25th Hellenic Society for Neuroscience Meeting

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Organized by the Hellenic Society for Neuroscience and the University of Patras Neuroscience Network

November 25 - 26, 2011
Conference and Cultural Centre of the University of Patras

Friday, November 25th, 2011 - pm

14:30 – 16:00 **Registration**
University of Patras Conference and Cultural Center

16:00 – 16:15 **Opening remarks**

16:15 – 18:00 *Chair: A. Dermon, G. Paxinos*

Michael G. Stewart Dept of Life Sciences, The Open University, UK
Quantitative electron microscopy (EM) in studies of an animal model of Down's syndrome (the Tc1 mouse): hippocampal investigations
Elias D. Kouvelas Dept. of Physiology, Medical School, University of Patras
Unconscious and plasticity: bridges between neuroscience and psychoanalysis

18:00 - 20:00 **HSN General Assembly**

20:00 - 21:00 G. Kostopoulos, E. D. Kouvelas, G. Papadopoulos
Book presentation: «Το σύμπαν των εγκεφάλων» συγγραφείς: Γιώργος Παπαδόπουλος, Ηλίας Κούβελας

Saturday, November 26th, 2011 - am

9:00 –10:30 *Chair: G. Papadopoulos, G. Panagis*

Konstantinos Moutoussis
Dept. of Philosophy and History of Science, University of Athens
Cognitive influences on visual percepion
Christina Dalla Dept. of Pharmacology, Medical School, University of Athens
Sex differences in models of depression
Georgia Gregoriou Dept. of Physiology, Medical School, University of Crete
Neural mechanisms of visual attention: Interactions between distant brain areas

10:30 - 12:00 **Coffee Break / Posters**

Saturday, November 26th, 2011 - pm

12:00 - 14:00 *Chair: S. Taraviras, F. Stylianopoulou*

Francois Tronche Laboratoire de Physiopathologie des Maladies du Système Nerveux Central, Université Pierre et Marie Curie, France
Stress, sex and transcription: Genetic dissection of steroid receptor gene function in behaviour
Theofilos Mantamadiotis Dept. of Physiology, Medical School, Univ. of Patras
New insights into the CREB signalling pathway in glioblastoma biology: lessons from zebrafish, mouse and human studies
Myrto Denaxa MRC National Institute for Medical Research, London, UK
An Lhx6-controlled gene cascade in cortical interneuron development

14:00 – 16:00 Lunch Break/ Posters

16:00 - 17:30 *Chair: F. Angelatou, K. Psaropoulou*

Antigoni Ekonomou King's College, London, UK
Pharmacological enhancement of endogenous neurogenesis in an animal model of amyloidosis - Novel therapy for neurodegenerative diseases?
Panagiotis Politis Biomedical Research Foundation, Athens
Prox1 regulates binary fate decisions in spinal cord neurons
Spyros Georgopoulos Biomedical Research Foundation, Athens
Cholesterol receptors modify amyloid deposition by regulating the inflammatory response

17:30 - 18:30 Concluding remarks - Poster Awards

Organizing committee: A. Mitsacou (president of the organizing committee), F. Angelatou, G. Voukelatou, P. Giompres, A. Dermon, S. Efthimiopoulos, D. Karagogeos (president of HSN), G. Kostopoulos (co-ordinator of NeuroNet), T. Mandamadiotis, N. Matsokis, M. Margariti, N. Panagopoulos, C. Papatheodoropoulos, S. Taraviras
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ABSTRACTS

ABSTRACTS

OF INVITED SPEAKERS

“Sex differences in animal models of depression”

Christina Dalla

Dep. of Pharmacology, Medical School, University of Athens, Greece

Many stress-related mental disorders, including depression occur more often in women than in men. Also, sex differences in emotional and cognitive processes are apparent in all rodent models of stress and depression, but this depends on the parameters that we study and on basal differences between males and females. During the last decade, we have studied sex differences in behavior, as well as in neurochemical, neurobiological and neuroplasticity indices in different models of depression. These include the forced swim test (FST), the chronic mild stress (CMS), and the learned helplessness model.

Following CMS, we have found decreased serotonergic activity in the hippocampus of female rats, as well as enhanced 5-HT_{1A} mRNA in the hippocampus of males only. Also, CMS decreased 5-HT_{1A} mRNA levels in the prefrontal cortex of males and this was reversed by clomipramine antidepressant treatment. Finally, CMS decreased 5-HT_{2A} mRNA levels in the prefrontal cortex of males, while increased it in female rats. As in CMS, in response to FST, serotonergic turnover ratio was also decreased in females and the levels of the 5-HT_{1A} mRNA were enhanced only in males. Interestingly, depression-like symptomatology in the FST was more evident in females, while this was not dependent on sex differences in HPA axis activity. Also, the effectiveness of the selective-serotonin-reuptake-inhibitor sertraline in the FST was influenced by the sex of the animal and the phase of the estrous-cycle. Finally, in the learned helplessness model females did not express learned helplessness behaviour in response to uncontrollable stress, as males do. Consequently, adult hippocampal neurogenesis was not decreased in response to stress in female rats.

Our data point to sex-differentiated neurobiological and behavioural alterations, which support a major role of serotonin in the mediation of the stress response. We conclude that each animal model of depression has limitations that need to be recognized, in order to use them effectively in the investigation of psychiatric disorders in men and women.

An Lhx6-controlled gene cascade in cortical interneuron development

Myrto Denaxa and Vassilis Pachnis

Division of Molecular Neurobiology, MRC, National Institute for Medical Research, The Ridgeway, NW7 1AA, London, UK

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 specification into defined neurochemical and electrophysiological subgroups. Nevertheless, despite considerable progress over the last several years, the transcriptional network that controls different aspects of cortical interneuron development is poorly understood.

Previous work from our lab has shown that the LIM-homeodomain factor Lhx6 is a key regulator of the migration and differentiation of PVA and SST expressing interneurons (Liodis et al., 2007). To identify molecular pathways which regulate interneuron differentiation and migration we have undertaken a genome-wide microarray approach, comparing gene expression profiles of brains derived from wild-type or Lhx6-deficient embryos. Our expression analysis identified a large number of 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 the AT-rich DNA binding protein Satb1, which have not been implicated previously in cortical interneuron development. Using in situ hybridization, immunohistochemistry and quantitative PCR, we initially verified that its expression is altered in Lhx6-deficient mice. Subsequently, we have carried out a series of gain- and loss-of-function experiments to address the role of Satb1 on interneuron development. Our studies provide insight into the molecular cascades, which are controlled by Lhx6 and regulate the migration, differentiation and maturation of specific subsets of cortical interneurons.

Pharmacological enhancement of endogenous neurogenesis in an animal model of amyloidosis - Novel therapy for neurodegenerative diseases?

Antigoni Ekonomou[1], Alyma Somani[1], Ivan Rattray[2], Po-Wah So[2], Mike Modo[2], Clive Ballard[1]

[1] Wolfson Centre for Age-Related Diseases and [2] Neuroscience Departments, King's College London, UK;

Alzheimer's disease (AD), the most common form of dementia, was first described at the beginning of last century. In 2006, there were 26.6 million sufferers worldwide, and it is estimated that this number will reach more than 106 million by 2050. AD is a neurodegenerative disease that results in loss of neurons and brain atrophy which are manifested by disturbances in memory, attention and orientation, changes in personality and impairments in gait and movement. As the population ages, AD is one of the most costly diseases to society with severe personal, social, psychological and physical consequences.

The currently used treatments for AD offer a small symptomatic benefit; a variety of clinical trials along with recommendations for a change in the lifestyle are also provided and recommended. As there is no cure or treatments available to delay or halt AD,http://en.wikipedia.org/wiki/Alzheimer's_disease - cite_note-pmid3776457-8#cite_note-pmid3776457-8 the development of new drugs/therapies/treatments which can be more beneficial than the existing ones are of paramount importance. A very promising one can be the manipulation of endogenous neural stem cells in the adult brain as a cell replacement strategy for patients with AD and other neurodegenerative diseases.

Adult neural stem cells (ANSCs) have been identified in many organisms, including humans, mainly in two brain areas: the subventricular zone (SVZ, also referred to as subependymal zone, SEZ) of the lateral ventricles and the subgranular layer (SGL) of the dentate gyrus. In the healthy brain, the progeny of the ANSCs from the SVZ differentiate into interneurons at the olfactory bulb, whereas new granule cells in the hippocampal dentate gyrus originate from the SGL. This endogenous neuron replenishing mechanism declines with age and may be one of the contributing factors to loss of plasticity and regenerative capacity of the ageing human brain. Interestingly, adult neurogenesis can be manipulated by different factors, some of them being already prescribed drugs, such as antidepressants and hormones.

In the present study, the effect of pharmacological manipulation of endogenous neurogenesis is studied in a transgenic animal model of amyloidosis, the TASTPM mouse. TASTPM animals carry the human APP Swedish and the PS1 M146V mutations identified in many familial cases of AD and start exhibiting amyloid deposits as early as two months of age and cognitive impairments at 6 months of age. Animals are administered the drugs either alone or in combination daily for two weeks starting at 4 months of age and are behaviourally assessed prior to and 6 weeks after the drug administration. After the end of the *in vivo* experiments, animals are sacrificed and one brain hemisphere is processed for immunohistochemistry and the other half is processed for protein analysis. The levels of endogenous neurogenesis during the drug administration and at autopsy are determined and are correlated to the cognitive performance, synaptic plasticity and the amyloidosis levels for each treatment group/individual animal in order to identify the most beneficial drug(s) treatment. Future proteomic analysis will also establish the micro-environmental and cellular changes attributing any beneficial role to the drugs used, leading to the possible establishment of new drug targets or improvement of the existing ones.

Cholesterol receptors modify amyloid deposition by regulating the inflammatory response.

Spiros Georgopoulos

Alzheimer's disease (AD), the major cause of dementia, is a progressive neurodegenerative disease that impairs basic cognitive functions, primarily memory. Cholesterol has been implicated in AD pathogenesis and statins, medicines that regulate cholesterol homeostasis have been shown to have a beneficial effect in AD. Cholesterol receptors regulate cholesterol homeostasis. SB-BI has been described as the HDL-cholesterol receptor and LDLR as the LDL-cholesterol receptor. Depletion of SR-BI or LDLR results in elevated cholesterol levels and atherosclerosis in transgenic mice.

Our hypothesis is that cholesterol-related genes as SR-BI and LDLR are involved in AD pathogenesis. We used a genetic approach by deleting SR-BI and LDLR endogenous genes in AD transgenic mice to evaluate the effect in the amyloid related phenotype.

We examined the role of SR-BI in the development of the amyloid related phenotype and CAA in a huAPP (Swe Ind) transgenic mouse. We showed that SR-BI regulates perivascular macrophages in the mouse brain. Reduction or deletion of SR-BI in heterozygous and homozygous mice caused a significant increase in perivascular macrophages. HuAPP/SR-BI heterozygous knock–out mice ($SR\text{-}BI^{+/-}$) developed a significant increase in amyloid plaque formation and vascular amyloid deposition in the brain and exacerbated learning and memory deficits compared to their J20 littermates. Our findings suggest that inactivation of a single SR-B1 allele is sufficient to impair perivascular macrophages response to Aβ and to enhance fibrillar amyloid deposition and CAA.

To investigate the role of LDLR in the development of the amyloid related phenotype we used an APP/PS1 transgenic mouse (5XFAD) that develops an AD-like pathology with amyloid plaques, astrocytosis and microgliosis. We found that 4 months old 5XFAD transgenic mice on the LDLR deficient background (LDLR-/-) have increased amyloid plaque deposition. This increase is associated with a significant decrease in astrocytosis and microgliosis in the 5XFAD/LDLR-/- mice. To further elucidate the role of LDLR in relation with ApoE we have generated 5XFAD transgenic mice on the ApoE deficient (ApoE-/-) or the ApoE/LDLR double deficient background (ApoE-/-/LDLR -/-). We have found that ApoE deletion in the 4 months old 5XFAD/ApoE-/- mice decreases amyloid plaque formation as expected, but has no effect on astrocytosis or microgliosis. By comparison 5XFAD/ApoE-/-LDLR -/- double deficient mice of the same age have increased amyloid deposition with decreased astrocytosis and microgliosis.

Our analysis shows that SR-BI and LDLR regulate the immune response in the mouse AD brain in AD transgenic mice and modify the amyloid related phenotype. We have shown that both SR-BI and LDLR are involved in AD pathogenesis in transgenic mice. These are important finding as they establish the importance of genes involved in cholesterol homeostasis, as SR-BI and LDLR in AD.

Neural mechanisms of visual attention: Interactions between distant brain areas

Georgia G Gregoriou

Lecturer in Physiology, Faculty of Medicine, University of Crete and

Collaborative Researcher, Institute of Applied and Computational Mathematics, Foundation for Research and Technology, Hellas

Abstract

Visual attention facilitates the selection and processing of stimuli that are relevant to behavior. When attention is guided by behavioral goals, rather than being captured automatically by salient stimuli, it is considered to be guided by "top-down" signals. It has been suggested that areas in the prefrontal (PFC) and posterior parietal (PPC) cortices provide top-down inputs to the visual cortex causing a selective enhancement of the representation of the behaviorally relevant stimulus.

To directly test the hypothesis that PFC is a source of top-down signals to extrastriate cortex we conducted extracellular recordings simultaneously in the frontal eye fields (FEF), an area within PFC, and visual area V4. FEF neurons showed earlier firing rate changes due to attention than neurons in area V4 in agreement with the proposal that FEF provides signals related to the allocation of attention to area V4. Moreover, attention increased neuronal synchronization between the two areas in the gamma frequency range (30-60Hz) with phase relationships that can facilitate neuronal communication and increase the impact of the top-down inputs to the visual cortex. Most importantly, we found that only visual FEF neurons, but not movement or visuomovement cells, showed enhanced gamma frequency synchronization with neural activity in V4 during attention, suggesting that only visual FEF cells are a source of top-down attentional feedback. This finding challenges motor theories of attention which suggest that attention reflects the preparation for an eye movement and that attentional processes are mediated by oculomotor signals. Finally, our results indicated that inputs from FEF to V4 were dominant at the onset of attention to a location possibly mediating attentional selection, whereas inputs from V4 to FEF predominated during sustained attention. These data provide direct evidence on the role of FEF in attentional selection and begin to reveal how the dynamics of neuronal interactions between distant brain areas can allow the effective communication of distinct neuronal populations.

