ABSTRACT BOOK

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Oral Presentations

1. A novel role for Corticotropin Releasing Hormone (CRH) in the regulation of neurogenesis

Yassemi Koutmani[a], Panagiotis K. Politis[b], Maximillianos Elkouris[c], George Agrogiannis[d], Maria Kemerli[d], Efstratios Patsouris[d], Eumorphia Remboutsika[c], Katia P. Karalis[a,e,1]

Departments of [a]Developmental Biology and [b] Center for Basic Research, Biomedical Research Foundation of the Academy of Athens, 115 27 Athens, Greece; [c] Department of Stem Cell Biology Laboratory, Biomedical Sciences Research Center "Alexander Fleming", 166 72 Vari-Attika, Greece; [d] 1st Department of Pathology, Medical School, University of Athens, 152 34 Athens, Greece; [e] Division of Endocrinology, Children's Hospital, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115

Neurogenesis during embryonic and adult life is tightly regulated by a network of transcriptional, growth and hormonal factors. Emerging evidence indicates that activation of the stress response, via the associated glucocorticoid increase, reduces neurogenesis and contributes to the development of adult diseases. Since Corticotropin Releasing Hormone or Factor (CRH/CRF) is the major mediator of adaptive response to stressors, we sought to investigate its involvement in this process. Accordingly, we found that CRH could reverse the damaging effects of glucocorticoid on neural stem/progenitor cells (NS/PCs), while its genetic deficiency results in compromised proliferation and enhanced apoptosis during neurogenesis. Analyses in fetal and adult mouse brain revealed significant expression of CRH receptors in proliferating neuronal progenitors. Furthermore, by using primary cultures of NS/PCs, we characterized the molecular mechanisms and identified CRH-R1 as the receptor mediating the neuroprotective effects of CRH. Finally, we demonstrate expression of CRH receptors in human fetal brain from early gestational age, in areas of active neuronal proliferation. These observations raise the intriguing possibility for CRH-mediated pharmacological applications in diseases characterized by altered neuronal homeostasis including depression, dementia, neurodegenerative diseases, brain traumas and obesity.

2. Protein-protein interactions between the cell cycle exit and neuronal differentiation protein BM88/Cend1, Ran-binding protein RanBPM and dual-specificity kinase Dyrk1B regulate cyclin D1 levels and cell cycle progression/exit in mouse neuroblastoma cells.

Konstantinos Tsioras, Florentia Papastefanaki, Rebecca Matsas and Maria Gaitanou

Laboratory of Cellular and Molecular Neurobiology, Hellenic Pasteur Institute, 127 Vassilissis Sofias Avenue, 11521 Athens, Greece.

BM88/Cend1 is a neuronal-lineage specific modulator implicated in coordination of cell cycle exit and neuronal differentiation of neural stem/precursor cells. In the current study we identified the signal transduction scaffolding protein RanBPM as a BM88/Cend1 binding partner. Association of BM88/Cend1 with RanBPM was confirmed by glutathione S-transferase pull down assays and co-immunoprecipitation experiments. RanBPM has been implicated in cell cycle progression of neuronal precursors, via an as yet unkown mechanism, while it has also been identified as a Dyrk1B kinase-binding protein in lung epithelial cells. In turn, Dyrk1B has been shown to target directly cyclin D1 in these cells thus triggering its nuclear export and subsequent proteasomal degradation. Here we showed that BM88/Cend1, RanBPM and Dyrk1B are expressed in mouse brain and can form complexes, *in vivo*. To elucidate a potential mechanism involving BM88/Cend1, RanBPM and Dyrk1B in cell cycle progression/exit, we transiently co-expressed these proteins in mouse neuroblastoma Neuro 2a cells. We found that the BM88/Cend1-dependent or Dyrk1B-dependent down-regulation of cyclin D1 is reversed following their interaction with RanBPM. More specifically, binding of RanBPM with either BM88/Cend1 or Dyrk1B stabilizes cyclin D1 in the nucleus and enhances cellular proliferation. However, when all three proteins are co-expressed Dyrk1B is rescued in the nucleus to target cyclin D1 and exert its antiproliferative function. Further, co-expression of RanBPM with either BM88/Cend1 or Dyrk1B also had a negative effect on Neuro 2a cell differentiation in the presence of retinoic acid as compared with cells expressing each protein separately. In order to examine if the protein interactions between Cend1, Dyrk1B and RanBPM observed in Neuro 2a cells have relevance *in vivo*, we analyzed the expression profile of these molecules in wild-type and Cend1 null mice at different postnatal ages. We found that in wild type mice Cend1 and Dyrk1B expression is strongly up-regulated with neuronal differentiation while RanBPM does not show significant variation. On the other hand, Dyrk1A is predominantly expressed in the embryonic brain and up to postnatal day 10, being significantly down-regulated thereafter. Interestingly in Cend1 null mice, which exhibit increased proliferation of progenitor cells associated with elevated cyclin D1 levels and impaired neuronal differentiation Dyrk1B expression is reduced as compared with wild-type mice of corresponding ages. This suggests that Cend1 contributes to maintaining physiological levels of Dyrk1B, in accordance with our findings in Neuro 2a cells. Our results suggest a novel regulatory mechanism involving Cend1, RanBPM and Dyrk1B which may be operative in neuronal precursors to control the balance between cellular proliferation and neuronal differentiation.

3. Synergistic action of the neurogenic molecules Cend1 and Neurogenin-2 in directing astrocytic reprogramming

Aravantinou Fatorou A.[1], Matsas R.[1], Berninger B.[2,3] and Thomaidou D.[1]

1-Hellenic Pasteur Institute, Athens, Greece; 2-Ludwig-Maximillians University, Munich, Germany; 3-Mainz University, Physiological Chemistry Department, Germany

Reprogramming resident astroglial cells in vitro towards neurogenesis and formation of functional, synapse-forming neurons (Heinrich et al., 2010) has been a great advance in regenerative medicine. Recent studies demonstrate that besides the well-documented neural stem cell properties of astrocytic populations of the two neurogenic regions of the adult brain (Doetsch et al., 2003), namely the subventricular zone (Doetsch et al., 1999) and the subgranular zone of the hippocampus (Seri et al., 2001), astroglial cells isolated from non-neurogenic brain regions have the potential to be reprogrammed into functional neurons through forced expression of specific transcription factors known to instruct neurogenesis during embryonic development (Heinrich et al., 2010; Heinrich et al., 2011). Based on our previous studies on the potential of the neurogenic gene Cend1 in directing neural stem/precursor cells to exit the cell cycle and acquire a neuronal phenotype, in parallel with evidence demonstrating direct activation of Cend1 expression by the bHLH proneural genes of the neurogenin family, we aimed to explore their combined effect on the proliferation and differentiation properties of postnatal cortical astrocytes. To this end, forced expression of either Cend1, Neurogenin-2 or both, resulted in an important increase of two subpopulations of morphologically distinct GFAP(-) cells with elongated morphology that strongly expressed the radial glial marker Glast 24h, 48h and 72h following transduction. More specifically, Cend1-overexpressing radial glia cells were bipolar, elongated cells, while Ngn2 overexpressing radial glia cells were mostly triangular "kite" like cells. A parallel decrease in the number of GFAP(+) astrocytes overexpressing both Cend1 and Ngn2 was noted. Further characterization revealed a subpopulation of cells differentiating towards the neuronal lineage, as they were exhibiting a differentiated neuronal morphology and expressed β-III tubulin(+), as well as neuronal subtype-specific markers, including GABA and TH. Surprisingly, only in the double-transduced cultures, colonies of small round Glast(+)/Nestin(+) cells were detected after 24h, which, a day later, formed three-dimensional spheres of high proliferative potential attached to the culture dish. When these 'astrospheres' were isolated and cultured under NSC conditions, they grew as neurospheres expanding in culture for over ten passages. Importantly, when astrospheres were cultured in the absence of growth factors, they differentiated into neurons, astrocytes and oligodendrocytes, implying that they have a pluripotent potential similar to that of the neural stem/progenitor cells. Moreover, studies using live cell imaging for longer time periods were performed to further investigate the proliferation and differentiation potential of these cells, as well as the combined role of Cend1 and Ngn2 on astrocytic reprogramming.

