in the patients in comparison with the controls (45.9%). Patients with MDD had a higher frequency of the Ala/Val and Val/Val genotype (47.1%) of MTHFR gene than the control group (38.1%). We observed positive association BDNF 66Val and MTHFR 677Val allele which was significantly associated with MDD risk. Allelic association analysis of XRCC1 and XPD did not show show significantly higher risk in patients with MDD. Conclusion: In central Slovak population are manifested a selective BDNF and MTHFR polymorphisms which are associated with an increased risk of MDD. This work was supported by MZ-2007/55-UK-16 grant and by project " Identification of novel markers in diagnostic panel of neurological diseases "code: 26220220114, co-financed from EU sources and European Regional Development Fund.

WE05-06

SYNAPTIC PROTEIN-PROTEIN INTERACTIONS AS POTENTIAL ALCOHOL TARGETS IN MOUSE CORTEX

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Protein-protein interactions (PPIs) are important for virtually every process in a living cell: They participate in many physiological processes, and are essential for neurotransmission in the brain. Growing evidences suggest that clusters of gene expression profiles correlate with and predict PPIs.

Alcohol exposure alters the expression pattern of several genes encoding proteins required for normal synaptic function and crucial for diverse synaptic events, including neurotransmitter vesicle transport and targeting, scaffolding, trafficking and targeting of synaptic proteins.

With the present study, we aim to define alcohol-sensitive synaptic PPIs and to determine if these complexes can be modified as a result of excessive alcohol consumption.

We used an interaction proteomics approach, (co-immunoprecipitation, immunoblotting, and LC-MS/MS mass spectrometry) to identify novel PPIs in cortical membranes prepared from alcohol-naïve C57BL/6J mice using calcium-activated potassium channel (BKCa), dynamin-1, syntaxin-1A, synaptosomal-associated protein of 25 kDa (SNAP-25), and synaptobrevin-2 (VAMP-2) as bait proteins.

Our results highlight novel important interactions among synaptic proteins, including the dynamin-1 associations with BKCa and with VAMP-2. Plus, we found that BKCa, SNAP-25 and VAMP-2 share many interacting protein partners encoded by genes which have been consistently reported to be differentially expressed following alcohol excessive consumption. We are currently processing cortices from C57BL/6J mice subjected to ''Withdrawal-Induced Drinking, 2-bottle choice'' (WID-2BC) alcohol dependence paradigm. Using co-immunoprecipitations followed by semiquantitative MS analysis (Isotope Tagging for Relative and Absolute Quantification, iTRAQ), we are testing the effect of the development of alcohol dependence on the identified synaptic PPIs.

Investigating alcohol action on multiple synaptic PPIs could provide new insights into cell function adaptations in the presence of alcohol. This study could potentially accomplish a wide ranging significance for neurochemistry and other areas of neurobiology and addiction research, and ultimately help to define new molecular sites for therapeutic interventions.

WE05-07

RELATIONSHIP BETWEEN CADPS2 SPLICING PATTERN AND SERUM BDNF LEVEL IN AUTISM

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Autism is a severe neurodevelopmental disorder, while its underlying molecular mechanisms remain largely unknown. A disturbance in Ca^{2+}-dependent activator protein for secretion 2 (CADPS2) -mediated BDNF release in the brain may contribute to autism susceptibility, because an aberrant alternatively spliced CADPS2 mRNA that lacks exon 3 was reported in some autistic patients, and BDNF plays a key role in many aspects of brain development and function, including the formation of synapses and circuits. We investigated the effect of CADPS2 mRNA splicing pattern on plasma BDNF level in children with autistic disorder. We also examined relationship between CADPS2 splicing pattern and behavioral characteristics of the autistic children. There was little relationship between the splicing pattern and plasma BDNF level. Though statistically insignificant, possible correlations between CADPS2 mRNA splicing pattern and characteristic behaviors in autistic children were suggested.

WE05-08

ANALYSIS OF CORTICAL DEVELOPMENT IN MICE LACKING DBZ

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DBZ (Disc1-Binding Zinc-finger protein) interacts with DISC1 (Disrupted-in-schizophrenia 1), a promising susceptibility gene for major mental illness. The DISC1/DBZ interaction regulates neurite outgrowth in primary cultured hippocampal neurons and PC12 cells. DBZ is a brain-specific protein and highly expressed in cerebral cortex, hippocampus and striatum in adult rodent brain. Here we examined the expression of DBZ mRNA in developing cerebral cortex from E10 to adult mice by in situ hybridization. DBZ mRNA started to express at E12 and increased gradually with the cerebral cortex development.

WE05-09

EFFECT OF ANTIPSYCHOTIC MEDICATION ON OLIGODENDROCYTE PROGENITOR CELL *IN VITRO*

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In the developing brain, oligodendrocyte progenitor cells (OPCs) proliferate, migrate, and differentiate into mature oligodendrocytes (OLGs) capable of myelinating axons. Recently, OPCs have been identified as an abundant and widespread population in the adult as well as in the developing animal. Current researches favor the hypothesis that these OPCs in the adult brain are able to proliferate and differentiate into myelinating OLGs as in development.

We previously reported OLG dysfunction in the animal model of schizophrenia (Makinodan et al.). . If altered OLG function is an etiological factor or involved in the pathogenesis of schizophrenia, OPCs may respond to antipsychotics during recovery process. In the present study, we used primary OPC cultures from optic nerve of P1-2 Wister rat pups to investigate the direct effects of haloperidol (typical antipsychotic) and olanzapine (atypical antipsychotic) on the proliferation and differentiation of OPCs. Our results showed that (1) olanzapine treatment significantly increased the viable cell number of OPCs when compared to the haloperidol treatment at relatively high concentrations, (2) olanzapine treatment also resulted in less expression levels of MBP mRNA than haloperidol treatment, (3) these pharmacological effects may be mediated via ERK signaling pathway.

Our findings suggest a new neural mechanism of antipsychotic action of olanzapine, leading to establish a role for oligodendrocyte-lineage cells in the etiopathology and treatment of schizophrenia.

WE05-10

DIFFERENTIAL ACTIVATION OF NUCLEUS ACCUMBENS SHELL VS. CORE DURING ACQUISITION OF COCAINE- VS. SOCIAL INTERACTION CPP

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Is social interaction as a non-drug (''alternative'') reinforcer able to help addicts reorient their behavior away from the drug of abuse? We could show (Fritz et al. 2011, Addiction Biology, in press) that social interaction is able to speed up extinction of cocaine conditioned place preference (CPP), reverses place preference even if cocaine CPP training is continued, and can prevent reacquisition

of cocaine CPP. In the present study, we investigated the contribution of the core (AcbC) and the shell (AcbSh) subregions of the nucleus accumbens, and of the basolateral amygdala (BLA) to the acquisition/expression of social interaction CPP vs. cocaine CPP.

Male Sprague–Dawley rats were single-housed and CPP-trained for cocaine (15 mg/kg i.p.) alone, for an i.p. saline injection paired with social interaction (weight- and gender-matched partner within the confines of the CPP chamber) alone, or concurrently for cocaine- vs. social interaction CPP. We performed excitotoxic lesions of the AcbC, the BLA, or the AcbSh. All groups were tested for CPP expression and Zif268 immunocytochemistry.

Lesioning the AcbSh before the concurrent acquisition and expression of social interaction CPP vs. cocaine CPP shifted the preference toward cocaine whereas AcbC and BLA lesions shifted the preference toward social interaction. These findings suggest that the inactivation of the AcbC or the BLA is sufficient to inhibit the incentive salience of drug-associated stimuli, and increases the motivation for the non-drug stimulus social interaction. In addition, different brain areas seem to be engaged during the acquisition and expression of social interaction CPP as compared to the reversal of reacquisition of cocaine CPP by social interaction.

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WE05-11

STUDIES OF THE LIFE AND DEATH OF SEROTONERGIC NEURONES IN A PRIMARY MURINE NEURONAL-GLIAL CULTURE

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While the essential roles of brainstem serotonin (5-HT) neurones are well recognized, they have attracted renewed interest because of new evidence for key roles in autism, sudden infant death syndrome and regenerative neurobiology. Remarkably there is a dearth of knowledge about the life and death of 5-HT neurones presumably because they have been so difficult to study in primary culture. Our aims were to develop methodology to allow the establishment of primary cultures containing 5-HT neurones, and to employ these cultures as a suitable model to evaluate the patterns of injury and death of 5-HT neurones. A coronal section of ventral brainstem, containing principally rostral groups of 5-HT-containing raphe nuclei, was dissected from the brains of E14-16 mice. Procedures for the digestion and isolation of cells were based upon those previously employed for mesencephalic dopamine cells (Mercer et al. 2005. Biochem Pharmacol, **69**: 339) wherein after resuspension in an optimized Neurobasal medium, cells were plated in 96 and 48 microwell plates, (densities $0.1–0.2 \times 10^6$ cells/well). Media changes were as previously described (Zagami et al. 2009. Glia, **57**: 119). Cultures contained increasingly mature neurones in the presence of astrocytes over the period of culture (1–12 days). Immunocytochemistry ($n = 6$) for 5-HT, microtubule associated protein-2 (MAP2) and glial fibrillary acidic protein (GFAP) identified neurones which were MAP-2 and 5-HT positive, presumed 5-HT neurones, and mature astrocytes. The neuritic tree and primary axons of 5-HT neurones became increasingly complex over the 12 days in culture. Analyses of cell viability ($n = 3$) and morphology (Hoechst & TUNEL staining), indicated that 5-HT neurones were sensitive to injury by oxidative stress (hydrogen peroxide EC50 100 μM) and autophagy (rapamycin EC50 15 μM) indicative of recruitment of different forms of programmed cell death. Our primary culture is amenable to expansion of the 5-HT neuronal sub-population and is suitable for dissection of the death modalities of 5-HT neurones.

WE05-12

COCAINE-INDUCED CHANGES IN MESOCORTICOLIMBIC TRACE METAL CONTENT AND DISTRIBUTION

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The trace metals zinc (Zn), iron (Fe), and copper (Cu) play an essential part in normal brain functioning as these metals are integral structural and biological components of many proteins responsible for proper neural physiology. Imbalances in brain trace metal content have been associated with diseases like Alzheimer's, Parkinson's, epilepsy, amyotrophic lateral sclerosis, prion diseases and ischaemia. The underlying cause associated with metal imbalance and disease has been attributed to oxidative stress and energy metabolism mechanisms but other mechanisms may be involved. In animals, Cu-chelating enzymes have been used for inducing states of demyelination and recent use of the Cu-chelating enzyme cuprizone in mice has been reported to induce schizophrenia-like behavior and neurobiological changes in dopamine (DA) which are reversed by antipsychotic treatment. In this paper we use ultra-sensitive and high-resolution synchrotron X-ray fluorescence microspectroscopy to present evidence of changes in brain content of the trace metals Zn, Fe, and Cu after repeated cocaine exposure and in response to genetic manipulation of the dopamine transporter gene (DAT). Cocaine (10 mg/kg i.p for 4 days) increased striatal Zn, Fe, and Cu content while DAT heterozygote and knockout mice showed decreased striatal Zn, Fe, and Cu content. DAT heterozygote and knockout mice also showed selective changes in one or more metals in cortical and other limbic regions. These findings show that disturbances in trace metals in reward-related brain areas can result from cocaine exposure and genetics that involve abnormal DA signaling. Such findings raise implications about the involvement of trace metal imbalances in DA-related neuropsychiatric disease and mood and motivational disorders like depression, schizophrenia, and addiction.

WE05-13

IDENTIFICATION OF MECP2-TARGET SYNAPTIC MOLECULES ASSOCIATED WITH PATHOGENESIS OF RETT SYNDROME

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Rett syndrome is an autistic disease caused by MECP2 mutations, encoding methyl-CpG-binding protein 2. MeCP2 protein is bound to the methylated promoters of genes to suppress their expression, indicating that pathogenesis of Rett syndrome is deregulation of the target genes in neurons. Although several genes are now thought to be target genes for MeCP2, the involvement of these genes in the classical neuropathology of RTT remains unclear. Using genome microarray approach, we found 22 genomic regions overlapped with MeCP2 binding, DNA methylation, and histone modification in human oral cancer cell lines. Of these regions, we confirmed MeCP2-binding and DNA methylation in the upstream regions in three genes, LIN7A, PCDHB1 and PCDH7, in SH-SY5Y cells derived from human neuroblastomas. PCDHB1 and PCDH7 were down-regulated by MeCP2 (but not by mutant or deleted MBD), up-regulated under MeCP2 reduction with siRNA in SH-SY5Y cells and in brains from Mecp2 KO mice and PCDHB1 was up-regulated in postmortem brains from Rett syndrome patients. On the other hand, Lin7a was down-regulated under MeCP2 reduction with siRNA in Neuro2a cells and in brains from Mecp2 KO mice. These results suggest that MeCP2 function as both an activator and a repressor of transcription. Since these three molecules are essential for synaptogenesis during brain development, abnormal regulation of synaptic molecules via MeCP2 deficiency may cause autistic features, as well as mutations of synaptic molecules.

WE05-14

EFFECT OF MELATONIN ON AMPHETAMINE-INDUCED CALPAIN-DEPENDENT TOXICITY IN SUBSTANTIA NIGRA OF POSTNATAL RATS

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Amphetamines are potent central nervous system stimulants that activate multiple signaling cascades, especially on the midbrain dopaminergic system, and may cause neuronal cell degeneration. It has been reported that the mechanism of amphetamine-induced neurotoxicity and neuronal cell degeneration involve multiple processes. Recent studies show that amphetamine can induce an increase in intracellular calcium (Ca^{2+}) concentrations. High levels of Ca^{2+} inside the cell activate several intracellular signaling cascades including calpain, a calcium-dependent cysteine protease. Moreover, calpain activity is also regulated by an endogenous calpain inhibitor named calpastatin. Therefore, we investigated the effect of amphetamine on the level of calpain, calpain-specific spectrin breakdown products (calpain-specific SBDP), and calpastatin in postnatal rat substantia nigra, where most of the dopaminergic neurons are located. Western blot analysis was used to determine whether repeated amphetamine administration altered the levels of calpain, calpain-specific SBDP, calpastatin, and phosphorylated tyrosine hydroxylase, a rate-limiting enzyme in dopamine biosynthesis. The present study shows that amphetamine decreases the level of tyrosine hydroxylase phosphorylation and calpastatin but increases calpain activation and formation of calpain-specific SBDP in substantia nigra of rats. Moreover, the protective effect of melatonin was also investigated in this study. Our results show that pre-administration with melatonin provides a protective effect against amphetamine-induced alteration caused by amphetamine, demonstrated by a restoration in tyrosine hydroxylase phosphorylation and calpastatin levels and reduction in calpain activation and formation of calpain-specific SBDP. In conclusion, the neurotoxicity caused by amphetamine leads to the degeneration in neuronal cells, whereas melatonin provides a protective effect against amphetamine-induced calpain-dependent death pathway in substantia nigra of rats.

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WE05-15

DOPAMINE D4 RECEPTOR EXON III POLYMORPHISM IN ATTENTION-DEFICIT/HYPERACTIVITY DISORDER

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Dopamine plays an important role in a modulation of behavior and cognition via fronto-striato-cerebellar circuits in which dopamine receptors represent an important link in dopamine signaling. In the third exon of the dopamine D4 receptor (DRD4) gene, there is a polymorphism consisting of a variable number of tandem repeats (VNTR). It was postulated that variations of DRD4 might result in differences in second messenger coupling or signal transduction. DRD4 variants with 6 or more repeats are assumed to be associated with different psychiatric disorders such as addictive behavior, novelty seeking and attention-deficit/hyperactivity disorder (ADHD). ADHD is a complex disorder diagnosed through the persistence of three behavioral symptoms: impulsivity, hyperactivity and/or inattention. Aim of our study was to determine the distribution of the DRD4 genotypes in children with ADHD and in healthy children in order to investigate and to clarify the role of DRD4 in the etiology of ADHD. DRD4 genotypes were determined using polymerase chain reaction (PCR) and agarose gel electrophoresis in 139 children with ADHD (according to DSM-IV criteria) and in 134 healthy, age matched, children. There was no gender difference in the DRD4 genotypes. DRD4 genotype frequencies differed significantly between healthy children and children with ADHD, and 7-repeats allele was found more frequently in ADHD children. There were no significant differences in DRD4 genotype frequencies between different types of ADHD. Children with predominantly inattentive type of ADHD had significantly higher frequency of 7-repeats allele compared to healthy children. These findings confirmed an important association between the DRD4 VNTR polymorphism and the expression of ADHD, and/or symptoms of inattention, suggesting that genetic variations in the

third exon of DRD4 gene may be, among other factors, a risk factor in the development of ADHD.

WE05-16

THE ASSOCIATION BETWEEN CATECHOL-O-METHYLTRANSFERASE GENE VARIANTS AND CHILDHOOD ATTENTION DEFICIT HYPERACTIVITY DISORDER

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Attention deficit hyperactivity disorder (ADHD) is a multifactorial, highly heritable developmental disorder characterized by behavioral symptoms of impulsivity, hyperactivity and/or inattention. The enzyme cathecol-O-methyltransferase (COMT), which is responsible for the degradation of catecholamines, could have an important role in genetic susceptibility to ADHD. COMT could play a significant role in modulating dopamine levels in the prefrontal cortex which was implicated in ADHD etiology. We aimed to analyze the association of COMT Val108/158Met (rs4680) polymorphism which affects COMT activity with ADHD features since genetic studies of the functional Val158Met polymorphism in ADHD have been inconsistent. The study included 113 medication free children with ADHD diagnosed according to the DSM-IV criteria and 187 children without psychiatric diagnoses and free of medication that served as control group. Genotyping was done using the TaqMan SNP Genotyping Assay. We found an association between Val108/158Met polymorphism and the symptoms of ADHD in male, but not in female children. Lack of association in female children is probably due to a small number of female patients with ADHD, which represents the limitation of this study. We also found an association with ADHD features when comparing Val carriers to Met/Met homozygotes in male children. These differences were especially significant when comparing male patients with combined type of ADHD with healthy control subjects. Our results confirmed the association between COMT variants and ADHD in male children, which was due to the higher frequency of Met/Met homozygotes in children with ADHD compared to healthy controls. These results suggest that carriers of the high activity COMT variant are less prone to develop ADHD. This study also suggests that COMT Val108/158Met polymorphism is associated with the ADHD combined subtype.

WE05-17

SERIAL EXPOSURE TO STRESS AND METHAMPHETAMINE ALTERS THE STRUCTURE AND FUNCTION OF THE BLOOD BRAIN BARRIER

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Methamphetamine (Meth) is a widely abused psychostimulant. Since drug abuse and stress are comorbid events, we believe it is important to study Meth in the context of stress. Stress and Meth have similar acute effects and in combination produce enhanced long-term damage to dopaminergic and serotonergic terminals. High doses of Meth alone have also been shown to increase blood brain barrier (BBB) permeability, but only at doses which result in hyperthermia and seizures. The BBB is comprised of endothelial cells, astrocytes, pericytes and the surrounding basement membrane. Endothelial transmembrane proteins, such as claudin-3, -5 and occludin, are responsible for the formation of endothelial tight junctions. In addition, astrocytic and endothelial transmembrane proteins, such as β-dystroglycan, stabilize tight junctions. Nothing is known with regard to the effects of Meth on structural components of the BBB or the effect of the combination of stress and Meth on the BBB. We hypothesized that serial exposure to mild stress and a moderate dose of Meth will alter the structural components and function of the BBB. We observed an increase in cleaved β-dystroglycan and a decrease in expression of the tight junction proteins 24 hours after treatment, indicating altered BBB structure. Interestingly, decreases in some BBB structural proteins persist up to 7 days after treatment. Furthermore, an increase in endogenous IgG staining persisted up to 7 days after Meth and stress, indicating altered BBB function. Future studies will investigate the mechanisms responsible for the BBB disruption. This is the first study illustrating that serial exposure of stress and Meth alters expression of tight junction proteins and BBB permeability. In addition, this is the first evidence of long-term BBB alterations in response to stress and Meth.

WE05-18

ALTERED MALE HYPOTHALAMIC-PITUITARY-TESTICULAR AXIS AS A CONSEQUENCE OF PRENATAL STRESS EXPOSURE

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Exposure to adverse events during early life can alter midbrain dopaminergic system (DA) activity. In man the onset ages of these disorders closely parallels the onset and decline ages of the reproductive period. Moreover androgens can influence forebrain DA system. Previously we demonstrated that prenatal stress (PS) exerts an impairment of DA metabolism in limbic brain areas especially after puberty indicating a particular sensitivity of DA system to variations in gonadal hormones peaks. The aims of this study was to evaluate aspects of the hypothalamic-pituitary-testicular (HPT) axis status of males rats exposed to PS. Stress consisted on a 3 daily- 45 minutes restraint session from day 14th of gestation to delivery. Follicle stimulating (FSH), Luteinizing (LH), Testosterone (T) and 5-a Androstane-3alpha, 17betha-Diol (DIOL) hormones serum levels of 28, 35, 45, 60 and 75 days old male progeny were determined by radioimmunoassay. Testes of 35 and 60 days old animals were processed for histological morphometric measures and for androgen receptor (AR) quantification by western blot technique. PS diminished FSH levels at post natal day (PND) 28 in comparison with control group (C) and reduces LH levels at PND 28 and 75. T serum levels were diminished at 75 days old PS rats. DIOL levels were increased at PND 28 and 45 on PS group.

Additionally, the rate of spermatogenesis was accelerated on PS rats. However, the mean Leydig cell's number was reduced on PS rats. These findings suggest that stress during gestation induces long term effects on the male progeny HPT axis. Since gonadal hormones can influence DA system development by inducing plastic changes in the brain, a disbalanced hormone milieu might be responsible for the DA metabolism alterations observed in our studies.

WE05-19

EFFECT OF MELATONIN ON METHAMPHETAMINE-INDUCED INDUCIBLE NITRIC OXIDE SYNTHASE OVEREXPRESSION IN SH-SY5Y DOPAMINERGIC CELLS

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Methamphetamine (METH) is a strong addictive drug and commonly abused worldwide. This psychostimulant drug caused the disturbances of dopaminergic and serotonergic neuron in several brain areas. Exposure to METH was found to induced oxidative stress, reactive oxygen species (ROS), reactive nitrogen species (RNS) and neuronal inflammation. The role of METH-induced neuroinflammation stills unclear. The expression of inflammatory genes and mediators such as inducible nitric oxide synthase (iNOS), cyclooxygenase (COX) -2, interleukin (IL)-1β, interleukin (IL)-6, Tumor necrosis factor (TNF)-α etc indicated the inflammatory state. All of these inflammatory mediators are known regulated by NF-κB transcriptional factor. In this study we investigated whether METH causes inflammatory effects of and the involvement of the NF- κB pathway. The result showed that METH significantly induced the iNOS expression in a dose-dependent manner and activated NF-κB phosphorylation. Furthermore, we also examined the anti-inflammatory property of melatonin. The results showed that melatonin significantly decreased iNOS protein expression through the inhibition of activated NF-κB. These results demonstrate the cellular mechanisms of neuronal inflammation induced via NF- κB -dependent pathway, and the potential role of melatonin on protection of neuroinflammation.

WE05-20

METHAMPHETAMINE TOXICITY INDUCES RAS SIGNALING CASCADES IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS

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Several pieces of evidence have been demonstrated that methamphetamine (METH) toxicity can induce neuronal cells degeneration in the brain. However, the molecular mechanisms underlying this degenerative process are still unclear. In addition, Ras signaling cascade modulates death processes in several cell types have been continually reported. In this study, Ras-dependent death signaling cascade was investigated in METH-induced neuronal cell death in dopaminergic SH-SY5Y cultured cells. The results of present study showed that METH significantly decreased cell viability and phosphorylation of tyrosine hydroxylase (phospho-TH) in SH-SY5Y cells. Conversely, METH-induced increase in phosphorylation of c-Jun (phospho-c-Jun) was observed in SH-SY5Y cells. An inhibitor of the enzyme that catalyzes the farnesylation of Ras proteins, farnesyltransferase inhibitor (FTI-277) was able to reverse the toxic effects of METH on reduction in cell viability and phospho-TH, and induction in phospho-c-Jun. These results might emphasize an involvement of Ras signaling cascades in METH-induced toxicity in dopaminergic cells.

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WE05-21

ALTERATION OF CIRCADIAN GENES EXPRESSION IN RAT HIPPOCAMPUS AFTER AMPHETAMINE INJECTION

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Amphetamine (AMPH) is a psychostimulant drug whose chronic abuse may be associated with impairment of memory and adaptive changes in gene expression. AMPH addicts often suffer from circadian rhythm disorders, paranoia and psychosis. The hippocampus is an important brain area that involves in memory, behavior and emotion. In addition, the circadian expression of many genes has been found in the hippocampus. In the present study, we investigated whether AMPH affected the expression of clock gene, *Period1 (Per1)*, brain-derived neurotrophic factor (BDNF) and melatonin receptor (MT1) in rat hippocampus. Rats were daily treated with saline or AMPH (5 mg/kg, i.p.) during the daytime for 7 days. The results from real-time polymerase chain reaction (PCR) showed that the circadian peak of *Per1* was 4 hours phase-advanced and the expression of BDNF shifted the highest peak delay by about 2 hours, whereas the circadian rhythm of MT1 was abolished after AMPH administration. These findings suggest that AMPH-induced alteration of *Per1*, BDNF and MT1 expression in hippocampus may be related to the mechanism for AMPH-induced behavioral changes and impairment of memory. The further investigation should be performed to clarify the mechanism of these effects that may help to identify novel targets for the treatment of addictions.

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WE05-22

REVERSAL OF COCAINE CONDITIONED PLACE PREFERENCE BY SOCIAL INTERACTION

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A main challenge in the therapy of drug dependent individuals is to help them reactivate interest in non-drug-associated activities. Among these activities, social interaction is doubly important because treatment adherence itself depends on it. We developed a rat

animal experimental model based on the conditioned place preference (CPP) paradigm in which only four 15-minute episodes of social interaction with a gender- and weight-matched male conspecific (i) reversed CPP from cocaine to social interaction despite continuing cocaine training and (ii) prevented the reinstatement of cocaine CPP (Fritz et al. 2011, Addiction Biology) . The reversal of CPP from cocaine to social interaction was enhanced by the sigma1 receptor antagonist BD1047 with an ED50 of 0.0036 mg/kg (i.p.) (Fritz et al. 2011. Pharmacology, **87**:45–48). Social interaction also reversed cocaine CPP-induced expression of the immediate-early gene zif268 in the nucleus accumbens shell, the central and basolateral amygdala and the ventral tegmental area (Fritz et al. 2011, Addiction Biology). These findings suggest that social interaction, if offered in a context that is clearly distinct from the previously drug-associated ones, may profoundly decrease the incentive salience of drug-associated contextual stimuli. In the present study, we investigated if the two subregions of the nucleus accumbens (Acb), the core (AcbC) and shell (AcbSh) would differentially affect CPP for cocaine vs. social interaction. Animals were concurrently trained for CPP to cocaine and social interaction (mutually exclusive stimulus presentation during training). We are currently investigating which type of neuron in the AcbC is affected by this reversal.

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WE05-23

5-MCA-NAT INCREASES ENDOGENOUS DOPAMINE LEVELS IN CHICK RETINA DEVELOPMENT

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Our best goal is investigate the physiology of the QR2 (NQO1) enzyme (NRH: quinone oxidoreductase II, EC 1.10.99.2), and the role of the melatonin binding site MT3 in this context. This enzyme is a dimeric flavoprotein highly homologue with QR1. Functional similarities have been postulated between QR1 and QR2, such as xenobiotic action. Only one *in vitro* work shows for QR2 a different function in relation to QR1, as being a cathecol quinone reductase. A role in catecholamines (adrenaline, noradrenaline and dopamine) metabolism could explain the relationship between QR2 and nuropsichiatrics disorders. Our published results show that the activation of the MT3 by 5-MCA-NAT (1–10 nM, concentrations selective to MT3) increases cAMP levels in embryonic and mature chick retinas. As the activation of the MT3 by 5-MCA-NAT does not modify the cAMP contend in CHOK1 cells that do not content dopamine, we hypothesized that the increase in cAMP rises observed in our experiments is in function of the 5-MCA-NAT binding MT3 /QR2 action on dopamine metabolism. Immunohistochemistry for QR2 enzyme in retinas from post-hatched chick retinass showed positive immunoreactivit to NQO1 (SC-1875 antibody), visualized with Texas red (Santa Cruz Biotechnology, Inc.). The dopamine accumulation in 10, 14 embryonic days old and mature chick retinas, incubated in DMEM (0.1 mM ascorbic acid), stimulated or not with 5-MCA-NAT (1, 3, 10 nM), plus/or not luzindole 5 μM, plus/or not 4-PPDOT 10 nM (pH 7.4, 37°C) was extracted and measured by Dopamine ELISA (GenWay) protocols. Our results showed an increase in endogenous dopamine accumulation in relation to control retinas by 5-MCA-NAT (1, 3 nM) that was blocked by luzindole, but not by 4-PPDOT. The magnitude of the 5-MCA-NAT effect was dependent on the developmental stage observed. Thus, we suggested that 5-MCA-NAT (1–3 nM) can be recovering dopamine in the retina.