UNCONSCIOUS AND PLASTICITY: BRIDGES BETWEEN NEUROSCIENCE AND PSYCHOANALYSIS

Elias D. Kouvelas

Department of Physiology, Medical School, University of Patras

When Sigmund Freud first explored the implications of unconscious neural processes to behaviour, he adopted a neural model of behaviour in an attempt to develop a scientific psychology. I am talking about the "Project for a scientific psychology". This book was written in 1895, few years after his magnificent publication on the structure of the neuronal cells. Freud himself, because of the immaturity of the brain science at the time, he abandoned this model for a mentalistic one, the psychoanalysis.

However, many years have passed and neuroscience today is in the cusp of a revolution, similar to the unraveling of human genome in 1990s. Therefore, terms like consciousness and unconscious can be discussed not only on a psychological or psychoanalytic basis but also on a neurobiological one.

Thus, about hundred years later, at the end of the 20th century, Eric Kandel suggested that part of our unconscious ego, what he calls procedural unconscious, has not been repressed and is concerned with unconscious habits, and perceptual and motor skills that are mapped into procedural (implicit) memory. These ideas are in agreement with the psychoanalytic work of the group of Louis Sanders and Daniel Stern in Boston, as well with the ideas expressed by psychoanalysts like Otto Kernberg and the late Mauro Mancia.

To my opinion, mirror neurons play also a key role in the establishment of inter-subjectivity between the mother and the infant during the pre-linguistic stages of life.
Francois Ansermet and Pierre Magistretti suggest that through the mechanisms of plasticity, through synaptic re-arrangements and re-associations with new traces that have been inscribed, an unconscious internal reality can be formed, which plays a key-role in the determination of the subject.

Furthermore, perception does not only originate from the external world through exteroceptive pathways but also through interoceptive pathways which inform the brain about the state of our body which is essential for our feeling of pleasure and displeasure.
The function of amygdala can be a good example in order to visualize some of the mechanisms of the unconscious association of an external stimulus with a somatic state.

New insights into the CREB signalling pathway in glioblastoma biology: lessons from zebrafish, mouse and human studies

Theo Mantamadiotis, Nikos Papalexis
Dept. of Physiology, Medical School, University of Patras

Gliomas are the most common malignant cancers of the nervous system. Unfortunately, they are also amongst the most difficult cancers to treat. The discovery that many primary tumours, including gliomas, develop from ‘cancer stem cells’ has advanced our understanding of tumour biology. Discovering the genes and pathways that regulate cancer stem cell specific survival and growth will aid in the development of novel and more effective treatments. The Cyclic-AMP Response Element Binding protein (CREB) is a serine/threonine kinase-regulated nuclear factor modulating the transcription of numerous genes in nerve cells and has various roles in neuronal function, ranging from survival to more complex brain functions. Our recent work has shown that CREB is required for the maintenance of normal brain development and neuronal expansion in both zebrafish and mouse. We further found that CREB regulates genes important for neural progenitor cell survival but it also likely regulates paracrine growth/survival factor expression in the neural stem cell niche. Given recent findings that aberrant CREB expression can impart oncogenic properties on various cell types, we explored the role of CREB in brain cancer biology by examining its expression in a panel of human patient brain tumour specimens. We show that both the level of expression and the number of cells expressing activated/phosphorylated CREB is markedly elevated in tumours compared with adjacent non-tumour control brain tissue. These observations are the first to highlight a link between CREB and brain tumours. Our hypothesis is that CREB has a role in brain tumour development/growth and that at least some of CREB’s neuro-oncogenic properties are due to its role in promoting brain tumour stem cell survival and growth. Here we present preliminary data and outline our ongoing investigations toward the dissecting the role of the CREB signalling pathway in glioma biology.

Cognitive influences on visual percepion

Kostas Moutoussis

Dept. of Philosophy and History of Science; University of Athens

Visual perception can be influenced by top-down control, coming from higher cognitive brain functions. In the present talk I will present data from two types of experiments, supporting this idea. In the first case, the effects of higher-order adaptation to the initial dominant percept in binocular rivalry were studied. We found that adaptation to faces or houses was able to bias perceptual selection towards the categorical direction of the adaptation stimulus. In the second case, we studied the effects of language on visual sensitivity. It was found that prior presentation of sentences, implying a particular orientation in space, was able to improved sensitivity in a contrast-detection task, in an orientation-specific way. Results from both types of experiments support the idea that, for a given visual system, the outcome of visual perception does not only depend on the properties of the visual stimulus but also on influences from higher brain functions.

Prox1 regulates binary fate decisions in spinal cord neurons

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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+ progenitors in the ventral spinal cord that defines the pMN domain. However, the upstream molecular mechanisms that control this neuronal specification are not well understood. Advances in our understanding of the gene regulatory networks controlling the acquisition of neuronal subtype identity will provide insights and practical information for applications of regenerative medicine**.** To this end, we have previously shown that Prox1, a transcription repressor and downstream target of proneural genes, regulates differentiation of neural stem/progenitor cells (NSCs) via direct suppression of *Notch1* gene expression. Active Notch1 signalling 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 NSCs destined to generate interneurons, and only transiently expressed into pMN domain during early stages of MN specification. Gain-and-loss of function studies in the chick neural tube and mouse NSCs show that Prox1 is sufficient and necessary for the suppression of MN identity in the spinal cord. Mechanistically, activated Notch1 signaling cannot rescue the negative effect of Prox1 on MN generation, suggesting an alternative mode of action independent of its role in *Notch1* gene regulation. In agreement, ectopic expression of Prox1 in the pMN domain is sufficient to suppress *Olig2*, which is a master regulator for the initial specification of pMN domain and MN identity. Conversely, shRNA-mediated knockdown of Prox1 in the chick neural tube indicates that Prox1 is required for the suppression of *Olig2* outside the pMN domain. Most important, chromatin IP analysis in the mouse embryonic spinal cord showed that endogenous Prox1 directly binds to the proximal promoter of the *Olig2* gene locus, as well as to the K23 enhancer, which specifically drives *Olig2* expression into the pMN domain. In accordance, by deleting DNA binding domain, Prox1 abolishes in vivo the ability to block MN fate acquisition and *Olig2* gene expression, further suggesting a direct action at the transcriptional level. Moreover, plasmid-based transcriptional assays in primary NSCs from mouse embryonic spinal cord suggest that Prox1 suppresses the activity of *Olig2* gene promoter and K23 enhancer. Collectively, these observations indicate that Prox1 is essential for the suppression of MN fate in NSCs via direct transcriptional repression of *Olig2*.

QUANTITATIVE ELECTRON MICROSCOPY (EM) IN STUDIES OF AN ANIMAL MODEL OF DOWN SYNDROME (THE Tc1 MOUSE): HIPPOCAMPAL INVESTIGATIONS

Prof. Mike Stewart
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Trisomy of human chromosome 21 (Hsa21) occurs in ~1 in 750 live births, and the resulting gene dosage imbalance gives rise to Down syndrome (DS), the most common known genetic form of mental retardation. Hippocampal pathology is likely to contribute to cognitive disability in Down syndrome (DS). However, the neural network basis of this pathology and its contributions to cognitive impairment are unclear. In this lecture I will describe results showing dysfunctional connectivity between dentate gyrus (DG) and CA3 networks in the transchromosomic **Tc1 mouse** model of DS, which carries > 75% of the ~250 known (Human) Hsa21 genes.

Studies have been carried out primarily using quantitative 3-dimensional morphometric methods at transmission electron microscope (TEM) level, and these have enabled us to describe the nature of changes in circuitry of the hippocampus of the Tc1 mouse, and specifically in DG and CA3. These investigations have informed further research by our collaborators using confocal microscopy and electrophysiology. Our data demonstrate that ultrastructural synaptic abnormalities culminate in impaired interactions between these two hippocampal subregions *in vivo*. These results parallel our research on the **Ts65dn mouse**, which is segmentally trisomic for a region of mouse chromosome 16 (*Popov et al., 2011*).

The data from both studies highlight the vulnerability of DG-CA3 networks to aberrant gene expression, and implicate hippocampal circuit abnormalities that may contribute to distinct cognitive phenotypes in DS.

Popov, VI, et al. (2011) Three-Dimensional Synaptic Ultrastructure in the Dentate Gyrus and Hippocampal Area CA3 in the Ts65Dn Mouse Model of Down Syndrome *Journal of Comparative Neurology 519(7):1338-1354.*

Stress, sex and transcription; Genetic dissection of steroid receptor genes function in behavior

Francois Tronche

Laboratoire de Physiopathologie des Maladies du Système Nerveux Central, Université Pierre et Marie Curie, France

The glucocorticoid receptor (GR) is a transcription factor mediating adaptation to environmental challenges and stress. Stress-induced glucocorticoid secretion by the adrenal gland activates GRs which in turn trigger changes in gene transcription that initially facilitate adaptation but that in chronic stress conditions lead to behavioral pathologies, such as addiction, anxiety and depression. Since GRs are expressed in most structures and cell types of the brain it remains currently unknown whether GRs influence different pathologies by acting on specific cellular targets or whether they non-specifically modify the reactivity of the entire brain by acting concomitantly on several brain structures. In this context, we investigated the potential cellular target of the influence of GR on vulnerability to drugs of abuse, anxiety and despair. We generated animals in which the GR, or associated proteins, were specifically absent in different component of the dopaminergic or the serotoninergic pathways. We found that elimination of GR proteins in the postsynaptic site of the dopaminergic transmission, but not in the pre-synaptic one, largely reduced the impulse activity of mesencephalic dopamine cells and the reinforcing effects of cocaine, but not morphine. In contrast other stress-related phenotypes normally influenced by GR activity, such as anxiety, were not modified in dopamine-selective GR gene mutants, whereas they are when GR gene is inactivated in 5-HT1A expressing neurones.

ABSTRACTS

FOR POSTERS

ARIEL, A LONG NON CODING RNA, WITH A POSSIBLE REGULATORY ROLE IN NEUROGENESIS

Antoniou D, Stergiopoulos A. and Politis P.K.

Center for Basic Research, Biomedical Research Foundation of the Academy of Athens, Greece

The 98% of human genome does not encode for proteins. It has now become evident that a significant percentage of these sequences code for numerous long-non-coding RNAs (lncRNAs). These RNAs are evolutionarily conserved in mammalian genomes and function in diverse biological processes, including stem cell pluripotency, cell-cycle regulation, organogenesis and cell-type specific differentiation. It is now known that at least some of them can positively or negatively affect the expression of nearby genes. Accordingly, we have identified by bioinformatic analysis a novel lncRNA, named Ariel (previously known as AK142161) which is transcribed in an anti-parallel manner to Prox1. Here, we have experimentally validated that Ariel is an antisense transcript to Prox1, consisted of two exons of 781 bp total length, with an overlapping sequence of 134bp at the 5'-UTR region of Prox1 mRNA. Prox1, the mammalian homologue of Prospero in Drosophila, is a homeobox transcription repressor with a key role in regulating neuronal differentiation of neural stem cells (NSCs). Here, we show that Ariel is broadly expressed during mouse central nervous system development. In particular, Ariel's expression is correlated with induction of neurogenesis and exhibits a pattern similar to that of Prox1. These observations suggest a positive role in post-transcriptional regulation of Prox1 gene. Consistently, plasmid-based luciferase assays indicate that Ariel may stabilize Prox1 mRNA through an interaction with the complementary and overlapping sequence at the 5'-UTR of Prox1 mRNA. We are currently investigating whether Ariel affects neuronal differentiation of NSCs via its ability to stabilize Prox1 mRNA expression.

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COMBINED ACTION OF THE NEUROGENIC MOLECULES CEND1 AND NGN2 IN DIRECTING CULTURED ASTROCYTES TOWARDS NEURONAL REPROGRAMMING

K. Aravantinou-Fatorou[1], R. Matsas[1], B. Berninger[2,3] and D. Thomaidou[1]
1 Hellenic Pasteur Institute, Athens, Greece
2 Ludwig-Maximillians University Munich
3 Institute of Stem Cell Research, Neuherberg, Gemany

Recent studies demonstrate that besides the well-documented neural stem properties of astrocytic populations of the two neurogenic regions of the adult brain, astroglia isolated from non-neurogenic adult brain regions has the potential to be reprogrammed into synapse-forming neurons upon forced expression of transcription factors known to instruct neurogenesis during embryonic development. Based on 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 bHLH proneural genes, we aimed to explore Cend1 and Ngn2 combined action in postnatal cortical astrocytes proliferation and differentiation properties. To this end, forced expression of Cend1, Ngn-2 or both, results to the significant increase of a subpopulation of elongated GFAP (-) cells, that strongly express the radial glial marker Glast. Additionally, a subpopulation of either Cend1- or Ngn2-overexpressing cells starts expressing markers of the neuronal lineage, such as Tuj1, GABA and TH, whereas the extent of morphological neuronal differentiation and the percentages of subtype-specific neuronal markers rise significantly upon over-expression of both neurogenic factors. Interestingly, in double-transduced cultures, colonies of small round Cend1(+)/Ngn2(+)/Glast(+)/Nestin(+) cells forming three-dimensional spheres of high proliferative potential are detected. Studies using live-cell imaging for longer time-periods and NSC 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.

Supported by FP7 REGPOT Project 264083 Neurosign

CB1R signalling and Cytoskeleton-dependence in neural cells

Olga Asimaki, Nikos Sakellaridis and Dimitra Mangoura

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Most G-protein coupled receptors mediate their effects on neuronal growth and differentiation through activation of ERK1/2. In analyzing the proximal signaling of cannabinoid receptors type 1 (CB1Rs) in primary cortical neurons, we have shown that Methanandamide (R(+)-MA) induced a biphasic ERK1/2 activation at 5 and 15 min, mediated by sequential activation of $G_{q/11}$/PLC/PKCε and transient complex formation of activated PKCε with Src and Fyn, and subsequent $G_{i/o}$/Src/Fyn/FGFR recruitment, respectively. Recruitment of molecules increased with time of exposure to R(+)-MA, suggesting that it also served trafficking of receptors, while these CB1R proximal signaling events were found to be organized in lipid rafts. Concurrently to these intermolecular signaling interactions, cytoskeleton associated proteins MARCKS and p120catenin were drastically modified by phosphorylation of PKCε and Src, respectively. We therefore investigated the role of cytoskeletal microfilaments and microtubules in the CB1R-dependent signaling of primary neurons using specific chemical disruptors (cytochalasin D and nocodazole, respectively). Our results showed that the presence of each cytoskeletal disruptor inhibited the second activation of ERK by CB1R at 15 min, but not the first, possibly indicating that cytoskeleton integrity is a prerequisite for CB1R recycling in membrane lipid rafts. These receptor-proximal signaling events correlated well with induction of neuritic outgrowth in the long term. Specifically, this induction reached significant levels by 48 h, when the average length of the major neuritic process in R(+)-MA-treated neurons was increased by 37.5% over the vehicle-treated. The significance of actin cytoskeleton as an integrator of CB1R signaling was further confirmed by studies with neuroglioma cell lines where R(+)-MA induced phenotypic differentiation of cells, which was effectively prevented by cytochalasin D. Taken together these results present evidence that the plasma membrane and the underlying cortical cytoskeleton play a central role in the regulation of CB1R signaling pathway, and may provide a molecular basis for the known regulation of neuronal function with chronic, in utero exposure to cannabinoids (support: PENED 03EΔ778/GGET/EU).