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4. Analysis of P Bodies in *C. elegans* reveals their pivotal role in neuronal cells implicated in several aspects of organismal physiology

Fivos Borbolis, Aris Rousakis, Anna Vlanti, Fani Roumelioti and Popi Syntichaki

Molecular Genetics of Aging Lab, Center of Basic Research II, Division of Genetics and Gene Therapy, Biomedical Research Foundation of the Academy of Athens, 115 27 Athens, Greece

Many studies have revealed the key role of specific cytoplasmic mRNP particles in the regulation of mRNA metabolism and gene expression in all eukaryotes. P Bodies are a distinct class of such mRNPs that exist in all eukaryotic cells and possess a key role in mRNA metabolism, by being involved in processes like mRNA degradation and surveillance, gene silencing and translational repression. Several components of PBs have also been observed in neuronal RNA granules, presumably regulating local mRNA metabolism at the synapses. Using *C. elegans* as a model organism we demonstrated that disruption of P Bodies' integrity and function has a negative impact on longevity, which is independent of developmental defects. Tissue specific studies revealed different levels of contribution for each tissue in lifespan determination and in often related processes, like fertility and stress resistance. Since the nervous tissue was identified as the major determinant, we carried out a preliminary attempt to identify the physiological mechanisms that underlie the relationship between mRNA metabolism and aging in the context of a whole organism

5. Differential role of neurofibromin isoforms during synaptogenesis

Sophia Karouzaki, Spyros Theofilopoulos, Emmanouella Tsirimonaki and Dimitra Mangoura

Centre of Preventive Medicine, Neurosciences and Social Psychiatry, Biomedical Research Foundation, Academy of Athens, Soranou Efesiou 4, 11527 Athens

Neurofibromin, a large mobile protein abundantly expressed in the CNS, is the product of the *Nf1* gene, mutaions of which cause the disease Neurofibromatosis. It is a Ras-GAP through its central domain termed GRD, and thus Ras hyperactivation has been thought as the underlying mechanism for the mental retardation, seen with large deletions of the gene. *Nf1* transcripts in the brain exist as GRDI and with the inclusion of exon 23a, as GRDII; the latter type is associated with significantly lesser RasGAP activity. In addition, we have identified and shown that neurofibromin contains a functional nuclear localization sequence (NLS) at its C-terminal helical domain, which is transcribed only in tissues involved in neurofibromatosis. Therefore to gain insights on the role of neurofibromin isoforms in neuronal differentiation we addressed the expression of GRDI, II, and NLS and resulting functional aspects. In a series of developmental studies we found that both mRNA and protein expression of neurofibromin gradually increases in all brain regions, albeit with different timetables. In every region, however, the onset of neuronal differentiation of early postmitotic neurons was marked by a peak in protein expression and switches in transcript expression. Specifically, GRDI transcripts become predominant over type II, while mRNA levels containing NLS decreased with differentiation as ΔNLS transcripts increased dramatically, possible indicating expression of neurofibromin variants with strong RasGAP activity and lesser nuclear availability for differentiation and synaptogenesis. Moreover, co-immunoprecipitations analyses of tissues (a) that predominantly expressed GRDI or GRDII showed that neurofibromin with GRDII had a higher affinity for actin than tubulin, while the opposite was true for type GRDI; (b) neurofibromin from stages expressing ΔNLS transcripts was poorly detected in post synaptic densities (PSDs), while in more mature tissues co-immunoprecipitated with Neuroligin 3 (NRL3), a protein that is enriched in PSDs and involved in cognition. To further evaluate this apparent tight regulation of neurofibromin isoforms embryonic development, we employed an established model of neuronal differentiation, namely treatment of SHSY-5Y neuroblastoma cells with retinoic acid for 72 hours. We found that RA, while increasing neuritic outgrowth and arborization, decreased and almost abolished the expression of GRDII and NLS transcripts and, more importantly, that only in differentiated, ΔNLS expressing cells neurofibromin co-immunoprecipitated with NRL3. Taken together, these data provide novel insights on a possible dominant negative effect of GRDI over GRDII and NLS transcript expression, suggest a possible mechanism for the differential localization and mobility of the protein during neuronal differentiation, and emphasize the role of neurfibromin in synapse formation and function.

6. Effect of enriched environment on tissue histamine levels in the visual system

Dimitrios P. Bessinis, Christina Dalla, C. Dioli, Maria Koronaiou, Zeta Papadopoulou Daifoti and Ekaterini Tiligada

Department of Pharmacology, Medical School, University of Athens, 11527 Athens, Greece

The purpose of the present study was to evaluate the effect of the environment in the histamine levels of the optic chiasm and the primary visual cortex in rats. Male and female Wistar rats, reared in standard laboratory or enriched environmental cages from birth were sacrificed during the critical period for visual development in pre-puberty at postnatal day 25 (p25) or in adulthood at p90. Additional groups of animals were born in standard conditions, exposed to enriched environment at p90 and sacrificed at p150. The optic chiasm and the visual cortex were dissected out and tissue histamine was quantified fluorophotometrically. The basal levels of histamine were higher in the optic chiasm of males in all ages studied. In the visual cortex significant sex differences were identified only in pre-puberty, where the histamine levels of males were higher than females. When environmental enrichment was introduced from birth, histamine levels decreased at p25 in the optic chiasm, whereas prolongation of enrichment to adulthood did not induce significant alterations in the tissue histamine levels. On the contrary, in animals housed in standard laboratory cages a decrease in the amine content of the optic chiasm was observed at adulthood. Increased amine levels were detected only in the optic chiasm of female rats exposed to enriched environment during adulthood. This study is the first to demonstrate the connection between histamine and the environment in the visual system and suggests that histamine may play a more dynamic role in the development and function of the visual system than previously considered believed[1, 2].

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7. Generation of patient-specific induced pluripotent stem cells for modeling Parkinson's disease

Georgia Kouroupi[1], Delphine Bohl[2], Gregoire Bisch[2], Kostas Vekrellis[3], Leonidas Stefanis[3,4], Jean-Michel Heard[2], Rebecca Matsas[1]

[1]Laboratory of Cellular and Molecular Neurobiology, Hellenic Pasteur Institute, 127 Vas. Sofias avenue, 11521 Athens, Greece ;[2]Unité Rétrovirus et Transfert Génétique, INSERM U622, Department of Neuroscience, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris cedex 15, France; [3]Division of Basic Neurosciences, Biomedical Research Foundation of the Academy of Athens, 4 Soranou Efesiou street, 11527 Athens, Greece ; [4] Second Department of Neurology, University of Athens Medical School, Athens, Greece

Stem cells offer a remarkable potential for studying human development, disease modeling and may have important implications in Regenerative Medicine, especially for cellular therapies of neurodegenerative diseases. Such prospects have been reinforced since the discovery that transgene expression of up to four defined factors is sufficient to reprogram adult somatic cells to a pluripotent stem cell-like state. The resulting induced pluripotent stem (iPS) cells resemble embryonic stem cells in their properties and potential to differentiate into a spectrum of adult cell types. Here we report the generation of iPS cells from skin fibroblasts of Parkinsonian patients with a genetic form of the disease first identified in the Greek population, and aged-matched unaffected individuals using the reprogramming technology. Parkinson disease (PD) is a common neurodegenerative disorder, characterised by progressive loss of midbrain dopaminergic neurons. Human fibroblasts from healthy individuals and PD patients carrying the A53T mutation in alpha synuclein were obtained from punch skin biopsies, following their informed consent. iPS clones were derived by co-transduction of human fibroblasts with retroviral vectors expressing the human cDNAs for the reprogramming factors *OCT4*, *SOX2*, *KLF4* and *C-MYC*, sub-cloned into the murine leukemia viral vector pMXs as previously described [1]. A cellular PD model is being created by directing these cells to differentiate into dopaminergic neurons, the cell type destroyed in Parkinson's disease (PD), for studying PD pathogenesis and discovering and testing new drugs and therapies.

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8. Spikes, LFP, and Spatiotemporal Response Fields

John S. Pezaris.

Massachusetts General Hospital / Harvard Medical School, Boston, Massachusetts, USA

Spike trains have long been used to determine the response characteristics of individual neurons through reverse-correlation mapping techniques, such as spike-triggered averaging, to create spatio-temporal response fields (STRFs). We describe how these techniques can be generalized from using spike trains as the input signal to using continuous-valued recordings such as the wideband counterpart to the LFP, that we call the WFP (Wideband Field Potential). Examining signals from two separate brain areas, we find the resulting spatio-temporal response fields reflect a robust signal within the WFP that carries more information than sorted spike trains from the same recording.

First, primate motor (MI) and dorsal pre-motor (PMd) cortex recordings made in the Hatsopoulos laboratory at the University of Chicago during a random reach task are examined. We find stronger evidence for gesture-based tuning than for velocity or acceleration tuning. Additionally, by tuning the WFP filters to different bands, we find apparently independent information streams in different frequency channels. Continuous signal response fields tend to have higher signal-to-noise than spike-based fields, but not overwhelmingly so.