WE05-24

MELATONIN ATTENUATES METHAMPHETAMINE-INDUCED DECREASE IN VESICULAR MONOAMINE TRANSPORTER 2 (VMAT2) MRNA IN SK-N-SH CELL LINES

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Methamphetamine (METH) is widely used stimulant drug with high abuse potential producing long term decreases in dopaminergic functions. Vesicular monoamine transporter2 (VMAT2) is a key protein in regulating intra-neuronal dopamine homeostasis and vesicular turnover. It packages cytoplasmic dopamine into synaptic vesicles for storage and subsequent release. In the present study we examined the effect of METH on VMAT2 in SK-N-SH dopaminergic cell line. By western blot analysis we found that VMAT2 protein expression in METH treated group was significantly reduced when compared to control group. In this study we also show neuroprotective effects of melatonin which acts as pervasive and powerful antioxidant. Pretreatment with melatonin prevents reduction in VMAT2 expression when compared to METH-treated group. To have a better understanding and to know whether this decrease in protein expression occurs due to changes at the transcriptional level, we analyzed the mRNA levels by RT-PCR and found marked reduction in VMAT2 mRNA levels in METH-treated group. On pre-treating with melatonin the decrease in VMAT2 mRNA level was prevented to a significant level showing the neuroprotective effects of melatonin. We also determined the interaction of VMAT2 with other drugs like lobeline and reserpine which interact at different sites of this protein affecting the dopamine homeostasis. As VMAT2 very well qualifies to be a marker of dopamine neuronal integrity, drug discovery targeting VMAT2 may provide new insights to the underlying neurochemical mechanisms of psychostimulant abuse and neurodegenerative disorders.

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WE05-25

KINETIC STUDY ON DNA METHYLATION OF AUTISM SUSCEPTIBILITY GENE, SHANK3, IN THE DEVELOPING MOUSE BRAIN

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Shank3, a multidomain protein containing SH3 and PDZ domains, is localized in the postsynaptic density, and interacts with various synaptic molecules including PSD-95 and glutamate receptors. Furthermore, SHANK3 gene is reported as a major

causative gene of 22q13.3 deletion syndrome, showing severe language and speech delay, mental retardation, hypotonia and autistic feature. Therefore, it has been thought that Shank3 plays important roles in the formation and function of synapse in the developing brain and is involved in higher brain function. The SHANK3 gene has five CpG islands (CpG island-P, CpG island-2, -3, -4, -5) whose methylation is involved in tissue specific expression of SHANK3 gene. However, DNA methylation of CpG islands in the developing brain remains unknown. In this study, we examined DNA methylation of five CpG islands in the developing mouse brains (E17-12w) by using Hpa II-McrBC-PCR method, and confirmed that CpG island-P was unmethylated and CpG island-3 was methylated in all developing stages. In contrast, the rate of methylation in CpG island-2, -4, and -5 significantly increased after postnatal day 7. To elucidate the related molecules involved in DNA methylation of SHANK3 gene, we here focused on methyl CpG binding protein 2 (MeCP2), which was identified as a responsible gene for Rett syndrome and has been thought to regulate gene transcription, mRNA splicing, and chromatin structure. We examined whether the MeCP2 binds to methylated CpG islands of SHANK3 gene using a chromatin immunoprecipitation (ChIP) assay, and found that MeCP2 bound to the CpG islands-2, -3 and -4 at postnatal day 14. We recently found mutations within a CpG island of SHANK3 gene in autistic patients with mental retardation. To clarify the effect of methylation of SHANK3 gene in the developing brain and the involvement in development disorders including autism and mental retardation, further study is now on going.

WE06 Mechanism of Neuroprotection

WE06-01

RESTORATIVE EFFECT OF ACETYL-L-CARNITINE ON BEHAVIORAL DEFICITS AND NEUROTOXICITY INDUCED BY A NEUROTOXIC DOSE REGIMEN OF METHAMPHETAMINE

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Methamphetamine (METH) is a highly addictive psychostimulant drug that injures monoaminergic neurons and results in behavioral impairments in humans and animals. Acetyl-L-carnitine (ALC) is an endogenous quaternary ammonium compound that forms part of the intracellular carnitine system, plays a vital role in the mitochondrial oxidation of fatty acids, and shows a protective and regenerative action profile on the nervous tissue after toxic or traumatic injuries. The purpose of this study is to examine whether ALC can reverse the METH-induced neurotoxicity and behavioral deficits. Male ICR mice were treated with METH (4%D75 mg/kg s.c., 2 hour apart) or saline. Behavioral tests including novel location recognition test (NLRT), novel object recognition test (NORT), and social interaction were examined 7 days later to validate the behavioral effects of METH. Subsequently, ALC (30 or 100 mg/kg, i.p.) was administered once daily for seven consecutive days. ALC significantly restored the cognition deficits, social withdrawal, and lower levels of tyrosine hydroxylase in the striatum after METH treatment. In addition, METH reduced glial cell line-derived neurotrophic factor (GDNF) expression in the hippocampus and this effect was reversed by ALC. These findings suggest that ALC might exert its neurorestorative effects, at least in part, through GDNF and have therapeutic potential for the treatment of behavioral abnormality in METH abusers.

WE06-02

SENP-1 IS INVOLVED IN THE REGULATION OF CELL DEATH IN OXYGEN/GLUCOSE-DEPRIVED NEURONES

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Post-translational modification by Small Ubiquitin-like MOdifier (SUMO) proteins is essential for the integrity of eukaryotic cells. Rapid changes in global SUMO conjugation occur in cells subjected to stress and it is hypothesised that this may represent a defence response. Here we show that metabolic stress caused by oxygen and glucose deprivation (OGD) leads to increases in SUMO-1 and SUMO-2/3 conjugation in cultured rat neurones. To investigate whether these changes contribute to neuroprotection we, overexpressed the catalytic domain of the deSUMOylating enzyme SENP-1 to down-regulate SUMOylation. Decreasing global SUMOylation in this way markedly increased OGD-induced cell death compared to overexpression of the inactive SENP-1 mutant (C603S). This is consistent with SUMOylation being involved in the protection of cultured neurones from OGD. Interestingly, while SUMO modification enhanced cell survival, OGD also decreased degradation of SENP-1 resulting in an overall increase in SENP-1 protein. Taken together these data indicate that the neuronal response to OGD involves both regulated SUMOylation and deSUMOylation. Given the net increase in global SUMO conjugation, the raised levels of SENP-1 may be required for maturation of SUMO proteins and also for stress-induced deSUMOylation of specific SUMO substrate proteins. **Support:** Royal Society.

WE06-03

PROX1 SUPPRESSES THE GROWTH OF NEUROBLASTOMA CANCER CELLS

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Neuroblastoma is a pediatric tumor that originates from precursor cells of the sympathetic nervous system. Notch1 signaling plays a critical role in neuroblastoma pathogenesis, being a target for a potential therapy, which is currently under intense pre-clinical and clinical investigation. Under physiological conditions, activated Notch signaling inhibits neuronal differentiation and maintains precursors of the autonomic and central nervous system in a proliferative state. We have recently shown that a cross-inhibitory interaction between Prox1, a homeobox transcription repressor, and Notch1 controls the fine balance between proliferation and differentiation of neural precursor cells during embryonic development (Kaltezioti et al., PLoS Biology, 2010) . Thus, Prox1 is a candidate gene with a critical role in modulating Notch1 signaling and could be involved in suppressing malignant transformation. Consistently, it was very recently reported that Prox1 is expressed in higher amounts in human neuroblastoma with favorable prognosis, while Notch1 expression shows exactly the opposite correlation. In agreement, here we provide evidence that Prox1 blocks proliferation and promotes differentiation of human and mouse neuroblastoma cells. By BrdU incorporation assays and Phospho-HistoneH3 immunostainings, we showed that Prox1 exerts a strong antiproliferative effect on Neuro2A, SH-SY5Y and primary neural precursor cells. Clonal analysis, TUNEL assays and FACS-based analysis demonstrate that Prox1 has an anti-apoptotic effect. Most important, the repressive function of Prox1 on Notch1 cannot fully explain its anti-proliferative effect on neuroblastoma cells, i.e. constitutive activation of Notch1 signaling only partially rescues the anti-proliferative effect of Prox1. To define the Prox1-induced genetic program that mediates these effects, we have constructed an inducible Prox1-overexpression system (Tet-On) in Neuro2A. We are currently using this system in conjunction with RNA-Seq technology to further analyze the full transcription program and signalling pathways regulated by Prox1. Moreover, we are currently studying the ability of Prox1 to modulate the migratory/metastatic properties of neuroblastoma cells *in vitro* and *in vivo* by allotransplantations. Elucidation of these mechanisms should provide useful insights into neuroblastoma carcinogenesis and treatment.

WE06-04

NDFIP1 PROTECTS NEURON FROM DEATH FOLLOWING BRAIN INJURY

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The removal of harmful proteins is an important mechanism for ensuring neuron survival following injury. Ndfip1 is an important player in the ubiquitination and degradation of target proteins through the Nedd4 ligase pathway. Therefore genetic manipulation of Ndfip1 expression would be expected to modify the animal response to brain injury. In this project, we examine the effect of brain injury on animals lacking a copy of the Ndfip1 gene. Ndfip1 heterozygous mice and wild-type littermates were used. The mice received a closed head injury using an electric weight-drop device and euthanized 24 hours later. The lesion volume was quantified in serial coronal sections, using 2,3,5-triphenyltetrazolium chloride (TTC) staining. There is significant increased of cortical lesion volume ($p < 0.05$) in Ndfip1-heterozygous mice ($n = 7$) compared to their wild-type littermates ($n = 7$). The reduction of Ndfip1 in the brains of heterozygous mice leads to increased susceptibility to injury, resulting in larger injury areas. Ndfip1 is an important player for neuron protection following brain injury.

WE06-05

NEUROPROTECTIVE EFFECTS OF NON-ACTIVATED N9 MICROGLIAL CELLS IN CO-CULTURE WITH SN56 CHOLINERGIC NEUROBLASTOMA CELLS

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Microglial cells, through the proinflammatory mediators are key elements initiating inflammatory reactions in the brain. They are linked with patomechanism of Alzheimer's and other neurodegenerative diseases. The aim of this work was to investigate modifying effects of non-activated migroglia on co-cultured cholinergic neuronal SN56 cells subjected to common neurotoxic signals. Chronic exposure of nondifferentiated SN56 cells (NC) to 0.175 mM Zn caused inhibition of puryvate dehydrogenase (PDH) activity (30%), as well as decrease of acetyl-CoA level (37%), choline acetyltransferase (ChAT) activity (23%) as well as loss of cell viability (36%). In differentiated (DC) cells Zn remaining detrimental effects on PDH but aggravated suppression of acetyl-CoA and inhibition of ChAT activity, increasing them to about 50%. Under same neurotoxic conditions, N9 microglial cells cultured on isoporated inserts and added to neuronal cultur dishes, overcame neurotoxic effect Zn maintaining control levels of cell viability, acetyl-CoA and PDH activity both in NC and DC. Suppresive effects of Zn on ChAT activity was partially reversed by inserts N9. Addition of resveratrol (0.005 mM) augmented beneficial effect of N9 cousing increase of ChAT activity to control levels. These data sugest that in some specific conditions non-activated microglia may protect neuronal cholinergic cells against neurotoxic insults. Supported by MNiSW NN401029937, IP2010035370, GUMed ST-57 and W-185, projects.

WE06-06

GLOBAL CHANGE IN HIPPOCAMPAL PROTEOME ON EXPOSURE TO ENRICHED ENVIRONMENT DURING HYPOBARIC HYPOXIA

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Exposure to an enriched environment promotes neurochemical, structural and neurophysiological changes in the brain and is associated with enhanced synaptic plasticity and improved hippocampal dependent learning. On the contrary hypobaric hypoxia is known to cause memory impairment and decreases synaptic plasticity. Using a global proteomics-based approach we have now been able to reveal the altered expression of a diverse range of hippocampal and cortical proteins following exposure to an enriched environment during hypobaric hypoxia. Sprague Dawley rats (8 weeks) were subjected to a 7 day hypoxic exposure in which they were housed in either nonenriched or enriched conditions. Whole protein extracts from pre frontal and cerebral cortex were then isolated and subjected to differential gel electrophoresis. Of the 1645 resolvable protein spots detected in this study, 34 spots (2.1% of the detectable proteome) were significantly altered in abundance following exposure to an enriched environment with significant fold change. Following in-gel tryptic digestion and Maldi-Tof/Q-star mass spectrometry, database searching revealed the identity of 25 protein spots displaying environmental enrichment-related modulation of expression. Identified proteins belonged to a variety of functional classes with gene ontology analysis revealing the majority (> 70%) of regulated proteins to be part of the energy metabolism, cytoplasmic organization/biogenesis and signal transduction processes.

WE06-07

NEUROPROTECTIVE EFFECTS OF ACETYL-L-CARNITINE (ALCAR) AFTER TRAUMATIC BRAIN INJURY IN YOUNG RATS

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Traumatic brain injury (TBI) is the leading cause of morbidity and mortality in children. Decreased oxidative glucose metabolism and impaired mitochondrial functioning occur after TBI, due to decreased activity of pyruvate dehydrogenase and the malate-aspartate shuttle. Acetyl-L-carnitine is metabolized directly to acetyl CoA and can be used as an alternative energy substrate in brain. We used the controlled cortical impact (CCI) model of TBI in 21–22 day old male rats to study injury and neuroprotection in developing brain. During the first 24 hours after injury both shams (craniotomy only) and TBI rats were treated with either ALCAR (4 doses, 100 mg/kg/dose) or with saline. On days 3–7 after surgery TBI + saline rats had more foot slips in beam walking compared to sham-operated rats (6 ± 1 SEM for TBI, vs. 2 ± 0.2 for shams, and TBI + ALCAR rats. The novel object recognition test demonstrated that TBI + saline rats spent less time exploring novel objects than sham rats or TBI + ALCAR (45 ± 5% for TBI + saline, compared to 65 ± 10%, and 68 ± 4% for shams and TBI + ALCAR rats, respectively). Our data show that treatment with ALCAR

during the first 24 hours after TBI improves structural integrity of the brain and behavioral outcomes after injury. The protective effect of ALCAR may be due in part to the ability of this compound to provide energy to brain after injury when the metabolism of glucose is impaired. Such metabolic neuroprotection is consistent with our recent report demonstrating that [2–13C] acetyl-L-carnitine in metabolized in astrocytes and GABAergic neurons, and via the pyruvate recycling pathway in developing brain.

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WE06-08

NEUROPEPTIDE Y ENHANCES NEUROGENESIS AND EXERTS A NEUROPROTECTIVE ROLE DURING TRIMETHYLTIN-INDUCED HIPPOCAMPAL NEURODEGENERATION

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The neurotoxicant trimethyltin (TMT) is considered a useful tool to obtain an animal model of neurodegeneration associated with cognitive impairment. Intraperitoneal injection of TMT causes in rodents massive neuronal death, selectively involving pyramidal neurons in definite hippocampal subfields. An increase in precursor cell division in the rat hippocampus, although without repair, accompanies neurodegeneration (review, 1). Neuropeptide Y (NPY), widely expressed in the nervous system, plays important roles in several physiological processes, including endogenous neurogenesis (review, 2). We used immunocytochemistry and molecular biology techniques to study the effects of NPY on hippocampal neurogenesis and neuronal death in TMT-treated rats. Rats received NPY intracerebroventricularly 4 days after treatment and were sacrificed 5 days after NPY administration. Bromodeoxyuridine (BrdU), which is incorporated in dividing cells, was administered for 4 days starting from NPY administration, in order to monitor neurogenesis. Quantitative analysis of BrdU-labelled cells in the subgranular zone revealed a significantly higher number of newly generated cells in the hippocampi of TMT+NPY-treated rats than in TMT-treated animals, while the number of BrdU cells double-stained with the markers Glial fibrillary Acidic Protein, Nestin or Doublecortin appeared unchanged. Quantitative analysis of fluorojade-stained degenerating neurons evidenced a significantly lower number of stained cells in the CA1 region of TMT + NPY-treated rats than in TMT-treated rats. Moreover, real time PCR analysis showed an increased expression of the neuroprotective agents Brain-Derived Neurotrophic Factor and Bcl-2 in TMT + NPY-treated rats. The present data offer information indicating that NPY exerts a proliferative effect on the neurogenic niche and a protective role in regard to TMT-induced neuronal damage.

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WE06-09

SP-8203 HAS NEUROPROTECTIVE EFFECT AND IMPROVES MEMORY DEFICITS IN BRAIN ISCHEMIC INJURY THROUGH NMDA RECEPTOR

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The extracts of earth worms, *Eisenia andrei*, have been used as a therapeutic agent for stroke in the traditional medicine. It is also reported that the protease fraction separated from the extracts has strong anti-thrombotic activity. Besides anti-thrombotic actions, we found that SP-8203, N-[3-(2,4-dioxo-1,4-dihydro-2H-quinazolin-3-yl)propyl]-N-{4-[3-(2,4-dioxo-1,4-dihydro-2H-quinazolin-3-yl)propylamino]butyl}acetamide, derived from the extracts of earth worms blocked N-methyl-D-aspartate (NMDA) receptor-mediated excitotoxicity in a competitive manner. The neuroprotective effects of SP-8203 were attributable to prevention of Ca^{2+} influx through NMDA receptors. The systemic administration of SP-8203 markedly reduced neuronal death following middle cerebral artery occlusion in rats. SP-8203 significantly improved motor coordination on the Rota rod and spatial learning and memory in the water maze test. These results provided strong pharmacological basis for its potential therapeutic roles in cerebral ischemia.

WE06-10

MELATONIN ATTENUATES METAMPHETAMINE-INDUCED MITOCHONDRIAL FISSION AND DEGENERATION IN NEUROBLASTOMA SH-SY5Y CELLS

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Methamphetamine (METH) is a psychostimulant drug that can cause the degeneration of the neuronal cells. It has been suggested that METH-induced nerve terminal degeneration and neuronal cell death involve in multiple pathways including mitochondrial-dependent death pathway. Several pieces of evidence have emphasized that mitochondrial fission play some roles in cell death processes. Melatonin is a natural occurring compound with the well known antioxidant properties. In the present study, we aimed to investigate the protective effects of melatonin in METH-induced toxicity and mitochondrial fission in neuroblastoma SH-SY5Y cultured cells. METH significantly decreased cell viability and increased the mitochondrial fission protein (Fis1) levels. Melatonin can reverse the toxic effects of METH-induced reduction in cell viability and induction in Fis1 levels. In addition, the morphological alteration of mitochondria was investigated in METH-treated cells with and without melatonin pretreatment using transmission electron microscopy (TEM). The results of the present study demonstrate the potential affects of melatonin on reduced cell death and restored mitochondrial function against METH-induced toxicity in neuronal cells.

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WE06-11

MECHANISMS FOR THE NEUROPROTECTIVE AND ANTIDEPRESSANT EFFECTS OF DHEA: RELEVANCE TO DEPRESSIVE SYMPTOMS IN PARKINSON'S DISEASE

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Dehydroepiandrosterone (DHEA) is a neuroprotective and antidepressant hormone. It modulates dopamine (DA), noradrenalin (NA) and serotonin (5-HT) systems, involved in Parkinson's disease (PD) and depression; some PD patients (40–50%) show depressive symptoms. However, the effect of DHEA on monoamine metabolism is not completely elucidated. We tested the effect of the steroid on monoamine metabolism in the corpus striatum (CS) and nucleus accumbens (NAc). DHEA was i.p. injected to male Wistar rats (30–120 mg/kg) that were sacrificed two hours later. The CS and the NAc were dissected out; monoamine content and turnover was determined by HPLC-ED; *in vivo* and *in vitro* monoamine oxidase (MAO) activity was determined fluorimetrically. DHEA reduced DA turnover in the CS, suggesting that the steroid reduced neurotransmitter release or catabolism; however, the steroid reduced MAO activity in the NAc only, suggesting that the effect on DA turnover in the CS does not involve MAO, and thus, may reflect reduced neurotransmitter release. *In vitro*, DHEA reduced MAO A and B activities in homogenates from both brain regions. Regarding the NAc, our results show that MAO inhibition does not alter DA metabolism and may possibly affect other neurotransmitters. The steroid increased 5-HT turnover in both the CS and the NAc, while it tended to increase NA content in the NAc only, suggesting that the inhibitory effect of DHEA on MAO activity in this region modulates NA metabolism. It may be suggested that DHEA inhibits DA release in the CS, reducing its catabolism and reactive oxygen species generation by MAO. In the NAc, DHEA may increase serotonin release, and also, stimulate NA neurotransmission by inhibiting MAO A. This may be involved in the antidepressant effect of the steroid, while inhibition of MAO B activity and DA turnover might be neuroprotective. It may be suggested that DHEA could be useful to reduce depression and neurodegeneration in PD.

WE06-12

PROTECTIVE EFFECT OF MELATONIN ON THE DOPAMINERGIC PATHWAY FROM AMPHETAMINE-INDUCED NEUROTOXICITY IN POSTNATAL RATS

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Amphetamine (AMPH) has been known to damage the dopaminergic nerve terminals by the formation of ROS and finally neuronal death. Melatonin, hormone from pineal gland, is well known for its antioxidant property. The developing fetus is substantially deficient in most antioxidative enzymes and risks from drug enhanced oxidative stress. The present study has been performed to investigate the effect of melatonin on the changing of tyrosine hydroxylase (TH), synaptophysin, and alpha-synnuclein in the dopaminergic structures; substantia nigra, ventral tegmental nucleus (VTA), striatum, nucleus accumbens and prefrontal cortex, of AMPH-treated postnatal rats. The postnatal rats at age 4 were injected with either AMPH alone or AMPH with 30 min-pretreated with melatonin daily for 7 days and sacrified on P10. The expression of these proteins has been detected by western blot analysis and immunohistochemical technique. The results showed that TH and synaptophysin levels were significantly decreased but alpha-synuclein increased in the AMPH-treated group. The immunoreactivity of TH decreased in the neurons of substantia nigra, VTA and that of TH and synaptophysin reduced in the terminals of striatum, nucleus accumbens and prefrontal cortex. Pretreatment with melatonin prior to AMPH administration prevented AMPH-induced reduction in TH, synaptophysin and induction of alpha-synuclein in different brain regions. These results suggest that melatonin provides a protective effect against AMPH-induced neurons and nerve terminal degeneration in the immature rat brain probably via its antioxidant properties.

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WE06-13

A CRUCIAL ROLE OF THE C-TERMINAL SEQUENCE FOR NEUROPROTECTIVE ACTIVITY OF PEPTIDE SEMAX

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Peptide MEHFPGP ((ACTH (4-7)-PGP or semax) has a well-marked cognitive and neuroprotective effects in laboratory animals and humans. Previously, we and other have shown that the major metabolite of semax peptide PGP was completely devoid of cognitive but not neuroprotective activity *in vivo*.

Objectives: The aim of this study was to compare the neuroprotective properties of peptides semax and some of its fragments in *in vitro* models of neuron injury.

Methods: Oxidative stress-induced necrotic cell death was accomplished by a 30-min incubation of PC12 cells with 1 mM H_2O_2. For modeling glutamate neurotoxicity cerebellar granule neurons were treated with 100 uM Glu/10 uM Gly in Mg^{2+}-free medium. Intracellular Ca^{2+} concentration and mitochondrial membrane potential were determined by simultaneous fluorescence imaging with fura-2FF and rhodamine-123. Apoptotic and necrotic cells were determined by staining with fluorescent dyes Hoechst/IP or Hoechst/Syto 13/EthD-1. Neurotrophin's mRNA was assayed by quantitative real-time PCR.

Results: It was shown that semax, and some of its C-terminal fragments at concentrations 1-1000 uM increase PC12 cell survival after H_2O_2-induced oxidative stress. However removal of the C-end Pro led to the complete loss of cytoprotective activity of the semax fragments. PGP demonstrated the highest cytoprotective activity of all peptides tested. In the Glu-excitotoxicity model semax and PGP

in concentration 100 uM both increased cell survival and delayed Ca^{2+}-deregulation/mitochondrial depolarization. It should be noted that in all cases PGP showed a stronger effect than the semax. Moreover PGP as well as semax stimulated BDNF and NGF genes expression in mixed neuronal cultures from rat cortex.

Conclusion: Based on these findings we suggest that the C-terminal sequence PGP is crucial for neuroprotective effect of peptide semax.

WE06-14

CALPASTATIN ATTENUATES CALPAIN AND CASPASE ACTIVATION IN METHAMPHETAMINE-INDUCED DEGENERATION IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS

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Methamphetamine (METH) is an abused psychostimulant drug that can cause neurotoxicity to dopaminergic cells. It has been demonstrated that METH can induce caspase- and calpain-dependent death cascades. The purposed of the present study was to investigate the functional role of calpastatin, a specific endogenous calpain inhibitor protein on caspase and calpain activation in METH-induced degeneration in neuroblastoma SH-SY5Y cell cultures. In this study, we found that METH significantly decreased cell viability, tyrosine hydroxylase phosphorylation and calpastatin levels. Supplementation of cells with exogenous calpastatin was able to reverse the toxic effect of METH on reduction in cell viability and tyrosine hydroxylase phosphorylation. METH also significantly increased calpain levels, the formation of calpain-specific breakdown products and cleaved caspase-3 levels and again these effects were diminished by pretreated the cells with calpastatin. These data point to the contribution of calpastatin as a potential regulatory factor for caspase- and calpain-dependent death processes.

WE06-15

GUANOSINE IS NEUROPROTECTIVE AGAINST OXYGEN/GLUCOSE DEPRIVATION: MODULATION OF OXIDATIVE STRESS VIA A1R, MAPK, NF-κB, INOS PATHWAY

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Guanosine (GUO) is an endogenous modulator of glutamatergic excitotoxicity and has been shown to promote neuroprotection in *in vivo* and *in vitro* models of neurotoxicity. The aim of this study was to evaluate the involvement of big conductance Ca^{2+}- activated K^+ (BK) channels, adenosine A1 receptors (A1R), PI3K, MAPK/ERK and NF-κB pathways in the mechanism of neuroprotection promoted by GUO in rat hippocampal slices submitted to oxygen/glucose deprivation (OGD). Previous studies from our laboratory have shown GUO protects hippocampal slices against OGD by increasing glutamate uptake via the involvement of BK channels and PI3K pathway. GUO effect on increasing glutamate uptake is also blocked by MAPK/ERK inhibition (by using PD98059, 25 μM) but not by A1R blockade with a specific antagonist (DPCPX, 250 nM). GUO also prevented ROS production and mitochondrial depolarization induced by OGD. This effect was not blocked by charybdotoxin (a BK channel blocker, 100 nM) or LY294002 (a PI3K inhibitor, 10 μM), but the presence of the MAPK/ERK inhibitor or the A1R antagonist inhibited this GUO effect. GUO also reduced the increased iNOS expression promoted by OGD. This effect was mediated by A1R activation, MAPK/ERK modulation and inhibition of NF-κB activation. In conclusion, the neuroprotective mechanism of action of GUO also involves reduction of ROS production, mitigates mitochondrial depolarization, inhibition of NF-κB activation and reduction of iNOS expression. Then, the neuroprotective effect of GUO seems to occur by two signaling pathways triggered by BK channels or adenosine A1 receptors activation. However, both pathways converge to MAPK/ERK activation which promotes the normalization of the unbalance oxidative status of the cells and glutamatergic transmission, thus affording neuroprotection.

WE06-16

POST-ISCHEMIC ISGYLATION OF PROTEINS IS NEUROPROTECTIVE

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Addition of a small peptide called ISG15 to proteins is known as ISGylation which is an ubiquitin-like post-translational modification. We currently show that focal ischemia induced by transient middle cerebral artery occlusion (MCAO) in adult mice significantly induces cortical protein ISGylation between 6 hours to 24 hours reperfusion. With 2-D Western blotting, 45 proteins were observed to be significantly increased in ISGylation (by 1.8 to 9.7-fold) after focal ischemia compared to sham control. Of those, MALDI-TOF analysis identified Enolase 1α, ATP synthase 5a1, phosphoglycerate mutase 1, glutamine synthase and α-internexin. Immunochemistry showed that ISGylated proteins are localized in both neurons and astroglia in the ipsilateral cortex of the ischemic mice. When subjected to transient MCAO, ISG15–/– mice showed increased mortality, exacerbated infarction and worsened neurological recovery compared to wild-type controls. In addition, mice lacking UBE1L (the first ligase of the ISGylation cycle) also showed bigger infarcts when subjected to transient MCAO. The rCBF or other physiological parameters were not significantly different in both the knockouts compared to wild-type controls. These studies indicate that increased protein ISGylation might be an endogenous neuroprotective adaptation to minimize the post-stroke brain damage. Funded by the American Heart Association.

WE07 Cell Death

WE07-01

THE EFFECTS OF JNK, GSK3β AND AKT ON ACTIVATION OF BAX-MEDIATED APOPTOSIS

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Neuronal apoptosis has been implicated in both chronic and acute neurodegenerative conditions such as Alzheimer's, Parkinson's and stroke. The intrinsic pathway of apoptosis is mediated by the Bcl-2 family of proteins and is initiated following several types of cell stress. Particularly important is the pro-apoptotic protein Bax which facilitates the release of pro-apoptotic factors from the mitochondria. Following several types of neuronal stress the activation of Bax is mediated by the pro-apoptotic, BH3-only protein Puma. Several kinases, cJun N-terminal kinase (JNK), glycogen synthase kinase 3 (GSK3) and AKT, have been shown to be important in signaling upstream of Bax activation. This study sought to characterize if this is the case in Puma-mediated apoptosis and through which targets these kinases affect apoptosis. Bax-dependent apoptosis was induced in cerebellar granule neurons (CGNs) by potassium withdrawal; a model which we have already found to cause significant Puma mRNA induction. Inhibition of JNK and GSK3 (pro-apoptotic pathways) and activation of AKT (pro-survival pathway) using pharmacological inhibitors resulted in decreased cell death and attenuation of Puma mRNA induction following potassium withdrawal. In the case of JNK and AKT this data was corroborated by molecular modulation of these pathways using adenoviral constructs. We have also examined the interaction between these pathways during apoptotic signaling and our results suggest that AKT functions upstream of GSK3 while JNK functions independently to mediate Puma induction. Finally, several potential downstream transcription factor targets have been examined to determine if they provide the link between kinase signaling and Puma transcription. Thus far, cJun, ATF3 and ATF2 all appear to be induced during apoptosis in a JNK dependent fashion. Downstream targets of GSK3 and AKT are still unknown. Our next step will be to link these transcription factors to the Puma promoter and to identify novel targets that may function downstream of the other two kinase pathways.