EFFECT OF LEAD ON ANXIETY AND ACETYLCHOLINESTERASE ACTIVITY OF SPECIFIC BRAIN REGIONS IN ADULT MICE

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It has been proposed that cholinergic neurotransmitter system may be involved in the mechanisms of lead (Pb) neurotoxicity mainly during development [1] and that Pb seems to be implicated in the aetiology of psychological pathologies [2]. The aim of the present study was to examine the effects of lead (500 ppm $Pb(CH_3CO_2)_2$/day, in drinking water, for 4 weeks) on **i)** anxiety and **ii)** the activity of the two acetylcholinesterase (AChE) isoforms [salt soluble (SS)- and detergent soluble (DS)-isoforms] of specific brain regions (cortex, cerebellum, hippocampus, striatum, midbrain), in adult male mice. Anxiety was assessed by measuring a) the percentage of time spent in the open arms of the elevated plus maze apparatus and b) the thigmotaxis time (time remaining close to vertical surfaces) spent in an open-field. The activity of both AChE isoforms was evaluated by Ellman's colorimetric method. Our results showed that lead-treated mice displayed a decrease (40.9 %) in the time spent in the open arms and an increase (13.45 %) in thigmotaxis time compared to their respective controls, suggesting thus, an anxiogenic-like behavior. Concerning the activity of AChE, lead caused significant inhibition in the enzyme's activity (in a range of 6.35-24.62 % in both isoforms) in all brain regions tested. Conclusively, our data indicate that lead exerts neurotoxic effects in adult mice, through behavioural and cholinergic disturbances.

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TRANSIENT AXONAL GLYCOPROTEIN 1 IS INDISPENSABLE FOR THE PROPER FUNCTION OF THE OLFACTORY SYSTEM IN RODENTS

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TAG-1(Cntn2), a neuronal recognition molecule of the immunoglobulin superfamily, plays important roles in neurite outgrowth, fasciculation, neuronal migration and axon guidance. In the olfactory bulb, mitral (MCs) and tufted cells comprise the main projection neurons that express TAG-1 during development. These cells project their axons to the olfactory cortex through the lateral olfactory tract (LOT). TAG-1 expression is first detected at E13.5 in newly born MCs, while it is absent from the mature olfactory bulb. The exact role of TAG-1 in the olfactory system has not yet been investigated.

Previous analysis of mice deficient for TAG-1 (Tag-1$^{-/-}$), revealed cognitive and motor deficits, including learning and memory defects as well as motor coordination and balance abnormalities. In our present study we further analyzed the behavioral properties of Tag-1$^{-/-}$ mice related with olfaction and social behavior. We demonstrate that homozygous mutants display severe alterations in several behavioral assays concerning olfactory memory, odor discrimination and social odor recognition. In order to investigate the molecular defects that could account for the previously mentioned behavioral phenotype, we performed a detailed immunohistochemical analysis of olfactory bulb development in the mutant mice. We observed significantly decreased numbers of projecting neurons in the olfactory bulb of adult and newborn Tag-1$^{-/-}$ mice compared to control animals. Subsequent experiments showed that the decrease in mitral and tufted cell numbers cannot be attributed to a proliferation defect, since the number of postmitotic cells that give rise to the projecting neurons, is unaltered in homozygous mutants. Analysis is in progress for the evaluation of migratory deficits or increased cell death as a result of the absence of TAG-1 protein in the olfactory bulb.

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NEUROGENESIS ALONG THE SEPTO-TEMPORAL AXIS OF THE ADULT RAT HIPPOCAMPUS

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The study of adult neurogenesis has been extremely popular among neuroscientists, mainly due to its implications in pathogenesis and therapeutics of severe brain pathological conditions, such as schizophrenia, temporal lobe epilepsy and Alzheimer' s disease. Today, it is widely accepted that there are two neurogenic regions in the adult mammalian brain: the subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus (DG) of the hippocampal formation.

The hippocampal formation of the mammalian brain exhibits structural, biochemical, molecular, functional and electrophysiological differentiation along its septo - temporal axis. The aim of this study was to examine any possible differentiation in the number and distribution of Brdu labeled newborn cells in the infrapyramidal and suprapyramidal blade of septal (dorsal/SH) and temporal (ventral/TH) part of the hippocampus in the young adult rat, over a time period of one month after their genesis.

A total of eighteen 3-month old male Wistar rats, weighing between 320 and 365 gr, were used in this study. In order to label adult born granule cells, all rats were given two i.p. injections of 5- Bromo-2′-deoxyuridine (Brdu; 20mg/ml solution in saline at a dose of 100mg/kg/injection), 12hours apart. At specific time points after the first Brdu injection (2 days, 5 days, 1 week, 2 weeks, 3 weeks and 4 weeks) animals were perfused transcardially with 0,9% saline followed by a 4% paraformaldehyde solution. Brains were then dissected out, processed routinely and embedded in paraffin. Ten μm thick coronal sections, along the entire septo - temporal axis of the hippocampus were collected. One in ten sections, 100μm apart from each other, from the SH and TH (-2,56 to -4,16mm, and -4,80 to -6,04mm, respectively, relative to bregma according to the atlas of Paxinos and Watson) were stained immunohistochemically against the antigen of Brdu.

Statistical analyses were performed using SPSS 19.0 software. Comparisons within groups were made using the Independent Samples T – Test**,** and One Way Anova (Bonferroni's test for post hoc analyses), with significance set at $P<0.05$, was applied for comparisons between groups.

Our data indicate that there are striking differences in the number of Brdu positive cells in the subgranular and granular cell layer of the DG, between SH and TH, at all studied time points. The population of Brdu positive newborn cells peaks at five days after Brdu administration (SH: 1450.00 ± 99.57 cells; TH: 663.33 ± 280.26 cells), decreases significantly seven days after Brdu administration (SH: 1178.3333 ± 121.75 cells; TH: 500.00 ± 181.44 cells) and shows a gradually reduction until one month after Brdu administration (SH: 678.33 ± 133.1 cells; TH: 330.00 ± 65.98 cells). No statistical significant differences in the number of Brdu positive cells of the DG between right and left hemisphere were found. At all time points studied, and both at SH and TH, a slightly larger portion of newborn granule cells was found in the suprapyramidal than in the infrapyramidal blade of the DG.

The above findings indicate that the well known structural and functional septo-temporal differences of the adult hippocampus are amplified by an ongoing uneven dentate granular cells genesis.

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THE ROLE OF THE RTK ALK AND ITS LIGAND JEB IN LONG TERM MEMORY

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Anaplastic Lymphoma Kinase (Alk) is a Receptor Tyrosine Kinase (RTK) activated in several human cancers, but with largely unknown physiological functions. The Alk gene is widely expressed in the adult central nervous system of Drosophila melanogaster and especially in mushroom bodies, neuronal structures implicated in olfactory learning and memory. It has already been demonstrated that the Drosophila ortholog dAlk is involved in body size determination and associative learning. Specifically, reduced neuronal dAlk activity increases body size and enhances associative learning. We examine the role of dAlk expression in the mushroom bodies on Long Term Memory, whereas the ablation of dAlk outside the mushroom bodies does not affect LTM. In addition, we are investigating the role of Jelly Belly (Jeb), a secreted protein which is the activating ligand of dAlk. Immunochemistry on sections of Drosophila adult brains have shown increased concentration of dAlk in mushroom bodies dendrites (calyces) and the co-localization of dAlk and its ligand Jeb at the calycal synapses. However, there is no evidence that Jeb is expressed inside the mushroom bodies. Consequently, we hypothesized that Jeb is delivered to the mushroom bodies calyces by another subset of neurons. Specific data will be provided to prove our hypothesis. Our results suggest a general role of dAlk as a negative regulator of neuronal functions.

AMYLOID PRECURSOR PROTEIN REGULATES CALCIUM HOMEOSTASIS

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The amyloid precursor protein (APP) is a type-1 transmembrane protein involved in Alzheimer's disease. In the last decade it has become increasingly evident that APP, its protein-protein interactions and its proteolytical fragments may affect the calcium homeostasis and vice versa. Previous studies in our laboratory have shown that APP interacts with the Homer 2 and Homer 3 proteins, and that this interaction could be inhibited by the release of calcium from intracellular stores. In the present study we are trying to investigate the impact of the presence/absence of APP on calcium homeostasis, and especially on the efflux of calcium from ER stores. For this cause we used 3 groups of SH-SY5Y cells: 1) SH-SY5Y cells in which the expression of APP is inhibited by shRNAs, (SY APP-), 2) naïve SH-SY5Y cells, (SY naïve) and 3) SH-SY5Y cells that overexpress the APP protein, (SY APP+). The levels of cytosolic Ca^{+2} after treatment with thapsigargin, activation of Capacitative Calcium Entry and treatment with SOCs (Store Operated Channels)-inhibitors (SKF) were measured by Ca^{+2} fluorimetry using the reagent fura-2AM. We found that the absence of APP caused a statistically significant greater response to thapsigargin than that of SY naïve cells. In the same cellular group, we also observed an increase in the CCE, comparatively to SY naïve cells. As far as the kinetics of SKF is concerned, SY APP- cells respond faster to this reagent in comparison to SY naïve cells. SY APP+ cells, on the other hand, show reduced response to thapsigargin and reduced CCE, although this reduction isn't statistically significant. This increase in calcium release from the ER in the absence of APP could be explained by an increase in the levels of calcium in the ER, or by a dysregulation of Ca^{+2} transfer through the ER membranes. In future experiments we will also attempt to clarify the effect of APP-Homer interaction on calcium homeostasis.

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CORTEX VS CEREBELLUM UNDER ADULT ONSET HYPOTHYROIDISM: METABOLOMIC ANALYSIS OF A MOUSE MODEL REVEALS SIGNIFICANT DIFFERENCES

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It has for long been known that adult onset hypothyroidism (AOH) is accompanied by movement, behavioral and mental dysfunctions, however the underlying changes in mammalian brain metabolic physiology have not yet investigated in a systemic and systematic way. This is true because the adult mammalian brain has long been considered metabolically nonresponsive to thyroid hormones (TH). However, about the TH role on brain function several studies now support the TH direct role on brain metabolism. Still, the current knowledge remains fragmented as extracted from studies with different experimental setups on various brain regions. Moreover, because AOH effects on brain metabolic physiology have been observed to be region-specific, a holistic view of metabolism in particular brain regions under AOH will enhance the knowledge about the disease. In the systems biology era a fingerprint of the metabolic physiology of a biological system is provided through metabolomic analysis. In this context the goal of the present study was the comparison of prolonged AOH on the cortex and cerebellum metabolic physiology using Gas Chromatography-Mass Spectrometry (GC-MS) metabolomics in a mouse model involving the 2-month administration of 1%w/v KClO4 in the drinking water of 60 days old male Balb-c/J mice based on the protocol described in [1].

The metabolic profile acquisition of the cortical and cerebellar tissues of both the euthyroid and hypothyroid mice was carried out as shown in [1]. The profiles were appropriately normalized and filtered from any experimental biases. Multivariate statistical analysis was used to extract biologically relevant conclusions [1]. Specifically, a general decrease in the metabolic activity of both brain regions under prolonged AOH was observed, however the effect was more acute in the cerebellum. The indicated lighter effect of prolonged AOH on the cortex metabolic physiology is in agreement with previous targeted biochemical and/or neurophysiological studies. However, no clear justification for this difference between the two brain regions currently exists. In our study the comparison between the metabolic profiles of the cortex and cerebellum of the euthyroid animals is indicated the ¨leaner¨ metabolic profile of the cortex compared to that of cerebellum as a potential reason for the observed brain regional variation [2].

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NEUROPROTECTIVE EFFECTS OF THE CANNABINOID HU-210 AGAINST 5,7-DIHYDROXYTRYPTAMINE SEROTONERGIC TOXICITY IN VIVO: EXTENSIONS IN MDMA NEUROTOXICITY

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Several cannabinoids have been shown capable of providing neuroprotection against different neurotoxic agents and therefore seem to be effective in the treatment of several neurodegenerative disorders. On the other hand, some substituted amphetamines, such as methamphetamine (METH) and 3,4-methylenedioxymethamphetamine (MDMA; 'ecstasy'), are strong serotonergic and dopaminergic neurotoxins. It is worth noting that neurodegenerative disorders and the substituted amphetamine-induced neurotoxicity provide similar pathogenic results. In this study, we examined the effects of chronic i.p. administration of HU-210, a potent cannabinoid agonist, after i.c.v. injection of 5,7-dihydroxytryptamine (5,7-DHT), a strong serotonergic neurotoxin. The serotonergic lesion was estimated by calculation of serotonin (5-HT) and its metabolite 5-HIAA levels using HPLC methodology. In addition, SERT (serotonin transporter) density was evaluated using [^{3}H]paroxetine binding. Our results showed that 5,7-DHT injection significantly reduced 5-HT content in hippocampus and striatum, 5-HIAA content and [^{3}H]paroxetine binding in hippocampus. In addition, it induced a significant increase in 5-HT turnover (5-HIAA/5-HT) in both brain regions mentioned above. Moreover, the chronic HU-210 treatment reversed all 5,7-DHT neurotoxic effects, restoring, in some cases, the levels of serotonergic indices. HU-210 treatment alone did not alter the levels of estimated indices indicating that the HU-210-induced serotonergic effect was the result of neuroprotection rather than a chronic administration up-regulatory effect. Our findings show for the first time that cannabinoids are capable of providing neuroprotection against 5,7-DHT-induced serotonergic degeneration and, therefore, may be tested in the future for the treatment of similar neurotoxic conditions in humans. Substituted amphetamines are extensively used among humans for recreational purposes often in combination of cannabis use. Our findings may, at least partially, explain in these cases the lack of evidence for serotonergic toxicity in humans.