Then, primate thalamic lateral geniculate nucleus (LGN) recordings made during a visual mapping task are examined. Again, we find clear evidence for strong response fields computed with WFP that have substantially higher signal-to-noise (SNR) than those from spike trains from the same recordings. Tantalizing hints were found for different response channels in different WFP frequency bands, although not as strong as for motor cortex.

Taking the relative strengths of the WFP and spike-based STRFs to be an indicator of the level of local similarity within an area, these findings suggest that there are differences in encoding locality and redundancy from one cell to the next in the two brain areas presented. Specifically, LGN carries much more encoding redundancy for information contained in the mapping task than the motor cortex does in the random reach task.

9. Serotonergic receptors' mRNA modulation by clomipramine treatment in the chronic mild stress model of depression: sexual dimorphism exposed

Pothitos M. Pitychoutis[1], Christina Dalla[1], Antonios C. Sideris[1], Panagiotis A. Tsonis[2] and Zeta Papadopoulou-Daifoti[1]

[1]Department of Pharmacology, Medical School, National & Kapodistrian University of Athens, 75 Mikras Asias Street, Goudi, 115 27, Athens, Greece ;[2]Center for Tissue Regeneration and Engineering and Department of Biology, University of Dayton, Dayton, OH, 45469-2320

It is well established that women experience major depression at roughly twice the rate of men. Clinical and experimental evidence show that the responsiveness of males and females to antidepressant pharmacotherapy, and particularly to tricyclic antidepressants (TCAs), is sexually dimorphic. Herein, we sought to investigate whether chronic treatment with the TCA clomipramine may affect serotonergic receptors' (5-HTRs) mRNA expression in a sex-dependent manner in the chronic mild stress (CMS) model of depression. Male and female rats were subjected to CMS for 4 weeks and during the next 4 weeks they concurrently received clomipramine treatment (10 mg/mL/kg). CMS and clomipramine's effects on 5-HT_{1A}R, 5-HT_{2A}R, and 5-HT_{2C}R mRNA expression were assessed by *in situ* hybridization histochemistry in selected subfields of the hippocampus and in the lateral orbitofrontal cortex (OFC), two regions implicated in the pathophysiology of major depression. CMS and clomipramine treatment induced sex-differentiated effects on rats' hedonic *status* and enhanced 5-HT_{1A}R mRNA expression in the *cornu ammonis* 1 (CA1) hippocampal region of male rats. Additionally, CMS attenuated 5-HT_{1A}R mRNA expression in the OFC of male rats and clomipramine reversed this effect. Moreover, 5-HT_{2A}R mRNA levels in the OFC were enhanced in females but decreased in males, while clomipramine reversed this effect only in females. CMS increased 5-HT_{2C}R mRNA expression in the CA4 region of both sexes and this effect was attenuated by clomipramine. Present data exposed that both CMS and clomipramine treatment may induce sex-differentiated and region-distinctive effects on 5-HTRs mRNA expression and further implicate the serotonergic system in the manifestation of sexually dimorphic neurobehavioral responses to stress.

10. Novel role for AD-related TAU protein – a link to affective disorders

Sotiropoulos I. [1,2], **Dalla C.** [1], **Daifoti Z.** [1], **Almeida O.F.X.** [3], **Sousa N.** [2]

1-Department of Pharmacology, Medical School, University of Athens, Greece ;2-ICVS/3Bs Associate Laboratory, Life and Health Sciences School, Portugal ;3-NeuroAdaptations Group, Max Planck Institute of Psychiatry, Germany

Improvements in lifespan over the last decades have, unfortunately, not been matched by improvements in the mental health span. W.H.O. estimates that the leading cause of mental disability in the coming years will be depression and Alzheimer's disease (AD), raising them as significant public health problem. Research studies focused on risk factors show a causal role of environmental parameters such as stress in pathogenesis of depression, while recent findings implicate stress in AD pathology. Furthermore, accumulating evidence has suggested a common neurobiological basis for depression and AD, with stress as "connecting factor". Stress and glucocorticoids (GC) are known to influence neuronal plasticity and brain function by inducing neuronal atrophy and/or dysfunction as well as cognitive and emotional deficits. Recent studies have shown that stress and GC trigger the two major pathogenic pathways of AD, TAU hyperphosphorylation and APP misprocessing towards the generation of Aβ. Based on the above, and the suggested common neurobiological basis of depression and AD, we hypothesize that TAU and Aβ may lie at the core of stress/GC actions towards brain pathology; thus, TAU hyperphosphorylation and APP misprocessing appear to be critical mechanisms through which stress and GC exert their detrimental effects upon the substrates of cognition, mood and emotion. Findings of this project implicate the involvement of TAU in stress-induced neuronal dysfunction, neurochemical and behavioral deficits possibly involved in anxiety and depression.

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11. An intact prefrontal cortex - hippocampal circuit is required for the expression of "depressive" behavior in male and female rats

V. Kafetzopoulos[1], C. Dalla[1], N. Kokras[1,2], A. Vasalou[1], and Z. Papadopoulou-Daifoti[1]

1. Department of Pharmacology, Medical School, University of Athens, Greece ; 2. First Department of Psychiatry, Eginition Hospital, Medical School, University of Athens, Greece

Depression is twice as much prevalent in women as in men, with important diversification in clinical symptomatology, outcome and response to treatment. The prefrontal cortex (PFC) and the hippocampus are involved in the pathophysiology of depression and in antidepressant response. However, the contribution of the circuit formed by their connection is not clear. Hippocampus is directly connected to the PFC, whereas the reverse connection relies mainly on a relay thalamus nucleus, the *nucleus reuniens* (RE) [1]. By lesioning the RE, the communication between the PFC and the hippocampus is partially disrupted, leaving those two regions otherwise intact. The Forced Swim Test (FST) is a widely used behavioral paradigm, which can identify treatments and manipulations with antidepressant potential. Immobility behavior during FST has been previously equated with "despair" in humans and is decreased by antidepressant treatment [2]. The present study aims to elaborate in both sexes on the contribution of the PFC-hippocampus circuit in the FST stressful experience, and in the behavioral effects of antidepressant treatment. Adult male and female Wistar rats weighting 250-300g received an excitotoxic RE lesion with 20% w/v NMDA solution, or a sham-operation. After recovery, rats were tested in the open field and 24h later in the modified forced swim test (FST). All rats were subjected to a 15min pre-test swim session and 24h later to a second 5min FST session. Duration of immobility, swimming and climbing behaviors was recorded. Half of the rats received a standard subacute treatment of sertraline (10mg/kg, 3 i.p. injections between the FST sessions), while the rest received vehicle. Female rat estrous cycle was evaluated daily via vaginal smears and all females underwent the FST test in the diestrous II cycle phase. RE-lesioned rats of both sexes had lower *immobility* duration than sham-operated rats. Sertraline treatment reduced immobility duration in both male and female sham-operated rats. The sertraline effect was substantiated only in female RE-lesioned rats, but not in males. RE-lesion enhanced *swimming* duration in both sexes. In males, sertraline enhanced swimming duration in sham-operated rats, but not in RE-lesioned animals. In contrast, sertraline treatment enhanced swimming duration in both sham and RE-lesioned female rats. *Climbing* duration was unaffected by RE-lesion and sertraline treatment. Our results show that the integrity of the PFC-hippocampus circuit is necessary in both sexes for the expression of passive behavior in the FST. Furthermore, present sertraline results imply that SSRI treatment mediates a shift in coping strategy similar to the one induced by the inhibition of the communication between PFC and hippocampus. Of interest is the divergent response of RE-lesioned males and females to the SSRI treatment. As previously described [3], such divergent response can be attributed to the sex-differentiated pre-treatment baseline. Thus, our results further indicate that disrupting the PFC-hippocampus circuit also affects the sex-differentiated response to SSRI treatment.