WE07-02

EFFECT OF MANGANESE ON PHOSPHATIDYLSERINE METABOLISM AND CELL DEATH IN PC12 CELLS

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Introduction: Manganese, which induces neurological disorders, causes PC12 cell apoptosis, associated with reactive oxygen species generation. Various apoptotic stimuli affect the metabolism of phosphatidylserine, whose oxidation contribute to externalization on apoptotic cell surface. In this study, the effect of $MnCl_2$ on phosphatidylserine metabolism in PC12 cells is reported.

Materials and Methods: PC12 cells were incubated (24–72 hours) with or without $MnCl_2$ (0.1 or 1 mM) Cell viability and apoptosis were evaluated by MTT assay and by flow cytometric method using annexin V-FITC apoptosis detection kit, respectively. PS metabolism was evaluated by adding [^{3}H] serine 1h before the end of the incubation; radioactivities into phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine were then measured. Expression of phosphatidylserine-synthezing enzymes were evaluated by Western-Blotting.

Results: After 24 hour of treatment, incorporation of [^{3}H] serine into phosphatidylserine was reduced by 1 mM $MnCl_2$, having 0.1 mM $MnCl_2$ no effect. Reduction of phosphatidylserine radioactivity was greater prolonging the treatment to 72 hours, a condition causing drastic cell death mostly due to apoptosis. The effect of 24 hours treatment with 1 mM $MnCl_2$ was further investigated measuring conversion of phosphatidylserine into phosphatidylethanolamine and phosphadylcholine. Newly synthesized phosphatidylserine was decarboxylated into phosphatidylethanolamine; percentage of radioactive phosphatidylethanolamine with respect to radioactive phosphatidylserine was 80% in $MnCl_2$-treated cells and 20% in controls. Total radioactivity into glycerophospholipids was lower in $MnCl_2$-treated samples respect to controls, confirming reduction of phosphatidylserine synthesis, in apparent contrast with the increased expression of phosphatidylserine-synthesizing enzymes.

Conclusion: $MnCl_2$ reduced phosphatidylserine synthesis in PC12 cells and increased phosphatidylserine decarboxylation, a mitochondrial process, in conditions that did not induce significant apoptosis and release of cytochrome c from mitochondria. Thus, these effects could represent early events of $MnCl_2$-induced apoptosis. The possibility that the greater expression of phosphatidylserine synthesizing enzymes could be a consequence of the reduced phosphatidylserine levels in the membrane is suggestive.

WE07-03

DEHYDROEPIANDROSTERONE (DHEA) PROTECTS THE RETINA FROM AMPA EXCITOTOXICITY *IN VIVO*: TRKA RECEPTOR INVOLVEMENT

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Retinal diseases, such as diabetic retinopathy, are characterized by a neovascular and neurodegenerative component. While available therapeutics target the neovascular aspects of the disease, novel therapeutic agents are essential for the treatment of the neurodegenerative component. The latter involves retinal ischemia and excitotoxicity which lead to visual impairment and blindness. The neurosteroids DHEA has been reported to have antiapoptotic and neuroprotective actions in different paradigms. It was recently shown to mimic NGF in protecting various neuronal cell types from apoptosis and directly interacting with prosurvival TrkA receptors. The aim of the present study was to investigate whether DHEA and NGF could protect retinal cells from cell death in an *in vivo* model of AMPA excitotoxicity. Sprague-Dawley rats were intravitreally administered AMPA (42 nmol/eye) or AMPA and DHEA (10-6, 10-7, 10-8 M) or NGF (60pg/eye) or vehicle. The TrkA receptor

inhibitor (Calbiochem 648450, 10-6, 10-5 M) was co injected with AMPA and DHEA (10-6 M) or NGF. Twenty four hours after treatment, eye cups were removed and prepared for immunohistochemistry. Antibodies raised against retinal markers such as cholineacetyl transferase (ChAT) and calbindin were employed, labeling cholinergic amacrine cells and horizontal, amacrine and cone bipolar retinal cells, respectively. Cleaved caspase-3 immunoreactivity and TUNEL assays were also employed for the detection of apoptosis. AMPA (42 nmol per eye) injections led to the attenuation of ChAT and calbindin immunoreactivity, twenty four hours after administration. Co- administration of AMPA and DHEA protected the retina in a dose dependent manner. NGF mimicked the DHEA neuroprotective effects, while the TrkA inhibitor reversed the neuroprotection afforded to the retina by DHEA (10-6 M) and NGF. These results support that the endogenous neurosteroid DHEA protects the retina from AMPA excitotoxicity via a mechanism involving the TrkA receptor. Further studies are essential in order to elucidate the mechanisms involved in DHEA's neuroprotection and to evaluate the therapeutic relevance of these results. [Partially funded by the Graduate Program of Neuroscience, University of Crete].

WE07-04

SUSTAINED ACTIVATION OF PI3K/AKT IS ACCOMPANIED BY REDUCED SURVIVAL OF HYPOXIC-ISCHEMIC SH-SY5Y CELLS

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Cell response during hypoxic conditions plays a crucial role in the central nervous system, as it is connected to a variety of dysfunctions, due to imbalanced stimulatory and inhibitory transmission mechanisms, leading to excitotoxicity. The present study was undertaken to assess the potential role of PI3K/Akt survival signalling pathway and its crosstalk with hypoxia-inducible factor HIF-1a, during hypoxic -in the presence of CoCl2- and ischemic -glucose deprivation- state, in human neuroblastoma cell line (SH-SY5Y). Cell survival was significantly decreased in the above conditions and as expected, CoCl2 (50–400 μM) caused a large increase on HIF-1a protein levels which was evident as early as 2 hours after CoCl2 addition. Qualitatively, the same results were obtained when CoCl2 was added in medium without glucose although HIF-1a protein levels remained markedly lower in this case. Interestingly, PI3K/Akt was clearly activated in both conditions, a finding that was also proven by the increased phosphorylation of GSK3, the direct target of p-Akt. Besides, a crosstalk between these two pathways was demonstrated, using wortmannin, the specific PI3K/Akt inhibitor. Next, the possible involvement of calcium (Ca^{2+}), as possible cell death cause, was considered, as activation of PI3K/Akt survival pathway could not explain the decreased cell survival in the above conditions. Remarkably, decreased Ca^{2+} concentrations in the endoplasmic reticulum and increased ion influx from the plasma membrane were detected in hypoxic-ischemic cells. Moreover, endoplasmic reticulum depletion by thapsigargin, a specific calcium pump inhibitor, was able to induce PI3K/Akt activation even though cell death was, as expected, inevitable. It seems therefore, that the measured calcium variations could account for impaired cell signaling and death, while, in particular, increased calcium influx could explain the sustained stimulation of PI3K/Akt, which, however, can not overmaster the apoptotic signals that lead to cell death.

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WE07-05

EFFECTS OF SECRETED WILD-TYPE α-SYNUCLEIN ON CA^{2+} HOMEOSTASIS IN NEURONAL CELLS

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α-Synuclein (SNCA) is an abundant presynaptic neuronal protein that is linked to Parkinson's disease (PD) pathogenesis. Recent data from our laboratory and others suggest that SNCA can be normally secreted from neuronal cells. To assess the effects of secreted SNCA on neuronal homeostasis, we used as a source of secreted SNCA inducible-SH-SY5Y cells expressing wild-type SNCA. We have shown that these cells readily secrete SNCA species (monomeric to high molecular weight forms) into their culture medium, partly via an exosome-mediated manner. Secreted SNCA forms were shown to be toxic to recipient neurons. This toxic effect did not involve the uptake of SNCA into neuronal cells. To investigate the possible mechanisms of extracellular SNCA-mediated neuronal death, we applied naturally secreted SNCA to differentiated SH-SY5Y cells. We found that such treatment altered Ca^{2+} homeostasis, in recipient neuronal cells, manifested by increased Ca^{2+} influx upon depletion of intracellular Ca^{2+} stores with thapsigargin. This effect was SNCA dependent since immunodepletion of SNCA from the conditioned medium reduced Ca^{2+} influx. The SNCA-induced Ca^{2+} influx was mediated via L-type channels, since their specific inhibition with nifedipine, abolished this effect. Differentiated SH-SY5Y cells incubated with nifedipine and extracellular or intracellular Ca^{2+} chelators are resistant to secreted SNCA-mediated toxicity. Our data suggest that secreted SNCA is toxic to recipient neuronal cells through engagement, at least partly, of the intracellular homeostatic Ca^{2+} machinery. Therefore, manipulating Ca^{2+} signaling pathways mitigates extracellular SNCA toxicity and may represent a potent therapeutic target for PD.

WE07-06

EFFECTS OF THYROID HORMONES, T4 AND T3, ON THE SURVIVAL AND NEURITE OUTGROWTH OF CEREBELLAR GRANULE CELLS IN CULTURE

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Thyroid hormone (TH) is essential for the proper development of the mammalian CNS. In the developing cerebellum, TH deficiency has been shown to cause defects in cell survival, differentiation, and migration. During the neonatal period, cerebellar granule cells (CGCs) contact and receive synaptic inputs from mossy fibers originating in pontine nuclei, which is essential to their survival. Primary cultures of CGCs require chronic depolarization in a medium with high KCl concentration (25 mM; K25), reflecting the activity-dependent cell survival observed *in vivo*. When cultured under usual low KCl conditions (5 mM; K5) for 24–48 hours, CGCs undergo apoptotic cell death. In the present study, we report the effect

of thyroid hormones, L-thyroxine (T4) and 3,3',5-L-triiodothyronine (T3), on the death of CGCs induced by low KCl. CGCs obtained from postnatal day 6 (P6) rat cerebellum were cultured in D-MEM/F-12 containing 10% fetal bovine serum and 25 mM KCl for 48 hours, and then transferred to serum-free medium containing 5 mM KCl with or without T4 or T3. Though both T4 and T3 induced up-regulation of TH-dependent genes in CGCs, only T4 was effective in inhibiting low KCl-induced cell death. To further understand the mechanism underlying this phenomenon, the effects of TH on neurite outgrowth and maintenance were analyzed. At 48 hours after serum withdrawal, CGCs in K5 with T4 had well-extended bipolar neurites indistinguishable from those of cells in K25 with or without TH. Proportion of polymerized actin or tublin was also increased by the addition of T4. Though many effects of TH are mediated through genomic action for which T3 is much more effective than T4, the effect of T4 observed in this study suggests its non-genomic direct action on the regulation of cytoskeletal dynamics leading to neurite outgrowth and cell survival.

WE07-07

NITRIC OXIDE REGULATES AKT PHOSPHORYLATION AND NUCLEAR TRANSLOCATION IN CULTURED AVIAN RETINAL CELLS

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Previous studies have shown that nitric oxide (NO) inhibits apoptosis of retinal neurons in culture through the canonic cyclic GMP/protein kinase G-dependent pathway but also involves multiple kinase pathways including PI3 kinase/AKT. Both NO and AKT have survival-promoting properties and have important roles in CNS development and plasticity. The purpose of this study was to explore the effect of endogenous NO produced from L-arginine or exogenous NO produced from SNAP on AKT phosphorylation in cultured chick retinal neurons. Our results demonstrate that either SNAP or L-arginine enhance AKT phosphorylation on ser-473 and thr-308 residues in a concentration and time-dependent manner. This effect was mediated by the activation of soluble guanylyl cyclase and protein kinase G, since it was blocked by the respective enzyme inhibitors ODQ (or LY83583) and KT5823, and mimicked by the respective enzyme activators YC-1 and 8-Bromo cyclic GMP, and also by the cyclic GMP phosphodiesterase inhibitor zaprinast. In addition, the effect of SNAP was suppressed by LY294002 or Wortmannin, indicating the involvement of phosphatidylinositol 3 kinase. Glutamate or NMDA also promote AKT phosphorylation and these effects were abrogated by the nitric oxide synthase inhibitor L-NAME, revealing a mechanism involving activation of NMDA receptors and NO production. We have also found that L-arginine or SNAP elicit AKT translocation into the nucleus of retinal neurons as well as of other neuronal cell lines. We then conclude that NO produced from endogenous or exogenous sources promotes AKT activation and shuttling to the nucleus probably participating in neuronal survival pathways important during CNS development.

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WE07-08

CARBON MONOXIDE PREVENTS NEURONAL APOPTOSIS BY ASTROCYTIC METABOLISM MODULATION: PURINERGIC SIGNALLING

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Low doses of carbon monoxide (CO) play a beneficial role in several cerebral models, including primary culture of neurons and astrocytes, as well as isolated non-synaptic mitochondria. CO generates small amounts of reactive oxygen species (ROS), which act as signalling intermediaries and induce a preconditioning state. Herein, a model of primary co-culture of neurons and astrocytes (2D) is used to explore the role of CO in cell-to-cell communication. Neuronal survival was assessed after being co-cultured with astrocytes pre-treated or not with CO. Neuronal cell death was triggered by tert-butylhydroperoxide (t-BHP), an oxidative stress inducer. CO-pretreated astrocytes reduced neuronal cell death in the co-culture systems. Since there is no physical contact between neurons and astrocytes, CO seems to induce the release of some neuroprotective factor from astrocytes. The classical energetic molecule ATP and its degradation product, adenosine, are the candidate molecules for being neuroprotective. ATP/adenosine content in the media after CO treatment was assessed by HPLC, showing an increase on its levels. Neuronal survival was determined under several conditions. Addition of adenosine or ATP resistant to degradation into the neuronal media increased neuronal survival, confirming its protective action in this model. Furthermore, several chemical inhibitors of purinergic receptors (both P1 and P2), namely PPADS, suramin or SCH 58261, partially reverted CO neuroprotection in co-cultures. Moreover, neuronal survival after astrocytic treatment with CO and with a blocker of connexin 43 (for preventing ATP release from astrocytes) was assessed. The adenosine receptor A2A involvement in CO mechanism was confirmed by siRNA silencing. Overall, one can conclude that CO neuroprotective role is not limited to a direct action into a single cell type; but this gasotransmitter also modulates purinergic signalling for increasing astrocytic protection against neuronal cell death.

WE07-09

NMDAR MEDIATES NEURONAL SURVIVAL BY ACTIVATION OF NURR1

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Cerebellar granule cells (CGCs) differentiate and maturate during their migration from the external granular layer (EGL) to the internal granular layer (IGL). Lack of excitatory inputs triggers their apoptotic death. NMDA receptor stimulation promotes the survival of these neurons during that process. It is possible to mimic this process *in vitro* by culturing CGCs in low KCl concentrations (5mM) in the presence or absence of NMDA. Using this

experimental approach, we have obtained whole-genome expression profiles after 3 and 8 hr of NMDA addition to identify genes involved in NMDA-mediated survival of CGCs. Gene ontology analysis of the results identified that most of the regulated genes were included in the anti-apoptotic, development or neurogenesis clusters. One of the identified genes was Nurr1, a member of the orphan nuclear receptor subfamily Nr4a. We show here that Nurr1 is induced by CREB activation in response to NMDA. Silencing Nurr1 by shRNA leads to a decrease in BDNF protein levels and a reduction of NMDA neuroprotective effect. Moreover, we report that Nurr1 is increases during cerebellum postnatal development and that Nurr1 and BDNF show a similar expression pattern during development. Thus we conclude that Nurr1 is necessary for NMDA-mediate survival of CGCs.

WE07-10

ACTIVATION OF P2X7 NUCLEOTIDE RECEPTOR INDUCES CELL DEATH OF DEVELOPING AVIAN RETINAL CELLS IN CULTURE

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ATP is a signaling molecule that via activation of P2Y receptors induces cell proliferation in retinal cultures. However, other effects of ATP during development of the retina are still poorly explored. Here, we investigated the effect of ATP on cell death in cultured chick embryo retina cells. In cultures obtained from retinas of 7-day-old chick embryos (E7) that were cultivated for 2 days (E7C2) both ATP and BzATP induced a decrease in cell viability characterized by the MTT method that was time- and dose-dependent and that could be blocked by 0.2 mM oxidized ATP or 0.3 μM KN-62. While incubation of cells with 0.5 mM H_2O_2, for 5 hours, decreased cell viability by 70% ($n = 3$), a maximal decrease of $\sim$ 30% ($n = 3$) was observed by incubating the cells for 3 hours with 3 mM ATP or 0.1 mM BzATP. An increase in cleaved caspase-3 levels and in the number of TUNEL positive cells was observed when cultures were incubated with 3 mM ATP. In contrast to H_2O_2 that induced a significant release of LDH, the release of this enzyme induced by ATP was very low. Cell death induced by ATP was observed between E7C1 and E7C5, the maximal levels being detected by E7C2. Nucleotides were able to increase neuronal ethidium bromide and sulforhodamine B permeability in purified neuronal cultures as well as in mixed cultures, an effect that was blocked by the P2X7 receptor antagonist BBG. Since nucleotide-induced cell death was observed only in mixed cultures with neurons and glial cells, but not in purified cultures of neurons or enriched glial cultures, our results suggest that ATP induces apoptosis in a population of chick embryo retinal neurons in culture through activation of P2X7 receptors. These data also suggest that P2X7-induced neuronal apoptosis occurs only when neurons are cultivated in the presence of glial cells, although purified neurons in culture do express P2X7 receptors.

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WE08 Animal Model of Neuropsychiatric Disorders

WE08-01

THE HYPERALGESIA INDUCED BY SLEEP DEPRIVATION IS NOT DECREASED BY DIAZEPAM AND IS ASSOCIATED WITH AN INCREASED NOS ACTIVITY IN PAG

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The periaqueductal gray matter (PAG) area of the brainstem is involved in pain and anxiety modulation. Paradoxical sleep deprivation (PSD) has been found to promote hyperalgesia, relieve symptoms of depression and trigger anxiety episodes. The mechanisms that govern these changes are poorly understood. One of the neurotransmitters related to these behavioral changes is nitric oxide (NO). The experimental methods of PSD have been linked to the generation of anxiety in animals, and the hyperalgesia is frequently associated to the anxiety generated by these methods. In this study we investigated the effects of the anxiolytic drug diazepam and the analgesic drug acetylsalicylic acid on anxiety and thermal nociceptive response in paradoxical sleep deprived rats; we also evaluated the activity of nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) an indicator of NO sinthase activity on the PAG. Rats were deprived from paradoxical sleep for 96 hours and submitted to the nociceptive test on the hot plate (46°C). The assessment of anxiety level was performed using the open field and the elevated plus maze tests. PSD animals showed an increased rate of locomotion (+ 314.8%, $p < 0.05$), increased entries number and time spent on open arms in elevated plus maze (+ 319.2%, $p < 0.05$; + 257.1%, < 0.05) and reduced hind paw withdraw latency on the hot plate (–64.2%, $p < 0.05$). Diazepam and acetylsalicylic acid did not influence the responses in PSD animals in the open field and hot plate tests. In addition, the number of NADPH-d positive cells in PAG was higher after PSD (+ 59%, $p \leq 0.05$). The results obtained in this work showed that the PSD method did not induce anxiety and neither the ansiolitic nor the analgesic drugs changed the hyperalgesia induced by PSP. Our data also suggest that the hyperalgesia observed in PSD are associated with increased NOS activity in PAG.

WE08-02

EMBRYONIC BORN MUSHROOM BODY INTRINSIC NEURONS ARE NECESSARY FOR ASSOCIATIVE OLFACTORY LEARNING IN DROSOPHILA LARVAE

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We analysed anatomically the Drosophila larval brain by using various Gal4 drivers in combination with hydroxyurea treatment to identify the embryonic or larval origin of the intrinsic mushroom body Kenyon cells with respect to associative olfactory learning. We confirmed that NP1131 Gal4 drives expression in embryonic born mushroom body neurons, important for the formation of appetitive olfactory associations. In addition we investigated whether the same neurons are necessary to form olfactory associations by using salt and electric shock as a negative reinforcer. Finally, we used different odor combinations to check whether the role of the embryonic born Kenyon cells we identified in associative learning is odor specific. Our data support the hypothesis that olfactory associations in Drosophila larvae are stored in a small set of embryonic born mushroom body intrinsic neurons and the participation of larval born Kenyon cells that are gradually integrated in the developing mushroom body during larval life is highly unlikely.

WE08-03

DIFFERENTIAL EFFECTS OF ANESTHETIC KETAMINE ON RAT'S RECOGNITION MEMORY. FUNCTIONAL INTERACTION WITH THE NITRERGIC SYSTEM

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There is poor experimental evidence concerning the effects of anesthetic doses of the non-competitive NMDA receptor antagonist ketamine on rodent's memory abilities. The present study was designed to investigate (a) the long-term consequences of pre-and post-training administration of anesthetic ketamine on rats' recognition memory; (b) to evaluate whether or not these effects are related to the hypothermic properties of ketamine; and (c) to evaluate whether or not the nitric oxide synthase inhibitor L-NAME (1, 3, 10 mg/kg, i.p.) was able to counteract the expected behavioural deficits produced by anesthetic ketamine. For this aim, the novel object recognition and the object location paradigm were selected. Pre and post-training administration of ketamine (100 mg/kg; i.p.) disrupted animals' performance, although to a different extent, in both these recognition memory paradigms. These findings indicate that anesthetic ketamine impaired both spatial and non-spatial recognition memory. Hypothermia-induced by this NMDA receptor antagonist and the type (spatial vs. non-spatial) of the behavioural paradigm utilized seem to affect rats' recognition memory abilities. Finally, L-NAME (1-3, but not 10 mg/kg) antagonized this deficit on cognition produced by anesthetic ketamine indicating that an NO component modulates these effects.

WE08-04

RECEPTORS FOR BIOGENIC AMINES DIFFERENTIALLY MODULATE THE STRESS RESPONSE IN DROSOPHILA MELANOGASTER

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In the Central Nervous System, Biogenic Amines (BAs), activate specific receptors, which are responsible for the cellular events that define the generation or modification of behaviors in an individual. In our laboratory, we use the fly *Drosophila melanogaster*, a well-known genetic model in neurobiology, to evaluate the contribution of specific BAs and their receptors to the generation of complex behaviors, including stress. This is a condition that determines the

failure of an individual to effectively respond to mental, emotional or physical demands, and it has been shown in both vertebrates and invertebrates that depends on BAs actions.

In this work, we evaluated the effect of two stress conditions, starvation (flies without access to food but with access to water) and desiccation (flies without access to food or water), on fly survival. We also assessed arousal by evaluating startle-induced locomotion. In order to evaluate the contribution of different BAs receptors to stress-induced effects, we expressed RNAi for several dopamine, octopamine (the functional homolog of noradrenaline in invertebrates) and serotonin receptors in Mushroom Bodies and the Pars Intercerebralis and studied the effects induced by this genetic manipulation on fly survival and arousal.

Our results show that OAMB and Oct2R octopamine receptors differentially modulate survival of flies under the stress conditions studied, while serotonin or dopamine receptors seem not to affect it. Moreover, all the BA receptors studied decrease arousal in desiccated flies.

These results show that BA receptors differentially modulate stress-induced effects in *Drosophila* flies.

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WE08-05

SEX DIFFERENCES IN RAT ANXIETY AND DEPRESSION TESTS: THE CONTRIBUTION OF CORTICOSTERONE

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Despite the fact that affective disorders are more common in women than in men, most relevant tests have been developed in male rats. Importantly, when females are compared to males in anxiety and depression tests, sex differences are identified in behavioural and neurochemical levels, as well as in the magnitude of drug response (Dalla et al 2010) . In the present study we sought to investigate whether sex differences in anxiety and depression tests can be attributed to sex differences in HPA axis, as reflected in corticosterone levels, which is higher in females than in males. In order to artificially equalize peripheral corticosterone levels between males and females, we subjected male and female adult rats in either adrenalectomy or sham-operation and we supplemented them with physiological steady doses of corticosterone. Following recovery, we subjected all rats to the open field test, the dark-light paradigm and the forced swim test. In the open field test, males were less explorative than females and adrenalectomy decreased activity only in males. Males were more anxious than females in the open field and light-dark paradigms, while adrenalectomy decreased indices of anxiety only in males. In the forced swim test, females exhibited higher ''depressive-like'' symptomatology than males and this was not abolished by adrenalectomy. Adrenalectomy again had an effect only in male's behaviour, since it decreased climbing and enhanced swimming only in males. These results indicate that the stress-induced rise in corticosterone is important for the male stress response in anxiety and depression tests, while this appears less important for the organization of the female response to behavioural stressors. Such hypothesis could explain the differential coping strategy under stress between males and females and the resulting sex-dependent response to antidepressant treatment.

WE08-06

METABOTROPIC GLUTAMATE RECEPTORS: A POSSIBLE MECHANISM OF ACTION FOR VALERIANA OFFICINALIS ANXIOLYTIC PROPERTIES

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Valerian extract is commonly used in alternative medicine for its anxiolytic and sedative properties. This study evaluated the anxiolytic properties of valerian extract in adult wild-type zebrafish (Danio rerio). Naïve zebrafish have a natural preference for dark places.The dark/light tank test was used to measure anxiety. Behavioral assays demonstrated that exposure of zebrafish to Valerian extract or valerenic acid increase their residence time in the white compartment by 85% and 58%, respectively, compared to naïve zebrafish (25%). LAP3 (mGluR I antagonist) and EGLU (mGluR II antagonist) inhibit the anxiolytic effects of Valerian and valerenic acid. This study demonstrated that Valerian and valerenic acid have anxiolytic properties in the zebrafish. Moreover, the selective interaction of valerian with mGluRI and II may represent an alternative explanation for the anxiolytic properties of this plant and support the role of mGluR in anxiety.

WE08-07

A REWARDING AND FRUSTRATING EXPERIENCE EARLY IN LIFE DIFFERENTIALLY REGULATES BRAIN MONOAMINES IN ADULTHOOD

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Early life experiences have been shown to induce prolonged effects on neuroendocrine and behavioral responses to stress. In an attempt to reproduce experimentally the effects of neutral, positive or negative neonatal experiences on adult behaviour and brain function we have developed a paradigm in the rat, in which mother contact is used as either a positive (rewarding) or negative (frustrative) reinforcer in a T-maze, during postnatal days 10–13. The present study was based on the hypothesis that this early life manipulation on the primary social factor i.e. mother-infant interaction may affect attachment, cause disruption of the normal social interaction in adolescence, and social conflict in adulthood, and alter brain monoamines. In order to test this, early life trained rats, as well as controls were subjected to a social interaction test in adolescence and to social defeat, in adulthood. Rats trained under frustration as neonates exhibited more aggressive-like behaviors during social play in adolescence and more proactive coping behaviors during social defeat in adulthood. Furthermore, we investigated whether these long-term effects of this early life experience on behavior are accompanied by changes in brain monoamine levels and turnover. HPLC analysis of biogenic amines from the brains of adult animals either subjected or not to the social defeat stress showed differences in the serotonergic and

dopaminergic system of the prefrontal cortex and amygdala. More specifically, in both the PFC and the amygdala, rats trained as neonates under frustration had lower serotonin (5-HT) levels. Furthermore, the prefrontal cortex of these animals also had lower dopamine levels. Social defeat resulted in increased serotonin and dopamine levels in the prefrontal cortex and amygdala of neonatally frustrated rats. These results indicate that long-lasting changes in social behavior following an early life rewarding or frustrating experience are accompanied by changes in brain monoaminergic activity.

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WE08-08

THE EFFECT OF ADOLESCENT TOLUENE EXPOSURE ON BEHAVIOURAL RESPONSES TO COCAINE IN ADULTHOOD: IMPACT OF DOSE AND EXPOSURE FREQUENCY

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The purposeful inhalation of volatile solvents is increasingly prevalent in adolescent populations, posing a significant risk to the maturing brain. Furthermore, adolescent exposure is thought to potentially predispose individuals to affective problems and substance abuse later in life. Utilising a rodent model of chronic intermittent toluene (CIT) exposure via inhalation we aimed to characterize the behavioural consequences of prior toluene exposure during adolescence, including cross-sensitization to cocaine. Adolescent male Wistar rats (PN 24) were exposed to either air or one of 2 CIT paradigms: 3000 ppm for 1hr per day for 5 days per week or 5000ppm for 1hr per day for 3 days per week for a total of 4 weeks ($n = 10$ per group). Behavioural tests including the Y-maze and light/dark test revealed no overt differences in spatial memory, response to a novel environment or anxiety. There was however a significant ($p < 0.05$) difference in the acute locomotor response to cocaine (20 mg/kg, i.p) 10 days after the last exposure, and the development of sensitization following repeated exposures to cocaine in rats exposed to 3000 ppm toluene 5 times a week compared to air controls. The same effect was not observed in rats exposed to 5000ppm 3 times a week. In addition, there was an exaggerated locomotor response following acute MK801 (0.5 mg/kg, i.p) in rats exposed to 3000 ppm toluene for 5 times a week compared to air controls, that was again not observed in the 5000 ppm for 3 times a week cohort. These data suggest that inhalant abuse during adolescence may result in neuroadaptations (including to the glutamatergic system) to neuronal pathways involved in the behavioural responses to other drugs of abuse, such as cocaine. They also highlight that the dose and frequency of exposure may play an important role in the impact of adolescent inhalant abuse on the maturing brain.