PROX1 SUPPRESSES THE PROLIFERATION OF NEUROBLASTOMA CELLS VIA A DUAL ACTION IN p27-KIP1 AND CDC25A

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Neuroblastoma is a pediatric tumor that originates from precursor cells of the sympathetic nervous system with less than 40% long-term survival in children diagnosed with high-risk disease. These clinical observations underscore the need for novel insights in the mechanisms of malignant transformation and progression. Accordingly, it was recently reported that Prox1, a homeobox transcription regulator, is expressed in higher levels in human neuroblastoma with favorable prognosis. Consistently, we have recently shown that Prox1 exerts a strong antiproliferative effect on neural precursor cells during embryonic development. Thus, *Prox1* is a candidate gene with a critical role in suppressing malignant neuroblastoma transformation. Here we provide evidence that Prox1 strongly suppresses the proliferation of mouse and human neuroblastoma cell lines by arresting the cell cycle in G1 phase, and blocks the growth of neuroblastoma tumors in SCID mice. Conversely, shRNA-mediated knockdown of basal Prox1 expression significantly induces proliferation, genomic instability, and the ability of neuroblastoma cells to form tumors. Mechanistically, analysis of an inducible Prox1-overexpressing Neuro2A cell line indicates that Prox1 is sufficient to suppress CyclinD1, CyclinA and CyclinB1, consistent with a role in cell cycle arrest. Surprisingly, Prox1 strongly induces CyclinE1 expression in the same system despite its action on blocking cell cycle progression, which could account for the context dependent oncogenic function of *Prox1*. Most importantly, Prox1 was sufficient to decrease Cdc25A and induce p27-Kip1 but not p21-Cip1 or p53. By alleviating the Prox1 action in Cdc25A and p27-Kip1 expression, we were able to rescue its effect on cell cycle arrest. Together these data suggest that Prox1 negatively regulates neuroblastoma carcinogenesis through suppression of Cdc25A and induction of p27-Kip1 to counteract CyclinE1 overexpression and block cell cycle progression. Furthermore, these observations render Prox1 a candidate target for the treatment of neuroblastoma tumors.

Inhibition of Acetylcholinesterase by Saffron Constituents: Molecular Docking & *In Vitro* Studies

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A variety of neurological and neuromuscular disorders, including Alzheimer's disease, involve a diminution of cholinergic activity, resulting in profound memory disturbances and irreversible impairment of cognitive function. Inhibitors of the acetylcholine breakdown by acetylcholinesterase (AChE) constitute one of the few available therapeutic modalities, despite their shortcomings. In order to find natural products which inhibit AChE, we investigated the effect on AChE structure and activity of saffron extract (styles of *Crocus sativus*) and its constituents by *in vitro* enzymatic and molecular docking studies; saffron is rich in mono- and di-glycosides of crocetin (crocins), and has been used in traditional medicine for mental disorders. Saffron extract showed moderate AChE inhibitory activity (up to 30 %) that was not dose-dependent. However, crocetin, dimethylcrocetin and safranal exhibited inhibitory activity to AChE with IC_{50} values of 96.33, 107.1 and 21.09 μM, respectively. The inhibitory activities of saffron's constituents were comparable to the standard drug galanthamine (IC_{50} of 1.93 μM). Enzyme kinetic analysis showed that the aforementioned compounds exhibited mixed type inhibition. The *in silico* docking studies resulted to be in good agreement with the in vitro testing, and contributed to better understanding the binding interactions of crocetin, dimethylcrocetin, crocin (di-gentiobiose ester of crocetin), picrocrocin and safranal within the binding site of the studied protein. These findings may be of value for the development of new therapeutic agents.

EAE INDUCED INFLAMMATION ALTERS HIPPOCAMPAL NEUROGENESIS IN ADULT MICE BRAIN.

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Neural Progenitor cells (NPCs) located in the subgranular zone (SGZ) of the dentate gyrus (DG) give rise to thousands of new hippocampal neurons every day. These NPCs differentiate to dentate granule cells and are integrated into existing neuronal circuits. The hippocampal neurogenesis has been proven to decline during aging and in chronic degenerative disorders such as Alzheimer disease, Parkinson disease and dementia. However, the role of inflammation in hippocampal neurogenesis is still unclear and the experimental evidence appears to be controversial. Here we demonstrate that the inflammatory environment prevailing in the brain of Experimental Autoimmune Encephalomyelitis (EAE) mice trigger increased proliferation of NPCs in SGZ and shift their differentiation pattern to an astrocytic fate, distorting neurogenesis.

Chronic EAE was induced to five to six-week-old C57Bl/6 female mice (*n*=8), immunized subcutaneously with MOG 35-55. Normal mice of the same age and sex were used as controls (*n*=6). EAE mice were examined daily for neurological signs, which reach the maximum clinical score 18-22 days after EAE induction. In order to label the proliferating NPCs, the immunized and control C57Bl/6 mice on days 18 and 21 after EAE induction received four injections (every two hours during a 6-h period) of BrdU (100mgr/kgr, i.p.; Sigma), a thymidine analogue that is incorporated into DNA synthesis, and sacrificed at the peak of the disease (day 23) under deep anesthesia with 4% paraformaldehyde solution in 0.1 M PB pH 7.4. Brains were post fixed in the same fixative overnight and 50μm vibratome coronal sections from the left brain hemispheres were cut and collected. The right hemispheres were used for the assessment of inflammatory infiltrates in mice brain. In free floating sections the following double-label fluorescence stainings were performed: BrdU/GFAP, BrdU/doublecortin, BrdU/Calretinin, BrdU/Fractin, after pretreatment with 2 N HCl for 20 min. The total number of BrdU+ cells in SGZ of dorsal hippocampus (located between 1.34 and 2.54 posterior to bregma) for each animal was calculated and the percentages of double-labeling coexpression in SGZ, granular cell layer (GCL) and hilus were estimated. Data were pooled to obtain the mean ± SE for each experimental group and statistical analysis was performed with the appropriate tests.

According to the results obtained, the number of BrdU+ cells in EAE mice at the peak of the disease was significantly higher in the DG of dorsal hippocampus compared to controls (1072.08±78.0 vs 643.4±65.7, P=0.005). A positive correlation between the number of proliferating cells in SGZ and the clinical score or brain inflammatory infiltrates of diseased animals was also detected. Inflammation triggered not only the proliferation, but also the migration of BrdU positive cells into the GCL. Animals with EAE exhibited higher numbers of BrdU/GFAP in the GCL and dentate hilus compared to controls. Minor apoptotic BrdU positive cells were detected in EAE and control animals.

According to our data, brain inflammation not only enhances mitotic activity of NPCs in the adult DG, but also modifies their differentiation pattern altering neurogenesis.

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Dose and sex-dependent behavioral effects of sertraline treatment in the forced swim test

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In affective disorders such as depression, sex is emerging as an important differentiating factor in the epidemiology of the disease and its treatment response. Animal models, and in particular the Forced Swim Test (FST), are currently used in elucidating potential sex differences in antidepressant response. In the present study, we sought to investigate a hypothesized sex- and dose-dependent interaction of antidepressant treatment with the FST behavioral indices.

Adult male and female Wistar rats were subjected to a 15min FST pretest session and then treated with three injections of sertraline 10mg/kg, 40mg/kg or vehicle at 0, 19 and 23 hours post-FST. Twenty-four hours later, they had a second 5min FST test session. Immobility duration was recorded as an index of passive behavior. Furthermore, swimming and climbing behaviors were recorded as indices of active serotonergic and noradrenenergic behavioral responses, respectively. Meanwhile, the estrous phase of female rats was determined, in order to test the females in two discrete phases of the estrous cycle.

In males, as expected, both high and low sertraline doses equally decreased immobility and climbing, while greatly increasing swimming duration. On the contrary, in females low sertraline dose effectively reduced immobility and increased swimming in diestrus II but was behaviorally ineffective in proestrus, probably due to the higher baseline swimming duration in proestrus females compared to males. High sertraline dose reduced immobility and increased swimming and climbing in diestrus II. However, in proestrus, high sertraline dose reduced immobility and increased climbing only.

In conclusion, present results indicate that a dose-dependent effect of increasing SSRI doses was evidenced in female but not in male rats in immobility and climbing behavior in the FST. Such dose-dependency suggests that female rats are more sensitive to the modulation of the catacholaminergic neurotransmission, in comparison to males after treatment with higher doses of setraline.

Serum Levels of S-100B after Recreational Scuba Diving.

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Recreational scuba diving is a sport of increasing popularity. Previous studies indicating subtle brain injury in asymptomatic divers imply a cumulative effect of minor neural insults in association with diving for professional and/or recreational purposes, over the long-term. This is the first study to investigate putative neural tissue burden during recreational scuba diving by measuring circulating levels of S100-B, a sensitive biomarker of brain injury. 5 male divers performed 3 consecutive dives under conservative recreational diving settings (maximum depth 15 m, duration of dive 56 min, ascend rate 1.15 m/min) with an interval of 12 h between each session. Although a small increase in serum S-100B levels after each dive was apparent, this increase did not quite reach statistical significance (p=0.057). Moreover, no abnormal S-100B values were recorded (mean baseline: 0.06 μg/L, mean post-dive: 0.086 μg/L) and no effect of the 3 consecutive dives on changes in S-100B levels was detected. These results suggest that under the experimental conditions tested, diving does not seem to have a discernible and/or cumulative impact on central nervous system integrity. The extent to which variable diving settings and practices as well as individual susceptibility factors underlie putative neural tissue burden in asymptomatic divers, remains to be established.

LONG-TEM EFFECTS OF NEONATAL T-MAZE LEARNING UNDER CONDITIONS OF REWARD OR DENIAL OF EXPECTED REWARD ON PREFRONTAL CORTEX STRUCTURE AND FUNCTION

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Early life experiences are considered as major determinants of adult behavior and brain function. Adverse events have a profound impact on the development of brain areas such as the prefrontal cortex and predispose individuals for maladaptive reactions and even psychopathology. We utilized the animal model of neonatal training in a T-maze under conditions of reward or denial of expected reward to investigate its long-term effects on prefrontal cortex function in the Attention Set Shift Task (ASST), a rat analogue of the Wisconsin Card Shorting Test for humans. In ASST, animals have to learn to predict the presence of hidden food based on a specific type of environmental cue (i.e. the type of texture of the material covering the food) while ignoring other types of cues (i.e. the odor of the material covering the food) and then learn by trial-and-error to reverse the predictive rule either intradimensionally (i.e. one type of texture over another) or extradimensionally (i.e. odor over texture).

Adult rats denied expected reward as neonates were deficient in the intra-dimensional rule reversal trials of the ASST, compared to both rewarded as neonates and control animals, indicating a prefrontal cortex malfunction. This behavioral deficit was accompanied by lower activation during ASST, as assessed by Fos immunoreactivity, of the medial orbitofrontal cortex, an area necessary for inhibiting learned reactions and impulses. Most interestingly, in the same prefrontal area, levels of phospho- and acetylated histone H3, determined immunohistochemically, were indicative of epigenetic changes in the chromatin structure lowering gene expression. In addition, neuronal morphology was altered showing aberrant dendritic arborizations, following Gogli-Cox staining. Moreover, as determined by HPLC, dopamine levels in the prefrontal cortex were lower in adult animals denied expected reward as neonates.

Our findings that the neonatal experience of learning under conditions of denial of expected reward had long-term effects on prefrontal cortex at the molecular, neurochemical, structural and functional level, support the concept that early-life experiences can program in an experience-specific way, adult brain function and thus behavior.

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Differential role of neuronal RASGAP neurofibromin Type I and Type II in developing neurons, and their association with the F-actin cytoskeleton

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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. Moreover, overpression of GRDI mimicked the developmental switch in transcript expression, as it lowered the expression of GRDII transcripts. 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.

GENERATION AND ANALYSIS OF A NOVEL TRANSGENIC MOUSE LINE WITH DEFECTIVE FIBER TRACTS AND CORTICAL LAMINATION DEFICITS

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Appropriate generation of cortical circuits plays a key role in the function of the cerebral cortex. The cortex receives major sensory input from the thalamus through thalamocortical axons (TCAs) and sends information to other structures via cortical efferents (corticothalamic axons or CTAs). Based on the "handshake" hypothesis, these two axonal systems are thought to constitute a case of codependent axonal growth and guidance. Early generated CTAs from the subplate pioneer neurons interact with TCAs to navigate correctly. However, we do not know yet if this interaction is essential for the navigation of both set of axons.

TAG-1(Cntn2), a neuronal recognition molecule of the immunoglobulin superfamily, is involved in neurogenesis, neurite outgrowth and fasciculation. Among other neuronal subpopulations, it is expressed early by pioneer neurons in the preplate and later on in the marginal zone and subplate of the developing cortex. In addition, the protein is a specific marker of CTAs. In order to study the formation and function of CTAs and TCAs *in vivo* we generated the transgenic mouse line *Tag1* loxP-GFP-loxP-DTA. This line expresses GFP under the *Tag-1* promoter also possessing the coding sequence of DTA (Diptheria Toxin subunit A) under quiescence. We crossed these mice to the *Emx1*Cre line, which expresses the Cre recombinase specifically in the embryonic cortex. Upon crossing, GFP expression is eliminated and the toxin starts being expressed in TAG-1^+ neuronal cells of the neocortex resulting in their death.

Analysis of the developing cortex reveals extensive cell death resulting in a significantly smaller cortex. Although some CTAs remain, they are significantly reduced. Postnatally, no anterior commissure, abnormal corpus callosum and a smaller hippocampal formation are observed. Moreover, cortical layering is disorganized. Therefore, the elimination of TAG-1^+ cortical neurons results in a more severe phenotype than expected. Analysis is in progress to reveal the full phenotype and understand the functional consequences of the lack of TAG-1^+ neuronal cells in the neocortex.

NEONATAL HANDLING INDUCES CHANGES IN AMPA RECEPTOR SUBUNIT EXPRESSION WITHIN RAT HIPPOCAMPUS, AMYGDALA AND CEREBRAL CORTEX

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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. A previous study has shown selective effects of neonatal handling on rat brain NMDA receptors [1]. 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 GluRA, GluRB, GluRC and GluRD, which combine to form tetramers. Most AMPARs are heterotetramerics, made of at least two of the four proper subunits GluRA-D. AMPA receptors that are permeable to Ca^{2+} lack the GluRB subunit, while both GluRA and GluRB subunits have an important role in AMPAR trafficking towards the synapse.