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12. Crocins, the active constituents of *Crocus sativus L,*. antagonized psychotomimetic effects produced by the NMDA receptor antagonist ketamine in rats

G. Georgiadou, V. Grivas[a], P.A. Tarantilis[b], N. Pitsikas[a]

[a] Department of Pharmacology, Medical School, University of Thessaly, Larissa, Greece ;[b] Laboratory of Chemistry, Department of Science, Agricultural University of Athens, Athens, Greece

Crocus Sativus L. is a plant cultivated in various parts of the world including Greece. Crocins are among the active components of this plant. Clinical findings revealed that *Crocus Sativus* L. and its extracts possess antidepressant properties, whereas preclinical research suggested their involvement in various neural processes including anxiety and memory. There is experimental evidence that the non-competitive NMDA receptor antagonist ketamine impairs cognition and produces psychotomimetic effects in rodents. The aim of the present study was to investigate the efficacy of crocins in antagonizing these ketamine-induced psychotomimetic effects in rats. Crocins (15 and 30 mg/kg) reversed ketamine (3 mg/kg)-induced recognition memory deficits. In addition, crocins (50 mg/kg) counteracted hypermotility, ataxia and stereotypies produced by ketamine (25 mg/kg). Finally, crocins (50 mg/kg) attenuated ketamine (8 mg/kg)-induced reduction of social interaction. Our findings indicate that crocins attenuate behavioural effects related to the hypofunction of the NMDA receptor proposing thus, a potential role for these compounds in the treatment of schizophrenia.

13. The role of β2 subunit of nAChR in cognitive functions and species-specific behaviour with aging

Eleni Konsolaki and Irini Skaliora

Neurophysiology Laboratory, Developmental Biology Division, Biomedical Research Foundation of the Academy of Athens, Greece

Mice lacking the β2 subunit (β2-/-) of the nicotinic acetylcholine receptor (nAChR) have been put forward as a model of attention–deficit/hyperactivity disorder and a possible model of accelerated cognitive aging. Furthermore, using these knockout mice it has been shown that the β2 subunit in medial prefrontal cortex controls higher cognitive functions such as attention. However, little is known about the role of this subunit in other frontal brain regions such as cingulate cortex, or about the effect of aging in higher cognitive functions. Moreover, species-typical behaviours in the form of activities of daily living (ADL) have not been reported in β2-/- mice. In the present study we studied the performance of these mice in one-hour open-field, novel object recognition test and Morris water maze task. In addition, we assessed species-typical behaviours, including burrowing, hoarding, nesting, and marble burying. In parallel, we examined the 3D morphology of pyramidal neurons in layer V in cingulate and visual cortex in adult and old β2-/- and wildtype mice using confocal microscopy. Analysis of neuronal and dendritic parameters revealed apparent deficits in cingulate cortex in β2-/- compared to wildtype mice. The results indicate that the lack of the β2-subunit may contribute to absence of habituation and lead to impairments in object recognition and reversal learning. Moreover, the marked reduction in burrowing and nest construction, in the absence of any detectable impairment of motor ability, reveal an additional role of the β2 subunit in these behaviours.

14. Social transmission of fear: the effect of *β*2 nAChR subunit on fear conditioning by-proxy

Zena Chalkea[1,2], Alexia Polissidis[1], Eleni Konsolaki[1], Mirto Panopoulou[1] and Irini Skaliora[1]

1. Neurophysiology Laboratory, Biomedical Research Foundation of the Academy of Athens ; 2. Basic and Applied Cognitive Science, Department of Philosophy and History of Science, University of Athens

How is information about indirect danger acquired and which factors determine its effect on subsequent behaviour? Applying models of observational fear learning to genetically modified rodents would facilitate the study of the neural mechanisms underlying the social transmission of fear-related information. Here, we examined fear conditioning by-proxy (FCbP) in animals lacking the β2 subunit of the nicotinic Acetylcholine receptor (nAChR). It has been suggested that these animals have deficits in executive functions such as conflict resolution and social interaction. Previous work in our lab had indicated specific morphological deficits in cingulate cortex, a brain region that has been associated with observational fear learning and processing of social information.

Naïve C57BL_6 and $\beta 2^{-/-}$ mice were exposed to a previously fear-conditioned cage mate during the presentation of the conditioned stimulus (tone; Day 2). On the following day both groups (FC and FCbP) were tested for fear reactions to both tone and context (Day 3). In addition, we assessed the contribution of several factors to the estimated fear response, such as the type and duration of social interaction between the two animals, and the amount of freezing displayed by each one during the second day.

Statistical analysis revealed no differences in fear conditioning or amount of social interaction between $\beta 2^{-/-}$ and wild type C57BL_6 mice. Although FCbP animals of both genotypes displayed no freezing to context, they showed significant differences in cued-fear: $\beta 2^{-/-}$ mice did not freeze to the stimulus, while one in three wildtype mice expressed cued fear. Interestingly, only mice that exhibited high social interaction with the FC animal during tone presentation (Day 2) expressed fear to the stimulus (Day 3).

These results suggest that (i) mice are able to acquire information about possible danger indirectly through social interaction; (ii) the efficiency of social transmission of fear depends on the amount of interaction between animals during cue presentation; and (iii) $\beta 2^{-/-}$ mice are unable to acquire such information, suggesting that executive functions are influenced by the absence of β2 subunit-containing receptors.

15. The Influence of the rs1358278A/G *FOXP2* Polymorphism on Gating, Cognition, Language/Thought and Affect in Healthy Males.

Manolis Pasparakis[1], Erasmia Koiliari[1], Stella SG Giakoumaki[2], PanosRoussos[1,3], Panos Bitsios[1].

1Department of Psychiatry and Behavioral Sciences, University of Crete, Heraklion, Crete, Greece; 2 Department of Psychology, University of Crete, Heraklion, Crete, Greece; 3Department of Psychiatry, Mount Sinai School of Medicine, New York, New York, United States

Introduction: Forkhead box P2 (*FOXP2*) encodes a transcription factor involved in speech and language and in the control of the corticobasal ganglia circuits (1) . There is also evidence supporting a role for the *FOXP2* locus in schizophrenia (2 , 4) , autism (1) and ADHD (3). Language impairment, inattention, impulsivity and abnormalities of corticobasal ganglia circuitry are central features of these disorders; however the impact of *FOXP2* risk polymorphisms on relevant intermediate phenotypes has not yet been studied. Here we selected the rs1358278A/G *FOXP2* non-coding polymorphism which has been associated with schizophrenia (4) .

Methodology: This polymorphism was analyzed in 829 healthy males, phenotyped for prepulse inhibition (PPI), cognition, schizotypy, emotional personality traits and affective startle modulation. Subjects were grouped according to genotype in three groups AA (n=437), AG (n=322) and GG (n=70) and ANOVAs or Kruskal-Wallis tests were used to analyse the phenotypic variables.

Results: The GG homozygotes had more Within- and Double-Errors in a Spatial Working Memory task ($p<0.001$) especially in the most difficult conditions ($p<0.001$) (trend $p=0.08$ for Between-Errors), an abnormal pattern in the Affective Startle paradigm (did not suppress startle in the pleasant picture viewing condition $p<0.05$), scored higher in Alexithymia (Difficulty Identifying Feelings $p<0.05$) and, interestingly, they showed an alexithymic profile in the Iowa Gambling Task in the absence of significant differences in this task.

Conclusions: The rs1358278G which was previously shown to be part of a risk haplotype for schizophrenia and was associated with speech incoherence in patients, shows evidence of abnormalities in working memory and the processing of emotional material in healthy male G homozygotes. Our results suggest that one way for this *FOXP2* polymorphism to increase risk for schizophrenia, may be through impairments in working memory and affective processes underlying response to linguistic, pictorial and reward stimuli.

Keywords: *FOXP2*, rs1358278, Schizophrenia, Intermediate Phenotypes, Association Study

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16. Over-expression of matrix metalloproteinase 9 results in enhanced release of neurotrophic sAPPα and protects against loss of cognitive abilities in a mouse model of Alzheimer's disease

Fragkouli Apostolia [(1),] Stylianopoulou Fotini [(2)], Stamatakis Antonis [(2)], Tsilibary Effie C [(1)] and Tzinia Athina [(1)]

[(1)] Laboratory of Cell &Matrix Pathobiology, Institute of Biology NCSR, Demokritos, Athens, Greece;
[(2)] Lab. of Biology-Biochemistry, Department of Nursing, University of Athens, Greece

Accumulation of Aβ peptide released from the amyloid precursor protein (APP) upon cleavage by β- and γ-secretases- is believed to play a critical role in the pathogenesis of Alzheimer's disease (AD). Alternatively, APP is cleaved within the Ab sequence by α-secretases, giving rise to the neurotrophic soluble fragment sAPPa (1). MMP9, a matrix metalloproteinase expressed at low levels in the brain plays a significant role in neuronal plasticity, and based on in vitro studies it also exerts Aβ-degrading, as well as α-secretase-like activity (2). We have previously shown that mice over-expressing MMP9 (TgMMP9) display enhanced neuronal plasticity, enhanced cognitive abilities, and increased levels of sAPPα in the brain, suggesting that MMP9 displays α-secretase-like activity in vivo (3).