WE08-09

INHIBITION OF PEROXYNITRITE BY CUII(ATSM) IS NEUROPROTECTIVE AND RESULTS IN IMPROVED MOTOR AND COGNITIVE FUNCTIONS IN ANIMAL MODELS

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Parkinson's disease is a debilitating disorder that affects up to 1% of the geriatric population. This neurodegenerative disorder is attributed to the loss of dopaminergic neurons within the substantia nigra pars compacta (SNpc) with subsequent impairment in fine motor control and coordination. In addition, as must as 70% of PD patients also suffer from cognitive decline. Whilst the exact pathological mechanism still evades researchers, evidence suggests that oxidative and nitrosative stresses contribute to neuronal death. Nitrosative stress occurs due to the over production of reactive oxygen species (RNS), particularly peroxynitrite (ONOO-). ONOO- is a highly reactive radical that is capable of cysteine thiol nitrosylation and tyrosine nitration on proteins, such as α-synuclein (α-syn), tyrosine hydroxylase (TH) and vesicular monoamine transporter-2 (VMAT2).

In vitro assays showed that CuII (ATSM) was able to enhance the degradation of ONOO- and, in doing so, inhibit the nitration of α-synuclein in a dose dependent manner. When administered into animal models (1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) and 6-hydoxydopamine (6-OHDA) lesioned mice and A53T α-syn transgenic mice), CuII(atsm) was found to be therapeutic in all the murine models, having improvements in motor function and rescued the loss of dopaminergic neurons in the SNpc. Improved motor function in the MPTP lesioned mice corresponded to increase levels of TH, VMAT2 and dopamine. Treatment also improved cognition and increased synaptophysin levels in A53T transgenic mice. Reductions in α-syn levels were seen in addition to reduced nitrosative stress (nitrotyrosine levels) and nitrated α-synuclein. CuII(atsm) was also able to rescue toxicity and inhibit increases in nitrotyrosine levels in differentiated SH-SY5Y cells treated with 3-morpholinosydnonimine (SIN-1), a compound that releases ONOO-.

The ability of CuII(atsm) to inhibit ONOO- and nitrosative stress leading to therapeutic outcomes in 4 murine PD models is very encouraging and therefore, seen as a novel and potential effective pharmacological compound in targeting PD pathology.

WE08-10

ADULT NEUROGENESIS IMPAIRMENT RESULTS IN ALTERATION OF HIPPOCAMPUS-DEPENDENT BEHAVIORAL TASKS BUT NO LEARNING DEFICITS

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The exact function of adult hippocampal neurogenesis remains elusive, although it has been suggested to play a role in learning and memory processes. In our studies, we employed cyclin D2 gene knock-out (cD2 KO) mice with almost complete deficiency of newborn neurons in the adult brain (Kowalczyk et al., J Cell Biol,

2004). These mice have also slight morphological abnormalities of the brain, including the hippocampal formation. Previously, we have shown for cD2 KO mice that new hippocampal neurons are not obligatory for memory formation (Jaholkowski et al. Learn. Mem, 2009). In the present study, the animals were subjected to hippocampus-dependant behavioural tests requiring and non-requiring learning component. cD2 KO mice showed significant impairment in such species-typical behaviours as nest construction, digging, and marble burying. They were building none or poorer nests, digging less robustly, and burying fewer marbles than WTs. Moreover, cD2 KO mice showed normal sucrose preference, however, in contrary to the controls, preceded by neophobia phase. cD2 KO animals were more active in the open field and automated motility chamber, and showed increased explorative behaviour in IntelliCage. On the other hand cD2 KO mice performed normally in the cue and context fear conditioning tasks. Presented results suggest that either morphological abnormalities of the hippocampal formation or adult brain neurogenesis impairment (or both) alter hippocampal-dependant behaviours without influencing learning abilities.

WE08-11

EFFECTS OF AGE ON PYRAMIDAL NEURON MORPHOLOGY, VASCULATURE AND BEHAVIOUR IN A MOUSE MODEL OF ACCELERATED COGNITIVE AGING

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The cholinergic system is involved in most cognitive tasks and is particularly vulnerable to the process of aging. In order to explore the process and variability of aging we have examined brain vasculature, neuronal morphology and behavioural parameters in mice lacking the beta-two subunit of the nicotinic Acetylcholine Receptor (nAChR). These animals show certain cognitive deficits in old age and hence have been considered a model of accelerated cognitive aging. In the present study we have examined brain vasculature and morphology of pyramidal neurons in two cortical areas (cingulate and primary visual cortex) and in four experimental groups: wildtype and beta-two knockout mice at two ages: adult (3–6 months) and old (18–24 months). All animals are YFP+. Furthermore, we have conducted behavioural experiments in the same age groups. As expected we found signs of morphological degeneration with aging in both WT and KO mice. However, we also found significant structural differences between wildtype and beta-two KO mice already from adulthood. Interestingly, this effect was significantly more pronounced in prefrontal cortex. In agreement with the morphological evaluation, behavioural tests revealed impairments in beta-two KO mice already from adulthood, in learning tasks that require high-level cognitive processing. On the other hand, in locomotor and anxiety tasks both genotypes only showed the expected effect of age. Taken together, these findings demonstrate that (i) the process of aging is not uniform but region-specific, (ii) the increased morphological degeneration in prefrontal cortex and the cognitive impairments of beta-two KO mice do not begin in old age, but are already present in adult mice, challenging the notion that beta-two KO mice are a model of accelerated cognitive aging. Our data suggest that beta-two subunit may have a specific role in cholinergic vulnerability related to cognition. Electrophysiological analysis of brain activity is currently in progress in order to complete this examination.

WE08-12

EFFECT OF HYPERICUM PERFORATUM INFUSION ON BEHAVIOR AND CHOLINERGIC SYSTEM OF ADULT MICE AFTER CO-ADMINISTRATION WITH CADMIUM

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Aim of this project was to study the possible positive effects of Hypericum perforatum infusion (2% w/v) on adult mice after simultaneous administration with cadmium (50 ppm/day, 3 weeks), by determining a) anxiety/fear behavior (thigmotaxis and elevated plus maze test); and b) the activity of two acetylcholinesterase (AChE) isoforms on mice brain regions (cerebral hemispheres, midbrain, cerebellum) and liver. The results indicated that: A) cadmium provide increased anxiety/fear behavior which was reversed in the animal group co-administered with the infusion (↓ 7.71% of thigmotaxis indicator and ↑ 42.92% of time remaining at open arms of the elevated plus maze device); and B) the activity of the two AChE isoforms (salt-soluble /SS and insoluble/DS) varied among the brain regions examined, after cadmium administration. Co-administration with Hypericum perforatum, showing also tissue-specificity.

WE08-13

POSSIBLE ROLE OF NARINGIN AGAINST COLCHICINE INDUCED COGNITIVE DYSFUNCTION AND OXIDATIVE DAMAGE IN RATS

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder affecting the elderly. Central administration of colchicine, a microtubule disrupting agent, is known to cause cognitive impairment, oxidative stress and neurofibrillary degeneration which simulate sporadic AD seen in humans. Present study was designed to evaluate the protective effects of naringin against the colchicine-induced cognitive impairment and oxidative stress in rats. Colchicine (15 μg/5μl) was administered intracerebroventricularly in rats. Rats were treated with naringin (per se; 40 and 80 mg/kg, p.o.) daily for a period of 25 days beginning 4 days prior to colchicine injection. On the 14th and 21st day after colchicine administration, behavioral studies were by Morris water maze paradigm, elevated plus maze task and locomotion (photoactometer). The rats were sacrificed on the 22nd day following the last behavioral test and various biochemical tests were performed to assess the extent of oxidative stress. Intracerebroventricular administration of colchicine resulted in poor retention of memory in Morris water maze, elevated plus maze task paradigms and caused marked oxidative damage. It also caused a significant increase in the acetylcholinesterase activity. Chronic administration of naringin caused an improvement in the cognitive dysfunction and significantly reduced the elevated malondialdehyde, nitrite concentration, acetylcholinesterase activity and restored SOD, catalase, glutathione-s-transferase and the reduced glutathione levels. The results of the present study suggest the neuroprotective activity of naringin against colchicine-induced cognitive impairment and associated oxidative stress.

WE08-14

CONDITIONED PLACE PREFERENCE FOR SOCIAL INTERACTION: CONTRIBUTION OF SENSORY MODALITIES

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A main challenge in the therapy of drug dependent individuals is to help them reactivate interest in non-drug-associated activities. We developed an animal experimental model based on the conditioned place preference (CPP) paradigm in which only four 15-minute episodes of social interaction with a gender- and weight-matched male Sprague Dawley rat (i) reversed CPP from cocaine to social interaction despite continuing cocaine training and (ii) prevented the reinstatement of cocaine CPP (Fritz et al. 2011, Addiction Biology, in press). The sigma1 receptor antagonist BD1047 (EC50, 0.0036 mg/kg i.p.) enhanced the reversal from cocaine CPP to social interaction CPP (Fritz et al. 2011. Pharmacology **87**:, 45–48). In the present study, we investigated which of the sensory modalities of the composite stimulus ''social interaction'' contributes most to the rats' preference for it. If touch was limited by a barred window running across the whole length of the partitioning, CPP was still acquired, albeit to a lesser degree. If both rats were placed on the same side of the partitioning, rats did not develop CPP for social interaction. Thus, decreasing the available area for social interaction from 750 to 375 cm^2 prevented the acquisition of CPP to social interaction despite the fact that animals could touch each other more intensely than through the bars of the partitioning. The other sensory modalities are currently being investigated. Supported by a Lise Meitner Fellowship (M1169-B189 to R.E.) and by the Verein fuer Experimentelle Psychiatrie, Psychotherapie und Pharmakologie (VEPPP).

WE08-15

HOW DOES DROSOPHILA SMELL VIBRATIONS?

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We use Drosophila melanogaster to investigate the contribution of molecular vibrations to olfaction. Drosophila is used based on its utility as a well understood sophisticated genetic system with a rich behavioral repertoire and highly advanced molecular genetics which permit rapid generation and analysis of mutants. A recent paper form the laboratory (Franco MI, Turin L, Mershin A, Skoulakis EM. 2011. ''Molecular vibration-sensing component in Drosophila melanogaster olfaction.'', Proc Natl Acad Sci USA, **108**: :3797–3802) indicated that Drosophila are capable of distinguishing a number of odoranst based on their molecular vibrations. Ongoing experiments to be presented, also use behavioral assays coupled with genetic manipulations aiming at establishing the generality of this vibrational phenomenon over a range of aversive and attractive odors. Another major hypothesis we are testing is whether the Drosophila olfactory system is designed to sense discrete frequencies (bandwidths) of vibrational spectra and to determine which of the known (or novel) olfactory receptors are ''tuned'' to the different vibrational frequencies and whether this is in addition to their ability to detect odorant shape. We will discuss our data in the context of our current hypothesis that a combination of vibrational and shape-sensing accounts for detection of odorants at least an order of magnitude greater than the available number of receptors.

WE08-16

BEHAVIORAL CHANGES AND NEURAL REGENERATION FOLLOWING *IN VIVO* TREATMENT WITH TRIMETHYLTIN IN MICE

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Our previous study showed that trimethyltin chloride (TMT) causes neuronal loss in the hippocampal dentate gyrus selectively 2 days later. To evaluate neural regeneration after hippocampal dentate granule cell loss, we examined behavioral changes and NMDA receptor signals in the dentate granule cells newly generated after neuronal damage induced by TMT. TMT (2.8 mg/kg) was i.p. injected into ddY male mice. NMDA (100 mg/kg) was injected on days 7 and 28 after TMT injection. NMDA-induced expression of c-Fos in the dentate granule cells was decreased on day 7 after TMT treatment. The decrease in NMDA-induced c-Fos expression was completely abolished on day 28 after TMT treatment. No significant change in locomotor activity was seen at least until day 56 after TMT treatment. However, forced swimming test revealed that depressive behavior was observed on day 14 after TMT treatment. Taken together, the dentate gyrus would be capable of regenerating functionally after the neuronal loss in mice. However, a transient depressive symptom could occur during neural regeneration after hippocampal dentate neuronal loss in mice.

WE08-17

RELATION BETWEEN PHYSICAL AND MENTAL FATIGUE IN URINE BIOMARKER, HYDROGEN PEROXIDE

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Oxidative stress is associated with fatigue. Therefore, biomarkers for oxidative stress may be available for those to fatigue. Biomarkers for oxidative stress have been found in serum, urine and brain; 8-hydroxy -deoxyguanosine (a marker of oxidative damage to DNA), L-carnitine, some oxidative stress biomarkers, isoprostanes, TBARS, protein carbonyls, catalase, glutathione peroxidase, and oxidized glutathione (GSSG), reduced glutathione (GSH) etc. However, whether biomarkers for oxidative stress are useful for those to fatigue, furthermore, relation between physical and mental fatigue in biomarkers is unknown. In the present study, we examined whether hydrogen peroxide (H_2O_2) is useful for biomarkers for physical and/or mental fatigue using urine from mice

and rats after treatment of physical and mental stress. We are also going to examine relation between physical and mental fatigue in a biomarker for mental fatigue by RT-PCR and Western blot analysis in brain. This study (No.22500614) is supported by Grant-in-Aid for Scientific Research (KAKENHI); Grant-in-Aid for Scientific Research(C) in 2010 and 2011.

WE08-18

STRESS-REACTIVITY AND BEHAVIORAL CONSEQUENCE OF SOCIAL STRESS IN NOVEL MODEL OF SOCIAL STRESS

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A novel model of social stress in rats was used that gives equal possibility to both animals to become a ''winner'' or ''loser''. Before the stress, animals were tested in Open Field (OF) test and Black-White box (BW) test to detect initial locomotor and anxiety level in rats. To evaluate stress-reactivity in ''winner'' and ''loser'' rats, the part of rats after social stress were subjected to acute foot-shock stress. Thereby, all rats were divided on 4 groups: control, acute foot-shock stress (approximately 1 mA, 20 minutes), social stress group (14 days of social stress) and combined stress group (rats were subjected to acute foot-shock stress after social stress). Elevated Plus maze (EPM), Social interaction test (SI), Radial maze (RM) were used to examine behavioral activity. Acute stressed rats demonstrated lower level of exploratory activity ($p = 0.03$) in EPM, decreased amount of time spent in contact with another rat (p=0.0067) and decreased vertical activity ($p = 0.0125$) in SI test. ''Winners'' from social stress group and combined group demonstrated higher total time spent in contact with another rat ($p = 0.001$ and $p = 0.00009$ respectively) in SI test, and elevated level of exploratory activity in EPM ($p = 0.02$). ''Winner'' rats from combined stress group demonstrated increased level of exploratory activity ($p = 0.03$) in EPM and increased vertical activity in SI test ($p = 0.05$). Decreased level of exploratory activity (0.04) in SI test and decreased level of emotionality ($p = 0.005$) in EPM were observed in ''loser'' rats from social stress group. Also, in social stress group more difference between ''winners'' and ''losers'' were observed in SI test, and in combined stress group more difference were obtained in EPM test. It was interesting that ''loser'' rats from social stress demonstrated decreased learning ability ($p < 0.05$), whereas ''loser'' rats from combined stress group didn't show such decreasing. So, one can conclude that acute stress differently affect loser and winner rats. In addition, it could be noted that acute non-social stress after social defeat can prevent learning impairment.

WE08-19

EEG: TO INVESTIGATE RECOVERY OF RAT BRAIN FUNCTION FOLLOWING ISCHEMIC STROKE

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EEG has a great importance in routine clinical diagnoses because of being economical and especially due to its role in detecting alterations in brain firing. Mathematical modeling of induced focal cerebral ischemia in animal model using EEG data was intervened and analysis was undertaken to ensure actual correlation of the different mathematical, physiological and biochemical paradigms. The EEG signals was analysed with the help of conventional and modern spectral analysis methods. Interpretation and performance of these methods were critically observed so that these can be further applied to research and clinical applications. The model developed was used to understand the pathophysiology of stroke and its diagnostic interventions. The altered firing of neurons which in turn were elucidated by analyzing the total electrical activity of the brain in the form of EEG. For this purpose, the EEG data was obtained from specific regions of rat brain and was processed by different modeling techniques with the help of MATLAB. The assessment of long lasting functional outcome, in addition to the prevalent classical approach to study stroke was necessitated and therefore highly recommended to evaluate the efficacy of therapeutic strategies in relation to EEG in animal model of brain stroke. Keeping in view the above facts strategies have been explored to develop an animal model which can manifest the true condition of brain stroke, related motor function deficit and cognitive impairment. Brain signals were recorded and analysed. Finally mathematical modelling specifically Power Spectrum Density analysis was done to correlate the prevalent condition with the normal condition and condition after pharmacological intervention. Taking into such considerations it was observed that there have been variations in PSD which can serve as one of the diagnostic tools for detecting brain stroke and its recovery.

WE08-20

INDIVIDUAL DIFFERENCES IN THE EFFECTS OF CHRONIC ANTIDEPRESSANT TREATMENT: SEX DIFFERENCES EXPOSED

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Partial response to antidepressant treatment is a common handicap in the pharmacotherapy of major depression and has been partly attributed to the idiosyncrasy of the patient. Experimentally, when naïve rats are exposed to the stress of a novel environment they display a highly variable exploratory activity; some rats are characterized by high rates of locomotor reactivity (HR), whereas others by low rates (LR). Recently, it was shown that individual differences, as assessed in this paradigm, may predict differential responses to sub-acute desipramine treatment in the forced swim test (FST) of behavioral despair. Intriguingly, recent clinical and experimental evidence point towards a sex-specific antidepressant response. Herein, we sought to investigate whether spontaneous reaction to novelty would serve as predictor of antidepressant efficacy in rats treated chronically with the tricyclic antidepressant clomipramine. Male and female Sprague-Dawley rats were assigned to HR/LR groups upon their locomotor performance in a novel open-field. Following the initial phenotypic characterization, HR and LR rats were chronically treated with clomipramine (i.p. 10 mg/kg; 28 days) or vehicle. Upon completion of the drug-administration regimen rats were subjected to FST. At 20 minutes following the FST probe, limbic brain regions implicated in the pathophysiology of MD were isolated and the monoaminergic status was appreciated by means of High Performance Liquid Chromatography (HPLC). According to our results, antidepressant efficacy was evident solely in male HR and female LR rats, as reflected in the

reduced time these rats spent floating. Further, neurochemical analyses showed that clomipramine treatment induced phenotype-specific serotonergic alterations upon FST exposure; in male HR rats serotonergic activity was boosted at all limbic regions examined, but was decreased in their LR counterparts. In female rats neurochemical alterations were modest. Overall, the current dataset lends further support that the male sex may benefit to a greater extent when treated with clomipramine and reveals that individual differences are associated with qualitative and quantitative sex-related behavioral and neurochemical manifestations in response to chronic antidepressant treatment.

WE08-21

BEHAVIORAL AND NEUROCHEMICAL ALTERATIONS IN ADULTHOOD PROVOKED BY INTERACTIONS OF NEONATAL AND ADOLESCENT EXPERIENCES

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It is well known that early life stressful events and social stress in adolescence are associated with the emergence of psychopathologies. Based on the above we developed an experimental model of a neonatal experience, in which during postnatal days 10–13 rat pups are exposed to a T-maze an arm of which leads to the mother. One group of animals is allowed contact with the mother (rewarded), while the other is denied (frustrated). For the adolescent experience we used a rat model of social stress: At the age of 30–38 days female rats were subjected daily to 1h of isolation and change of cage partner. In adulthood rats were exposed to 2 days of forced swimming test (FST), and immobility time was measured. Following the second day of exposure to the FST, as well as under basal conditions, rats were sacrificed and biogenic amines were determined by HPLC. Under basal conditions in both the PFC and the AMY, the neonatally frustrated rats had lower 5-HT levels. In the absence of stress during adolescence, rats exposed to the neonatal experience, either under reward or frustration, had longer immobility times than the controls, during the first day of forced swimming. On the second day, neonatally rewarded rats had longer immobility times than the neonatally frustrated or the controls. When rats were exposed to the adolescent stress, the neonatally frustrated had longer immobility times than either the rewarded or controls, only on the first day of the test. Exposure to adolescent stress affected only the neonatally frustrated animals, resulting in longer immobility times, increased metabolism of 5-HT in the PFC and the HIP while 5-HT itself was decreased in the AMY. Our results support that the neonatal experience interacts with that during adolescence to determine adult brain neurochemistry and the behavioral response to stress.

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WE08-22

LRRK2 OLIGOMERIZATION AND THE RECRUITMENT OF FADD AND CASPASE-8 TO OLIGOMERIC CELL-DEATH SIGNALING COMLEXES

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Mutations in leucine rich repeat kinase 2 (LRRK2) are the most common cause of familial Parkinson's disease (PD). Since LRRK2-related PD is clinically indistinguishable from the sporadic form, and because mutations in LRRK2 can also be found in a percentage of sporadic cases, understanding the mechanisms of mutant LRRK2-induced neurotoxicity can provide insight into the pathogenesis of PD in general. Recent work has demonstrated that LRRK2 interactions with FADD as well as caspase-8 are both critical for its neurotoxic effects; however, the precise mechanism by which these events trigger neuronal death is not fully understood. We have examined the oligomerization of mutant LRRK2 by size exclusion chromatography (SEC). Mutations in LRRK2 caused a clear shift to higher molecular weight fractions. Moreover, a point mutation that inhibits the major shift in elution of mutant LRRK2 also blocked neuronal death, suggesting that the formation of higher-order LRRK2 oligomers is a critical step in the induction of neuronal death by mutant LRRK2. In parallel experiments, we expressed the individual domains of LRRK2 (N-terminal, LRR, ROC, COR, kinase, and WD40) and mapped the domain responsible for the interaction with FADD to the large N-terminal region upstream of the LRR. In fact, when normalized to the expression level of full-length wild type LRRK2, the isolated N-terminal domain interacts even more strongly with FADD than full-length wild type LRRK2. Importantly, in primary cultures of cortical neurons, co-expression of this N-terminal fragment together with full length mutant LRRK2, prevented neuronal apoptotic death caused by mutant LRRK2 alone, suggesting it does so by binding endogenous FADD in a dominant negative fashion and preventing the caspase-8 dependent apoptotic cascade we have described previously. Preliminary evidence points to a potential cleavage step that may be critical in the formation of higher molecular weight oligomeric complexes containing LRRK2; and thus, this step may also be required for the induction of apoptotic death in neurons expressing mutant LRRK2.

WE08-23

NPF AS MOTIVATIONAL MODULATOR IN OLFACTORY LEARNING IN DROSOPHILA LARVAE

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How does an animal ensure that an appropriate motor action is expressed at the right time?

Similar to humans Drosophila larvae and flies use past experience to predict the future, be it the consequence of an animal's own actions or upcoming external events. These predictions then can contribute to the selection of behaviour which is in addition under motivational control. The mechanisms of motivational control have been widely studied; however the molecular and neuronal basics remain rather unknown.

Drosophila is an ideal model to investigate this issue as well-established learning paradigms exist. One prominent paradigm that

is used in flies and larvae uses learned representations of olfactory cues associated with food. Hereby activation of the neuropeptide F (NPF) was shown to mimic food deprivation and promotes memory performance in satiated flies. As larvae are constant feeders and therefore differ in their naïve food-seeking behaviour to flies we investigated the action of NPF in larval associative olfactory learning by spatio-temporal decrease and increase of the peptidergic NPF signalling. In contrast to adult behaviour larval learning performance decreased with higher NPF levels, suggesting a different function of NPF in the larval brain.

WE08-24

ANTICONVULSANT PROPERTIES OF CITRUS AURANTIUM IN ZEBRAFISH

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Leaves of *Citrus aurantium* (CA, bitter orange) are used in folk medicine to treat anxiety, insomnia and epilepsy. Essential oils from the peel of the fruit have shown to increase seizure latency induced by pentylenetetrazole (PTZ) and maximal electroshock seizure in mice. *In vivo* anticonvulsant properties of CA extracts were tested using adult zebrafish (*Danio rerio*). Zebrafish were exposed to various CA extract concentrations before exposure to PTZ 3 mg/ml. CA extracts 28.00 mg/ml increased seizure latency by 62%. The effects of CA extract on metabotropic (mGluR) and ionotropic (iGluR) receptors were examined through [^{3}H] Glutamate binding ([^{3}H]). CA extract showed an EC50 of 0.1967 mg/ml and reduced [^{3}H] Glu binding from 0.42mg/ml to 5.60mg/ml. In the presence of glutamate ligands CA extract showed selectivity for iGluR and mGluR groups II and III. To study the *In vivo* interaction of CA extract with mGLuR, zebrafish were treated with various glutamate receptor antagonists prior exposure to CA extract. Pretreatment with PHCCC potentiated the effect of CA in seizure latency suggesting an interaction between CA extract and mGluR I. Also exposure to EGLU followed by CA extract 28mg/ml abolished the increase in seizure latency caused by CA exposure, suggesting an interaction between CA extract and mGluR II receptors. Exposure to CPPG prior to CA extract 28mg/ml had no significant effect on the change in seizure latency caused by CA extract. Taken together, these experiments suggest a possible interaction of CA extract with mGLUR. Approved by IACUC protocol #3180110.

WE08-25

EFFECTS OF PARADOXICAL SLEEP DEPRIVATION ON PAIN SENSITIVITY AND ITS RELATIONSHIP WITH DOPAMINERGIC SYSTEM

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Paradoxical sleep deprivation (PSD) increases pain sensitivity and reduces morphine antinociception. Because dopaminergic neurons in the periaqueductal gray matter (PAG) participate in pain modulation and opioid-induced antinociception, we evaluated the effects of PSD on thermal pain sensitivity, morphine- and L-DOPA-induced antinociception and dopaminergic functionality in the PAG by assessing tyrosine hydroxylase (TH) immunoreactivity. Rats that were subjected to 96 hours of PSD received vehicle, morphine (2.5, 5 or 10 mg/kg, i.p.), L-DOPA (50 or 100 mg/kg, i.p.) or L-DOPA (50 mg/kg) + morphine (2.5 and 5 mg/kg) and were tested with a 46 °C hot plate 1 hour after the injections. The paw withdrawal latency response to the hot plate was decreased in the PSD rats and was modified by the highest dose of morphine, L-DOPA and L-DOPA + morphine. The analgesic effects were observed in control groups for all morphine doses, 100 mg/kg of L-DOPA and L-DOPA (50 mg/kg) + morphine (5 mg/kg). The number of cell bodies that were immunopositive for TH in the PAG was reduced in PSD rats. In conclusion, the increased thermal sensitivity was reversed by L-DOPA and could be caused by a reduction in PAG TH level. Our data also suggests a relationship between central dopaminergic network and opiate-induced analgesia in rats.

WE08-26

CIRCADIAN HYPOACTIVITY IN RELAXIN-3 KNOCKOUT MICE AND ACTIVITY/FEEDING EFFECTS FOLLOWING PHARMACOLOGICAL RXFP3 MANIPULATION

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Relaxin-3 is a newly identified neuropeptide, which is enriched within neurons of the nucleus incertus that widely innervate forebrain regions containing high levels of the relaxin-3 G-protein coupled receptor, RXFP3; and which has been implicated in the control of a range of behaviours such as arousal and feeding. In the present studies, C57BL/6J backcrossed relaxin-3 knockout (KO) mice were observed to run 30–40% less distance than wildtype (WT) controls on home-cage running wheels during the dark (active) phase ($p = 0.009$), and spent more time sleeping. Further evidence linking relaxin-3 with arousal was achieved in a separate cohort of WT mice subjected to food restriction, whereby acute icv infusion of an RXFP3 antagonist (0.9 nmol) decreased the climbing behaviour exhibited by mice ($p = 0.012$) during the light phase food anticipatory activity period before meal time. In a separate set of experiments, in contrast to rat studies which have demonstrated relaxin-3 to be potently orexigenic, increased consumption of regular chow was not observed in WT mice following acute icv infusion of 0.9 nmol mouse relaxin-3 ($p = 0.52$) or a specific RXFP3 agonist ($p = 0.59$), and similar results were obtained using relaxin-3 KO mice. Interestingly however, acute icv infusion of an RXFP3 antagonist (1.8 nmol) was shown to decrease the consumption of palatable food ($p = 0.008$). These studies suggest that while RXFP3 is able to modulate feeding behaviour under certain conditions in mice, this role does not appear as pronounced as in rats. Taken together, these studies provide further evidence that relaxin-3 can modulate arousal and feeding behaviours, which are often perturbed in a range of psychiatric disorders.