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 synaptic properties 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 and in cases sexually dimorphic changes were observed in AMPA receptor subunit expression, depending on brain region and AMPAR subunit, which imply an early experience-dependent selective modulation of brain circuits. Supported by Polembros Shipping Limited.

[1] Stamatakis et al. Neuroscience 164, 1457-1467, 2009.

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.

Mechanism of nucleocytoplasmic shuttling of neurofibromin in post-mitotic neurons

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Learning difficulties and development of benign and malignant tumors of the nervous system are clinical features of the autosomal dominant, progressive disorder Neurofibromatosis 1 (NF1) ; yet, the current knowledge on the function of the NF1 gene product, neurofibromin, remains limited. Neurofibromin is a RasGAP, that also contains a functional NLS, and we have previously reported its PKC phosphorylation-dependent subcellular shuttling in neuroblastoma cells and in neurons. In chick embryo neurons in vivo and in culture, this PKC substrate is highly deteced in the nucleus and, we have begun to address the mechanism of it's nucleocytoplasmic shuttling in this post-mitotic environment. To investigate the role of PKC phosphorylation in both the cyto-nuclear shuttling of neurofibromin and its nuclear degradation, we used a dephosphorylation assay and a phospho-sensitive antibody (JNC 2009) to detect relative abundance in nuclear and cytosolic fractions. As PKCε is the primary isoform in these neurons, we treated the cultures with the PKCε-selective peptide inhibitors (εV1-2) and activators (ψεRACK) and activators and found that PKCε-induced phosphorylation increased 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 regulates the nucleocytoplasmic shuttling of neurofibromin by retaining PKCε-phosphorylated neurofibromin inside the nucleus of post mitotic neurons. These effects were further enhanced in the presence of phosphatase inbitors like calyculin A. Using Leptomycin B (LMB), a specific inhibitor of CRM1-dependent pathway, and immunodetection in subcellular fractions of cultured neurons, we found that the abundance of neurofibromin was reduced, possibly due to degradation. Further investigations that this stabilization was proteasome-independent. Yet 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).

BEHAVIORAL AND PHYSIOLOGICAL EFFECTS IN MICE WITH DEVELOPMENTALLY DECREASED INHIBITION

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The mammalian cortex is composed of both pyramidal neurons, found in layers II, III and V, and interneurons, found in all 6 layers. Interneurons comprise about 25% of all cortical neurons, however, they are very heterogeneous. The vast majority of studies regarding cortical function and behavior have focused on the pyramidal neurons because they represent the output neurons from different cortical regions. On the other hand, the role of interneurons in shaping cortical activity, plasticity and ultimately behavior has largely been unexplored. In our study, we use a mouse genetic model that displays reduced number of inhibitory neurons in order to determine how decreased inhibition alters synaptic physiology and plasticity in the cortex, as well as its effects on behavior. The mouse model used has been shown to express 50% less interneurons in cortical areas, due to the loss of the Rac1 protein from MGE-derived interneurons (Vidaki et al, 2011, Cereb Cortex, doi: 10.1093/cercor/bhr145; Tivodar et al, HSN abstract). The 'missing' interneurons are known to express either the parvalbumin or the somatostatin proteins. We find that the mice that survive past 45 days have an increased anxiety phenotype when tested in the open field. Specifically, $Rac1^{Fl/Fl}$/$Nkx2.1^{+/cre}$ mice spend less time in the center, exhibit more grooming and decreased movement compared to their respective controls in the first five minutes tested in the open field. In addition, we examined the short-term and long-term plasticity induction in the prefrontal cortex, an area shown to be involved in anxiety behaviors. Our preliminary data indicate a defect in both short and long-term plasticity in the layer II prefrontal cortical networks, suggesting that this could contribute to the increased anxiety seen in these mice. In addition, 80% of the young mice (18-35 days old) that died showed extremely violent tonic-clonic seizures before their death. In the older $Rac1^{Fl/Fl}$/$Nkx2.1^{+/cre}$ mice that survived, we did not observe spontaneous tonic-clonic epileptic seizures, although about 50% of these mice showed signs of absence epileptic behavior. Electrophysiologically, some spontaneous epileptiform activity and increased stimulus-induced epileptiform activity was observed in $Rac1^{Fl/Fl}$/$Nkx2.1^{+/cre}$ compared to the wild-type mice. The above data may suggest a possible reduced threshold for induced-epileptic seizures. In addition, our data show that the $Rac1^{Fl/Fl}$/$Nkx2.1^{+/cre}$ mice could be used as a model to study the underlying mechanisms for the co-morbidity of anxiety and epilepsy, in a more subtle phenotype of epilepsy.

ADAPTOR PROTEIN DRK IS REQUIRED FOR ANESTHESIA RESISTANT MEMORY

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Adaptor Protein DRK (downstream of receptor kinase) is one of the primary molecules participating in the activation of MAPK (mitogen-activated protein kinase). The protein is preferentially distributed in the adult mushroom bodies, centers of olfactory learning and memory, and by binding to SOS (son of sevenless), leads to the activation of RAS in the MAPK cascade. The important role of DRK in learning and the formation of 90min memory has already been demonstrated as drk mutant heterozygotes exhibit deficits in olfactory learning and memory under limited training conditions. The aim of this poster is to present data underlining the important role of DRK in the formation of ARM (Anesthesia Resistant Memory), a unique form of memory characterized in Drosophila. We will demonstrate that flies with reduced DRK levels within the mushroom bodies as well as drk mutant heterozygotes produce deficient ARM after associative olfactory training. Results of this work will further contribute to better understanding the formation of ARM, a type of consolidated memory little understood and an area yet to be explored.

INCREASED SEIZURE LATENCY AND DECREASED SEVERITY OF PTZ INDUCED SEIZURES IN MICE AFTER ESSENTIAL OIL ADMINISTRATION: PRELIMINARY DATA

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AIMS AND SCOPE: Plant essential oils and their drastic components have long been evaluated for treatment of several conditions of neurologic origin or neurodegenerative diseases as a more "natural" alternative to artificial drugs expressing anxiolytic, sedative and antioxidant properties. The aim of the present study is to evaluate the essential oils of eight aromatic plants in the seizure latency and severity of PTZ-induced seizures in mice.
METHODS: Balb-c mice were divided in eight groups. 200μl of eight aromatic plant essential oils namely *Rosmarinus officinalis, Ocimum basilicum,Mentha spicata, Mentha pulegium Lavantula angustifolia, Mentha piperita, Origanum dictamnus and Origanum vulgare*, isolated from the respective aromatic plants, were administered 30min prior to intraperitoneal injection of a lethal dose of PTZ (80mg/kg). A ninth group received only one i.p. PTZ injection and was the control group. The development of tonic clonic seizures, seizure latency and severity as well as the percentage of lethality were determined for each group.
RESULTS: All groups of mice treated with the essential oils showed reduced activity and stability after the administration of the oils, except from those treated with Origanum vulgare (75% mortality after the administration of the oil). After administration of PTZ mice from the different groups showed different levels of reactions: increased latency of the onset of seizures, reduced severity of seizures (ranging from simple twitches to complete seizures). Mice who had received Mentha piperita essential oil showed the lowest percentage of lethality and seizures.
CONCLUSION: The administration of the tested essential oils had different effects on PTZ induced seizures in mice indicating that perhaps their different drastic component could account for this anticonvulsant effect. Further experiments incorporating the administration of the drastic component of the oils showing the most prominent anticonvulsant effect are needed to establish its possible use in the treatment of epipetic conditions.

L-TYPE CALCIUM CHANNELS MODULATE HIPPOCAMPAL SHARP WAVE-RIPPLE ACTIVITY.

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Sharp wave-ripples (SWRs) are a prominent and endogenous network activity in the hippocampus occurring during slow-wave sleep, and awake immobility, and it is thought to play a critical role in the process of memory consolidation which involves transfer of information from the hippocampus to neocortex. In the present study using recordings of field potentials from the CA1 field of ventral hippocampal slices we examined the effect of nifedipine, a blocker of voltage-dependent calcium channels of L-type, in the activity of sharp wave-ripples (SWR). We observed that nifedipine significantly increased the amplitude of sharp waves and ripples, reduced the incidence of SWR episodes and the average number of episodes. The drug did not significantly change the duration and frequency of the ripple oscillation. These results indicate that activity of voltage-dependent calcium channels of L-type play an important role in the modulation of SWRs. This action might underlie the effects of nifedipine and related drugs on hippocampus-dependent learning and memory.

THE MECHANISMS THAT GOVERN SELF-RENEWAL AND DIFFERENTIATION DECISIONS OF THE EMBRYONIC CORTICAL PROGENITORS ARE REGULATED BY GEMININ

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Cortical neurogenesis is a highly ordered process, requiring coordination of proliferation and differentiation of cortical neural progenitors in order to generate the complex array of neurons and glial cells in the adult mouse brain. At early stages of neurogenesis there are three distinct types of neuronal progenitor cells that reside in the neurogenic layers of the cortex, the ventricular and subventricular zone. The neuroepithelial (NE) and radial glial cells (RG) that divide in the ventricular zone of the embryonic cerebral cortex are divining either symmetrically in order to expand their number or asymmetrically in order to differentiate. At the subventricular zone there are the basal progenitor cells (BP) that divide mostly symmetrically towards neuronal differentiation. The timing of the decision of a progenitor cell to stop proliferating and exit from the cell cycle so as to generate a differentiated cell type is a crucial step in the correct development of the mammalian cerebral cortex.
Geminin has been suggested to play an important role in regulating the mechanisms that govern cell division and differentiation. To gain insight into the *in vivo* role of Geminin in the regulation of 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. Moreover, Geminin overexpression in cortical progenitor cells reduces cortical progenitor cell population and exhibit premature cell cycle exit towards neuronal differentiation.

SOCIAL HIERARCHY IN MALE ZEBRAFISH (DANIO RERIO): EFFECTS ON ADULT BRAIN PROLIFERATION PATTERN

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Teleost fish, as well as mammals, are known to develop well established social hierarchies, with the dominant individuals being more active and aggressive in the aquarium, showing their dominance by chasing and biting the subordinate fish. The present study questioned whether such stressful social interactions have an effect on brain neurogenesis, that in teleosts is known to persist throughout life. For this purpose, size-matched male zebrafish were either kept in pairs and allowed to establish stable social hierarchies or kept in social isolation. Behavior was recorded twice daily for 7 days and data of swimming and aggressive behavior (attacks, bites) were collected. Video analyses were used to estimate the dominance index.

Following 7 days of expressing stable social interactions, the animals were allowed to swim in a BrdU solution (5mM) for six hours to label the cycling proliferating cells. BrdU immunohistochemistry and double immunofluorescence were used to determine the number and type of the proliferating cells within the social groups studied, that is, dominant, subordinate and isolated zebrafish. Stereological disector analysis revealed important effects of the hierarchical behavior on the number of newborn cells in cerebellar and telencephalic regions. Our data suggest the significant impact of social interactions in the neurochemical profile and proliferative potential of adult male zebrafish brain and support that non-mammalian vertebrates represent an important complement to mammalian models.

This research has been co-financed by the European Union (European Social Fund-ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF)-Research Funding Program: Heracleitus II. Investing in knowledge society through the ESF.

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A CASE OF TRAUMA-INDUCED GLOBAL APHASIA SHOWING SIGNIFICANT IMPROVEMENT OF COGNITIVE FUNCTION AFTER A DECADE

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Trauma-induced cognitive or other neurological deficits, if reversible, typically show improvement in the first few months or few years after the event. Here we present the case of a 17-year-old female who suffered severe brain damage after a motorcycle accident, but showed continued improvement in cognitive functions up to ten years after the event.

Computed tomography immediately after the event showed right temporo-parietal skull fracture associated with acute subdural hematoma, subarachnoid hemorrhage and multiple hemorrhagic contusions of both hemispheres, but most pronounced in the left frontal and temporal lobe. On clinical examination, the patient's main neurological deficit was global aphasia.

The first four years after the accident, there had been no intervention for rehabilitation of cognitive functions and the patient showed only minor improvement in these domains. Four years after the event, evaluation of speech and language skills revealed persistent global aphasia with severe apraxia of speech and oral-motor apraxia (Boston Diagnostic Aphasia Examination). Specifically, the patient showed mutism and severe difficulties in understanding and perception of speech, with inability to read or write. Seven years after the accident, there was some improvement in understanding simple commands. Mutism persisted; however there was mild improvement in oral-motor apraxia.

During the last three years, a treatment program was initiated, focusing on the difficulties of perception and understanding of speech, as well as on orofacial motion programming optimization. As a result, the patient gradually started producing phonemes and morphemes, suggesting that oral-motor apraxia and apraxia of speech improved.

Currently, ten years after the insult, clinical evaluation shows a significant amelioration in understanding and perception of speech. The patient is able to distinguish, categorize and perform simple and complex commands. Furthermore, she is now able to repeat words and short sentences, naming objects after semantic or phonological help. Finally, the patient's reading and writing skills have improved.

This is a case of global aphasia due to severe traumatic brain injury which showed significant recovery up to ten years after the event. This is unusual, as the recovery usually presents in the first years after the insult, and highlights the importance of brain plasticity even after a considerable period of time, especially in young patients.

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MINING HUMAN BRAIN IMAGE DATA

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Understanding patterns and discovering associations, regularities and anomalies between anatomical structures and normal or abnormal function of the human brain is a fundamental goal in the neuroscience community. Current advances in brain image acquisition techniques have made available enormous amounts of remarkable high-resolution three-dimensional (3-D) image data. The availability of this data has already facilitated many advances in human brain mapping during the last decade. In addition to the continuous development of improved brain imaging techniques, greater computer capabilities and improvements in normalization techniques are leading to the creation of large databases of structure/function information. The analysis and exploitation of such large collections of brain images still remains a problem though. Major issues in the current attempts for managing this data are the efficiency, effectiveness and robustness of the database and data mining tools used to extract knowledge (in the form of patterns, associations, etc). New tools for content-based retrieval, association mining, classifications, etc, can have significant impact in this endeavor.