In order to examine whether MMP9 is able to improve the AD-related pathology in vivo, we have generated double transgenic mice by crossing TgMMP9 mice with the AD mouse model 5XFAD, which develops the major features of AD amyloid pathology (4). Our study revealed that as compared to their 5xFAD littermates, TgMMP9/5xFAD mice display increased levels of neurotrophic sAPPα inthe brain, not accompanied by changes in the levels other α-secretases, such as ADAM10. Furthermore, the double-transgenic animals had increased amounts of the post-synaptic protein PSD-95 suggesting enhanced plasticity, and of brain-derived neurotrophic factor (BDNF) compared to the 5XFAD mice models of AD. No major changes were detected in amyloid plaque load between the two animal groups. Moreover, although MMP9 is known to exert pro-inflammatory properties (5), no change in gliosis or neuro-inflammation were observed in either the 5xFAD or TgMMP9/5xFAD mice. Finally, our behavioral analysis revealed that as compared to their 5xFAD littermates, TgMMP9/5xFAD mice displayed a superior cognitive performance,that was comparable to that of their wild-type littermates. Overall then, our observations suggest that MMP9 exerts several beneficial effects, perhaps playing a protective role during progression of AD.

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17. Regulation of Amyloid Precursor Protein interactions by calcium

Elli Kyratzi and Spiros Efthimiopoulos

Department of Biology, Division of Animal and Human Physiology, University of Athens, 157 84 Panepistimiopolis, Ilisia, Greece

Ca^{2+} dysregulation is an important factor implicated in Alzheimer's disease (AD) pathogenesis whereas familial AD is caused by mutations in the Amyloid Precursor Protein (APP) or proteins that regulate its metabolism. However, there are no conclusive data about the relationship between the regulation of Ca^{2+} homeostasis and APP metabolism, function and protein interactions. Previous work in our laboratory has shown that APP interacts with Homer2 and Homer3 intracellular proteins and their expression inhibits APP processing towards Aβ. The focus of our research is to investigate the role of Ca^{2+} homeostasis alterations in APP/Homer protein interaction.

To investigate the effect of Ca^{2+} dysregulation on APP/Homer3 interaction, we first caused extracellular Ca^{2+} influx into HEK293 cells using the calcium ionophore A23187 or with a Ca^{2+} add-back protocol and monitored APP/Homer3 interaction by co-immunoprecipitation (co-IP) at various time points. In both cases, we observed reduced co-IP of Homer3 after immunoprecipitation of APP following extracellular calcium entry. We also tested the effect of ER Ca^{2+} store depletion in the same cells with thapsigargin, an inhibitor of the SERCA pump of the ER, which leads to Ca^{2+} efflux from the ER and rise of calcium in the cytosol, either in the presence or in the absence of extracellular Ca^{2+}. In the presence of extracellular Ca^{2+}, ER Ca^{2+} store depletion decreased APP/Homer3 interaction. In contrast, there was no effect on APP/Homer3 interaction when extracellular Ca^{2+} was chelated with EGTA suggesting that influx of extracellular calcium in response to ER Ca^{2+} store depletion disrupts APP/Homer3 interaction. Incubation with thapsigargin in the presence of the inhibitor of store-operated channels (SOCs), SKF96365, restored the interaction indicating that this type of channels mediating extracellular Ca^{2+} influx after depletion of intracellular Ca^{2+} stores seems to play an important role in the regulation of the interaction under these conditions. Interestingly, when we used the phospholipase C (PLC) stimulator m-3M3FBS, which generates IP_3 and causes IP_3-induced ER Ca^{2+} release, we also observed dissociation of APP/Homer3 complex. In the presence of m-3M3FBS, we applied inhibitors of IP_3-mediated ER Ca^{2+} release, specifically xestospongin C, a blocker of IP_3Rs, and inhibitors of kinases that could be induced through this pathway by Ca^{2+}, such as the inhibitor of CaMKII, KN-62. In both cases, there was restoration of the interaction showing that both the release of Ca^{2+} from the ER through IP_3Rs and the induction of kinases possibly phosphorylating the interacting proteins might play a role in the regulation of the APP/Homer3 interaction. Finally, in a more neuronal context, we performed immmunoprecipitation experiments in SH-SY5Y neuroblastoma cells after depolarization, a physiological stimulus introducing calcium in neurons, and we were able to show that APP/Homer3 interaction is also decreased upon elevation of cytosolic calcium under these conditions.

This is the first study showing that the interaction between APP and Homer3 altering Aβ production could be modulated by calcium proposing that the known effects of Ca^{2+} on APP metabolism regarding Aβ production could be mediated, at least in part, by the disruption of APP/Homer3 interaction. These data indicate Homers as key players in AD pathology opening new perspectives for the design of therapeutics to target Aβ production and contribute to the knowledge regarding the regulation of APP processing by Ca^{2+} and, in general, the role of Ca^{2+} dysregulation in AD.

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18. Deletion of tumor necrosis factor-alpha (TNF-α) reduces amyloid pathology in the 5xFAD model of Alzheimer's disease

Evaggelia Paouri and Spiros Georgopoulos

Department of Cell Biology, Biomedical Research Foundation of the Academy of Athens, Greece

Tumor necrosis factor-alpha (TNF-α) is a potent pro-inflammatory cytokine, which is secreted as a 51kDa soluble circulating trimer and exerts its signaling functions through engagement of its cell surface receptors [1-3]. In the central nervous system (CNS), TNF-α can be synthesized by microglia, astrocytes and some populations of neurons [1]. The first indication of a contribution for TNF-α signaling in Alzheimer's disease (AD) was the presence of TNF-α in amyloid plaques in post-mortem analysis of AD brains [1]. TNF-α is up-regulated in both CSF (cerebrospinal fluid) and serum of AD patients, and its levels have been shown to correlate with disease severity [2]. However, the role of TNF-α in the course of Alzheimer's disease is still unclear and its pathophysiological actions in AD have been reported to be controversial, as they have appeared to be either neurotoxic or neuroprotective [3].

In the present study, we studied the role of TNF-α in the pathogenesis of Alzheimer's disease in AD mice by inactivating the mouse TNF-a gene in a transgenic model of the disease. To achieve this we crossed 5xFAD transgenic mice, an established AD mouse model, with TNF-α deficient mice (TNF-$\alpha^{-/-}$), in order to obtain descendants with the 5xFAD TNF-$\alpha^{-/-}$ combination in their genotype. Analysis of brain sections from 5xFAD TNF-$\alpha^{-/-}$ mice revealed a significant decrease in amyloid plaques both in the hippocampus and cortex compared to controls. Furthermore, immunohistochemical analysis showed that deletion of TNF-α leads to significant decrease of Aβ deposition in the hippocampus and cortex. Genetic inactivation of TNF-α also affected astrocytic and microglial response in the 5xFAD brain. 5xFAD TNF-$\alpha^{-/-}$ mice displayed decreased astrocytosis and microgliosis in the subiculum of the hippocampus and cortex compared to control mice. These results demonstrate that the absence of the pro-inflammatory cytokine TNF-α ameliorates the disease phenotype in the 5xFAD model, and support the use of anti-TNFα therapy for human treatment of AD. Thus, these data confirm the importance of TNF-α in the pathogenesis of Alzheimer's disease.