WE08-27

RESTORING DA CLEARANCE MECHANISMS REDUCES ABNORMAL INVOLUNTARY MOVEMENTS THAT ACCOMPANY L-DOPA THERAPY

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Parkinsonian symptoms result from insufficient dopamine (DA) supply to the striatum. L-DOPA treatment initially improves Parkinson's disease (PD) symptoms. However, its beneficial effect is marred by the emergence of complex excessive involuntary movements (dyskinesia). Previously, using *in vivo* microdialysis and high performance liquid chromatography (HPLC), we showed that in dyskinetic rats, striatal DA levels are abnormally high during a dose of L-DOPA, and that DA levels directly correlate with dyskinesia. Our current work tested whether normalising striatal DA levels in PD rats following a dose of L-DOPA prevents dyskinesia. Our approach was to reinstate DA clearance mechanisms, i.e. the DA transporter, into the striatum, which are lost as a consequence of PD. Rat PD models were created by injecting 6-hydroxydopamine into the medial forebrain bundle. Four months later, L-DOPA methyl ester (6 mg/kg) and benserazide (15 mg/kg) were administered daily for 14 days, during which abnormal involuntary movements (AIMS) were measured. AIMS were classified into four subtypes: forelimb; orolingual; axial; and locomotive. The full length rat DAT cDNA was cloned, and we developed a B1a fibroblast cell line that expressed DAT. When a stable level of AIMS was established, these DAT-expressing fibroblast cells were transplanted into the striatum of dyskinetic rats. Following grafting of DAT-expressing fibroblasts, L-DOPA was administered for a further 10 days, during which AIMS reduced by 31 ± 10%. No significant reductions in AIMS were observed in dyskinetic rats that received non-DAT-expressing fibroblasts cells. Currently, microdialysis and HPLC experiments are being performed to confirm whether grafting of DAT-expressing fibroblasts reduces DA levels in the striatum of PD rats following L-DOPA treatment. Our results suggest that preventing excess DA levels in the PD-affected striatum, by restoring DA clearance mechanisms, reduces L-DOPA induced dyskinesia. This approach to manipulating DAT to achieve stable DA levels may point to a potential therapy for preventing dyskinesia.

WE08-28

ABSTANTICONVULSANT PROPERTIES OF VALERIAN EXTRACTS AND VALERENIC ACID IN ADULT ZEBRAFISH (*DANIO RERIO*)RACT TITLE

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Valeriana officinalis extracts have been attributed anxiolytic, sedative and anticonvulsant effects. Our aim was to examine the anticonvulsant properties of valerian extracts in adult zebrafish (*Danio rerio*). Zebrafish were pretreated with anti-epileptic drugs (AEDs), valerian extracts or valerenic acid and then exposed to Pentylentetrazole (PTZ 3 mg/mL) in the water tank. After exposure to PTZ naive fish swim in circles, dart about the enclosure; make a wild jump and loose posture. The latency period was defined as the wild jumping immediately followed by the loss of posture. Zebrafish pretreated 1 hour with AED such: clonazepam, phenytoin, gabapentin or valproate significantly increased ($p < 0.001$) latency when exposed to PTZ (3 mg/ml). Valerian extract, valerenic acid and AED had similar convulsion progression. Ethanolic valerian extract (0.50–2 mg/ml) significantly increased latency. Both extracts, showed similar latency of phenytoin, gabapentin and valproate in zebrafish exposed to PTZ. However, 5 mg/ml aqueous valerian extract were required to obtain a significant increase on latency. Valerenic acid increased latency at lower (20–80 μg) doses compared with valerian extracts. Our data demonstrated that valerian extract had similar effect to AED. Ethanolic valerian extract are 10 times more potent than aqueous extracts. Valerenic acids are at least 25 times more potent compared with AED and valerian extracts. Approved by IACUC protocol #3180110 on May 28, 2010.

WE08-29

THE ROLE OF THE VASOPRESSIN IN ALZHEIMER RELATED DEMENTIA

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Despite the growing number of patients and the potential danger what these patients mean to themselves or others, the treatment of dementia (as a phenomenon of the Alzheimer disease) is still an unsolved problem. The positive effects of vasopressin on the memory are well known, therefore we examined if its role changes with age and if these changes are correlated with the level of the cerebral amyloid precursor protein mRNA levels (APP; marker of Alzheimer disease). We compared the vasopressin deficient homozygote recessive Brattleboro rats with normal homozygote dominant animals in adult (3 months) and in old age (12 months). To receive a comprehensive picture about their memory we observed their social discrimination, object discrimination, and conditioned learning abilities (Shuttle box). The first two tests model a short-term, stress free learning process, while the Shuttle box test is a long-term, stressful examination. We measured the APP mRNS levels with quantitative PCR. Although the aged animals was less interested in examining their environment, but there was no difference between the memory of adult and aged control groups. The vasopressin deficient rats at both ages showed a weaker performance in the course of the social and object discrimination tests. During the shuttle box test the vasopressin deficient animals were more active. In adult rats the vasopressin deficiency worsened the memory, however in the aged rats this difference disappeared. The APP mRNS levels of the elder animals were higher, than the levels of the adults, and the presence of the vasopressin did not influence the elevation. We can conclude that the 1 year old rats do not show spontaneously memory deficit, although the results of the APP marker measurement are already pathological. The positive roles of the vasopressin in short-term memory tests are observable in both age groups, although it does not go parallel with the levels of the APP marker. So vasopressin does not seem to have a role in the development of Alzheimer related dementia.

WE08-30

PHARMACOLOGICAL RESCUE OF LEARNING DEFECTS IN NEUROFIBROMATOSIS TYPE 1 MUTANT FLIES

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Neurofibromatosis Type 1 (NF1) is a devastating disease affecting 1 in 3500 children. In addition to a heavy tumor burden and physical abnormalities, 55% of children have difficulties with visuospatial and learning tasks. Mouse NF1 mutants show spatial learning defects in a Morris water maze that can be genetically and pharmacologically rescued. Fly NF1 mutants also show learning defects using a classical learning protocol that pairs odor with shock. The NF1 protein regulates two signaling pathways known to be required for learning and memory tasks in flies. The central Ras GAP domain (GRD) of NF1 down regulates Ras and downstream MAPK and PI3K/mTOR pathways required for long term memory. The C-terminus of NF1 activates an adenylyl cyclase to increase cAMP levels required for learning. We are using three FDA approved drugs to alter signaling in these pathways: lovastatin, an HMG-CoA reductase cholesterol lowering drug; rolipram, an anti-inflammatory anti-depressant drug that inhibits cAMP phosphodiesterase; rapamycin, an anti-tumor drug that inhibits mTOR. We hypothesize that these drugs should improve learning of NF1 mutant flies. To test learning, groups of adult flies are exposed to two odors in succession, the first paired with electric shock. When given a choice between the odors, wild type flies avoid the odor that was paired with shock. Using this paradigm, we have shown improved learning of NF1 mutants after overnight treatment with lovastatin and rolipram, but not rapamycin. Individual adult flies can also be trained to associate a visual stimulus with heat punishment in a flight simulator apparatus. We found that NF1 mutant flies show a visual learning defect, which is improved after overnight treatment with lovastatin. Testing with the other two drugs is underway. We plan to determine whether the GRD or C-terminus of NF1 is regulating visual learning by expressing transgenic RNAi and human NF1 fragments in discrete areas of the fly brain. Successful completion of these experiments will inform and expedite mouse and human research.

WE08-31

INTERACTIONS OF OPIOID RECEPTORS WITH SPECIFIC AND NONSPECIFIC ION CHANNELS

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In the last decade more data were published on relationships between opioid receptors, different specific channel VDCC (N-type voltage-dependent Ca^{2+} channel, Heinke et al.2011), and non-specific ion channels: such as ASIC1 (acid sensing ion channel1, Mazzuca et al.2007) and TRPV1 (transient receptor potential channel vanilloid1, Zoellner et al.2004). Among opioid receptors mu-ligands (morphine, DAMGO, endomorphin1) were established to inhibit TRPV1 and VDCC channels,or to activate ASIC1a channel through the endogeneous kappa-ligand dynorphin (Sherwood et al.2009). The inhibition of the mu-ligands on TRPV1 was also observed using *in vitro* assays (Endres-Becker et al.2007,Vetter et al.2008).Furtheron the TRPV1 ligand capsaicin inhibited the endomorphin1 binding and the effect on Gi-protein of the mu-opioid receptor (Wollemann et al.2008), yet no direct evidence was found to clear the molecular mechanism of these actions. An increased level of met-enkaphalin was observed after tartantula mPcTx1 injections against pain via ASIC1a channel (Mazzuca et al.2007), The inhibition of the cAMP activated protein kinase A was suggested by Vetter et al.(2008) as a possible mechanism.No direct action of capsaicin on opioid receptor binding was found in our experiments of the reverse reaction (Wollemann et al.2008).These results let us to study the effect of protein kinase A and C inhbitors on these interactions between opioid receptors and ion channel molecules.

Thursday Oral Sessions
Plenary Lecture 5

PL5

MOTOR NEURONS AND THE SENSE OF PLACE

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The formation of selective synaptic connections is a defining moment in the construction of neural circuits, providing a foundation for network activities that govern behavior. The spinal monosynaptic reflex arc is one of the most intensively studied circuits in the CNS, given its crucial role in the coordination of motor behavior. The exquisite selectivity of sensory-motor connections can be revealed in the circuits that control limb movement – the fifty or so muscles that control modular elements of joint motion are each innervated by dedicated sets of sensory and motor neurons. A challenging developmental task confronts the sensory neurons that supply the spindles of an individual muscle: the necessity of forming strong connections with motor neurons that innervate the same muscle, weaker connections with motor neurons that innervate synergist muscles, and the avoidance of motor neurons that innervate antagonist muscles. The matrix of sensory input specificity is intertwined with spatial features of motor neuron organization. Motor pools that control muscles with related functions are organized into longitudinally arrayed columels that reflect the primary axes of limb construction.

To examine whether spatial features of motor neuron organization influence sensory-motor circuit assembly we analyzed connectivity patterns in mutant mice that scramble motor neuron position. The emergent connectivity patterns indicate that key aspects of sensory-motor connectivity are programmed through the targeting of sensory afferents to discrete dorsoventral domains, independent of motor neuron subtype. This strategy may: i/ provide a purpose for the stereotypic clustering of motor neurons, ii/ imply that many molecular features of motor neuron subtype are devoted to the precision of motor neuron positioning, and iii/ permit sensory afferents to capture neuronal modules that direct elemental units of motor behavior without the molecular burden inherent in allocating intricately matched surface recognition profiles. The idea that neuronal position determines input specificity in the absence of target recognition could have relevance for circuit assembly in other regions of the mammalian CNS.

Supported by HHMI, NINDS, ProjectALS and The Mathers Foundation.

Plenary Lecture 6

PL6

IP_3 RECEPTOR/CALCIUM CHANNEL AS A SIGNALING HUB: ROLE IN DEVELOPMENT, BRAIN FUNCTION AND BRAIN DAMAGE

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IP_3 receptor (IP_3R) is an IP_3-ligand gated Ca^{2+} release channel localized on the ER. We discovered IP_3R as a P400 protein missing in the Purkinje cell-deficient mutant mice (Develop Neurosci. 1989). We cloned and determined the whole sequence (monomer 313K Dalton)and it forms tetramer (Nature 1989). We solved 3D structure by cryo EM and X-ray crystalographic analysis. IP_3-ligand binding site comes close to the channel pore region and N-terminal region binds to the loop region of transmemebrane near the channel pore indicating unique channel gating. Many molecules bind to the region near the channel pore: GRP78 (Chaperone). ERp44 (Redox sensor), GIT1, NMDA-R, mGluRI, AMPA receptor, CaM, 4.1N, and homer etc. IP_3R moves dynamically inside the cell not only on the meshwork of ER, it moves along microtubles carrying vesicular ER, and clusters on the ER. mRNA of IP_3R moves along the microtubles. These various movements make the dynamic function regulation by IP_3R. We found a pseudo-ligand of IP_3R [we named it IRBIT (IP_3 receptor binding protein released with IP_3)] which binds to the IP_3 binding pocket of IP_3R. Phosphorylation of IRBIT is essential for binding to IP_3R. IRBIT not only inhibits IP_3-induced Ca^{2+} release but also binds and activates pancreatic type Na, bicarbonate cotransporter 1 and CFTR essential for pH balance. It also regulates polyA elongation. IRBIT binds signal recognition particle which is important for protein synthesis. All these data suggest that IP_3R works as a signaling hub for cell function. We found that IP_3R is essential for production of Ca^{2+} oscillation. It is involved in cell division, dorso-ventral axis formation, neural development, neurite extension, neuronal plasticity, spine specificity, cardiogenesis, osteoclast formation and exocrine secretion. We further found that IP_3R protects ER stress-induced brain damage. IP_3R/ Ca^{2+} channel are important in both physiological and pathophysiological functions.

ISN Young Scientist Lecture 2

YSL4

ARC – A MASTER CONTROLLER OF EXPERIENCE AND SYNAPTIC-DEPENDENT PLASTICITY

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Long-term storage of information in the brain is dependent on rapid, de novo RNA and protein synthesis. Similar synthesis is essential for long-term forms of synaptic plasticity such as long-term potentiation (LTP) and depression (LTD). Efforts to identify molecules that underlie transcription-dependent plasticity have revealed a set of immediate early genes that target to excitatory synapses. Among these, Arc is the most tightly coupled to behavioral encoding of information in neuronal circuits. Arc affects the trafficking of AMPA type glutamate receptors (AMPARs) by directly interacting with the endocytic machinery and homeostatically regulates surface AMPAR levels. However, very little is known about Arc's function at the level of neuronal circuits or its precise *in vivo* role in mediating information storage. The visual cortex provides an ideal preparation to investigate the mechanisms that underlie experience-dependent plasticity and the *in vivo* role of Arc, because of the ease of manipulating visual experience. Using chronic visually evoked potential recordings; we find that Arc deficient (KO) mice exhibit deficits in ocular dominance (OD) plasticity, due to deficient depression of the deprived eye responses. Consistent with this, we find deficits in layer 4 LTD in cortical slices. Furthermore, Arc KO mice do not exhibit open eye potentiation after longer periods of deprivation. A newly discovered form of experience-dependent plasticity, stimulus-specific response potentiation (SRP), seems to result from LTP-like mechanisms *in vivo*. Arc KO mice also show deficits in this form of plasticity. Despite the lack of experience-dependent plasticity in Arc KO mice, visual acuity is normal suggesting that the fundamental wiring of the visual system is intact. These data suggest that in the absence of Arc, synapses in the visual cortex are rendered insensitive to the effects of both experience and deprivation. The main conclusion garnered from this body of work is that Arc is a critical effector molecule downstream of many molecular signaling pathways and that dysregulation of Arc expression can have dire consequences for normal brain function.

Symposium 26
Proliferation Versus Differentiation of Neural Stem Cells: From CNS Morphogenesis to Pathophysiology and Treatment

S26-01

ESSENTIAL ROLES OF NOTCH SIGNALING IN EMBRYONIC AND ADULT NEURAL STEM CELLS

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To determine a role for Notch signaling in the telencephalic neural stem cells, we generated tamoxifen-inducible conditional knock-out mice that lacked Rbpj, an intracellular signal-mediator of all Notch receptors. When Rbpj was deleted in the embryonic brain, almost all telencephalic neural stem/progenitor cells prematurely differentiated into neurons and were depleted. When Rbpj was deleted in the adult brain, all neural stem cells differentiated into transit-amplifying cells and neurons. As a result, neurogenesis increased transiently, but three months later all neural stem cells were depleted and neurogenesis was totally lost. We also obtained the same results when the Notch effectors Hes1, Hes3, Hes5 and Hey1 were inactivated. These results indicated an absolute requirement of Notch signaling for the maintenance of neural stem cells and a proper control of neurogenesis in both embryonic and adult brains.

S26-02

CONDITIONAL EXPANSION OF NEURAL STEM CELLS IN THE ADULT MAMMALIAN BRAIN

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Neural stem cells (NSC) in the adult mammalian brain generate neurons and glia throughout life. However, the physiological role of adult neurogenesis and the use of NSC for therapy are highly controversial. One factor hampering the study and manipulation of neurogensesis is that NSC, like most adult somatic stem cells, are difficult to expand and their switch to differentiation is hard to control. Our laboratory has found that acute overexpression of the cyclin-dependent kinase 4 (cdk4) cyclinD1 complex in the adult mouse hippocampus cell-autonomously increases the expansion of NSC while inhibiting neurogenesis. Importantly, we developed a system that allows the temporal control of cdk4 cyclinD1 overexpression, which can be used to increase the number of neurons generated from the pool of manipulated NSC. Beside providing a proof-of-principle that expansion versus differentiation of somatic stem cells can be controlled *in vivo*, our study describes the first acute and inducible temporal control of neurogenesis in the mammalian brain, which may be critical for identifying the role of adult neurogenesis, using NSC for therapy, and, perhaps, extending our findings to other adult somatic stem cells.

S26-03

COORDINATE REGULATION OF CELL CYCLE PROGRESSION/ EXIT AND DIFFERENTIATION OF NEURAL STEM CELLS

Matsas, R., Tsioras, K. and Gaitanou, M.

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Studying the molecular and cellular basis of interactions taking place in the developing nervous system has important implications for designing innovative therapeutic strategies for the diseased or injured nervous system. We have been interested in the processes that regulate neural stem/progenitor cell proliferation, exit from the cell cycle and differentiation to neuronal phenotypes. Manipulating these processes holds promise for developing efficient stem cell therapies for the treatment of neurodegenerative diseases and neurotrauma (Makri et al. 2010 Stem Cells 28:127–39). We have thus identified BM88/Cend1 as a molecular target that participates in the complex processes by which a neural stem/progenitor cell becomes a mature neuron (Politis et al. 2007 PNAS 104:17861–6). By using gain- and loss-of-function approaches in cell lines, neurosphere cultures and *in vivo* in the developing chick neural tube and the mammalian brain, we demonstrated that its effect is achieved through a) activation of the p53-p21-pRb signalling pathway that controls the balance between cell cycle progression and exit (Georgopoulou et al. 2006 J Biol Chem, 281:33606–20; Katsimpardi et al. 2008 Stem Cells 26:1796–807); b) interference with sonic hedgehog signal transduction resulting in cyclin D1 down-regulation and cytoplasmic sequestration (Sergaki et al. 2010 Mol Cell Neurosci 44:15–29); and c) downregulation of the Notch signalling pathway (Politis et al. 2007 PNAS 104:17861–6). Using yeast two-hybrid analysis we recently identified RanBPM as a direct partner for BM88/Cend1. RanBPM is a scaffolding protein shuffling between the nucleus and the cytosol and acting as an adaptor molecule between the cell adhesion machinery and different intracellular signalling pathways. One such pathway in cancer involves the mirk/dyrk minibrain kinases that control cyclin D1 stability. Using co-transfection experiments to co-express BM88/Cend1, mirk/dyrk and RanBPM we show that a similar pathway may operate to control cyclin D1 levels and thus cell cycle progression and exit in neural stem/progenitor cells.

Supported by FP7 REGPOT Project 264083 Neurosign – Development of a Center of Excellence in Neurosignalling.

S26-04

REGULATION OF NEURONAL CONNECTIVITY BY CELL CYCLE UBIQUITIN LIGASES

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The assembly of neural circuits is essential for the development and function of the brain. Axon and dendrite morphogenesis

culminating in synapse formation represent critical developmental events that orchestrate the establishment of neuronal connectivity. Our studies using the rodent cerebellar cortex as a model system suggest that components of the cell cycle machinery play crucial roles in neuronal connectivity in post-mitotic neurons. We have identified a function for the cell cycle-regulated ubiquitin ligase Cdh1-APC in the control of axon growth and patterning in post-mitotic neurons. We have also discovered that the major mitotic ubiquitin ligase Cdc20-APC promotes the formation and elaboration of dendrites in post-mitotic neurons in the mammalian brain. Cdc20 is concentrated at the centrosome in neurons, and the centrosomal localization is critical for Cdc20-dependent dendrite development. We have also found that the centrosome-associated protein HDAC6 promotes the ubiquitinated state of Cdc20 and thereby stimulates Cdc20-APC activity and dendrite growth. In other studies, we have discovered that Cdc20-APC drives the differentiation of pre-synaptic sites in post-mitotic neurons. Our findings highlight the importance of cell cycle ubiquitin ligases in the control of neuronal morphogenesis and connectivity, with important implications for brain development and plasticity.

Symposium 27
Cyclic GMP-Phosphodiesterase Inhibitors as Therapeutic Agents in Nervous System Disorders

S27-01

CYCLIC GMP-PHOSPHODIESTERASE INHIBITORS AS THERAPEUTIC AGENTS IN NERVOUS SYSTEM DISORDERS

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In this presentation, I will describe the therapeutic effects of sildenafil treatment of ischemic stroke in young and aged rodents. Treatment of stroke initiated days and weeks after onset of stroke significantly improves neurological outcome without affecting or altering the volume of cerebral infarction. Sildenafil treatment of stroke in rodents significantly induces brain plasticity, neurogenesis and angiogenesis. I will also show that MRI can non-invasively monitor therapeutically relevant restorative processes induced by sildenafil, including angiogenesis, enhancement of local CBF and axonal remodeling. In addition to its therapeutic benefit for injury to the central nervous system, sildenafil will be shown to have a beneficial effect on the treatment of diabetic peripheral neuropathy. Remyelination of axons, neurite outgrowth and enhanced angiogenesis form a therapeutic constellation that significantly improves neurological function in the diabetic animal. The molecular pathways driving the sildenafil induced recovery for both stroke and peripheral neuropathy will also be described.

S27-02

CYCLIC GMP-PHOSPHODIESTERASE INHIBITORS AND COGNITION ENHANCEMENT

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Our understanding of the neurobiological processes underlying cognition is continuously improving, leading to the identification of targets for the development of cognition-enhancing drugs. One class of drugs is the phosphodiesterase (PDE) inhibitors, which have been identified as possible cognition enhancers about a decade ago. PDEs differ in the substrate, i.e. cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP), being hydrolyzed. Since these cyclic nucleotides have been suggested to play specific roles in processes of memory, selective PDE inhibitors preventing the breakdown of cAMP and/or cGMP could improve memory. Studies with different timing of treatment with specific PDE inhibitors indicated that distinct underlying signaling pathways for early and late consolidation processes exist corresponding to specific time-windows for memory consolidation. There is evidence that the underlying mechanisms of PDE inhibition on the observed behavioral effects are independent of possible cerebrovascular effects. Most likely the underlying mechanisms are a cGMP/PKG pathway for early consolidation processes and a cAMP/PKA pathway for late consolidation processes. Recently, the effects of specific PDE inhibitors are explored on other cognitive domains including acquisition processes/short-term memory and information processing. It will be shown that elevation of central cGMP levels as well as cAMP levels after treatment with a specific PDE inhibitor improve acquisition processes/short-term memory. The effects of specific PDE inhibitors on information processing by using a sensory gating paradigm indicate that elevation of cGMP as well as cAMP with a specific PDE inhibitor improves sensory gating, whereas elevation of cGMP alone has no effect. In a translational approach we also investigated the effect of PDE5 inhibition on cognition in humans. Within the context as described above, the latest results of specific inhibitors of PDE2, PDE4, PDE5 and PDE9 on cognitive processes will be presented and discussed.

S27-03

PHOSPHODIESTERASE 5 INHIBITORS AS THERAPEUTIC AGENTS IN ALZHEIMER'S DISEASE

Puzzo, D.[1], Privitera, L.[1], Staniszewski, A.[2], Deng, S.X.[3], Liu, S.[2], Zhang, H.[2], Palmeri, A.[1], Landry, D.W.[3] and Arancio, O.[2]

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Over the last few years there has been an increasing number of studies on phosphodiesterases inhibitors (PDEs), therapeutic agents able to modulate cyclic nucleotides cascade. Several PDE inhibitors have been developed and, sildenafil, an inhibitor of PDE5, has been widely used in the treatment of erectile dysfunction and pulmonary hypertension. We have studied the use of sildenafil as possible therapeutic agent in Alzheimer's disease (AD). Indeed, amyloid-beta (Aβ), a peptide thought to play a crucial role in AD pathogenesis, down-regulates the nitric oxide/cGMP pathway leading to an impairment of synaptic plasticity, reduction in cGMP levels and in phosphorylation of the transcription factor and memory molecule CREB.

Given that PDE5 inhibitors are known to enhance cGMP levels and CREB phosphorylation, we have tested whether these drugs might be beneficial against an AD mouse model (APP/PS1 mice). We assessed the short- and long-term effects of sildenafil on synaptic plasticity, memory, CREB phosphorylation and Aβ levels.

Sildenafil produces an amelioration of synaptic function, CREB phosphorylation and memory and a decrease in Aβ levels in APP/PS1 mice. Most importantly, the inhibitor exerted its effect not only immediately, but also for a prolonged period beyond the drug administration.

Inhibition of PDE5 might be a novel target for therapies wishing to lower Aβ levels in AD. Moreover, the long-term effect shown in our experiments could be very useful to improve the compliance of these compounds and their administration to a senile population

such as AD patients. Thus, existing PDE5 inhibitors or new compounds have potential for the prevention and treatment of AD and other diseases associated with elevated Aβ levels.

S27-04

ANTI-INFLAMMATORY AND NEUROPROTECTIVE EFFECTS OF CYCLIC GMP PHOSPHODIESTERASE INHIBITORS IN ANIMAL MODELS OF CNS INJURY

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CNS injury elicits a potent inflammatory response, comprising recruitment of inflammatory cells, reactive gliosis and release of pro-inflammatory cytokines and inflammatory mediators. NO, one of these mediators, stimulates cGMP formation in neurones and astrocytes but little is known about the role of this pathway in neuroinflammation. Natriuretic peptide release is also up-regulated during CNS injury and can increase cGMP formation in both astroglia and microglia/macrophages. *In vitro* both cGMP-mediated pathways have been shown to regulate glial inflammatory responses. We have recently obtained evidence that increasing cGMP by administration of phosphodiesterase 5 (PDE5) inhibitors regulates neuroinflammation and is neuroprotective *in vivo*. Using a cortical cryoinjury model in rodents we have shown that treatment with zaprinast or sildenafil enhances astrogliosis around the lesion while decreasing recruitment and activation of microglia/macrophages, protein oxidative stress and neuronal cell death. Additionally, PDE5 inhibitors promoted angiogenesis by inducing VEGF expression in reactive astrocytes and potentiated expression of the antioxidant proteins metalothioneins, effects that will contribute to neuroprotection. In a mouse model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE) induced by immunization with a myelin oligodendrocyte glycoprotein peptide (MOG35-55), daily administration of sildenafil after disease onset was shown to rapidly ameliorate clinical symptoms by preventing axonal damage and promoting remyelination. After only 3 days of treatment sildenafil significantly decreased leukocyte infiltration, microglial/macrophage activation and ICAM-1 expression while increasing Foxp3+-T regulatory cells. Highly reactive astrocytes forming scar-like structures were observed around infiltrates suggesting a role in restricting the spread of leukocytes from damaged into healthy parenchyma. Later on during recovery and in contrast to other beneficial treatments for EAE, widespread astrogliosis was observed in spinal cord grey matter of sildenafil-treated mice. These results strongly support the anti-inflammatory and neuroprotective potential of PDE5 inhibitors and suggest that regulation of glial reactivity may be an important component of the mechanisms involved in neuroprotection. This work was supported by MICINN and AGAUR grants.

Symposium 28
Assembly and Function of the Active Zone of Neurotransmitter Release

S28-01

ULTRASTRUCTURAL ORGANIZATION OF THE ACTIVE ZONE

Marty, S.[1,2,3], Siksou, L.[1,2,3], Varoqueaux, F.[4], Pascual, O.[1,2,3], Horellou, S.[1,2,3], Triller, A.[1,2,3] and Brose, N.[4]

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Neurotransmitters are released by fusion of synaptic vesicles (SVs) with the plasma membrane (PM) at an area called the active zone (AZ). To allow fast release in response to calcium influx, SVs must be docked to the PM, and maintained close to voltage-gated calcium channels. To investigate how it could be achieved, we analyzed the organization of the AZ of small spine synapses maintained in a close to native state. Hippocampal slice cultures were rapidly frozen ($\asymp$10 ms) under high pressure to avoid aldehyde-associated artifacts. After cryosubstitution, synaptic profiles were analyzed at high resolution ($\asymp$5 nm) using electron tomography. At the AZ, SVs are apposed to, but not hemifused with the PM to which they are linked by small filaments. Docked and undocked SVs are spatially segregated, since no undocked SVs are observed at 5–15 nm from the PM. In Munc13 KO mice, SVs are not docked, but rather located at 6–8 nm form the PM to which they remain linked by filaments. Docked SVs are grouped around clusters of fine filaments that emerge from the PM and do not form a regular grid. Immunocytochemistry indicates that Bassoon and CAST/ERC2 are concentrated in focal spots near the PM. Lateral interactions are observed between the docked SVs and the filaments emerging from the AZ. These studies indicate that a SNARE-dependent docking of SVs at the AZ may render SVs ready to be released. Further, groups of filaments at the AZ may link docked SVs to calcium channels. We are currently studying if modifications of SV docking underlie changes in the probability of neurotransmitter release.