Here, we present our experiences and results from a neuroinformatics project supported by the Human Brain Project initiative funded by the National Institutes of Health in the US. The goal of this project was to overcome the aforementioned problems and address the great need for developing efficient brain data mining tools for the analysis and management of large collections of brain images (from various imaging modalities) and associated clinical data. These automated tools enable interoperable brain image data representation that is easy to search. The main focus is on the management of the spatial regions of interest (ROIs) through a general *unified* framework regardless of whether these are lesions, tumors, areas of brain activation, or regions of (normal/abnormal) morphological variability of a variety of brain structures.

The work focuses on (a) developing efficient methods for feature extraction and classification of ROIs in brain images, (b) developing fast and effective database techniques supporting efficient retrieval of similar regions of interest in large brain image databases, (c) developing spatial and temporal data mining tools for discovering patterns and associations between anatomic and other variables such as function, pathology, or response to drugs and (c) integrating the above techniques with morphological analysis tools to correlate morphological changes to changes of other measurements such as functional, physiological, etc. The classification, similarity searching and data mining techniques are evaluated and validated using real and simulated data and their utility in the analysis of large data sets from a number of epidemiological studies of brain morphology and function is demonstrated.

We expect that this work will be useful to the neuroscience community advancing our ability to analyze brain image data and to discover associations between structural and functional data obtained using brain imaging techniques and other variables obtained through clinical assessment, as well as spatial and temporal patterns, anomalies or normal variations.

DEFINING DEVELOPMENTAL APRAXIA OF SPEECH: GREEK SLTs' PERCEPTIONS OF SYMPTOMS

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Objective

The clinical symptoms of Developmental Apraxia of Speech (DAS), as well as the neuromotor and linguistic deficits involved in this clinical entity are still a relatively controversial issue. The nature of DAS deficits is still under investigation and no firm conclusions have been reached to date. In the present study, the objective is to investigate the most common and the least common characteristics describing DAS symptomatology as perceived by SLTs working with Greek children. Factors which may influence the SLTs judgment regarding these disorders, such as, continuing education, studies, work experience, were also investigated.

Methods

A questionnaire describing and categorizing the clinical symptoms of DAS was designed by the research team and administered electronically to all registered SLTs (N=965). The questionnaire which was anonymous included information concerning specialization, years of study and experience. A sample of 114 SLTs completed the questionnaire (100 female, 14 male).Data was analysed with the statistical package SPSS 17.0.

Results

The most common and least common DAS symptoms found in Greek children were ascertained through the SLTs' responses. These characteristics were the same as those found by other investigators in other western countries. No relationship was found between specialization, years of study and experience in the SLTs' judgement and perceptions of DAS.

Conclusion

Greek SLTs show adequate knowledge regarding this diagnostic category, since they detect the same DAS symptoms as those found in other studies. It is noteworthy that this result is regardless of the SLT's specialization or years of experience. Further study of the most efficient intervention methods for these complex disorders is needed.

NEUROPROTECTIVE EFFECT OF DHEA-S ON SUBSTANTIA NIGRA AND STRIATUM OF THE WEAVER MOUSE – A GENETIC MODEL OF PARKINSON'S DISEASE

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The weaver mouse (wv/wv) is a genetic model of Parkinson's disease, which carries an autosomic recessive mutation that leads (among others) to progressive dopaminergic neurodegeneration in substantia nigra (S. Nigra), starting at postnatal day 7 (P7) and reaching 50% at P21, a fact that makes this animal model ideal for neuroprotection studies.

In this study, we administrated the neurosteroid dehydroepiandrosterone sulphate (DHEA-S) in wv/wv mice from P1 to P22 (wv/wv DHEA-S) and then we measured neuronal survival in S. Nigra.

Using western blot analysis and a specific antibody against TH our results:

i. have confirmed that in the wv/wv mesencephalon (an area that includes S. Nigra) there is neurodegeneration of about 50% at P22 compared to control (+/+) mice of the same age, that is reflected in striatum by a reduction of the tyrosine hydroxylase (TH) level of about 25%.

ii. have shown that in wv/wv mesencephalon, DHEA-S induces an increase in TH level of about 27% compared to wv/wv which had received saline (wv/wv NaCl) and that in striatum, DHEA-S has a neuroprotective effect of about 18%, bringing the TH level almost to normal.

Using immunohistochemical experiments, with the same TH-specific antibody we found that DHEA-S had very important neuroprotective effect (increase of the dopaminergic cell survival by 90%) in the wv/wv S. Nigra compared to wv/wv NaCl mice, which brings the number of the dopaminergic neurons almost to the control levels.

Those results suggest that DHEA-S represents a very important neuroprotective agent for the weaver S. Nigra, leading to almost normal dopaminergic neuron number in S. Nigra. It is also important to notice that the induced neuronal survival is reflected in the weaver striatum.

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CANNABIDIOL INHIBITS THE REWARD-FACILITATING EFFECT OF MORPHINE

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Cannabidiol (CBD), the main non-psychotomimetic constituent of *Cannabis sativa*, displays a plethora of pharmacological effects, including anticonvulsive, anxiolytic, antidepressant, antipsychotic, antiinflammatory and neuroprotective properties. In the present study we utilized the intracranial self-stimulation (ICSS) paradigm to examine the effects of CBD (5, 10 and 20mg/kg) on brain reward function and on the reward-facilitating effect of morphine (10mg/kg) and cocaine (5mg/kg). Male Sprague-Dawley rats were implanted with stimulating electrodes into the medial forebrain bundle (MFB) and were trained to respond for electrical stimulation using a rate-frequency paradigm. The highest doses of CBD tested significantly increased the threshold frequency required for MFB ICSS. Both cocaine and morphine produced a significant decrease in ICSS threshold. CBD inhibited the reward facilitating effect of morphine, but not cocaine, at a dose that did not by itself affect brain reward function. The effect of CBD on morphine was reversed after pretreatment with the 5-HT_{1A} receptor antagonist WAY100635. The present results indicate that CBD does not exhibit reinforcing properties in the ICSS paradigm over a range of doses tested, but rather have an inhibitory influence on reward mechanisms at high doses and even reduce the reward-facilitating effects of morphine. These effects of CBD were probably mediated by activation of 5-HT_{1A} receptors. The present data indicate that CBD interferes with brain reward mechanisms responsible for the expression of the acute reinforcing properties of opioids, such as morphine, and provide evidence that CBD may be clinically useful in attenuating the rewarding effects of opioids.

RGS2 AND RGS4 PROTEINS: NOVEL MODULATORS OF KAPPA OPIOID RECEPTOR SIGNALING

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Regulators of G protein Signaling (RGS) comprise a large multifunctional protein family that accelerate GTP hydrolysis of Gα subunits of G proteins, thus modulating G protein coupled receptor (GPCR) signaling. We have previously demonstrated that RGS4 directly interacts with mu (μ-OR) and delta (δ-OR) opioid receptors to regulate their signaling (1,2). To deduce whether there is selectivity in RGS-opioid receptor interaction we tested the ability of members of B/R4-RGS family to interact with the kappa opioid receptor (κ-OR). Pulldown experiments using GST fusion peptides encompassing the carboxyl terminus of κ-OR indicated that RGS2 and RGS4 interact within this receptor subdomain. Using a truncated version of RGS4 that lacks the N-terminus (ΔNRGS4) we also demonstrated that this region is responsible for κ-OR interaction. Co-immunoprecipitation studies indicated that RGS2 and RGS4 associate with κ-OR constitutively and upon receptor activation to confer selectivity for coupling with a specific subset of G proteins. Functional assays have shown that both members of B/R4-RGS family differentially modulate κ-OR mediated cAMP accumulation and ERK1,2 phosphorylation, without altering the internalization fate of the κ-receptor. Collectively, our results demonstrate that RGS2 and RGS4 are new interacting partners and negative modulators of κ-OR signaling.

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THE CYCLIC-AMP RESPONSE ELEMENT BINDING PROTEIN (CREB) AND ITS ROLE IN NEURAL PROGENITOR CELLS AND BRAIN CANCER

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ABSTRACT:

Gliomas are the most common malignant cancers of the nervous system. Unfortunately, they are also amongst the most difficult cancers to treat. The discovery that many primary tumours, including gliomas, develop from 'cancer stem cells' has advanced our understanding of tumour biology. Discovering the genes and pathways that regulate cancer stem cell specific survival and growth will aid in the development of novel and more effective treatments. The Cyclic-AMP Response Element Binding protein (CREB) is a serine/threonine kinase-regulated nuclear factor modulating the transcription of numerous genes in nerve cells and has various roles in neuronal function, ranging from survival to more complex brain functions. Our recent work has shown that CREB is required for the maintenance of normal brain development and neuronal expansion in both zebrafish and mouse. We further found that CREB regulates genes important for neural progenitor cell survival but it also likely regulates paracrine growth/survival factor expression in the neural stem cell niche. Given recent findings that aberrant CREB expression can impart oncogenic properties on various cell types, we explored the potential role of CREB in brain cancer biology by examining its expression in a panel of human patient brain tumour specimens. We show that both the level of expression and the number of cells expressing activated/phosphorylated CREB is markedly elevated in tumours compared with adjacent non-tumour control brain tissue. These observations are the first to highlight a link between CREB and brain tumours. Our hypothesis is that CREB has a role in brain tumour development/growth and that at least some of CREB's neuro-oncogenic properties are due to its role in promoting brain tumour stem cell survival and growth.

TAU-DEPENDENT NEURONAL TOXICITY IN VIVO CAN BE DIRECTLY CORRELATED WITH ITS PHOSPHORYLATION AT SERINE 238

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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 fruit fly Tauopathy model, we study the biochemical alterations on Tau that result in neuronal 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: Ser^{238} and Thr^{245}. 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. For that reason, we decided to generate phosphoantibodies that specifically target these two residues separately, starting from Ser^{238}. We investigate the correlation between occupation of that site in flies expressing wild-type or mutant Tau isoforms and Tau associated neuronal toxicity. Mutations include FTDP-17 associated sites or phosphorylation-incompetent forms of Tau in which single or a pair of phosphorylation sites are replaced by alanines. Our collective results reveal that phosphorylation at Ser^{238} is a critical mediator of Tau neurotoxicity *in vivo*.

Idas and Lynkeas/GemC1: two novel Geminin related proteins in mouse embryonic brain development

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Normal development requires a strict balance between cell proliferation and differentiation. Geminin is a bifunctional protein that participates in both cell cycle and developmental controls. During the cell cycle, Geminin interacts with Cdt1, a major component of the pre-replicative complex that licenses origins for replication. In development, balanced interactions between Geminin and transcriptional or chromatin remodelling factors determine proliferation versus differentiation decisions
Herein we introduced two novel paralogues, Idas and Lynkeas, which are both phylogenetically conserved in vertebrates and constitute two different genes with homology to Geminin's coiled coil, a necessary domain for most of Geminin's interactions. Idas and Lynkeas mRNA localization was studied by *in situ* hybridization in different developmental stages of the mouse embryonic brain. In contrast to Geminin, which is expressed throughout the ventricular and subventricular zones of the embryonic telencephalon, Idas and Lynkeas were found to be specifically expressed in the cortical hem and choroid plexus epithelium of the developing mouse telencephalon. Idas and Lynkeas expression was also detected in the developing mouse hindbrain where they were specifically expressed in the choroid plexus epithelium. Interestingly, in the hindbrain choroid plexus Idas exhibits a restricted expression pattern in the progenitor domain of the epithelium, as marked by the absence of sonic hedgehog expression.
The above results indicate that Geminin, Idas and Lynkeas constitute a Geminin superfamily implicated in mouse embryonic brain development.

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α5GABA$_A$ RECEPTORS MODULATE THE THRESHOLD FOR LONG-TERM SYNAPTIC PLASTICITY DIFFERENTLY BETWEEN VENTRAL AND DORSAL CA1 HIPPOCAMPAL SYNAPSES

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The hippocampal synapses display conspicuous ability for long-term plasticity. In addition, this ability differs along the long axis of the structure with the ventral CA1 hippocampal synapses displaying remarkably lower ability for long-term potentiation compared with their dorsal counterpart following high-frequency stimulation[1]. Recently, it has been shown that blockade of GABA$_A$ receptors containing the alpha5 subunit and low-frequency stimulation (10Hz) uncovers long-term potentiation at the CA1 synapses[2]. Taking into account that the ventral compared with dorsal CA1 field displays higher levels of alpha5-GABA$_A$ receptors[3] and using extracellular potentials recorded from adult rat hippocampal slices, we examined whether blockade of α5GABA$_A$ receptors and 10 Hz stimulation affects differently the ability for long-term plasticity between the dorsal and the ventral CA1 synapses. We found that 900 pulses at 10 Hz produced a higher long-term potentiation in dorsal compared with ventral synapses. Remarkably, 10 Hz stimulation, under blockade of 5-GABA$_A$ receptors, significantly facilitated long-term potentiation in ventral synapses but uncovered long-term depression in dorsal synapses. We hypothesize that α5GABA$_A$ receptors modulate the threshold for long-term synaptic modifications differently in ventral and dorsal hippocampus.

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The serotonergic system in the brain of female rats exposed to neonatal, adolescent and adult stress.

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The serotonergic system is known as an important modulator of emotional balance in both normal and pathological states such as depression and anxiety for which females are more vulnerable. Adverse experiences during critical periods such as the neonatal and adolescence are aetiopathogenetic factors for adult psychopathologies. Based on the above we determined 5-HT, 5-HIAA and 5HT1A receptor levels in the PFC, the AMY, and in the HIPP of adult females exposed to a neonatal experience and either stressed or not as adolescents. During the neonatal period (PND 10-13) rat pus were exposed to a T-maze one arm of which lead to the mother. One group of animals was allowed the contact with the mother (rewarded) while the other was denied (the expected reward-DER). HPLC analysis revealed that in both the PFC and in AMY, neonatally rewarded animals had higher basal 5-HT levels. Furthermore, in the AMY of this group of animals, higher levels of 5HTIA receptors were detected by western blot analysis. Another cohort of animals exposed to the neonatal experience was either subjected or not during adolescence (days 30-38) daily to 1 hour of isolation and cage partner stress. In adulthood rats were exposed to Forced Swimming Stress (FSS). Following the adult stress neonatally frustrated animals had higher 5HT in the AMY, and lower 5HIAA in the PFC and HIPP when not stressed in adolescence than when stressed. The adult FSS resulted in increased 5HT- compared to basal levels - in the AMY in animals not subjected to any neonatal experience. In contrast in the PFC the adult FSS resulted in decreased (compared to basal levels) 5HT in all animal groups. 5HT1A receptor levels in the AMY of the neonatally rewarded animals were decreased following FSS in adulthood compared to the basal condition. Since the serotonergic system is intimately involved in the control of HPA axis reactivity, which is determined by early experiences we also measured basal plasma corticosterone and following the FSS. Neonatally DER animals who had also been exposed to the adolescent stress exhibited the most effective stress response, lowering the stress induced corticosterone levels faster.
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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 a slow oscillation. The cellular correlates of the slow oscillation 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 slow oscillation 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.