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19. Boosting Chaperone-Mediated Autophagy as a means to mitigate alpha-synuclein-mediated neurotoxicity

Maria Xilouri[1], Oystein Rod Brekk[1], Natalie Landeck[2], Pothitos M. Pitychoutis[3], Themistoklis Papasilekas[1], Zoi Papadopoulou-Daifoti[3], Deniz Kirik[2] and Leonidas Stefanis[1,4]

1: Division of Basic Neurosciences, Biomedical Research Foundation of the Academy of Athens (BRFAA), Athens, Greece; 2: Brain Repair and Imaging in Neural Systems, Department of Experimental Medical Science, Lund University, Lund, Sweden; 3: Department of Pharmacology, Medical School, University of Athens, Athens, Greece; 4: Second Department of Neurology, University of Athens Medical School, Athens, Greece

Aberrant alpha-synuclein (AS) is linked to the pathogenesis of Parkinson's Disease (PD) and other neurodegenerative conditions termed synucleinopathies. One of the critical factors that determine the neurotoxic potential of AS is the protein levels. Excess of AS leads to PD in humans and to abnormal protein deposition and neurodegeneration in experimental animal models. Levels of AS are in part regulated by mechanisms of protein degradation. We have previously shown that Wild Type (WT) AS is degraded in part by the lysosomal process of Chaperone-Mediated Autophagy (CMA), while PD-associated mutant AS forms are not degraded by this process, and in fact impede degradation of other CMA substrates. Furthermore, evidence suggests a decrease of CMA components in PD brains. We reasoned that a possible therapeutic avenue against the toxic effects of AS would be induction of CMA, so as to, on the one hand, accelerate AS clearance, and, on the other, attenuate its toxic effects on lysosomes. To this end, we first created stable SH-SY5Y neuroblastoma cell lines, which express empty vector or the lysosomal transmembrane protein Lamp2a, the rate-limiting step in the CMA pathway. Lines expressing Lamp2a showed a significant induction of CMA activity compared to empty vector controls. Steady-state AS levels did not differ, but the half-life of AS was significantly shorter in the Lamp2a-expressing lines. Importantly, lines expressing Lamp2a were specifically protected against adenoviral WT AS-mediated neurotoxicity. Similar findings were achieved in primary rat cortical neuron cultures, where we induced CMA activity by infecting cultures with adenovirus expressing Lamp2a. To extend these findings *in vivo*, we overexpressed Lamp2a concurrently with WT AS via rAAVs (recombinant adeno-associated viruses) in the rat substantia nigra (SN), in a well established human rAAV-WT AS model of synucleinopathy. We observed robust co-localized expression of both transgenes in the dopaminergic neurons of SN, with Lamp2a expression effectively ameliorating AS-induced neurotoxicity observed in GFP+AS injected animals -as measured by an unbiased stereological estimator. This protective effect was also evident at the level of the nigrostriatal terminals, with an almost complete rescue of the dopaminergic phenotype - estimated by measuring dopamine levels by HPLC and tyrosine hydroxylase (TH) immunoreactive intensity by densitometry. Nigral overexpression of human WT AS resulted in accumulation of phosphorylated (S129) and SDS-soluble high-molecular weight (HMW) AS species in the GFP+AS injected animals. Importantly, both monomeric- and HMW AS species were profoundly reduced in the nigral fractions of AS+Lamp2a animals, with a definitive trend, albeit non-significant, for reduction of phosphorylated AS species. Taken together, we have provided proof-of-concept data for targeting the CMA pathway as a means to protect against AS-related toxic insults in neuronal cultures and in the living brain. The present study pinpoints for the first time an important *in vivo* role of CMA in WT AS pathobiology, and provides further impetus for the idea of modulating the CMA pathway as a novel therapeutic approach in PD and related synucleinopathies.

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20. Identification of novel molecular modifiers of Neurofibromatosis Type-1 (NF1) learning disabilities in the fruitfly *Drosophila Melanogaster*

Jean Y. Gouzi[1,2], James A. Walker[2], Robert Maher[2], Eirini-Maria Georganta[1], Mikela Mpouraimi[1], Anastasios Moressis[1], André Bernards[2] & Efthimios M.C. Skoulakis[1]

1. Institute of Cellular and Developmental Biology, Biomedical Sciences Research Centre "Alexander Fleming," Vari, Greece; 2. Center for Cancer Research, Massachusetts General Hospital - Harvard Medical School, Charlestown, MA

Neurofibromatosis 1 (NF1) is a common inherited disorder, affecting 1 in 3,000 worldwide. Among its hallmarks are tumors of the nervous system, short stature and learning disabilities. In the absence of treatment so far, the need to identify novel specific therapeutic targets has become a high research priority. NF1 is caused by loss-of-function mutations in neurofibromin, a negative regulator of Ras. *Drosophila melanogaster l*acking a conserved NF1 ortholog (*dNf1$^{-/-}$ mutant flies*) are reduced in size and display learning defects, both resembling human NF1 symptoms. In addition, its loss affects both the Ras/ERK and cAMP/PKA signaling pathways. We recently identified in our lab the neuronal Receptor Tyrosine Kinase *dAlk* and its activating secreted ligand *jelly belly* (jeb), as rate limiting upstream activators of *dNf1*-regulated Ras/ERK signals responsible for both organismal growth and olfactory learning defects. We further demonstrated that genetic or pharmacological inhibition of *dAlk* function using a selective human ALK inhibitor rescued both *dNf1$^{-/-}$* defects in flies. To identify additional rate-limiting components of *dNf1* pathways, we conducted a genetic screen for dominant modifiers of the *dNf1$^{-/-}$* growth defect. Identified suppressors confirmed the previously implicated *dAlk* and *jeb*, among other proteins involved in Ras/ERK signal transduction. Other modifiers include members of neuropeptide/cAMP/PKA pathways, components of the synaptic machinery, and proteins involved in vesicular transport. However whether these candidates can also modify *dNf1$^{-/-}$* learning defect remains unknown. Hence, we proposed to use the extant modifiers of the *dNf1$^{-/-}$* size defect to launch a systematic investigation of whether they also modify the learning deficits. Specifically, we are using an arsenal of individual single-gene mutants and specific RNAi lines that modify the *dNf1$^{-/-}$* size defect and we are currently in the process of testing them using the classical Pavlovian associative olfactory learning model. When possible, we will use pharmaceuticals to specifically modulate target activity *in vivo* in an attempt to reverse the *dNf1$^{-/-}$* learning deficiencies. Based on the fact that many of the identified modifiers of the *dNf1$^{-/-}$* size defect are known to play crucial roles in neuronal function, we anticipate that a substantial number of them will also modify the *dNf1$^{-/-}$* learning defects. From this unique comprehensive and multidisciplinary study we expect to provide promising perspectives towards the development of highly-selective pharmaceuticals for the amelioration of behavioral/cognitive impairments associated with NF1. We will present and discuss our preliminary results obtained from this ongoing targeted behavioral screen.

21. DnaJC11 is involved in mitochondrial cristae structure and neuromuscular disease in mice

Ioakeimidis Fotis[1,2], Rinotas Vagelis[1,2], Fasseas Constantinos[1], Kollias George[2] and Douni Eleni[1,2,*]

[1] AUA, Department of Agricultural Biotechnology, Laboratory of Genetics, Iera Odos 75, 11855, Athens;
[2] B.S.R.C. "Alexander Fleming", Division of Immunology, Fleming 34, 16672, Vari

Using N-ethyl-N-nitrosourea (ENU), a mutagen that causes nuclear point mutations at random, we have generated a mouse model of a neuromuscular syndrome. This syndrome manifests at 10 days after birth with abnormal hind limb posture and continuous with abnormal gait, tremors, muscle atrophy, blood abnormalities, growth retardation and premature lethality at one month of age. This is an autosomal recessive trait with complete penetrance. Histological analysis and transmission electron microscopy revealed severe vacuolation of the motor neurons of the spinal cord originating from mitochondria that have lost their cristae. By genetic mapping and sequencing we have found the causal mutation to be located within an intron of a novel member of the DnaJC family of co-chaperones, DnaJC11, affecting its splicing. We have verified the causal role of this mutation in rescue experiment by expressing the human ortholog.

The DnaJC11 protein has previously been suggested to be localized in the mitochondria of human and mouse tissues. We further support this suggestion by confocal microscopy and western blot analysis. Although this DnaJC member is of unknown function, it has been found to co-immunoprecipitate along with a number of proteins, some of which having been shown to play a role in the maintenance of cristae morphology. This is in line with our electron microscopy findings. Our current work is focused on mitochondrial functional assays and more extent electron microscopy to better characterize the mitochondrial morphology phenotype in muscle and the Central Nervous System of our mutants. Our findings reveal a novel mitochondrial protein that plays an important role in proper mitochondrial structure and neuromuscular function and given the functional redundancy between the mouse and human gene that we have shown, the DnaJC11 protein might prove a contributor in human neuromuscular diseases.

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 European Social Fund.

22. Addressing the Tau enigma:protein phosphorylation, neuronal toxicity and memory dysfunction.

Papanikolopoulou Katerina and Skoulakis Efthimios

BSRC Alexander Fleming, 34 Fleming street, 16672, Vari, Greece

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 combine histological, behavioural and biochemical approaches in order to determine the role of different disease-associated phospho-epitopes, including pS238, on Tau associated neuronal dysfunction and toxicity. Analysis was performed on wild-type Tau, FTDP-17 associated mutants as well as phosphorylation-incompetent forms of Tau in which single or a pair of serines and threonines were replaced by alanines. Our collective results revealed that phosphorylation at Ser^{238} controls Tau neurotoxicity in vivo.