S28-02

MOLECULAR MECHANISM OF ASSEMBLY AND ACTIVITY-DEPENDENT REMODELING OF THE PRE-SYNAPTIC ACTIVE ZONE

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Excessive excitation or inhibition induces compensatory changes in efficacy of synaptic transmission. This homeostatic plasticity serves to keep network activity within a physiologically meaningful frame – a pre-requisite for effective learning-induced plasticity. Homeostatic adaptations are of tremendous importance during development of the nervous system, in experience-dependent plasticity and under pathological conditions. They are achieved by modulation of the pre-synaptic neurotransmitter release machinery and by changes in neurotransmitter sensing by post-synaptic elements. Proteins of the pre-synaptic cytomatrix at the active zone (CAZ) are important modulators of release efficiency during Hebbians' plasticity; however, to date their role in homeostatic plasticity was neglected. In this study we have investigated whether and how the altered synaptic network activity influences the molecular composition of the pre-synaptic active zone (AZ). We used primary cultures of cortical neurons as a convenient model system allowing pharmacological manipulations and found significant down-regulation of cellular expression levels of pre-synaptic scaffolding proteins Bassoon, Piccolo, ELKS/CAST, Munc13, RIM, liprin-α and synapsin upon prolonged (48–h) activity depletion. This was accompanied by a general reduction of Bassoon, Piccolo, ELKS/CAST, Munc13 and synapsin levels at synaptic sites. Interestingly, RIM was up-regulated in a subpopulation of synapses. Analysis at the level of individual synapses revealed that RIM quantities correlated well with synaptic activity and that a constant relationship between RIM levels and synaptic activity was preserved upon activity silencing. Silencing also induced synaptic enrichment of other previously identified regulators of pre-synaptic release probability, i.e. synaptotagmin-1, SV2B and P/Q-type calcium channels. Seeking for responsible cellular mechanisms we revealed a complex role of the ubiquitin-proteasome system in functional pre-synaptic remodeling upon silencing. Only for some substrates including Bassoon or liprin-α silencing effects on the degradation rates were observed. Altogether, our data indicate a significant molecular reorganization of the pre-synaptic release apparatus during homeostatic adaptation to network inactivity that underlies the main silencing-induced functional hallmark at pre-synapse, i.e. increase of probability of release.

S28-03

LIPRIN-α2 REGULATES PRE-SYNAPTIC PLASTICITY BY CLUSTERING MULTIPLE PRE-SYNAPTIC COMPONENTS

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Neurons communicate via chemical transmission at structurally and functionally asymmetric synapses. In typical excitatory synapses in the brain, the pre-synaptic terminal releases vesicles containing the neurotransmitter glutamate, which diffuses across the synaptic cleft to bind to and activate glutamate receptors at the post-synaptic membrane. At the pre-synaptic plasma membrane, an electron-dense structure called the active zone is almost exclusively designated for synaptic vesicle exocytosis and neurotransmitter

release. The architecture and molecular organization of the active zone plays an important role in guiding synaptic vesicles to their docking and fusion sites and is optimized for rapid and regulated neurotransmitter release.

Here we focus on the role of the scaffold protein liprin-α in pre-synaptic function using a knock-down approach in mature dissociated and autaptic hippocampal neurons. We show that cells lacking liprin-α2 have a reduced evoked release, less paired-pulse depression and reduced RRP size suggesting a role for liprin-α2 in maintenance of synaptic vesicle pool size and pre-synaptic plasticity. Additionally, while reducing liprin-α2 in mature neurons does not affect synapse number or neuron morphology it does lead to a broadening of active zones and mislocalization of pre-synaptic components such as CASK and RIM. We propose a model in which liprin-α2 coordinates the clustering of pre-synaptic protein complexes at mature synapses, thereby regulating synaptic vesicle release.

S28-04

MOLECULAR DISSECTION OF ACTIVE ZONE FUNCTIONS IN NEUROTRANSMITTER RELEASE

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Synaptic transmission enables neurons to pass on signals to their targets within milliseconds. Calcium induces neurotransmitter release in the nerve terminal, where pre-synaptic active zones from hotspots for docking, priming and calcium-triggering of synaptic vesicle fusion.

A fundamental question we have addressed is how calcium channels are molecularly coupled to neurotransmitter release. Extensive and beautiful functional work has shown that calcium influx is highly concentrated at sites of synaptic vesicle exocytosis, but the underlying molecular mechanisms of localizing calcium influx to release sites remained unknown. Using mouse genetics combined with biochemical and functional experiments, we found a mechanism by which RIM proteins tether N- and P/Q-type calcium channels to pre-synaptic active zones. Furthermore, we also discovered that RIMs provide a molecular switch to activate Munc13, which in turn promotes synaptic vesicle priming. Thus, RIM proteins tether calcium influx to the priming machinery at sites of synaptic vesicle release. In contrast to RIM's facilitating roles, we observed that the recently identified active zone member ELKS2α inhibits neurotransmitter release by controlling the readily releasable pool size. Together, these findings suggest that active zones may act as modules for bi-directional regulation of synaptic strength in the nerve terminal. They also lead to the exciting hypothesis that active zones embody a molecular platform in neuronal circuits at which activity can be regulated through structural and functional plasticity.

Symposium 29
Molecular Mediators of Psychiatric and Cognitive Symptoms in Huntington's Disease

S29-01

DIFFERENCES IN THE TIME COURSE OF COGNITIVE DEFICITS IN TWO LINES OF HUNTINGTON'S DISEASE KNOCK IN MICE

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We have characterized a knock-in (KI) mouse model of Huntington's disease (HD) with a chimeric human/mouse exon 1 containing 140 CAG repeat and the human polyproline region in the mouse Hdh gene (CAG140 KI; Menalled et al., 2003; Hickey et al., 2008). In contrast to the Hdh(CAG)150 knock in mice that have an expanded CAG repeat inserted into the Hdh gene with a murine polyproline domain (Lin et al., 2001; Heng et al., 2007), the CAG140 KI mice show behavioral and neuropathological deficits from an early age. To further examine the differences in time course between these two models, we compared fully backcrossed CAG140 KI and Hdh(CAG)150 side by side and confirmed the differences in the time course of motor phenotypes (pole test, open field, running wheel), with CAG140 showing deficits as early as 1 month in some tests. In addition, both models show marked differences in the time course of huntingtin aggregates detected with several antibodies, including pEM48 (Li et al., 1999), MW8 (Ko et al., 2001), S830 (Sathasivam et al., 2001) and goat anti-Htt (Santa Cruz Biotechnology, CA), with CAG140 KI mice already showing diffuse nuclear staining, microaggregates, nuclear inclusions and neuropil aggregates in the striatum and cortex, at 4 months of age, whereas these pathological hallmarks occur much later in Hdh(CAG)150 mice. Furthermore, CAG140KI mice also show early (3 months of age) deficits in spontaneous alternation in the Y-maze while, Hdh(CAG)150 mice were not impaired in this test at the same ages. However, Novel Object Recognition performance was impaired in both lines by 4 months of age. The data suggest that the differences in constructs used to generate these KI lines markedly influence the severity of the phenotype, including cognitive deficits in the Y maze but not in the novel object recognition task that appears the most sensitive test to detect early HD-like cognitive impairments. Supported by a Hereditary Disease Fellowship to NRF, CHDI, and gifts to MFC.

S29-02

EARLY ONSET BEHAVIORAL AND MOLECULAR CHANGES IN TRANSGENIC MODELS FOR HUNTINGTON'S DISEASE: REVERSAL BY HDACI

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder due to expanded CAG repeats in the huntingtin gene. Though the resulting mutant huntingtin (htt) protein is already expressed in embryonic development, the disease usually manifests in the fourth decade of life. Our characterization of transgenic HD rats and mice yielded in the identification of post-natal behavioral changes comprising reduced ultrasonic vocalization, loss of pre pulse inhibition, and increased risk taking. In addition to disturbances of dopaminergic and glutaminergic regulation we provide first evidence of reduced neuronal differentiation capacity in the subventricular zone of the post-natal rat brain. Low dose treatment of this pre-HD-syndrome with the histone deacetylation inhibitors led to a marked improvement of these behavioral changes *in vivo* as well as a complete reversal of the aberrant neuronal differentiation capacity *in vitro*. We conclude that these phenotypic observations along with successful intervention by low dose non-specific HDACi are indicative for very early transcriptional dysregulation in HD; the consequences of which – if also is present in human gene carriers – should either be well compensated, only cause minor, clinically irrelevant alteration or even may provide the correlate of the postulated 'benefit' of glutamine storage in the CNS.

S29-03

LONG-TERM MEMORY DEFICITS IN HUNTINGTON'S DISEASE: SEARCHING FOR NOVEL THERAPEUTIC TARGETS

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Cognitive deficits are clinical features of Huntington's disease (HD) that are present in early stages, when motor symptoms are not yet evident. In pre-symptomatic HD patients, anatomic and functional brain atrophy has been observed in regions involved in cognitive function although molecular mechanisms underlying these deficits are largely unknown. Here, we have analyzed the onset and progression of cognitive dysfunction and the underlying molecular mechanisms in a transgenic exon-1 and in a knock-in full-length HD mouse models. Spatial and object recognition memories were evaluated by the Morris water maze and the novel object recognition task. Our data demonstrate impaired spatial and recognition long-term memory in both HD mouse models prior to the onset of motor disturbances. Biochemical analysis revealed dysfunctional activation of two important pathways involved in memory consolidation; the cAMP-dependent protein kinase (PKA) and the CREB/CBP pathways. Consistent with a role of PKA and CBP in cognitive deficits in HD, administration of either PKA or histone deacetylase inhibitors rescues recognition memory deficits in HD mouse models. All together our findings suggest that the precise pharmacological modulation of PKA and/or CBP pathways could be of benefit for treatment of early cognitive deficits in HD.

This work was supported by Fondo de Investigaciones Sanitarias (Instituto de Salud Carlos III, PI10/01072 and RETICS: RD06/0010/0006), and Ministerio de Educación y Ciencia (Grants SAF2008-04360 and SAF-2009 07077). A.G. was supported by Ministerio de Educación y Ciencia, Spain, and Generalitat de Catalunya (2009SGR-00326). A.S. was supported by a post-doctoral fellowship from Fundação para a Ciência e Tecnologia, Portugal (SFRH/BPD/47435/2008) and CIBERNED (CB06/05/0054), Spain. M.P. has a contract from the CHDI foundation.

S29-04

GENE-ENVIRONMENT INTERACTIONS MEDIATING AFFECTIVE AND COGNITIVE ENDOPHENOTYPES IN A TRANSGENIC MOUSE MODEL OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a fatal neurodegenerative disorder involving a triad of psychiatric, cognitive and motor symptoms. HD is caused by a CAG repeat expansion encoding a polyglutamine tract in the huntingtin protein. In a transgenic mouse model of HD (R6/1 line) we have characterized progressive onset of affective (depression-like) and cognitive endophenotypes, which precede motor deficits. We have also demonstrated that environmental enrichment (which enhances sensory stimulation, cognitive activity and physical exercise) can delay onset of these endophenotypes. The molecular mechanisms mediating affective and cognitive symptoms in HD, and their modulation by environmental stimuli, has been investigated. A key aspect of HD pathogenesis appears to be selective transcriptional dysregulation. We recently performed microarray analyses that indicated early gene expression changes are occurring in multiple brain regions of the HD mice, including the hippocampus and neocortex, associated with onset of the cognitive and affective signs. The hypothesis that environmental enrichment and wheel running exert their beneficial effects via selective regulation of gene expression was also explored. We found that even at an early pre-motor symptomatic age, gene expression was dysregulated, with regional and sex-specific differences apparent, including specific components of the serotonergic, dopaminergic and glutamatergic systems. The molecular effects of environmental enrichment and wheel running exhibit temporal specificity, regional selectivity and sexually dimorphism, and in some cases help explain the experience-dependent plasticity observed at cellular and behavioural levels. In addition to RNA changes, other neurochemical deficits were identified. These findings suggest that disruption of specific neuromodulatory systems occurs early in particular brain regions of HD mice, and may be involved in the affective and cognitive endophenotypes we have described. Our results also suggest that the timing and duration of these environmental manipulations are critical in terms of their ability to modify gene expression. These findings may inform the development of 'enviromimetics' and other therapeutic approaches for HD.

Symposium 30
Molecular Mechanisms of Synapse Development and Remodeling

S30-01

STRUCTURAL PLASTICITY OF HIPPOCAMPAL MICROCIRCUITS IN THE ADULT

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Learning is correlated with the assembly of new synapses, but the roles of synaptogenesis processes in memory are poorly understood.

I will discuss our recent work relating learning to the assembly of identified new synapses in the mouse hippocampus, and demonstrating functional roles for the new synapses in further learning, and in the behavioral expression of the learning. Taken together, the results begin to reveal principles of how widespread but specific long-lasting circuit remodeling processes are recruited to support learning and memory in juveniles and adults.

S30-02

INHIBITORY CUES PATTERNS SYNAPSES IN C. ELEGANS

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Cellular interactions between neighboring axons are essential for the stereotyped positioning of individual axons and topographic map formation. So far however, it is not known how axons communicate with each other at the level of synapse formation. In other words, how do axons divide up the target area when they choose synaptic targets? To answer this question, we focused on two closely related cholinergic motor neurons, DA9 and DA8. Both DA8 and DA9 neurons extend their axons through a commissure into the dorsal nerve cord, where they proceed anteriorly to form a series of en passant synapses. Although those axons largely overlap in the dorsal cord, they form synapses only at the specific area. DA9 forms about 25 synapses onto the dorsal muscles in its axon in the posterior-most domain (DA9 synaptic domain). Interestingly, DA8 axon has no synapses in DA9 synaptic domain, and starts to form synapses just anterior to DA9 synaptic domain. Therefore, DA8 and DA9 form 'tiled' synaptic domains leaving no apparent gap or overlap between them. We first used axon guidance mutants to show that contact between DA8 and DA9 axons appears critical.

From a forward, visual-based genetic screening, we identified that two transmembrane Semaphorins (smp-1, smp-2) and their receptor Plexin (plx-1) are essential for the synaptic tiling between DA8 and DA9. Interestingly, cell specific rescue and mosaic experiments suggested that both ligands and receptor function in cis in DA9. We also found that functional PLX-1:GFP fusion protein is localized at the anterior edge of the synaptic domain of DA9 in a Semaphorin and axon-axon interaction dependent manner. Deletion and mutational analyses of PLX-1 indicate that PLX-1 cytoplasmic RasGAP domain is essential for its function. Consistently, constitutive active Ras mimicked plx-1 loss of function phenotype. We propose that contact-dependent PLX-1 subcellular localization sets up the synaptic boundary between DA8 and DA9 by restricting the synaptic domain via inactivation of Ras.

S30-03

CONTROL OF EXCITATORY SYNAPSE DEVELOPMENT BY DYNAMIC RAC GTPASE SIGNALING

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Most excitatory synapses in the brain are located on small actin-rich protrusions called dendritic spines. Spines undergo activity-dependent changes in shape and number thought to be important for learning and memory. Furthermore, spine abnormalities are frequently associated with brain disorders including mental retardation, mental illness and Alzheimer's disease, suggesting that their development and remodeling are critical for normal cognitive function. The Rho family GTPase Rac plays a central role in promoting the formation, growth and maintenance of spines by modulating actin dynamics. Rac requires precise spatio-temporal regulation to function properly, however, the mechanisms that regulate Rac activity at synapses remain poorly understood. We previously identified the Rac-specific guanine nucleotide exchange factor (GEF) Tiam1 as a critical mediator of NMDA receptor- and EphB receptor-dependent spine development. To further characterize the mechanism by which Tiam1 regulates Rac-dependent spine development, we recently identified several novel Tiam1-interacting proteins including the Rac-specific GTPase activating protein (GAP) Bcr. We have confirmed that Bcr interacts and colocalizes with Tiam1 in neurons and blocks Tiam1-induced Rac signaling. The complex between Tiam1 and Bcr may serves as an 'on-off switch' that dynamically regulates Rac activity in spines. To investigate this possibility, we have examined Bcr's role in neurons and found that both *in vitro* and *in vivo*, Bcr and the highly homologous protein Abr appear to restrict spine and synapse development. The exuberant spine phenotype caused by blocking Bcr/Abr function in neurons was rescued by inhibiting Tiam1's function, suggesting that Bcr and Tiam1 do act together as a binary switch to tightly regulate Rac activity at synapses. Interestingly, EphB receptor tyrosine kinases appear to regulate this switch by binding to and phosphorylating Tiam1 and Bcr and transiently disrupting their interaction in neurons. Furthermore, like Tiam1, Bcr is required for proper EphB receptor-mediated spine development. Taken together, these results suggest that Bcr plays a critical role in excitatory synapse development by counterbalancing Tiam1's function, resulting in precise spatio-temporal regulation of Rac signaling at synapses.

S30-04

ACTIVITY-DEPENDENT SIGNALING PATHWAYS THAT REGULATE NEURONAL CONNECTIVITY

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The overall structure and function of circuits in the central nervous system is established by a variety of carefully orchestrated processes, including the formation of synapses and the morphogenesis of the dendritic arbor of individual neurons. These processes are subject to influence by sensory experience via changes in an individual neuron's activity. Using an RNAi-based approach in cultured hippocampal neurons, we discovered that knock down of the activity-regulated GTPase Rem2 causes an increase in dendritic branching while causing a decrease in excitatory synapse density. Rem2 is a member of the Rad/Rem/Rem2/Gem/Kir (RGK) protein family, a Ras-related subfamily of small GTPases, and is the only member of this family that is highly expressed in the central nervous system. We have demonstrated that both Rem2 transcription and translation is rapidly up-regulated in response to neuronal depolarization. Further, we have begun to elucidate the signal transduction mechanisms that govern Rem2 function in neurons and have demonstrated that binding to the calcium binding protein calmodulin (CaM) is required for Rem2 regulation of dendritic branching but is completely dispensable for its function in synapse formation. Thus, our experiments to date support a model in which Rem2 acts to constrain dendritic branching in response to increased neuronal activity by binding to and sequestering calcium-bound CaM away from CaMK family members. Importantly, we believe that Rem2 may represent a key molecule through which external stimuli mediate direct effects on neuronal architecture and connectivity.

Symposium 31
Alternate Brain Energy Substrates in Relation to What, Where, and When of Functional Energetics

S31-01
ROLE OF MONOCARBOXYLATE TRANSPORTERS IN BRAIN ENERGY METABOLISM

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Monocarboxylate transporters (MCTs) are proton-linked carriers for lactate, pyruvate and ketone bodies. Three isoforms, MCT1, MCT2 and MCT4, are predominant in the central nervous system. MCT1 expression is strong on endothelial cells that compose blood vessels where it likely plays a key role for uptake of blood-borne monocarboxylates. It is also expressed by different glial cells (e.g. oligodendrocytes and astrocytes) as well as by small populations of neurons. MCT2 is the predominant neuronal monocarboxylate transporter allowing significant lactate uptake and utilization by this cell type. MCT4 is exclusively found on astrocytes and facilitate lactate release in conditions of high glycolytic activity. If transport via monocarboxylate transporters is considered as a limiting factor for energy substrate fluxes in and out of cells, as suggested both by experimental and modeling studies, mechanisms must exist to control MCT activity and/or expression levels. Indeed, recent studies have highlighted different means to modify the capacity of brain cells to enhance either uptake or release of monocarboxylates. Thus, it was observed that different neuroactive substances including brain-derived neurotrophic factor (BDNF) cause an enhancement of MCT2 protein levels in neurons within a few hours via a translational regulation involving activation of the PI3K/Akt/S6 pathway. A similar effect was observed after intrahippocampal BDNF injection *in vivo*. In addition, it was also demonstrated that neuronal signals such as BDNF induces a translocation of MCT2 proteins from an endogenous pool into the cell membrane within minutes. As a result, lactate transport in neurons was increased by 80%. Recent investigations performed on cultured astrocytes revealed that glial MCTs are also subject to regulation. It was observed that nitric oxide leads to a striking enhancement in MCT4 expression levels, an effect obtained via a transcriptional regulation. Increased MCT4 expression induced by nitric oxide was accompanied by greater lactate transport capacity and lactate release by astrocytes. As a recent study demonstrated the involvement of MCTs in long-term memory, our data may provide some of the mechanisms by which MCTs ensure adequate energy supply in such conditions.

S31-02
LACTATE MUSCLES ITS WAY INTO CONSCIOUSNESS

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Blood-borne glucose is the major fuel for activated brain, but the pathways, processes, and cell types that consume the additional glucose metabolized above that in the resting state are not known. Acoustic stimulation of normal conscious rats increases the rate of glucose utilization in activated inferior colliculus, but the relative rise in the quantity of labeled products of (6-^{14}C)glucose retained in activated tissue (~30%) is much less than that with (^{14}C)deoxyglucose (~70%), suggesting that labeled glucose metabolites are quickly released from activated tissue. The fractional flux of glucose carbon into the pentose phosphate shunt pathway is increased from about 7–25% during acoustic stimulation, thereby increasing label loss from (1-^{14}C)glucose. (^{14}C)Lactate formed from (3,4-^{14}C) glucose microinfused into tissue and recovered in the microdialysate accounted for about 80% of the label compared to 20% for $^{14}CO_2$, indicating that lactate released to extracellular fluid is not taken up and rapidly oxidized by cells in activated zones. Deoxyglucose-6-phosphate was found to be a poor substrate for astrocytic gap junctions, but lactate transporter inhibition and blockade of astrocytic gap junctions reduced metabolite loss from activated tissue. Spreading of labeled lactate and glutamine from a point source increased during activation compared to rest, and about 30% of the label derived from (^{14}C)glucose microinfused into the inferior colliculus was recovered in dissected meninges, suggesting efflux via perivascular pathways. The rate and capacity for lactate uptake into astrocytes from extracellular fluid in brain slices and for trafficking of lactate to other astrocytes via gap junctional channels is several-fold greater than neuronal lactate uptake or transfer of lactate from astrocytes to neurons. Exchange of labeled lactate in brain for unlabeled lactate in blood was negligible during sensory stimulation and spreading cortical depression, but labeled lactate was quickly released from brain to blood during spreading depression. Together, these findings suggest that production and release of labeled lactate are upregulated during acoustic activation. Astrocytes probably have a major role in clearance of lactate, which is unlikely to be a major fuel.

S31-03
MYSTERIOUS ASPARTATE. AN IMPORTANT METABOLITE WITH MUCH TO REVEAL

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Aspartate is a putative neurotransmitter active at NMDA receptors as well as a metabolite present at millimolar concentrations in the brain yet the role it plays remains poorly understood. For various reasons it is a difficult molecule to study. It is scarcely mentioned in the literature and yet it is one of the most highly correlated molecules in metabolism. It is a reported neurotransmitter and yet is released in high amounts in hypoglycaemia with no apparent NMDAR activation. The major transporter used by aspartate is unknown but its very high level of correlation with other amino acid levels such as glutamine, glutamate, GABA and alanine suggest it is a highly ubiquitous and quickly transported molecule likely to be moved about via an exchanger rather than a specific uptake mechanism. It is often reported as being concentrated in neurons, yet has been shown to be part of a tightly

compartmentalised glial metabolon (Griffin JL et al. 2003). It is plainly an important, although largely overlooked molecule and there is much work still to be done to elucidate the role it plays in cerebral metabolic and functional activity.

Reference:

1. Griffin JL, Keun H, Richter C, Moskau D, Rae C & JK Nicholson. (2003) Compartmentation of metabolism probed by (2-^{13}C)alanine: Improved 13C NMR sensitivity using a Cryo-Probe detects evidence of a glial metabolon. Neurochem Int 42, 93–99.

S31-04

INTERACTION BETWEEN BRAIN AND MUSCLE METABOLISM: MUSCLE LACTATE AS BRAIN SUBSTRATE

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The human brain releases a small amount of lactate at rest, and even an increase in arterial blood lactate during anesthesia does not provoke a net cerebral lactate uptake. However, during cerebral activation associated with exercise involving a marked increase in plasma lactate (>15 mM), the brain takes up lactate in proportion to the arterial concentration. Cerebral lactate uptake, together with glucose uptake, is larger than the uptake accounted for by the concomitant O2 uptake, as reflected by the decrease in cerebral metabolic ratio (CMR) [the cerebral molar uptake ratioO2/(glucose + 1/2 lactate)] from a resting value of 6 to <2. The CMR also decreases when plasma lactate is not increased, as during prolonged exercise, cerebral activation associated with mental activity, or exposure to a stressful situation. The CMR decrease is prevented with combined 1- and 2-adrenergic receptor blockade but not with 1-adrenergic blockade alone. Also, CMR decreases in response to epinephrine, suggesting that a 2-adrenergic receptor mechanism enhances glucose and perhaps lactate transport across the blood-brain barrier. The pattern of CMR decrease under various forms of brain activation suggests that lactate may partially replace glucose as a substrate for oxidation.

S31-05

CONTEXT-DEPENDENT USAGE OF CARBOHYDRATES IN HUMAN BRAIN

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Accumulating evidence over the past 30 years supports that the cerebral cortex has an obligatory need for glucose as a metabolic substrate. However the absolute requirement for glucose versus alternate substrates such as lactate, ketones, and acetate/fatty acids is not known as well as the specific cellular functions supported by different substrates. This presentation will review results obtained in studies of human cerebral cortex in which cell type specific (neuron, glia) fuel oxidation was quantified using Magnetic Resonance Spectroscopy in health and disease. The results will be interpreted from the standpoint of current models of brain energy metabolism and the coupling of specific fuel metabolism to function.

Symposium 32
Cys-Loop Receptors: From Atomic Structures to Drug Design

S32-01

INSIGHT IN NACHR LIGAND BINDING FROM ACHBP CRYSTAL STRUCTURES

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Nicotinic acetylcholine receptors (nAChRs) display a broad variety of subtypes, which in turn present a complex subcellular and regional expression pattern in the brain, as well as a specific pharmacological profile. The association of these nAChRs with different types of brain disease has turned them into interesting drug targets for the treatment of Alzheimer's disease or schizophrenia, or for anti-smoking compounds among others. However, to date no high-resolution structural data is available on functional pentameric forms of membrane bound nicotinic receptors. Characterization of ligand binding and selectivity profiles of different nicotinic receptor subtypes, enabling efficient drug design, is an important issue. Over the last eight years, tenths of high resolution structures of acetylcholine binding protein (AChBP), which is homologous to the extracellular ligand binding domain of the nicotinic acetylcholine receptor, have been obtained. AChBPs in complex with different ligands have provided detailed insight into the neurotransmitter binding site of nicotinic acetylcholine receptors. I will discuss the crystallization and mutagenesis efforts towards rationalizing subtype specificity in these receptors through the structural studies of acetylcholine binding protein-ligand complexes.

S32-02

X-RAY STRUCTURES OF GENERAL ANAESTHETICS BOUND TO A BACTERIAL CHANNEL-RECEPTOR HOMOLOGOUS TO NACHRS

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General anaesthetics have enjoyed long and widespread use but their molecular mechanism of action remains poorly understood. There is good evidence that their principal targets are pentameric ligand-gated ion channels (pLGICs) such as inhibitory $GABA_A$ (γ-aminobutyric acid receptor A) and excitatory nicotinic acetylcholine receptors (nAChRs), which are respectively potentiated and inhibited by these allosteric effectors. The bacterial homologue from Gloeobacter violaceus (GLIC), whose X-ray structure was recently solved, is also sensitive to clinical concentrations of general anaesthetics. We recently solved the crystal structures of the complexes propofol/GLIC and desflurane/GLIC. These reveal a common general-anaesthetic binding site which pre-exists in the apo-structure in the upper part of the transmembrane domain of each protomer. Both molecules establish van der Waals interactions with the protein; propofol binds at the entrance of the cavity whereas the smaller, more flexible, desflurane binds deeper inside. Mutations of some amino acids lining the binding site profoundly alter the ionic response of GLIC to protons, and affect general-anaesthetic pharmacology. Simulations of molecular dynamics, performed on the wild type and two GLIC mutants, highlight differences in mobility of propofol in its binding site and help to explain these effects. These data provide a novel structural framework for the design of general anaesthetics and of allosteric modulators of brain pLGICs, including nAChRs. In addition, they give insights into the gating mechanism occurring in this family of channel that involve transmission of conformational reorganization from the extracellular domain to the transmembrane domain.

S32-03

5-HT3 RECEPTORS: FROM STRUCTURE TO THERAPY

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5-HT3 receptors are member of the Cys-loop family of receptors, and are involved in fast synaptic neurotransmission in the central and peripheral nervous systems. To date, genes for five different 5-HT3 receptor subunits have been identified (A–E). Receptors can be composed of A subunits alone, but can also combine with subunits B–E to produce functional heteromeric receptors. There are a range of 5-HT3 receptor agonists and competitive antagonists; a number of the latter are used to treat a range of conditions including irritable bowel syndrome, post-operative, chemo- and radio-therapy induced emesis. A wide range of substances, including alcohols, steroids and anaesthetics also modulate 5-HT3 receptors. Here we consider the current information regarding the structure and function of 5-HT3 receptors and their therapeutic potential.

S32-04

$GABA_A$ RECEPTOR STRUCTURE AND PHARMACOLOGY

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$GABA_A$ receptors are the site of action of a variety of clinically important drugs, such as benzodiazepines, barbiturates, steroids, anesthetics and convulsants. These receptors are composed of five subunits that can belong to different subunit classes, giving rise to an enormous heterogeneity of these receptors. Most of them, however, are composed of two alpha, two beta and one gamma subunit. So far, no crystal structure of the $GABA_A$ receptor is available. By using various crystal structures of the ligand bound acetylcholine binding protein and structures of the nicotinic acetylcholine receptors, we constructed multiple homology models of $GABA_A$ receptors. We then exploited the structure of diazepam and its derivatives to select those models best accommodating these compounds. Using a novel, widely applicable computational workflow we for the first time identified the diazepam bound

structure of the benzodiazepine binding site of $GABA_A$ receptors. This structure is consistent with a wide variety of experimental evidence. This model structure can now be used for identifying novel classes of ligands for the benzodiazepine binding site, and for modelling of benzodiazepine binding sites of other $GABA_A$ receptor subtypes. Docking of subtype-selective ligands into these binding sites will finally lead to appropriate structural hypotheses that allow lead optimization and fragment-based drug design, and thus, will dramatically speed up the development of subtype-selective drugs. In another study we identified a novel drug binding site at the extracellular alpha+beta- interface of $GABA_A$ receptors and identified the first class of compounds interacting with this site. These drugs cannot directly activate these receptors but allosterically modulate $GABA_A$ receptors in a way similar to benzodiazepines. Drugs indiscriminately acting at all alpha+beta- interfaces will have a much wider action than benzodiazepines and might be suitable for the treatment of epilepsy. Drugs that can distinguish between different alpha+beta- interfaces will exhibit quite specific actions mediated by different $GABA_A$ receptor subtypes.