FRAGILE X SYNDROME, cAMP AND LEARNING IMPAIRMENT.

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Fragile X syndrome (FXS) is the most common known cause of inherited mental retardation and is caused by alterations of the FMR1 gene. This 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. Several RNA-binding motifs associated with FMRP function were found, including two KH domains (hnRNP-K homology) and an arginine and glycine-rich motif (RGG box), sequence motifs that are common to RNA-binding proteins. It is known that cyclic AMP (cAMP), contributes to FXS neuropathophysiology, as there is a robust defect in cAMP production in FMRP mutants. Since the homolog of FMRP in Drosophila melanogaster has a high degree of amino acid sequence identity/similarity with the human FMRP, in this study we focus on the effects of cAMP levels in learning in drosophila dFMRP mutant heterozygots and in drosophila overexpressing the wild type dFMRP. Moreover, it is found that patients with a single point mutation in the *FMR1* gene in a conserved hydrophobic residue in the KH2 domain, exhibit a particularly severe Fragile X phenotype. Through mutations in KH1 (I244N substitution) and KH2 (I307N substitution) of dFMRP we research the learning processes of wild type flies when they overexpress mutated KH dFMRP domains. In this way we try to determine the importance of dFMRP function of RNA metabolism in the learning performance and the effect of this function in cAMP levels.

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STUDY OF THE ROLE OF NICASTRIN IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE IN A TRANSGENIC MOUSE MODEL OF THE DISEASE

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Amyloid β peptide (Aβ), responsible for the pathology of Alzheimer's disease (AD), is produced by the proteolytic processing of the amyloid precursor protein (APP) by γ-secretase. γ-secretase is a complex composed of four transmembrane proteins: the catalytic subunit Presenilin, Nicastrin, Aph-1, and Pen-2. In the present project we studied the role of Nicastrin, in the pathogenesis of Alzheimer's disease, via conditional inactivation of its gene in the mouse brain, using the Cre/loxP system. The conditional Nicastrin knock-out mice present intense neurodegeneration that leads to shrinkage of the volume of cortex and hippocampus (atrophy) with simultaneous considerable enlargement of lateral ventricles. Responding to the extensive neuronal death, a strong inflammatory reaction begins from brain astrocytes and microglia that are activated and increase numerically. To evaluate the role of Nicastrin in APP processing by γ-secretase, Aβ production and amyloid-related pathology, we crossed the conditional Nicastrin knock-out mice with the 5XFAD Alzheimer's disease transgenic mouse model. Our results indicate that Nicastrin knock-out 5XFAD mice produce Aβ, however in much lower amounts compared to 5XFAD mice of the same age. The produced Aβ forms aggregates that appear to be mainly intraneuronal and are probably found inside vesicles. In conclusion, we suppose that Nicastrin holds an important role in the pathological processing of APP while its lack strongly decreases the total levels of Aβ, limiting its production mainly inside neurons.

THE ROLE OF INTRINSIC EXCITABILITY IN MEMORY ALLOCATION

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Memory allocation is the process by which neurons are selected to encode a specific memory episode. Previous results have shown that increasing the levels of the transcription factor CREB using viral vectors in a subpopulation of neurons in the lateral amygdala increases the possibility that these neurons will encode a fear memory episode, during which an auditory stimulus is associated with a fearful event (i.e. electric shock) (Han *et al., Science* **316**, 457, 2007). Increased excitability could serve as a mechanism by which CREB might favor recruitment of neurons in the fear memory trace, since CREB-transfected neurons have increased levels of excitability (Zhou *et al., Nat Neurosci* **12**, 1438, 2009). Since CREB can bias and facilitate neuronal selection in a fear memory trace, we hypothesized that formation of a specific memory, which is known to increase CREB levels in a subset of neurons, would increase neuronal excitability and facilitate recruitment of the same neurons to a second memory trace, within a specific temporal window (because CREB increases for a specific time after memory formation). In order to test our hypothesis, we trained mice in auditory fear conditioning and performed patch-clamp recordings at different time points (1-3hrs or >4hrs) following training from amygdala pyramidal neurons. We measured properties of intrinsic excitability (input resistance, number of spikes, action potential properties) and found that neuronal excitability was increased 1-3 hours post-training and returned to baseline levels after 4hrs. In addition, we performed behavioral tests in order to test whether this increase in excitability could modulate memory performance on a different task, in this case conditioned taste aversion (CTA). We found that CTA training 3hrs (but not 5hrs) following initial fear conditioning training enhanced memory performance. Finally, we used a computational modeling approach that allows us to test possible mechanisms by which the increased excitability in pyramidal neurons could enhance memory, when a second training follows within a specific time window. Our model suggests that changes in excitability could modify the size of memory traces, which is also dependent on a balance of excitatory and inhibitory connection strength. Collectively, our results show that both neuronal excitability as well as population dynamics could interact to facilitate the process of memory allocation and enhance memory performance.

ROLE OF GEMININ IN SELF-RENEWAL AND DIFFERENTIATION OF ENTERIC NERVOUS SYSTEM PROGENITOR CELLS

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The enteric nervous system (ENS) is the largest and the most complex subdivision of the peripheral nervous system (PNS). The ENS is derived from the neural crest (NC), a migratory cell population that is generated along the axis of the neural tube shortly after neural tube closure. Three distinct NC populations give rise to the ENS: the vagal neural crest cells (somites 1-7) that gives rise to the majority of the ENS, the sacral NCCs (originating posterior to somite 28), and a subpopulation of anterior trunk NCCs (somites 6-7). In mice the colonization of the gastrointestinal tract by vagal NCCs starts at approximately embryonic day 9.5 (E9.5) and is complete by E14.5. 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 aim is to investigate how cell cycle control is integrated with signaling cues that promote differentiation in the developing ENS. Towards this direction we are studying the role of Geminin on the maintenance 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. Briefly, we have generated and analyzed mice that lack Geminin specifically in neural crest cells and our findings show that Geminin is necessary for survival and maintenance of neural crest cells. More specifically, the development of the ENS of mice that lack Geminin expression is defective, possibly due to the reduced ability to generate committed differentiated cells in the absence of Geminin.

THE EXPRESSION PATTERN OF SNCA ALLELES IN PARKINSON'S DISEASE PATIENTS, CARRIERS OF THE c.157G>A MUTATION

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The detection of a point mutation in the SNCA gene (MIM163890) in Parkinson disease (PD) patients, marked a turning point in the genetics of PD. This mutation was a c.157G4A base substitution (formerly reported as G209A) in the fourth exon of the SNCA gene, leading to p.Ala53Thr missense mutation. It was first identified in a large Italian PD family and three Greek PD families, over a decade ago, [Polymeropoulos et al., 1997] and led to the discovery of more missense mutations in the α-synuclein gene (SNCA) in PD, all in early onset PD families, accompanied by autosomal dominant inheritance. Following this, worldwide efforts have shown that genetic alterations in SNCA gene, including point mutations, gene multiplications and sequence variations, are of central importance in the pathophsysiology of Parkinson's disease (PD). It is considered that such alterations may be involved in PD pathogenesis through overexpression of α-synuclein protein leading to protein aggregation or through impaired expression of the functional gene. However, regulation of the SNCA wild-type and mutated gene is poorly understood.

We have recently shown that there is imbalance of allelic expression of SNCA gene in a PD patient carrying the c.157G>A mutation. In particular, the mutated allele in this patient is silenced epigenetically, while the wild-type allele is over-expressed, that is it is expressed to a higher level than in the steady-state condition of the two normal alleles combined, in a non-carrier matching control. The question that arises is to what extend this situation exists within the SNCA alleles, amongst c.157G>A mutation carrying patients.

We studied the expression levels of SNCA alleles in carriers of the c.157G>A mutation from two families (GR8 and GR15) presenting with autosomal dominant inheritance and early onset PD, originating from Achaia and Ilia. The aim of this study is to investigate the relative levels of expression of mutant versus the wild-type alleles, in these PD patients. Eight subjects which were carriers, heterozygous for the mutation from families GR8 and GR15 were included in this analysis. Of these, three are PD patients, four are non-patients, under the expected age of onset for the disease, while one individual is non-patient, over 80 years old. Four more subjects from the same families, non-carriers and non-patients, were used as matching controls. DNA and mRNA that was extracted from peripheral blood of the donors was used for the detection of the c.157G>A mutation, by PCR, RT-PCR and RFLP analysis after restriction enzyme Tsp45I digestion. Our results reveal that all carriers of the c.157G>A mutation express the mutated allele. In particular, the mRNA expression level is comparable between the mutated and the wild type allele, in any one heterozygous individual tested. This shows that the expression of the mutated allele of the SNCA gene is not the sole contributor in the development of the PD condition. Further work on the total SNCA expression in carriers and in normal controls is expected to clarify the point of possible of SNCA overexpression in these c.157G>A mutation carriers.

SYNERGY BETWEEN NR5A2 AND PROX1 PROMOTES NEUROGENESIS VERSUS ASTROGLIOGENESIS AND INHIBITS SELF-RENEWAL OF NEURAL STEM CELLS

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NR5A2/LRH-1 is an orphan nuclear receptor that has been associated with liver differentiation and function. It is essential for embryogenesis since knockout mice die at E6.5. Additionally, current evidence suggests that NR5A2 can replace Oct4 in the reprogramming of murine somatic cells to pluripotent cells. We have also recently shown that NR5A2 is involved in the Prox1-mediated suppression of Notch1 expression during neuronal differentiation. However, the physiological function of NR5A2 in nervous system remains elusive. To this end, we show here that NR5A2 is widely expressed in various regions throughout neuronal lineage, exhibiting higher levels in neurons than neural stem cells (NSCs), suggesting a correlation to neuronal differentiation. Accordingly, gain-of-function experiments in primary embryonic NSCs and Neuro2A neuroblastoma cells suggest that NR5A2 is sufficient to arrest proliferation and self renewal of NSCs, possibly via down-regulation of G1 cyclins, namely D1 and E1. Most important, NR5A2 misexpression in NSCs promotes neurogenesis at the expense of astrogliogenesis. In agreement, *in vivo* and *in vitro* expression studies showed that NR5A2 endogenous expression is totally excluded from mature astrocytes and limited to neuronal lineage. Mechanistically, NR5A2 is able to strongly induce Prox1, which directly binds to NR5A2 and acts as transcriptional co-repressor for this nuclear receptor. Furthermore, co-overexpression studies suggest that these transcription regulators act synergistically to promote neurogenesis and suppress astrogliogenesis. Collectively, these observations indicate an important function of NR5A2 in nervous system development. Moreover, the recent discovery of pharmacological agonists for NR5A2 renders it a candidate gene for applications of regenerative medicine.

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Peri-operative microdialysis: outcome prediction in brain tumor surgery

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Background: This study aimed in monitoring brain tissue metabolism in brain tumor surgery via microdialysis and in assessing whether putative peri-operative metabolic changes correlate with clinical outcome.
Materials and methods: 34 patients, ASA I-III, diagnosed with brain tumors were included in the present study, after written informed consent was obtained. Following induction of anesthesia, craniotomy was performed and a microdialysis catheter (CMA 70) was inserted into peritumoral tissue (<1cm from visible pathological tissue). Sampling started more than 1h after the insertion of catheters and continued at 30 min intervals during surgery and every 2 hours postoperatively for a period of 24h. Microdialysis samples were analyzed for glucose, pyruvate, lactate and glycerol. Patient's clinical outcome was assessed at the time of hospital discharge using a summarized Karnofsky scale (unfavourable: 0-70%, favourable: 80-100%). Data analysis was performed using student's t-tests, the Spearman correlation factor (r) and the Mann-Whitney test.
Results: Compared to patients with a favourable outcome, patients with an unfavourable outcome demonstrated significantly lower levels of glucose during the first 8h after surgery (1 ± 0.2 vs. 2.6 ± 1.3; $p=0.049$). Patients with an unfavourable outcome also demonstrated significantly elevated levels of lactate during hours 8-16 postoperatively (10.5 ± 1.5 vs. 5.7 ± 2.9; $p=0.036$). Similarly, patients with an unfavourable outcome were shown to have significantly higher lactate/pyruvate ratios throughout the first 24h, postoperatively ($p<0.001$). No correlation was found between changes in intra-operative values and patients' clinical outcome.
Conclusions: The present findings indicate that there is a correlation between a patient's clinical outcome and changes in metabolic conditions detected postoperatively in peritumoral tissue via microdialysis. Monitoring of such changes could thus allow the prevention of adverse events leading to patients' neurological deterioration. Collectively, the present findings indicate that monitoring of energy metabolites via intracerebral microdialysis may represent a valuable prognostic tool in brain tumor surgery.

CHOLESTEROL RECEPTORS MODIFY AMYLOID DEPOSITION BY REGULATING THE INFLAMMATORY RESPONSE

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Alzheimer's disease (AD) is a progressive neurodegenerative disease that impairs basic cognitive functions. Cholesterol has been implicated in AD pathogenesis and statins, agents that regulate cholesterol levels have been shown to exert a beneficial effect on AD. SR-BI and LDLR have been described as the HDL and the LDL-cholesterol receptors respectively, that regulate cholesterol homeostasis. Depletion of SR-BI or LDLR results in elevated cholesterol and atherosclerosis in transgenic mice. Our hypothesis is that cholesterol-related genes as SR-BI and LDLR are involved in AD pathogenesis. We used a genetic approach by deleting SR-BI and LDLR genes in AD mice to evaluate the effect on the amyloid phenotype. We examined the role of SR-BI in the development of AD and cerebral amyloid angiopathy (CAA) in a huAPP transgenic mouse. We showed that SR-BI regulates perivascular macrophages in the mouse brain. HuAPP/SR-BI$^{+/-}$ mice developed a significant increase in amyloid plaques and CAA in the brain and exacerbated learning and memory deficits. Our findings suggest that inactivation of a single SR-BI allele is sufficient to impair perivascular macrophages response to Aβ and enhance fibrillar amyloid deposition and CAA. To investigate the role of LDLR in the development of the amyloid related phenotype we used an APP/PS1 transgenic mouse that develops AD pathology with amyloid plaques, astrocytosis and microgliosis. We found that 4 months old APP/PS1/LDLR$^{-/-}$ mice have increased amyloid plaque deposition. This increase is associated with a significant decrease in astrocytosis and microgliosis. To further elucidate the role of LDLR in relation with ApoE we have generated APP/PS1/ApoE$^{-/-}$ and APP/PS1/LDLR$^{-/-}$/ApoE$^{-/-}$ mice. We have found that ApoE deletion in the 4 months old APP/PS1/ApoE$^{-/-}$ mice decreases amyloid plaque formation as expected, but has no effect on astrocytosis or microgliosis. By comparison APP/PS1/LDLR$^{-/-}$/ApoE$^{-/-}$ mice of the same age have increased amyloid deposition with decreased astrocytosis and microgliosis. Our analysis shows that SR-BI and LDLR regulate the immune response in the mouse brain of AD transgenic mice and modify the amyloid phenotype. These findings further establish the important role of SR-BI and LDLR in AD.