23. Molecular mechanism of ataxin-1 protein aggregation in the polyglutamine disease SCA1.

Spyros Petrakis[1], Tamás Raskó[1], Jenny Russ[1], Ralf P. Friedrich[1], Martin Stroedicke[1], Sean-Patrick Riechers[1], Katja Muehlenberg[1], Angeli Möller[1], Anita Reinhardt[2], Arunachalam Vinayagam[1], Martin H. Schaefer[4], Michael Boutros[3], Hervé Tricoire[2], Miguel A. Andrade-Navarro[4] and Erich E. Wanker[1].

[1] Neuroproteomics, Max Delbrueck Center for Molecular Medicine, 13092 Berlin, Germany
[2] Unité BFA (EAC 7059), Université Paris Diderot-Paris7/ CNRS, 75205 Paris Cedex 13, France
[3] Division of Signaling and Functional Genomics, German Cancer Research Center, 69120 Heidelberg, Germany.
[4] Computational Biology and Data Mining, Max Delbrueck Center for Molecular Medicine, 13092 Berlin, Germany

Spinocerebellar ataxia 1 (SCA1) is a neurodegenerative disease caused by trinucleotide (CAG) expansions in the coding region of ataxin-1 (ATXN1). This expansion results in longer polyglutamine chains in the relevant protein. Mutant proteins misfold into toxic oligomers and form intranuclear aggregates that cause neurodegeneration in the cerebellum. In the last decade a fairly large number of proteins has been reported to influence toxicity and aggregation of polyQ ATXN1 protein in yeast, worm or flies. However, it is not clear whether their human homologues are relevant for SCA1 pathogenesis.

To answer this question we systematically screened 250 human modulators for their effect on the toxicity/aggregation of human ATXN1. We identified 21 human proteins that influence polyQ-induced ataxin-1 misfolding and proteotoxicity in cell model systems. By analyzing the protein sequences of these modifiers, we discovered a recurrent presence of coiled-coil (CC) domains in ataxin-1 toxicity enhancers, while such domains were not present in suppressors. This suggests that CC domains contribute to the aggregation- and toxicity-promoting effects of modifiers in mammalian cells.

We focused our research on MED15 modifier gene which directly interacts with ataxin-1. We observed that MED15 protein, computationally predicted to possess an N-terminal CC domain, induces polyQ protein aggregation while no such effect was observed with the truncated protein MED15ΔCC, lacking a CC domain. Moreover, we inserted MED15CC domain into PUM1, an aggregation suppressor, generating a hybrid PUM1-MED15CC protein. We observed that PUM1-MED15CC promotes polyQ ATXN1 aggregation in cell model systems. In strong contrast, wild-type PUM1 had no such effect. These results indicate that MED15 seeds polyQ ATXN1 protein aggregation through its CC domain. Protein-protein interaction studies showed that the CC domain of MED15 is also necessary for its binding on ATXN1. Therefore, blockade of MED15-ATXN1 interaction may inhibit the aggregation of polyQ ATXN1. Our results indicate that proteins with CC domains are potent enhancers of protein misfolding and highlight them as novel targets for therapeutic intervention in polyQ diseases.

Poster Presentations

Poster Session I

1. Ariel, a novel long non coding RNA, regulates differentiation of neural stem cells

Daphne Antoniou, Athanasios Stergiopoulos and Panagiotis K Politis

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

Approximately 98% of the human genome does not encode for proteins. Extensive research has shown that a significant percentage of these sequences code for numerous long-non-coding RNAs (lncRNAs). These RNAs are evolutionarily conserved and function in diverse biological processes, including stem cell pluripotency, cell-cycle regulation, organogenesis and cell-type specific differentiation. Many lines of evidence suggest that lncRNAs can affect cell properties by regulating the expression of nearby protein-coding genes. Interestingly, preliminary data from our group indicate that Ariel, a novel lncRNA (previously known as AK142161), is transcribed from the *Prox1* genomic locus in an anti-parallel manner during central nervous system (CNS) development. We have very recently shown that *Prox1* has a key role in the regulation of neurogenesis during CNS development, suggesting that Ariel might be involved in the molecular mechanisms that control neural differentiation. *Ariel* contains two exons of 134 bp and 697 bp length, respectively, with an overlapping sequence of 133 bp at the 5'-UTR of the Prox1 mRNA. *Ariel* was originally identified in high-throughput genome-wide expression screens for lncRNAs. However, its detailed expression pattern and function remain elusive. To this end, our preliminary expression studies and functional experiments suggest an antagonistic relationship between these two genes of the *Prox1* locus. Specifically, *Ariel* is broadly expressed during mouse CNS development and is enhanced upon astrocyte differentiation of embryonic neural stem cells (NSCs), whereas Prox1 is down-regulated. This expression pattern is consistent with a possible role of Ariel in inducing astrogliogenesis and regulating neurogenesis. Accordingly, adenoviral-mediated gain-of-function experiments suggest that *Ariel* is sufficient to lead NSCs towards the acquisition of mature astrogliogenic identity at the expense of neuronal differentiation. On the contrary, Prox1 overexpression in the same system promotes neurogenesis and blocks astrogliogenesis. From a mechanistic point of view, *Ariel* is able to negatively regulate Prox1 both at mRNA and protein levels. We are currently investigating whether Ariel affects neuronal differentiation of NSCs via a negative loop at the *Prox1-Ariel* genomic locus.

Taken together, these observations raise the intriguing possibility that this specific genomic locus may act as a molecular switch between astrogliogenesis and neurogenesis in NSCs. Moreover, we believe that the understanding of the exact mechanistic basis of *Ariel* function in NSCs will offer a pioneering paradigm of how this novel class of regulatory molecules can control gene expression and tissue development.

2. The cell adhesion molecule TAG-1 is an important regulator of the olfactory bulb function in rodents

George Bastakis[1], Maria Savvaki[1], Antonis Stamatakis[2], Domna Karagogeos[1]

[1]Department of Basic Science, University of Crete Medical School and Institute of Molecular Biology and Biotechnology, Heraklion, Crete 711 10
[2]Laboratory of Biology-Biochemistry, School of Nursing, University of Athens, Athens, Greece

The main projection neurons of the olfactory bulb (OB) (mitral and tufted cells) and their axons, express the cell adhesion molecule TAG-1 during development. TAG-1 is cell adhesion protein of the immunoglobulin superfamily that plays an important role in neurite outgrowth, fasciculation, neuronal migration and axon guidance. Its expression is first detected at E13.5 in newly born mitral cells (MCs), while it is absent from the mature olfactory bulb. The exact role of TAG-1 in the olfactory system is currently unknown.

Homozygous mutant mice (*Tag-1*$^{-/-}$) display severe alterations in several behavioral assays concerning olfactory memory, odor discrimination, social and non-social odor recognition. In order to investigate the molecular defects that could account for this behavioral phenotype, we performed a detailed immunohistochemical analysis of OB development in these mutant mice. In adult and newborn *Tag-1*$^{-/-}$ mice, we observed a significantly decreased number of MCs in the mitral cell layer (MCL) of the main olfactory bulb (MOB) compared to age-matched control animals. This defect is specific for the projection neurons of the OB that are born at E11,5 (30% of the total) and can be attributed either to increased apoptosis or to other deficits during development of the olfactory bulb (such as proliferation and/or migration).

TUNEL assay and caspase 3 immunohistochemistry at E13,5-E18.5 did not reveal changes in the number of apoptotic cells between mutant and control animals, indicating that the reduction of MCs in adult mice is probably not an outcome of cell death. Using Tbr-1 as a post-mitotic neuronal marker for mitral and tufted cells, we did not observe differences at E14,5 *Tag-1*$^{-/-}$ OB regarding the pool of postmitotic Tbr-1+ neurons, excluding a proliferation defect. We extended our analysis beyond the MOB, to the accessory olfactory bulb (AOB), which receives input from the vomeronasal organ. We observed an increase in the number of the projection neurons (born at E11.5) in the AOB of *Tag-1*$^{-/-}$ mice, that was equivalent to the reduction previously observed in the MOB. This finding suggests that in the absence of TAG-1, the projection neurons of the MOB are misdirected possibly due to defects in migration. This hypothesis is currently under investigation in our lab.