Financial support by the EC-FP7 grant HEALTH-F4-2008-202088, by the grant P19653 of the Austrian Science Fund and by a DOC-fellowship of the Austrian Academy of Sciences, is gratefully acknowledged.

S32-05

EXOGENOUS TOXINS AND ENDOGENOUS PROTOTOXINS INTERACTING WITH NICOTINIC ACETYLCHOLINE RECEPTORS

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Snake venom protein neurotoxins are playing an important role of accurate tools in studies on nicotinic acetylcholine receptors (nAChRs). Recently we used Alexa-α-bungarotoxin for reliable detection of α7 nAChR in mouse DRG, employing as control α7 knock-out mouse or preincubations with different toxins (Shelukhina et al., 2009). Studying α-conotoxins from poisonous Conus mollusks, we synthesized α-conotoxin ImII, its W10Y mutant and radioiodinated derivative, and by combination of radioligand analysis and electrophysiology found that this atypical α-conotoxin intreracts at the classical agonist/competitive antagonist sites with α7 nAChR, but has distinct additional sites at the muscle-type nAChRs (Kasheverov et al., 2009). While α-conotoxins and alpha-neurotoxins are 'exogenous' toxins, the Lynx 1 protein expressed in mammalian brain which modulates nAChR activity, presumably has the same fold as α-neurotoxins but is membrane-tethered by GPI anchor. Lynx 1 represents an endogenous 'prototoxin'. We expressed in E.coli the water-soluble domain of human Lynx1 lacking a GPI anchor (ws-lynx1) and determined its NMR structure. Indeed, it has a three-finger fold but mostly disordered C-terminal loop and in general shows a higher conformational mobility than alpha-neurotoxins. Ws-lynx1 competed with radioiodinated α-bungarotoxin for binding to the acetylcholine-binding proteins (AChBP) and to Torpedo nAChR. At 1 microM ws-lynx1 enhanced the response to acetylcholine for α7 nAChR expressed in Xenopus oocytes and at 10 microM inhibited α4β2 and α3 β2 nAChRs. A common feature for ws-lynx1 and Lynx1 is a decrease of nAChR sensitivity to high acetylcholine concentrations. Thus, ws-lynx1 is an appropriate model to shed light on the mechanism of Lynx 1 action. Computer modeling, based on ws-lynx1 NMR structure and AChBP X-ray structure, revealed a possible mode of ws-Lynx 1 binding: in general, similar to that of α-neurotoxins, but contacting the target with the other side of the relatively flat molecule (Lyukmanova et al, 2011).

Symposium 33
Noncoding RNAs and Synaptic Plasticity

S33-01

REGULATING THE REGULATORS: ACTIVITY-DEPENDENT REGULATION OF NEURONAL MICRORNAS

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Our research group is interested in the role of miRNAs in synapse development and plasticity, as well as the potential impact of miRNA regulation on higher cognitive functions and neurological disease. During the last five years, we have identified key neuronal miRNAs and their targets that are involved in dendrite and spine morphogenesis. However, how miRNA activity in neurons itself is regulated in response to environmental signals is still largely unknown. Activity-dependent alterations in miRNA function are likely important for the bi-directional regulation of synaptic strength underlying learning and memory. Here, I will present our current efforts to systematically investigate activity-dependent miRNA regulation at different levels along the miRNA biogenesis pathway. Main topics will include the regulation of miRNA transcription and its relevance for network homeostasis, the mechanism of miRNA transport to the synapto-dendritic compartment, as well as the identification of novel neuronal miRISC interacting proteins and their role in activity-dependent target gene regulation. Our results elucidate novel mechanisms for the regulation of microRNA function, with likely implications for our understanding of memory-related processes and neurological disease.

This work is supported by grants from the DFG (SFB593) and the EU (ERC StG 'NeuroMir')

S33-02

NON-CODING RNA TRAFFICKING BETWEEN NEURAL STEM CELLS

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The mammalian genome is abundantly transcribed as complex interleaved networks of non-coding RNAs (ncRNAs). Many of these ncRNAs are expressed in the brain, including in specific neuroanatomical regions, cell types, or subcellular compartments. Furthermore, ncRNAs are expressed in response to neuronal activity and often in concert with adjacent or overlapping neuronal genes. We are now beginning to appreciate the central role this novel transcriptional class fulfils in regulating epigenetic and transcriptional programs and more broadly genome biology. Given such critical roles, the intercellular exchange of ncRNAs would permit their regulatory functions to be transferred to and impact on other proximal recipient cells. We show that neural stem cells secrete exosomes laden with a wide range of ncRNAs. This exportation process is highly selective, with alternative ncRNA classes being carefully sorted for export, and sensitive to external stimuli such as inflammatory cues. The receipt of exosomes imparts broad changes on the gene expression and cellular features of recipient cells. We propose the extracellular transfer of RNA between cells comprises a novel mechanism of intercellular communication, thereby aiding in the coordination of multicellular populations.

S33-03

MICRORNA REGULATION IN LONG-TERM POTENTIATION *IN VIVO*

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Regulation of microRNA (miRNA) expression and function in the context of activity-dependent synaptic plasticity in the adult brain is little understood. Here, we examined miRNA expression during long-term potentiation (LTP) in the dentate gyrus of adult anesthetized rats. Microarray expression profiling identified a subpopulation of regulated mature miRNAs 2 h after the induction of LTP by high-frequency stimulation (HFS) of the medial perforant pathway. Real-time polymerase chain reaction analysis confirmed modest upregulation of miR-132 and miR-212, and downregulation of miR-219, while no changes occurred at 10 min post-HFS. Surprisingly, pharmacological blockade of N-methyl-d-aspartate receptor (NMDAR)-dependent LTP enhanced expression of these mature miRNAs. This HFS-evoked expression was abolished by local infusion of the group 1 metabotropic glutamate receptor (mGluR) antagonist, (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA). AIDA had no effect on LTP induction or maintenance, but blocked activity-dependent depotentiation of LTP. Turning to the analysis of miRNA precursors, we show that HFS elicits 50-fold elevations of primary (pri) and precursor (pre) miR-132/212 that is transcription dependent and mGluR dependent, but insensitive to NMDAR blockade. Primary miR-219 expression was unchanged during LTP. In situ hybridization showed upregulation of the pri-miR-132/212 cluster restricted to dentate granule cell somata. Thus, HFS induces transcription miR-132/212 that is mGluR dependent and functionally correlated with depotentiation rather than LTP. In contrast, NMDAR activation selectively downregulates mature miR-132, -212 and -219 levels, indicating accelerated decay of these mature miRNAs. This study demonstrates differential regulation of primary and mature miRNA expression by mGluR and NMDAR signaling following LTP induction, the function of which remains to be defined.

S33-04

INVOLVEMENT OF MICRORNAS, ENDOGENOUS SIRNAS, AND OTHER NOVEL SMALL RNAS IN SYNAPTIC PLASTICITY AND LEARNING.

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Genomics studies have stimulated a revolution in our appreciation of the diversity and roles of RNAs. It is now clear that the number of non-coding RNAs in the mammalian genome exceeds the number of protein-coding RNAs by several-fold, and it is also clear that nearly all classes of RNAs can be processed to form small RNAs. However, what is NOT clear is what these small RNAs do,

or how, in the context of synaptic plasticity. Initially, it appeared that microRNAs and siRNAs primarily act post-transcriptionally to inhibit the translation and/or stability of specific mRNA targets. However, many additional mechanisms of action have emerged, and several new classes of small RNAs have been discovered that may not fit well into this mold. In the face of this uncertainty, my laboratory has deferred studies of 'function' and has instead focused on neurochemistry: identifying components of the small RNA machinery that are enriched in synaptic fractions of adult mouse forebrain, and that are modulated by synaptic activity or in behavioral paradigms (olfactory discrimination training in mice and repeated inescapable shocks producing learned helplessness in rats). I will review evidence that microRNA precursors and sense-antisense transcript pairs are expressed in synaptic fractions, together with the enzymes needed to process them. Deep sequencing studies have confirmed that mammalian brain expresses endogenous siRNAs, particularly those that arise from inverted repeats encoded within introns of genes that regulate synaptic plasticity. Abundant non-coding RNAs also give rise to diverse small RNAs, a subclass of which show extremely large changes during the onset of learning. Behavioral paradigms elicit global changes in abundance of small RNA populations (including microRNAs, endogenous siRNAs and the novel non-coding subclass alike), suggesting that small RNA function involves more than just individual small RNAs interacting with their specific targets.

Workshop 7
Monitoring and Manipulation of Neuronal Functions with Light

W07-01

GENETICALLY ENCODED PROBES FOR MONITORING OF CL AND CL-SELECTIVE CHANNELS

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Genetically encoded probes have become powerful tools for non-invasive monitoring of ions, distributions of proteins and the migration and formation of cellular components. Discovery that the native fluorescence of yellow fluorescent protein (YFP) is quenched by anions led to the development of genetically encoded intracellular halide sensors. We developed several molecular probes for non-invasive fluorescent monitoring of intracellular Cl [(Cl)i] and the functioning of Cl-selective ionotropic receptors for glycine (GlyR) and GABA. The first probe, termed Cl-Sensor, is a ratiometric CFP-YFP based construct, with relatively high sensitivity to Cl (Kapp about 30 mM). It allows estimating the (Cl)i distribution in small neuronal compartments such as dendritic spines. The second construct, termed BioSensor-GlyR, is a GlyR channel with Cl-Sensor incorporated into the cytoplasmic domain. This is the first molecular probe for spectroscopic monitoring of the functioning of receptor-operated channels. Using the similar strategy we dveloped also BioSensor-GABA, for monitoring activity of $GABA_A$ receptors. These types of probes offer a means of screening pharmacological agents and monitoring Cl under different physiological and pathological conditions. It also permit spectroscopic monitoring of the activity of Cl-selective channels expressed in heterologous systems and neurons.

W07-02

CONFORMATIONAL CHANGES IN GLYCINE RECEPTOR CHLORIDE CHANNELS PROBED USING VOLTAGE-CLAMP FLUOROMETRY

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Glycine receptor (GlyR) chloride channels are Cys-loop receptors that mediate inhibitory neurotransmission. We have previously employed voltage-clamp fluorometry (VCF) to investigate conformational changes associated with GlyR activation. Here we employed VCF to probe conformational changes associated with desensitization and phosphorylation of α1 and α3 GlyRs. GlyRs incorporating cysteines at locations of interest were expressed in Xenopus oocytes, labeled with rhodamine and studied using simultaneous voltage-clamp and microfluorometry. Oocytes were removed from anaesthetized frogs by procedures approved by the University of QLD Animal Ethics Committee. Although α1 and α3 GlyRs share identical amino acid compositions in the M2-M3 loop and surrounding domains, the fluorescence changes reported by a rhodamine label attached to the M2-M3 domain of the α3-R19'C GlyR were much smaller (~20%) and much slower to return to baseline after glycine removal than the corresponding signals in the α1-R19'C GlyR. By investigating a series of α1-α3 chimeras, we found that the intracellular M3-M4 loop was responsible for this difference. Given that α3 GlyRs are phosphorylated by protein kinase A at S346 in the M3-M4 domain, whereas α1 GlyRs are not, we investigated whether phosphorylation also induced a conformational change in the α3 GlyR M2-M3 domain. Indeed, treatment of oocytes expressing α3-R19'C GyRs with forskolin reversibly inhibited glycine-activated fluorescence. This effect was absent in the α1-R19'C GlyR and the S346A mutant α3-R19'C GlyR. This result indicates that the 19' residue in the α3 GlyR M2-M3 loop experiences a conformational change resulting from the phosphorylation of a distant intracellular site. This provides a possible basis for understanding how phosphorylation alters GlyR gating properties. Using a similar approach, we showed that conformational changes mediating α1 GlyR desensitization were detectable in loop 2 and in the pre-M1 domain, but not in the ligand-binding domains. Together, these findings provide new insights into the structural basis of GlyR desensitization and modulation.

W07-03

OPTOCHEMICAL GENETICS

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Transmembrane receptors allow a cell to communicate with its environment in response to a variety of input signals. These can be changes in the concentration of ligands (e.g. hormones or neurotransmitters), temperature, pressure (e.g. via acoustic waves or touch), transmembrane potential, or light intensity. Many important receptors have now been characterized in atomic detail and our understanding of their functional properties has markedly increased in recent years. As a consequence, these sophisticated molecular machines can be reprogrammed to respond to unnatural input signals. In this review, we will show how voltage-gated and ligand-gated ion channels, as well as metabotropic receptors, can be endowed with synthetic photoswitches. The resulting artificial photoreceptors can be used to optically control neurons with exceptional temporal precision. They work well in animals and might find applications in the restoration of vision and the optical control of other sensations. The combination of synthetic photoswitches and receptor proteins contributes to the field of Optogenetics and adds a new functional dimension to Chemical Genetics. As such, we propose to call it 'Optochemical Genetics'.

W07-04

SIMULTANEOUS CHLORIDE AND PH MEASUREMENT *IN VIVO* WITH A GENETICALLY-ENCODABLE OPTICAL PROBE

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A new method allowing the non-invasive measurement of combined pH-chloride maps in real-time will be presented. The procedure is based on a ratiometric biosensor comprising a highly-sensitive GFP variant fused to a reference fluorophore (DsRed). The construct is genetically encodable and can be selectively targeted to the desired intracellular compartment. As a first application I shall demonstrate high chloride concentration in dense-core-exocytosis granules of secretory pathways in cells under culture.

Operation under two-photon excitation will also be presented and its impact on *in vivo* studies will be discussed.

W07-05

OPTICAL CONTROL OF NEUROSECRETION

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Exocytosis is a key process in neurotransmission. It has been studied in great detail in neuroendocrine cells like chromaffins, because their size and shape provide good experimental access. However, in more conventional and relevant examples like hippocampal neurons, terminal size is about one micron and limits exocytosis studies with the current techniques. Thus, new methods for directly and accurately triggering exocytosis at a synaptic terminal are necessary and an appealing possibility is to use photoswitchable ion channels like the light-gated glutamate receptor (LiGluR), because light stimulation is non-invasive and provides spatiotemporal control at the level of individual synaptic terminals and single action potentials. We used amperometry on LiGluR-expressing chromaffin cells to show that UV illumination can trigger secretion repeatedly in individual cells. We took advantage of the graded behavior that the MAG photoswitch elicited on LiGluR channels, to define a three-gear control of neurotransmission where neurosecretion was stopped at 500 nm, and driven to a low or high frequency at 410–420 nm or 410–380 nm illumination, respectively. Individual vesicle fusion events are comparable to those obtained by depolarization. However, amperometric recordings showed that light triggered secretion occurred with a delay not observed with depolarizing stimuli. Release probability in a neuroendocrine cell is greatly determined by the spatiotemporal characteristics of calcium influx. Voltage gated calcium channels (VGCCs) are clustered and effectively determine secretion, while such level of organization was not expected for LiGluR channels. Exocytosis was further compared for calcium influxes through the two channel types using membrane capacitance measurements. We observed that capacitance increase was twofold larger when calcium ions permeated via VGCCs, than when did it through LiGluR, suggesting that secretion was more efficiently triggered by the VGCCs. These results were confirmed by limiting the intracellular diffusion of calcium with EGTA, which has a significantly stronger impact on LiGluR than on VGCCs.

Workshop 8
Cytoskeletal/Membrane Interactions in the Regulation of GPCR Signaling

W08-01

ENDOCYTOSIS AND RECYCLING DEPENDENT ENHANCEMENT OF 5-HT2 RECEPTOR SIGNALING BY CRF

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Depression is a widespread and serious disorder that affects the daily lives of 10–15% of Canadians. There are multiple potential causes of depression, including genetic pre-disposition, but the majority of cases are influenced by environmental factors. Anatomical localization studies have shown that both serotonin (5HT) 2A receptor (5HT2AR) and corticotropin-releasing factor receptor 1 (CRFR1) are appropriately localized within the brain to regulate each other's function at the molecular level. Moreover, several recent studies have reported effects of CRF peptide on 5HT mediated function and signal transduction. However, there is still a lack of information regarding how CRF modulates the 5-HT system at the molecular level. The current presentation will overview the role of GPCR endocytosis, and recycling in the regulation of 5HT2AR signalling following the prior activation of CRFR1. We find that CRFR1 activation leads to the co-internalization of both 5-HT2AR and 5-HT2CR, whereas 5-HT2AR activation does not stimulate CRFR1 endocytosis in either HEK 293 cells or primary mouse (E15–E16) cortical (10 DIV) neurons. We also show that the activation of the CRFR results in a 40 and 47% augmentation in the VMAX for 5-HT-stimulated IP formation in human embryonic kidney (HEK 293) cells co-expressing either human 5-HT2AR or human 5-HT2CR, respectively. This CRFR1-mediated increase in 5-HT receptor signalling is also observed following the activation of endogenous CRF receptors in primary mouse cortical neurons. The augmentation of 5-HT receptor signalling is dependent upon endocytosis as it is not observed in cells expression a dominant-negative dynamin mutant. In addition, CRFR1-mediated increases in 5-HT receptor signalling were blocked by the overexpression of a dominant-negative Rab4 mutant to block receptor recycling. Injection of CRF peptide into the pre-frontal cortex of mice 30 min prior to challenge with the 5-HT2 receptor agonist DOI also results in enhance anxiety-related behaviours when compared to mice treated with either CRF or DOI alone. Thus, we conclude that the observed CRFR1-mediated increase in 5-HT2AR signalling requires both the endocytosis and recycling of the 5HT2AR and represents a novel mechanism for the regulation of GPCR signal transduction that may be involved in stress induced anxiety-related behaviour responses.

W08-02

THE OTHER SIDE OF OPIOID RECEPTOR SIGNALING: REGULATION BY PROTEIN-PROTEIN INTERACTION

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Ample experimental evidence has demonstrated that opioid receptors can physically interact with a variety of accessory proteins, confirming that signal transduction of these receptors is not restricted to heterotrimeric G protein activation. Such interactions can alter the effectiveness of agonist-driven cell signaling, determine the signals generated and alter the trafficking, targeting, fine tuning and cellular localization of these receptors by providing a scaffold that links the receptors to the cytoskeletal network. In the present study emphasis will be given in unconventional interacting partners such as the Regulators of G protein Signaling (RGS4, RGS2) and Signal Transducers and Activators of Transcription 5A and B (STAT5A/B), which associate with μ and δ-opioid receptors. Evidence will be presented on how RGS4 and RGS2 confer selectivity to the opioid receptors to select a specific subset of G proteins; how activation of opioid receptors results in recruitment of RGS proteins to the plasma membrane in HEK293 cells; and how RGS2 and RGS4 exert a differential modulatory effect in ERK1,2 phosphorylation, agonist-driven inhibition of adenylyl cyclase and the internalization fate of μ- and δ-opioid receptors. Other studies will clarify how STAT5B associates with the δ-opioid receptor, is phosphorylated by c-Src kinase upon receptor activation and forms selective pairs with selective Gα and Gβγ subunits to regulate the transcription of STAT5B-dependent genes. Finally, it will be shown how activation of the δ-opioid receptor with selective agonists promotes a multi-component signaling complex consisting of specific Gαi/o proteins, the STAT5B transcription factor and other signaling intermediates to mediate neuronal survival and neurite outgrowth. Understanding the mechanisms by which these interactions are regulated is expected to address problems related to phenomena such as pain perception, tolerance and dependence that occur upon chronic opiate administration and define whether disruption of such interactions may contribute to the development of novel therapeutic strategies.

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W08-03

CB1R SIGNALLING COMPLEX FORMATION WITH PROXIMAL KINASES PKCε, SRC AND FYN, AND TRANSACTIVATION OF FGFR, EMANATES FROM LIPID RAFTS

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Signalling events, pivotal to neuronal function, may both be initiated and enhanced on lipid rafts, which represent plasma membrane microdomains enriched in cholesterol, glycosphingolipids, and lipid-modified signalling molecules on their cytoplasmic face. The heptahelical, transmembrane, Type 1 (CB1) cannabinoid receptors, play important roles in the regulation of dendritic branching, synapse formation, and synaptic transmission but the

proximal signaling interactions or their topology remain unclear. We now show in primary telencephalic chick embryo neurons, that CB1Rs associate with lipid rafts for subcellular trafficking in response to agonist stimulation for a biphasic activation of ERK. More specifically, the majority of CB1Rs associates with the actin-interacting PKC isoform PKCε at basal conditions; upon agonist stimulation, a sequential activation of Gq/PLC/PKCε leads to activation of Src/Fyn/ERK. Concurrently, CB1R was acutely and distinctly recruited to lipid rafts; upon longer treatment CB1R was primarily detected to subcellular fractions which contain ER and Golgi membranes, and by 20 min of stimulation, CB1R recycled back to lipid rafts. Moreover and within the context of lipid rafts, CB1Rs interacted via Src and Fyn with FGFR-tyrosine to transduce a second, additional peak of ERK1/2 activation. Agonist-induced activation of ERK and CB1R-FGFR interactions were abolished by the specific FGFR inhibitor PD173074, specific cannabinoid receptor antagonists, while lipid raft and actin cytoskeleton integrity was a pre-requisite for CB1R-dependent complex formation. These receptor-proximal signaling events correlated well with induction of neuritic outgrowth in the long term. Our studies suggest that in neuronal cellular backgrounds, the CB1R intracellular signaling engages transactivation of FGFRs after intramolecular interactions within the lipid rafts, and that lipid rafts may also serve as vehicles for endocytosis (support: PENED 03EΔ778/GGET/EU to DM).

W08-04

ROLE OF CYTOSKELETAL AND SCAFFOLDING PROTEINS IN LIPID RAFT TARGETING OF G PROTEINS

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Membrane targeting of Gsalpha has been attributed to several factors, but it is clear that in order to be oriented in the membrane for appropriate access to GPCR and adenylyl cyclase, Gsalpha must be palmitoylated. Removing palmitoylation sites prevents membrane targeting and adding both palmitoylatoin and myristoylation sites increases association with both plasma membrane and intracellular membrane and disrupts trafficking into lipid rafts subsequent to Gs alpha activation. Tubulin, both on the membrane (particularly in lipid rafts) and in microtubules appear to be binding substrates for Gsalpha, and the targeting to membrane tubulin versus microtubule tubulin have very different effects on cellular activity.

Workshop 9
Neurobiology and Pathology of Spine Formation in Mental Retardation Mice

W09-01

ABNORMAL DENDRITIC SPINE FORMATION IN *ATRX* METAL RETARDATION MODEL MICE

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Alpha-thalassemia X-linked mental retardation (ATRX) syndrome is a severe, non-progressive mental retardation that is frequently associated with various clinical manifestations including severe mental retardation, genital abnormalities and epileptic seizures. We generated *ATRX* mutant mice ($ATRX^{\Delta E2}$) lacking of exon 2 with mild mental retardation, which is accompanied with impaired cognitive functions. We here addressed causative mechanisms of abnormal spine formation in the medial prefrontal cortex (mPFC) observed in $ATRX^{\Delta E2}$ mice. Consistent with other mental retardation model mice, the mutant mice showed remarkable morphological changes in the dendritic spines that are long and thin without change in the number of spine on the dendrites. Interestingly, an aberrant increased calcium/calmodulin-dependent protein kinase II (CaMKII) activity was observed in the prefrontal cortex of $ATRX^{\Delta E2}$ mice. The increased CaMKII autophosphorylation was associated with increased phosphorylation of Rac1-guanine nucleotide exchange factors (GEFs), Tiam1 and Kalirin-7. Since Tiam1 and Kalirin-7 are substrate for CaMKII, thereby activating p21-activated kinases (PAKs), we confirmed increased phosphorylation of Tiam1, Kalirin-7 and PAK1-3 in extracts from the mPFC. Taken together, the aberrant CaMKII activation likely mediates abnormal spine formation in the mPFC and the elevated Rac1-GEF/PAK signaling in $ATRX^{\Delta E2}$ mice accounts for mental retardation syndrome with concomitant abnormal spine formation.

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W09-02

SYNAPTIC INTEGRATION OF NEW NEURONS GENERATED IN THE ADULT HIPPOCAMPUS

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The discovery of neuronal stem cells in the adult mammalian brain raised new perspectives on brain plasticity and for the treatment of neurodegenerative diseases. Using a viral-based gene delivery approach, we identified new hippocampal granule neurons generated during adulthood and analyzed their synaptic integration ultrastructurally. We observed that while these cells integrate into the hippocampal network, they preferentially contact pre-existing synapses, thereby forming synaptic contacts with multiple partners. Over maturation, the connectivity of new neurons changes in favor of individual synapses. This change suggests that adult neurogenesis modifies the connectivity of the existing neurons and that synapse replacement may occur in the adult hippocampus.

W09-03

ALTERATIONS OF SPINES DYNAMICS AND NETWORK DEVELOPMENT BY THE RHO GTPASE EFFECTOR PAK3

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Confocal imaging techniques applied to living neurons have shown that excitatory synapses on dendritic spines are dynamic structures that undergo a continuous turnover. This structural plasticity is particularly high during development and contributes to the establishment of brain circuits. Using hippocampal slice cultures and long-term repetitive imaging, we have shown that learning related activity patterns promote an increase in this turnover and operate as a selection process that switches activated synapses to a long-term stable state. This is done through a competitive mechanism that promotes a selective stabilization of synapses activated by a learning paradigm and a replacement of non-activated inputs by new spines. Interestingly these new spines tend to cluster around activated synapses, promoting a localized structural plasticity. Thus learning related paradigms play a major role in shaping the structural organization of synaptic networks by increasing their specificity. In this context, we investigated whether and how the mental retardation protein PAK3, which is a downstream effect of the Rac/Cdc42 signaling pathway, affected spine dynamics. Evidence will be presented that expression of a mutant variant of PAK3 or inhibition of PAK activity through an inhibitory TAT-peptide or a pharmacological antagonist leads to an enhanced growth of new protrusions. These new spines appear in clusters and are regulated by activity. Furthermore activity promotes a recruitment of PAK3 in spines and inhibition of PAK3 prevents the mechanisms of activity-mediated spine stabilization. These results suggest that PAK3 signaling plays an important role in activity-mediated synapse remodeling and that mutation of PAK3, by interfering with these mechanisms, leads to an excessively dynamics synaptic network lacking specificity and modulation by activity.

W09-04

ABNORMAL BEHAVIORS AND SPINE MORPHOLOGY IN HEPARIN-BINDING EPIDERMAL GROWTH FACTOR-LIKE GROWTH FACTOR (HB-EGF) KNOCKOUT MICE

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Purpose: Recently, neurotrophic factors and cytokines have been shown to be associated with psychiatric disorders. Heparin-binding epidermal growth factor-like growth factor (HB-EGF), a member of epidermal growth factor (EGF) family, is a potent mitogenic peptide for various types of cells. In the brain, it serves as a neurotrophic molecular and plays a significant role in cell proliferation, neural differentiation, and synaptic plasticity. However, little is known about the relationship of HB-EGF and mental illness.

Method: We generated mice in which HB-EGF activity is disrupted specifically in ventral forebrain, by using conditional gene-targeting techniques and its region specific deletion of the HB-EGF was confirmed by immunostaining and in situ hybridization. We performed some kinds of neurobehavioral tasks and measured monoamine contents in various region of the brain. We also analyzed the morphology of pyramidal neurons in cortical layer III and measured long term potentiation (LTP) in hipocampus of HB-EGF KO knockout mice.

Results: HB-EGF knockout mice exhibited (i) behavioral abnormalities similar to those described in psychiatric disorders, which were ameliorated by typical or atypical antipsychotics, (ii) altered dopamine and serotonin levels in the brain, (iii) decreases in spine density in neurons of the prefrontal cortex, (iv) reductions in the protein levels of the NR1 subunit of the N-methyl-D-aspartate (NMDA) receptor and post-synaptic protein-95 (PSD-95), (v) decreases in the EGF receptor, and in the calcium/calmodulin-dependent protein kinase II (CaMK II) signal cascade. HB-EGF KO mice also showed (vi) decreased hipocampal LTP and (vii) impaired hippocampus dependent memory formation.

Conclusion: The current study demonstrates that HB-EGF KO mice exhibited the behavioral abnormalities and impaired spine morphology, reflected in a comprehensive spectrum of psychomotor and cognitive dysfunctions. These results suggest the alterations affecting HB-EGF signaling could comprise a contributing factor in psychiatric disorder.

W09-05

THE ROLE OF SHANK PROTEINS IN REGULATING SPINE FORMATION AND SYNAPSE FUNCTION

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Shank families of proteins are among the major scaffold proteins that organize the post-synaptic density (PSD) at the excitatory synapses. Shank1-3 proteins are large scaffold proteins that contain ankyrin repeats, an SH3 domain, a PDZ domain, a proline-rich domain and a SAM domain. They are associated with the NMDA receptor-PSD-95 complex by their binding to C-terminal GKAP, and with type I mGluRs via an interaction with Homer in the proline-rich domain. In the last few years we have contributed in showing that Shank promotes the accumulation of post-synaptic density proteins in dendritic spines and that Homer and Shank form a mesh-like matrix structure in the PSD working as an assembly platform for other PSD proteins.