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ROLE OF HIPPO PATHWAY IN MALIGNANT MENINGIOMAS

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Meningiomas are the most frequently diagnosed primary brain tumor accounting for 33.8% of all central nervous system tumors (Wiemels et al. 2010). They are thought to originate from arachnoid cap cells. Most of them are benign (WHO Grade I), but 6-8% are atypical (Grade II) or anaplastic/malignant (Grade III) (Pham et al 2011). High grade (II, III) meningiomas can reveal more aggressive behavior, invasiveness in the surrounding tissues, and recurrence after resection.

The hippo pathway originally defined in *Drosophila Melanogaster,* is conserved in mammals and plays a critical role in cell proliferation and apoptosis (Zeng et al. 2008, Chan et al 2010). Inhibition of the hippo signaling pathway leads to translocation of YAP and TAZ transcription co-activators into the nucleus. This results in over expression of their target genes with anti-apoptotic, cell contact inhibition and proliferative function (Halder et al 2011). CD44 is the major cell-surface HA binding protein (Arufo et al 1990) and thought to play role in tumor invasion and metastasis (Ariza et al 1995). CD44 is connected with the Hippo pathway through merlin, the NF2 gene product (Striedinger et al 2008). So CD44 can be considered as an upstream molecule of the Hippo pathway.

In order to investigate the role of the Hippo signaling pathway in the malignant behavior of meningiomas, the expression of CD44 evaluated by immunohistochemistry on formalin-fixed paraffin-embedded tissue samples from 34 cases of human meningiomas. Nineteen (n=19) cases out of 34 were grade I and 15/34 (n=15) grade II or grade III. Cytoplasmic and/or membranous CD44 expression in the tumor cells was found in 13/15 (86.6%) of high grade tumors. Only 1/19 (5.2%) low grade meningiomas showed CD44 immunopositivity.

Our preliminary results indicate that increased expression of CD44 probably suggests inhibition of hippo pathway that may play a role in invasiveness of high grade meningioma. Additional studies are required in order to evaluate the precise role of hippo pathway in malignant meningiomas behavior.

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THE ABSENCE OF SMALL RhoGTPases LEADS TO DEFECTS IN CELL CYCLE EXIT AND MIGRATION OF CORTICAL INTERNEURONS

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The proper function of the CNS requires the correct connection between glutamatergic neurons and GABAergic interneurons. Cortical GABAergic interneurons are characterized by extraordinary neurochemical and functional diversity. Although recent studies have uncovered some of the molecular components underlying interneuron development, including the cellular and molecular mechanisms guiding their migration to the cortex, 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. We have addressed the specific role of Rac1 in interneuron progenitors originating in the medial ganglionic eminence, using Cre/loxP technology. Our analysis indicates that 50% of Rac1-deficient GABAergic interneurons originating in the MGE fail to migrate towards the cortex, due to an intrinsic defect. The progenitors of these cells show a delay in cell cycle exit. This is likely due to their inability to progress through the restriction point within the G1 phase of the cell cycle. 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. In addition, MGE cells grown in vitro, show cytoskeletal alterations such as a significant reduction in growth cone formation in the absence of Rac1 protein. We are currently characterizing the downstream effectors of Rac1 signaling in these cells. Additional studies are aimed at addressing the role of Rac3, the neuronal-specific Rac member. Our aim is to determine whether the phenotypes of double Rac1 and Rac3 mutants reflect qualitatively-distinct effects of these Rho GTPases or rather quantitative effects of their combined activities.

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A NEUROPHYSIOLOGICAL STUDY OF THE DEEP BRAIN STIMULATION EFFECT ON THE AUTONOMIC FUNCTION IN PARKINSON'S DISEASE

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Despite the extended scientific investigation on the motor impairment in Parkinson's disease (PD), several aspects of the autonomic dysfunction's pathophysiology remain to be cleared out. Deep Brain Stimulation of the Subthalamic Nucleus (STN-DBS) has been established as an effective treatment of the motor symptoms of PD, but its influence on the autonomic function is less well studied. The purpose of our study was to assess the impact of STN-DBS on the autonomic function in patients with advanced PD. Twenty-four patients with idiopathic PD (mean age±SD, 62.1±9.4 years old) were examined 3 days before and 6 months after DBS. Each neurophysiological examination session included a. recording of sympathetic skin response (SSR) from both palms and a sole and b. time domain analysis of RR interval variation during normal and deep breathing (Rest RR IV and DB RR IV respectively), during Valsalva manoeuvre (Valsalva ratio) and during tilt test (Tilt ratio). Subsequently, an off-line frequency domain analysis of heart rate variation was performed so that the Total Power, the Low Frequency band, the High Frequency band and their normalized units were estimated. These neurophysiological measurements were compared to those of 24 healthy controls matched for age and sex. There was no statistical difference between SSR measurements between patients before and after surgery. Six out of 24 pre DBS patients and 7 post DBS patients had abnormal or absent SSR (χ^2, p=0.114). No change was found in the post DBS values of Rest RR IV, DB RR IV, Valsalva ratio & Tilt ratio compared to the pre DBS values (p>0.050), whereas the postoperative measurements of the above parameters were significantly lower in the patients than in the controls (p<0.050). Spectral analysis showed a. significantly reduced LF in the patients after surgery than in the patients before DBS and b. significantly lower postoperative LF, TP and LF_{norm} in the patients than in the healthy controls. In conclusion, based on the neurophysiological assessment presented here, STN-DBS induced no effect on SSR measurements, as well as on the balance of sympathetic and parasympathetic function.

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EFFECT OF HYPOTHYROIDISM ON THE DOPAMINERGIC AND NORADRENERGIC SYSTEMS OF THE MOUSE BRAIN

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Thyroid hormones (T_3 and T_4) are widely distributed in the brain and have a multitude of effects on the central nervous system. Adequate thyroid function is essential for normal brain development and mental functioning in adulthood. It is known that the dopaminergic system of the basal ganglia plays a central role in most movement, cognitive and emotional functions. In addition, the noradrenergic innervation of the frontal cortex is essential for the performance of higher brain functions. In the present study, we examined the effect of hypothyroidism on both dopaminergic and noradrenergic systems in the striatum, substantia nigra and frontal cortex of mouse adult brain, in order to clarify the mode of action of thyroid hormones. For this purpose adult *balb c* mice were divided into two groups where the first was granted tap water while the second was granted $KClO_4$ 1% solution in tap water in order to become hypothyroid. Eight weeks later mice were euthanized, brains removed, sectioned in a cryostat and stored until they were processed for autoradiographic binding studies for D_2 dopamine receptors, dopamine (DAT) and norepinephrine transporters (NET) as well as for in situ hybridization studies for the mRNA of D_2 receptors. Autoradiography showed a statistically significant reduction in the levels of D_2 dopamine receptors in both striatum and substantia nigra of hypothyroid mice compared to the euthyroid. A significant decrease was also observed in the levels of DAT in striatum and NET in frontal cortex. In situ hybridization studies showed a statistically significant reduction in the mRNA levels of D_2 receptors in both striatum and substantia nigra of hypothyroid mice. Our results provide insight into the possible ways thyroid hormones interact with the dopaminergic and noradrenergic neurotransmitter systems and exert their effects in the central nervous system.

ADENOSINE/GLUTAMATE INTERACTIONS IN HIPPOCAMPUS INVOLVE PHOSPHORYLATION OF NMDAR AND ERK1/2 KINASES INDUCED BY mGluR5 RECEPTOR STIMULATION

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Hippocampal metabotropic glutamate 5 receptors (mGluR5), regulate both physiological and pathological responses to glutamate. Because mGluRs activation enhances NMDA-mediated effects and given the role played by NMDA receptors in synaptic plasticity and excitotoxicity, modulating mGluR5 may influence both the physiological and pathological effects elicited by NMDAR activation. Furthermore in Hippocampus mGluRs have been shown to interact with adenosine A2A receptors in regulating NMDA receptor currents.

In order to further understand the molecular basis of mGluR5/NMDA interactions, we have investigated in the present study the effect of the "in vitro" mGluR5 and Adenosine 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 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 results showed that when ZM 241385, a selective antagonist of A2A receptors, was co-administrated at the concentration of 1μM, abolished the CHPG evoked phosphorylation of NR2B subunit (tyr-1472) , bringing it to the basal levels.

In conclusion, the mGluR5 receptor mediated phosphorylation of NR2B subunit at tyr-1472, probably through Src kinases, might underlie the enhancement of mGluR5 receptor evoked currents of NMDA receptors, shown by electrophysiological studies. Furthermore, the fact that ZM 241385 totally abolished the CHPG-induced phosphorylation of NR2B (tyr 1472) subunit, indicates that in hippocampus Adenosine A2A receptors regulate mGluR5 function by permitting them to interact with NMDA receptor, thus enhancing their currents. 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.

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PHYTOCHEMICAL COMPOSITION OF " MOUNTAIN TEA" FROM *SIDERITIS CLANDESTINA* SUBSP. *CLANDESTINA* AND EVALUATION OF BEHAVIOURAL AND CEREBRAL BIOCHEMICAL PARAMETERS IN ADULT MICE FOLLOWING TEA CONSUMPTION

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The goal of this study was to evaluate the phytochemical composition of *Sideritis clandestina* subsp. *clandestina* tea, and study the effect of 6-week tea consumption on behavioural parameters, cerebral oxidant/antioxidant indices and activity of two acetylcholinesterase (AChE) isoforms in adult male mice. The phytochemical profile of the *Sideritis* tea was determined by liquid chromatography-UV diode array coupled to ion-trap mass spectrometry with electrospray ionization interface. Seventeen phenolic compounds have been tentatively identified in the tea by liquid chromatography. The identified compounds (17) were classified in the quinic acid, iridoids, phenylethanol glycosides and flavonoids natural product classes. The effects of *Sideritis* tea (2% & 4% w/v, daily) intake on anxiety-like state of the mice were assessed by elevated plus-maze test and thigmotaxis test. Our results showed that only the 4% Sideritis-treated group exhibited significantly decreased thigmotaxis time, while both treated groups showed increased time remaining in the open arms of the elevated plus-maze apparatus. Learning/memory ability, evaluated by step-through passive avoidance task, did not differ significantly among the animal groups. The oxidant/antioxidant status of brain regions after *Sideritis* tea consumption, was also studied via measurement of malondialdehyde (MDA) and reduced glutathione (GSH) levels with fluorometric assays. Consumption of both doses of *Sideritis* infusion caused a significant dose-dependent increase in GSH and decrease in MDA levels of cerebellum and midbrain, whereas cerebral cortex remained unaffected. Regarding the activity of the two isoforms of AChE [salt soluble (SS) and detergent soluble (DS) fractions], the intake of both tea doses inhibited AChE activity of total brain, cerebral cortex, hippocampus and striatum compared to the control untreated group. Conclusively, mountain tea consumption prevents anxiety-related behaviours, confers antioxidant protection and inhibits the activity of AChE in rodent tissues in a region-specific, dose-dependent manner and its phytochemical constituents are shown for the first time.

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ROLE OF THE JUXTAPARANODAL CELL ADHESION MOLECULE TAG-1 DURING 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 fiber, responsible for the rapid propagation of action potentials is severely impaired during the course of the disease. Consequently, the molecular organization of myelinated fibers is disrupted during the onset and the progression of MS. Our analysis is focused on the adhesion molecule TAG-1 (Cntn2), expressed both by axons and glial cells in the juxtaparanodes. We have previously shown that TAG-1 is responsible for the molecular organization of the juxtaparanodal domain, where it interacts with Caspr2 and voltage gated potassium channels. Moreover, the glial form of TAG-1 is sufficient to restore the phenotype of homozygous mutants which include the hypomyelination of the optic nerve. TAG-1 has also been identified as an autoantigen in MS patients and subsequent experiments in experimental autoimmune encephalomyelitis (EAE) animals have implicated it in white and grey matter pathology. We would like to test whether the *Tag-1* deficient mice as well as transgenic animals that express TAG-1 exclusively from oligodendrocytes are differentially affected upon de-and remyelination. Furthermore we would like to address the possible neuroprotective role of the glial form of TAG-1. For this reason we use the cuprizone model of toxic demyelination, an established animal model for the elucidation of MS pathology that excludes the involvement of the immune system. The specific model resembles type III and IV MS lesions where oligodendrocyte death occurs. In the context of our experimental analysis, we subjected *Tag-1* $^{+/-}$, *Tag-1* $^{-/-}$ and transgenic animals expressing TAG-1 only in oligodendrocytes (*Tag-1*$^{-/-}$*; plp*$^{Tg(rTag-1)}$) to a 6 week toxin treatment. This is the time period needed for the reversible demyelination of the CNS corpus callosum. Via immunohistochemical analysis in both brain and cerebellum cryosections we examined the levels of demyelination, astrogliosis and oligodendrocyte population using specific markers (MBP, PLP, Olig2, GFAP). All genotypes exhibit demyelinating lesions and increased astrogliosis in the lesion areas after 6 weeks of treatment. *Tag-1* $^{-/-}$ animals display more severe demyelinating phenotype than Tag-*1*$^{-/-}$*; plp*$^{Tg(rTag-1)}$ animals. Moreover, the oligodendrocyte population shows greater reduction in the corpus callosum of *Tag-1* $^{-/-}$ than of *Tag-1* $^{+/-}$ and *Tag-1*$^{-/-}$*; plp*$^{Tg(rTag-1)}$ mice. The analysis is still in progress and is expected to provide a detailed characterization of the role of TAG-1, especially the glial form, during the de- and re-myelination processes.

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