Human Shank3 gene deletion and mutations are associated with cognitive impairment ranging from mental retardation to autism. We used RNA interference (RNAi) to knock down Shank3 expression in hippocampal cultures and showed that this treatment specifically reduced the synaptic expression of the metabotropic glutamate receptor mGluR5 but did not affect the expression of other major synaptic proteins. As a functional consequence of Shank3 RNAi knockdown, we found impaired mGluR5-dependent synaptic plasticity (DHPG-induced long-term depression), and impaired mGluR5-dependent modulation of neural network activity. We also found morphological abnormalities in the structure and number of dendritic spines and impaired glutamatergic synaptic transmission. However, pharmacological augmentation of mGluR5 activity using CDPPB (3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide) as the positive allosteric modulator of these receptors restored mGluR5-dependent signaling and normalized glutamatergic synaptic transmission.

These data demonstrate that restoring mGluR5-dependent synaptic plasticity using CDPPB could represent a new strategy for pharmacological treatment of patients with Shank3 mutations.

Workshop 10
Cracking the Cerebral Codes in Different Ways

W10-01

METABOLIC FLUX ANALYSIS APPROACHES TO INVESTIGATE BRAIN METABOLISM IN CELL CULTURES

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Metabolic modeling has been useful to elucidate important aspects of brain metabolism and the biochemistry underlying some diseases. This talk will provide an overview on the application of metabolic flux analysis (MFA)-based approaches to estimate intracellular fluxes of cultured brain cells. MFA provides a steady-state flux distribution based on the metabolic network's stoichiometry, and using information from uptake/production rates of metabolites measured in culture supernatant. ^{13}C NMR data can be additionally used to distinguish flux ratios through parallel reactions. We have applied this methodology to characterize the metabolic effects of *in vitro* ischemia in cultured astrocytes, and of hypoglycaemia in cultured cerebellar neurons. In particular, astrocytes up-regulated glucose utilization by 30% and the pentose phosphate pathway and TCA cycle fluxes by three and twofold, respectively, in the first hours post-ischemia. Moreover, a 2–5 fold increase in branched-chain amino acids catabolism suggested the additional importance of anaplerotic molecules to the fast recovery of energetic status (1). In cerebellar neurons, a 12 h hypoglycaemic insult revealed an adaptation to glutamine metabolism concomitantly with the up-regulation of the pyruvate recycling (PR) pathway (2). This study also suggested that the PR pathway appears to be significant in these cells even under normal conditions. Finally, we have recently implemented a more robust and powerful MFA approach – isotopic transient ^{13}C MFA – allowing to estimate a higher number of fluxes of cultured astrocytes, including the malate-aspartate shuttle flux and glutamate/α-ketoglutarate exchange rate. This method explores the information provided by ^{13}C-labeling dynamics time-courses in intracellular metabolites after administration of a ^{13}C-labeled substrate. In conclusion, these methodologies are valuable for the investigation and development of new therapies targeting specific metabolic pathways in brain cells.

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W10-02

REDUCTIONISM: A BRAIN SLICE MODEL OF CORTICAL METABOLISM AND COMPARTMENTATION

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Here, we describe use of a reductionist brain model, the brain tissue slice, to generate snapshots of functional metabolism in response to a pharmacological (GABAergic) perturbation. Tissue slices prepared from Guinea pig cerebral cortex were incubated for 1 h in the presence of (3-^{13}C)-pyruvate and ligands with affinity for GABA receptors. The resultant patterns of ^{13}C flux and metabolite levels were measured by ^{13}C/^{1}H NMR spectroscopy, generating 'metabolic fingerprints' for each ligand. Effects of agonists and effectors at GABA receptors (A, B, and C types) were examined, compared to those of exogenous GABA and evaluated using multivariate statistical models. Data clusterings did not directly correlate with GABA receptor types but produced at least seven distinct groups ranked according to their affinity for GABA. As our experimental model retains, to a large extent, the structure and function of normal brain tissue, the generated database can be used to assess GABAergic ligands and make unique inferences relevant to their modes of action in brain. Here, we will show applications of this to approach with GHB and alcohol.

W10-03

EX VIVO MODELING OF CEREBRAL METABOLISM

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13C turnover studies using ^{13}C NMR have provided considerable insight into cerebral energetics and neuroglial compartmentation *in vivo* and *in vitro*. However, the ^{13}C turnover approach remains limited by the reduced sensitivity and temporal resolution of the ^{13}C NMR method. This precludes the adequate observation of metabolic processes faster than the tricarboxylic acid cycle, thus precluding investigation of many mitochondrial-cytosolic metabolite exchanges, transminations and hydration-dehydration or hydrogen transfer reactions. Hydrogen turnover is considerably faster than ^{13}C turnover and consequently would allow to resolve in time faster metabolic processes. This lecture describes progress made using 1H-2H exchange as a complementary tool to 13C turnover. We investigated by ^{13}C NMR the turnover of the H3 deuterons of (2-^{13}C) glutamate and (2-^{13}C) glutamine in the brain of partially deuterated rats. Adult animals (150–200 g) fed *ad libitum* received 50% $2H_2O$ or tap water 9 days before infusing (1-^{13}C) glucose or (2-^{13}C) acetate for 5, 10, 15, 30, 60, or 90 min. The brains were then funnel-frozen and acid extracts were prepared and analyzed by high-resolution ^{13}C NMR. The deuteration of one or the two H3 hydrogens of (2-^{13}C) glutamate or glutamine resulted in single (−0.07 ppm) or double (−0.14 ppm) isotopic shifts upfield of the corresponding C2 perprotonated resonance, demonstrating two sequential deuteration steps. The faster monodeuteration generated 3R or 3S (2-^{13}C, 3-2H) glutamate or glutamine through the alternate activities of cerebral aconitase or isocitrate dehydrogenase, respectively. The slower process produced bideuterated (2-^{13}C, 3,3'-2H2) glutamate or glutamine through the consecutive activity of both enzymes. The kinetics of deuteration was fitted to a Michaelis-Menten model including the apparent K(m)' and Vmax' values for the observed deuterations. Our results revealed different kinetic constants for the alternate and consecutive deuterations, suggesting that these processes were caused by the different cytosolic or

mitochondrial isoforms of aconitase and isocitrate dehydrogenase, respectively. The deuterations of (2-^{13}C) glutamate or glutamine followed also different kinetics from (1-^{13}C) glucose or (2-^{13}C) acetate, revealing distinct deuteration environments in the neuronal or glial compartments.

W10-04

COMPARTMENTAL ANALYSIS AND SENSITIVITIES OF KINETIC CARBON-13 LABELING STUDIES

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The quantification of excitatory and inhibitory neurotransmission and the associated energy metabolism is crucial for a proper understanding of brain function. Although the detection of glutamatergic neurotransmission *in vivo* by ^{13}C NMR spectroscopy is now relatively routine, the detection of GABAergic turnover *in vivo* has remained elusive because of the low GABA concentration and spectral overlap. Using 1H-(^{13}C) NMR spectroscopy at high magnetic field in combination with spectral modeling and the use of different substrates, ^{13}C-glucose and ^{13}C-acetate, it is shown that GABAergic, as well as glutamatergic neurotransmitter fluxes can be detected non-invasively in rat brain *in vivo*. The analysis of GABA turnover requires a three-compartment model to incorporate glia and the glutamatergic and GABAergic neurons. The sensitivities of the analysis to model parameters, including anaplerosis and the dilution of glutamine, influence the results significantly and will be discussed in terms of effect and physiological basis.

W10-05

IN VIVO METABOLIC MODELING, THE TWO COMPARTMENT MODEL

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In vivo ^{13}C NMR spectroscopy has the unique capability to measure metabolic fluxes non-invasively in the brain. Quantitative measurements of metabolic fluxes require analysis of the ^{13}C labeling time courses obtained experimentally with a metabolic model. Metabolic modeling of ^{13}C NMR spectroscopy (^{13}C MRS) data using two-compartment neuronal-glial models enabled non-invasive measurements of the glutamate-glutamine cycle rate (V(NT)) in the brain *in vivo*.

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LB01 Late Breaking Abstracts

LB01-01

DOPAMINE D2 RECEPTOR MODULATES AKT SIGNALING AND ALTERS GABAERGIC NEURON DEVELOPMENT AND MOTOR BEHAVIOR IN ZEBRAFISH LARVAE

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An imbalance in dopamine-mediated neurotransmission and neurodevelopmental abnormalities are features of schizophrenia. The main target of antipsychotics, the dopamine D2 receptor, modulates the activity of Akt, which has an important role in the regulation of cellular processes that are critical for brain development, and it is known to be downregulated in the brain of schizophrenic patients. Thus, it is possible that altered D2-dependent Akt signalling leads to abnormal neurodevelopment in schizophrenia. To address this hypothesis, we first evaluated if dopamine modulates Akt signalling in the developing brain. We treated 3 days post fertilization (dpf) and 5dpf zebrafish larvae with dopamine. Our results indicate that dopamine causes specifically dephosphorylation at threonine 308 (T308). We treated 5dpf larvae with dopaminergic agonists and antagonists and demonstrated that dopamine regulates Akt activity by D2 receptors, but not by D1 receptors. In order to investigate the role of dopamine in the neurodevelopment, we examined dlx6:gfp transgenic 3dpf larvae, which express GFP in several forebrain GABAergic neurons, chronically exposed to dopamine. This treatment resulted in region specific alterations in the number of GABAergic neurons, but not the total number of cells or the proportion of glial cells, suggesting a specific effect on neuronal differentiation. Furthermore, we observed that dopamine affects motor behaviour in 3-5dpf larvae. Together, our data suggest that dopamine signalling represses Akt signalling and leads to defects in GABAergic neuronal differentiation in the zebrafish larval brain. Furthermore, the alterations in forebrain GABAergic neurons are correlated with altered context-dependent motor behaviour. Thus, with this model system, we could holistically assay the biochemical, morphological, and behavioural consequences of altered dopamine signalling during a 3-5 day developmental window. These results will help shape our understanding of the role of dopamine in brain development and provide new mechanistic insight for further assessing the neurodevelopmental origin model of schizophrenia.

LB01-02

NAADP-MEDIATED CALCIUM SIGNALLING IN ASTROCYTES

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Intracellular calcium signals and calcium waves spreading from astrocyte to astrocyte provide these cells with a specific form of excitability allowing them to integrate information and regulate synaptic transmission and plasticity. In this study we reassessed the calcium mobilization mechanisms in cortical astrocytes. Using NAADP-AM, a membrane-permeant analogue of the new second messenger Nicotinic Acid-Adenine Dinucleotide Phosphate (NAADP), we demonstrated that intracellular NAADP is capable to produce calcium responses in astrocytes. Furthermore, disruption of either NAADP or lysosomal signalling by a) the blockade of NAADP receptors, b) prevention of acidification or c) osmotic lysis of lysosome-related intracellular vesicles, reduced calcium responses induced by extracellular application of ATP but not bradykinin. ATP increased endogenous NAADP levels and, likewise, interfering with NAADP signalling diminished the magnitude of intercellular calcium waves, a process in which release of endogenous ATP is a key element. Overall, our data provide evidence for NAADP being an intracellular second messenger that releases calcium from lysosomes in astrocytes and acts in coordination with inositol 1,4,5-trisphosphate (IP3) to grant specificity to calcium signals.

LB01-03

INVOLVEMENT OF ASTROCYTES IN STRESS-INDUCED NEURODEGENERATION

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Neuroinflammation is considered to be involved in the pathogenesis of neurodegenerative disorders. Glial cells are among main players in neuroinflammation. The aim of the study was to access the involvement of glia in stress-induced neuroinflammation. Two series of experimental neurosis (chronic pain-emotional stress) were performed. Male Wistar rats were subjected to combined effects of white noise and foot shock reinforced by light flashes with different probability for 15 days. The probability of current reinforcement by light was 50% in the first experiment and 25% in the second one, the unexpectedness being higher in the first experiment. After the stress, rats were sacrificed, brains were fixed in AFA fixative, then paraffin embedded. For Nissl staining and immunohistochemical study, 10 μm thick frontal sections were prepared. Hippocampal subfields CA1, CA3 and dentate gyrus hilus were regions of primary interest. In the first experiment, most neurons displayed pathological changes (up to 98%), and a moderate neuronal loss was evident (about 7%; $p<0.05$ vs. control group). In the second experiment, the changes were much less expressed. Immunohistochemical analysis revealed changes in GFAP-positive cells after chronic stress

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exposure. The number of astrocytes in the hippocampal subfield CA3 decreased in the first experiment ($p<0.05$). In the dentate gyrus hilus the averaged area of the GFAP-positive cell increased in both experiments ($p<0.05$). Astrocytes in the CA1 appeared to remain unaffected by stress exposure in both experiments. We conclude that glial alterations in response to chronic pain-emotional stress are not closely related with the extent of neurodegeneration. Rather, the response of astrocytes may reflect adaptational processs. Supported by Russian Foundation for Humanities, grant # 10-06-01316

LB01-04

TETANUS AND BOTULINUM TOXINS NEED DOUBLE ANCHORAGE TO THE MEMBRANE AND INTACT DISULFIDE BOND FOR LOW PH INDUCED ENTRY INTO NEURONS

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Tetanus and botulinum neurotoxins are di-chain proteins that cause paralysis by inhibiting neuroexocytosis. These neurotoxins enter into nerve terminals via endocytosis inside synaptic vesicles, whose acidic pH induces a structural change of the neurotoxin molecule that becomes capable of translocating its L chain into the cytosol, via a transmembrane protein-conducting channel made by the H chain. This is the least understood step of the intoxication process primarily because it takes place inside vesicles within the cytosol. We describe how this passage was made accessible to investigation by making it to occur at the neurons surface. The neurotoxin, bound to the plasma membrane in the cold, was exposed to a warm low pH extracellular medium and the entry of the L chain was monitored by measuring its specific metalloprotease activity with a ratiometric method. We found that the neurotoxin has to be bound to the membrane via at least two anchorage sites for a productive low-pH induced structural change to take place. Moreover, this process can only occur if the single inter-chain disulfide bond is intact. We determinate that the conformational change pH range of neurotoxin B, C and D is similar (4.5-6) and it comprises that present inside the synaptic vescicles. Furthermore, we studied the protein sequence conservation of tetanus and botulinum neurotoxin different serotypes by alignment analysis. Using PROPKA3.0 software we found that they share a pool of conserved acid residues, that are predicted to protonate in the pH range in which the structural change occurs. We speculate that these residues could be involved in the initial steps of the pH dependent conformational change, proposing a stepwise sequence of events that lead from toxin binding to membrane insertion.

LB01-05

STUDY OF NITRIC OXIDE SYSTEM IN THE BRAIN OF RATS SUBMITTED TO A MODEL OF ISCHEMIC-HYPOBARIC HYPOXIA

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Nitric oxide (NO) is a molecule that depending both on its concentration and tissue redox status, can develop a dual neuroprotective/ neurotoxic function. Specifically, in the brain, NO acts as an inter- and intracellular messenger, triggering protective functions such as vasodilatation, an essential process involved in the developing of hypoxic insults that severely affect the central nervous system (CNS). Considering this facts, we have optimized an experimental model of ischemia followed by a hypobaric hypoxia (HH) period resembling high altitude environmental conditions in order to study the NO system response to a hypoxic situation. Thus, in this study we analyze the amount of NO (measured as nitrate/nitrite and S-nitroso compounds [NOx]) and the localization and activity of the three nitric oxide synthase isoforms in the brain of rats submitted to the above mentioned model after two reoxygenation periods (0 and 2 hours). Our results show that nNOS and iNOS in situ expression increases after the 2 hours period, fact that correlates with the results of NOx and NO activity (measured as NADPH-diaphorase activity). On the other hand, eNOS isoform expression shows higher levels immediately after the HH insult (0 hours). In summary, in our experimental model of ischemic-hypobaric hypoxia, the NO activity found, seems to be related mainly to the nNOS and iNOS isoforms, although deeper investigation needs to be carried out. Supported by MICINN (SAF2008-03938).

LB01-06

REGULATION OF ERK PATHWAY BY D1-LIKE AGONIST IN THE MOUSE DENTATE GYRUS

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Activation of dopamine D1 receptors (D1Rs) has been shown to induce epileptiform activity. In this study we have characterized the molecular changes occurring in the mouse hippocampus in response to the administration of a D1-like family agonist. Systemic injection of SKF 81297 at 5.0 mg/kg induced behavioural seizures-like events and transient electrophysiological discharges in the dentate gyrus (DG). Seizures were prevented by administration of the D1-type receptor antagonist, SCH 23390, or of the cannabinoid CB1 receptor agonist, CP 55,940. The effect of SKF 81297 was accompanied by increased phosphorylation of the extracellular signal-regulated protein kinases 1 and 2 (ERK) in the granule cells layer and by reduced ERK phosphorylation in the polymorphic layer. Moreover we found that both CB1 and D2 receptor agonists strongly reduced ERK activation in the DG suggesting a modulatory effect on SKF 81297-induced ERK phosphorylation. In this study we have also assessed the eventual involvement of glutamatergic modulation blocking either the ionotropic or the metabotropic glutamate receptors.

Although the role of dopamine in the hippocampal formation has been established, the neuronal population expressing dopamine receptors in the DG is poorly characterized. Using drd2- and drd1a-EGFP BAC transgenic mice, we show a clear segregation between neurons expressing D1Rs and D2Rs in the mouse DG. Among all dopaminergic agonists tested, only the D1R-like agonist, SKF 81297, induces ERK phosphorylation which regulates Zif268 and Arc/Arg3.1. This activation depends on D1R stimulation and involves intact metabotropic glutamatergic transmission. Additional experiments suggest the involvement of the hilar mossy cells which amplify ERK activation in granule cell layer through a positive feedback.

LB01-07

CRY ME A RIVER: DEPRESSION-LIKE BEHAVIOUR IN CIRCADIAN-DEFICENT CRY-NULL MICE

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Major depressive disorder (MDD) is the most common psychiatric illness and a major cause of disability (Ustun et al. 2004). Biological rhythm-related symptoms, such as disturbances in the sleep-wake cycle, diurnal mood changes and periodic patterns of symptom recurrence and remission are common in patients with MDD. These observations suggest a direct link between defects in the cellular machinery generating circadian rhythms and MDD. Moreover, a significant association was found between single nucleotide polymorphisms of the circadian clock genes cryptochrome 1 (cry1) and cryptochrome 2 (cry2) and unipolar MDD (Soria et al. 2010; Lavebratt et al. 2010). We used mice lacking both cry1 and cry2 (cry-null mice) to further investigate the association between these circadian clock genes and depression. Previous studies showed a relatively intact rest-activity pattern but arrhythmic physiology and metabolism when these mice are kept in a standard 12h-12h light-dark cycle (van der Horst et al. 1999). We here demonstrate that Cry-null mice display several depression-associated behaviours including cognitive deficits in the object recognition task, increased anxiety in open field and elevated plus maze tests, and a lower responsiveness to the psychotropic effects of cocaine, suggesting anhedonia. These behavioural changes were independent of perturbation of the rest-activity cycle. Further investigations are ongoing to establish whether depression-like behavior in cry-null mice is related to a central dysregulation of the hypothalamus-pituitary-adrenal axis or a direct consequence of increased expression and activity of adrenal 3β-hydroxysteroid-dehydrogenase, a key enzyme in the synthesis of the stress hormone corticosterone.

LB01-08

GENERAL CONTROL NONREPRESSED 2 (GCN2) KINASE PARTICIPATION IN THE COURSE OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

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Central nervous system (CNS) demyelination is a condition observed in some important human diseases, such as multiple sclerosis (MS). EAE (experimental autoimmune encephalomyelitis) is an animal model for the study of MS. Evidences suggest that infiltrating Th1 and Th17 lymphocytes are important players leading to CNS demyelination in EAE. However, many aspects involved in the pathogenesis of the process are still poorly understood. Recent researches demonstrated that chemically induced amino-acid starvation response might suppress CNS immune activity. However, the exact mechanisms by which it occurs are still to be determined. Moreover, whether or not the starvation response could be physiologically involved in the modulation of the CNS inflammation during EAE is still an open question. The aim of this study is to verify the participation of the general control nonrepressed 2 (GCN2), a key regulator kinase of the amino-acid starvation response, in the course of the EAE. By immunizing wild type C57BL/6J (WT) and GCN2 knock-out mice (GCN2-KO) with myelin oligodendrocyte glycoprotein (MOG 35-55), it was noted that GCC2-KO showed higher clinical scores, specially during the remission phase of EAE - 21st day post-immunization ($p<0,001$). At this time point, GCN2-KO mice had higher levels of CNS inflammatory infiltration and demyelination in histological analysis. In these animals, real-time PCR (qPCR) of sorted CD3+ CD4+ infiltrated T lymphocytes showed higher levels of IFNg, T-bet, IL-17 and RORc and lower levels of Foxp3 mRNA ($p<0,0001$, comparing to WT group). Moreover, qPCR of the WT spinal cord showed higher levels of IL-10. The results obtained indicate that, in the remission phase of EAE, while WT animals show an anti-inflammatory profile, GCN2-KO mice have a prevalent Th1/Th17 response, associated with more severe symptoms. It is possible to suggest that the presence of GCN2 kinase, due to the activation of the amino-acid starvation response, contributes to the EAE remission phase by suppressing CNS inflammation and probably promoting T regulatory differentiation.

LB01-09

NITRIC OXIDE MODULATES ASCORBATE UPTAKE AND SVCT-2 EXPRESSION VIA PROTEIN KINASE G AND NUCLEAR FACTOR κB (NF-κB)

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Ascorbate is an important antioxidant which also displays important functions in neuronal tissues, including the retina. Of major importance is also nitric oxide(NO) which is considered a key messenger in the nervous system. Retinal cultures also express a high-affinity transport system for ascorbate, and the release of this molecule is delicately regulated by glutamate. Therefore, we asked whether there is any interplay between the ascorbate transport system and the NO signaling pathway in retinal cells. SNAP (100μM), a NO donor, was capable of increasing ascorbate uptake in cultured retinal cells. Given that NO can activate soluble guanylyl cyclase (sGC), with concomitant production of cGMP and PKG stimulation, we used LY83583(10μM) and ODQ(10μM), inhibitors of sGC, and found that both inhibit SNAP-induced ascorbate uptake. Moreover, KT5823 (500nM), a potent PKG inhibitor, also blocked SNAP effect. It has already been shown that NF-κB may regulate SVCT-2 expression; hence, we decided to investigate whether SNAP-induced modulation in SVCT-2 expression could be mediated by NF-κB. We observed, using cellular fractionation assays, that SNAP can induce NF-κB translocation from cytoplasm to the nucleus. Next, we evaluated the relation between NF-κB and the modulatory effect played by NO in SVCT-2 expression and ascorbate uptake. SNAP-induced increase in SVCT-2 expression is regulated by NF-κB since it was blocked by the NF-κB inhibitors PDTC (100μM) and sulfasalazine(100μM), indicating that NF-κB signaling may control the expression of SVCT-2 and, as a result, the uptake of ascorbate in retinal cells. Overall, we demonstrate here that ascorbate uptake is tightly controlled by NO and that this molecule utilizes NF-κB pathway to modulate both SVCT-2 expression and ascorbate uptake. These results demonstrate that the NO signaling system exerts a fine-tuned control of ascorbate availability to cultured retinal cells and strongly reinforces ascorbate as an important bioactive molecule in retinal tissue.

LB01-10

ABETA25-35 INDUCES IRON DEPENDENT ROS BURST IN MICROGLIA CELLS

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Increased iron and oxidative stress detected in brains of Alzheimer's patients may well participate in the pathology of the disease. It is not established by what mechanisms the iron levels are increased and it is still unclear how the redox balance gets impeded. Abeta can bind iron and other metals to the hydrophilic 1-16 domain and generate ROS. The cytotoxic effect of Abeta through ROS and metals are mainly studied in neuronal cells using the full length Abeta1-40/42 peptide. Here we study the cell response in microglia Bv2 cells to the non-metal associated part of Abeta25-35 to clarify if it also connects to iron dependent cytotoxicity and mediates iron dependent ROS production. We analyze the initial ROS production and its influence on the cell viability. Both analyses are performed in the absence or presence of iron chelators localizing to different cellular compartments, Ferrozine, being cell impermeable and Deferoxamine (DFO) and Deferiprone (DFP), both being cell permeable. In addition we test the Abeta25-35 effect on NADPH oxidase induced ROS production by pre-treating cells with Diphenyleneiodonium (DPI), an inhibitor of NADPH oxidase. Abeta25-35 induces rapid and strong iron dependent ROS production in Bv2 cells. Both extra- or intracellular iron depletion by the addition of different compartmentalized iron chelators as well as DPI can, with different efficiencies, inhibit the Abeta25-35 induced ROS burst. Abeta25-35 decreases the cell viability after 16 hours by over 50%. Despite that Ferrozine, DFO, DFP and DPI strongly reduce Abeta25-35 induced ROS they still fail to rescue the cells against Abeta25-35 toxicity as measured by MTT. We suggest that different cytotoxic mechanism and/or different cell signaling events is initiated from different domains of the Abeta peptide. The hydrophilic Abeta 1-16 might directly interact with metals catalyzing oxidative stress whereas the more hydrophobic Abeta25-35 interferes with the cell membrane components and can initiate iron dependent ROS production through e.g. NADPH oxidase as well as iron independent cellular signaling thus initiating apoptotic pathways.

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Front cover: The images show a series of neuronal development stage (from stage 1 to stage 3) and on the right are the matured neurons (DIV21).

Stage 1 to 3 neurons were observed at DIV2. All of them were double-fluorescent labeled (red for F-actin and green for drebrin). Red indicates the presence of F-actin and yellow indicates the presence of drebrin-bound type F-actin. All images were showed using the same scale. Original images, related into a study published in Mizui et al. (2011). *J. Neurochem.* **109**: 611–612.

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Future Meetings

SEPTEMBER 3–7, 2011 | PARIS, FRANCE
24th ECNP Neuropsychopharmacology Congress www.ecnp.eu

SEPTEMBER 5–6, 2011 | CAMBRIDGE, UK
Cambridge Neural Stem Cell Symposium http://bit.ly/944vcl

SEPTEMBER 5–8, 2011 | BORDEAUX, FRANCE
8th Conference of Mitochondrial Physiology and Pathology www.mitophysiology.org

SEPTEMBER 10–13, 2011 | BUDAPEST, HUNGARY
15th European Federation of Neurological Societies Congress www.efns.org

SEPTEMBER 11–14, 2011 | MELBOURNE, AUSTRALIA
World Congress on Huntington's Disease www.worldcongress-hd2011.org

SEPTEMBER 13–17, 2011 | PRAGUE, CZECH REPUBLIC
EuroGlia www.euroglia2011prague.cz

SEPTEMBER 21–24, 2011 | LODZ, POLAND
10th International Congress of the Polish Neuroscience Society www.pns2011.eu

SEPTEMBER 21–24, 2011 | TUBINGEN, GERMANY
56th Annual Meeting Germany Society for Neuropathology and Neuroanatomy www.clocate.com/Conference/56th-Annual-Conference-of-the-German-Society-for-Neuropathology-and-Neuroanatomy-2011/11659/

SEPTEMBER 22–23, 2011 | SAN FRANCISCO, USA
5th Neurodegenerative Conditions Research and Development Conference www.gtcbio.com/newsletter/NeuroI-w.htm

SEPTEMBER 22–24, 2011 | AMSTERDAM, THE NETHERLANDS
European Retina Meeting 2011 www.retina-meeting.eu

SEPTEMBER 29–OCTOBER 2, 2011 | ANTALYA, TURKEY
Novel Therapeutic Strategies and Targets for the Treatment of Stroke www.stroke-turkey2011.org

OCTOBER 20–23, 2011 | RIGA, LATVIA
7th International Congress on Vascular Dementia www2.kenes.com/Vascular2011

NOVEMBER 12–16, 2011 | WASHINGTON DC, USA
41st Annual Meeting of the Society for Neuroscience www.sfn.org

NOVEMBER 13–17, 2011 | ORLANDO, USA
AHA Annual Meeting http://scientificsessions.americanheart.org

DECEMBER 3–7, 2011 | DENVER, USA
Cell Biology www.ascb.org

DECEMBER 11–14, 2011 | SHANGHAI, CHINA
19th WFN World Congress on Parkinsonism and Related Disorders www.kenes.com/parkinson

JANUARY 31–FEBRUARY 3, 2012 | NEW ORLEANS, USA
International Stroke Conference http://my.americanheart.org/professional/Sessions/InternationalStrokeConference

MARCH 3–7, 2012 | BALTIMORE, USA
43rd Annual Meeting of the American Society for Neurochemistry www.asneurochem.org

MARCH 22–25, 2012 | ISTANBUL, TURKEY
3rd World Congress on Controversies in Ophthalmology www.comtecmed.com/cophy

APRIL 17–20, 2012 | MONTEREY, USA
10th International Conference on Brain Energy Metabolism www.ncire.org/ICBEM2012

APRIL 21–25, 2012 | SAN DIEGO, USA
FASEB Meeting www.faseb.org

MAY 2–5, 2012 | POTSDAM, GERMANY
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JULY 14–18, 2012 | BARCELONA, SPAIN
8th FENS meeting http://fens2012.neurosciences.asso.fr

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OCTOBER 10–13, 2012 | BRASILIA, BRAZIL
8th World Stroke Congress www.world-stroke.org

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