3. Intracellular mediators of cortical interneuron development

Katerina Kalemaki[1,2], Simona Tivodar[1,2], Zuzana Kunupa[1,2], Marina Vidaki[1,2], Victor Tybulewicz[3], Nicoletta Kessaris[4], Vassilis Pachnis[5], and Domna Karagogeos[1,2]

1: Department of Basic Science, Faculty of Medicine, University of Crete, Heraklion, Greece
2: Institute of Molecular Biology & Biotechnology (IMBB, FORTH), Heraklion, Greece
3: Division of Immune Cell Biology, Medical Research Council, National Institute for Medical Research, London, UK
4: Wolfson Institute for Biomedical Research and Department of Cell & Developmental Biology, University College London, UK
5: Division of Molecular Neurobiology, Medical Research Council, National Institute for Medical Research, London, UK

The correct function of cortical microcircuits depends on GABAergic interneurons that provide the main source of inhibition. Impaired interneuron function results in severe neurodevelopmental disorders such as schizophrenia, epilepsy and autism. Although some of extracellular cues crucial for interneuron development have been revealed, the intracellular components involved are still unknown. Here we are examining the role of Rac proteins in this process. Rac proteins are RhoGTPases that integrate multiple extracellular signals required for essential processes in diverse cell types as cytoskeleton organization, vesicle trafficking, transcription, cell cycle progression, and apoptosis. We are currently examining the roles of Rac1 and Rac3 specifically in interneurons derived from the medial ganglionic eminence (MGE), a population comprising the majority of cortical interneurons. Recent data from our team (Vidaki et al., 2011) using Cre/loxP technology points to a cell autonomous and stage-specific requirement for Rac1 activity within proliferating interneurons for the co-ordination of cell cycle progression with differentiation and migration. The majority of these mice die after 4 weeks of age due to epileptic seizures due to the fact that 50% of GABAergic interneurons are absent from the postnatal cortex. Our data demonstrate that Rac1 is necessary for the transition from G1 to S phase, at least in part by regulating CyclinD levels and Rb phosphorylation. MGE-derived interneurons missing both Rac1 and Rac3 proteins show an even more severe defect (80% of cortical interneurons are absent, especially the parvalbumin and somatostatin subpopulations) and the mice die even earlier than the Rac1 single mutants. The progenitors of these cells also show a delay in cell cycle exit. In addition, MGE cells in vitro show cytoskeletal alterations such as a significant reduction of the leading process length and in growth cone formation in the absence of Rac1 protein, while Rac1/Rac3-deficient MGE cells show different, even more pronounced cytoskeletal defects. The absence of Rac1/3 could affect the stabilization of microtubules resulting in improper formation of leading processes. Indeed, the neurites of Rac1/3-deficient MGE-derived cells in culture are severely impaired. Our aim is to decipher the molecular mechanisms underlying the observed defects in the mice lacing both Racs from their cortical interneurons.

4. Prox1 controls binary fate decisions in spinal cord neurons

Valeria Kaltezioti[1], Ismini Rozani[1], Hermann Rohrer[2] and Panagiotis K. Politis[1]

[1]Center for Basic Research, Biomedical Research Foundation of the Academy of Athens, 4 Soranou Efesiou Str, 115 27, Athens, Greece
[2]Department of Neurochemistry, Max-Planck Institute for Brain Research, 60528 Frankfurt/Main, Germany

Specification of spinal cord neurons depends on gene regulation networks that impose distinct fates in neural progenitor cells (NPCs). Motor neuron (MN) progenitors express *Olig2* to coordinate the gene expression program for MN specification and inhibit V2 interneuron identity. However, the upstream molecular mechanisms that control this binary specification are not well understood. Here we demonstrate that Prox1, a transcription repressor and downstream target of proneural genes, is involved in the molecular mechanisms of ventral spinal cord patterning by refining the p2/pMN boundary. First, Prox1 is strongly expressed in V2 interneuron progenitors and largely excluded from MN progenitors. Second, Prox1 directly suppresses *Olig2* gene expression to repress MN and promote V2 interneuron identity. Gain-and-loss of function studies in chick neural tube and mouse NPCs show that Prox1 is sufficient and necessary for the suppression of *Olig2* gene expression and proper control of V2 interneuron versus MN identity. Mechanistically, Prox1 interacts with the regulatory elements of *Olig2* gene locus in vivo and it is critical for proper *Olig2* transcription regulation. In particular, chromatin immunoprecipitation 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. Moreover, plasmid-based transcriptional assays in mouse NPCs from embryonic spinal cord suggest that Prox1 suppresses the activity of *Olig2* gene promoter and K23 enhancer. These observations indicate that Prox1 controls binary fate decisions between MNs and V2 interneurons in NPCs via direct repression of *Olig2*.

5. Spatiotemporal pattern of external granule cell proliferation and role of glutamate receptor antagonists in cerebellar foliation

Kommata Vasiliki, Tzanotis Nikos, Argyrousi Elentina, Dermon Catherine

Lab Human &Animal Physiology, Dept Biology, Univ. Patras, Patras, Greece.

The avian cerebellum, a highly conserved structure of vertebrate brain, is a morphologically unique laminated structure made up of a set of ten folia separated by fissures. Proliferation of the external granule layer (EGL) is a major event in embryonic development that coincides with the fissure formation. Recent studies have implicated glutamate, an excitatory amino acid neurotransmitter, in the regulation of neural progenitor cell proliferation. The present study aims to investigate the temporal pattern of granule cell production and the role of glutamate in the cerebellar folia formation. For this, we studied embryonic EGL patterns of cell proliferation and migration, and the effects of glutamate receptor antagonists in the normal development of chick cerebellar fissures. Specifically, the proliferation pattern in the EGL of the developing embryonic chick cerebellum was mapped during embryonic stages E12 to E15, by means of 5-bromo-2'-deoxyurinine (BrdU) immunohistochemistry. The ratio of active fast (BrdU) versus slow (proliferating cell nuclear antigen, PCNA) cycling cells was determined, using double immunofluorescence In addition, experimental animals were injected with the non-NMDA (non-N-methyl-D-aspartate) glutamate receptor antagonist CNQX (6-cyano-7-nitroquinoxaline-2,3-dione, 5mM) followed by BrdU injection (80μg/gr egg weight in physiological saline). Following different survival times (short-term; 3 hours), long-term; 24 hours), embryos were fixed, cryo-protected and serial cerebellar sagittal sections were analysed to determine proliferation and migration patterns by image analysis. Within the EGL, the double-labeled BrdU / PCNA cells ranged from 40-90% of the BrdU positive cells, depending on the folia topography (wall, apex, floor) and the developmental stage, with the higher rate observed at stage E15. This pattern of active proliferating cells and the granule cell production was modified in the embryonic stages studied, by blocking glutamate AMPA/kainate receptors. These data provide important information on the cycling parameters of the granule cell precursors during late developmental stages of avian cerebellum.

6. Age-related differences in prefrontal cortical function and mouse behavior

Xanthippi Konstantoudaki[1] and Kyriaki Sidiropoulou[1,2]

[1]Departmentt of Biology, University of Crete
[2]Institude of Molecular Biology and Biotechnology/Foundation for Research and Technology- Hellas, Vassilika Vouton, Heraklio, Greece

Several processes, such as proliferation and migration of cells, changes in synaptic plasticity and molecular makeup of the cells, take place during development of the nervous system, primarily before birth. However, neuronal changes continue during the early postnatal days all the way until adulthood in several brain regions. The prefrontal cortex (PFC) is a brain area involved in higher order cognitive functions, such as attention, working memory, and behavioral inhibition and shows changes in cellular function throughout the juvenile and adolescent period, considerably longer compared to primary sensory areas. It is notable that these cognitive functions mediated by PFC, change dramatically as a function of age throughout childhood and adolescence, together with its cellular function. Furthermore, the PFC has been implicated in various neurodevelopmental disorders, such as schizophrenia, attention deficit hyperactivity disorder, depression, autism, anxiety and bipolar disorder, which emerge before adulthood, either in childhood or adolescence. However, we have very little knowledge for the underlying cellular, network and physiological mechanisms during the different stages of postnatal development that seem to contribute significantly in the emergence of mental diseases. Thus, the goal of our study is to understand the postnatal development, of PFC neuronal processes, as well as, of behaviors that are mediated by PFC.

To this end, we have initiated a systematic study of the development of cortical network activity and performance in cognitive and emotional behavioral tasks in mice of three different developmental stages: a) juveniles b) adolescents, and c) adults.

We performed electrophysiological recordings in brain slices taken from mice of the above-mentioned age groups. Specifically, field excitatory postsynaptic potentials were recorded from layer II of the prefrontal or barrel cortex in response to electrical stimulation of layer II, in an effort to record the local cortico-cortical responses. We find that basal synaptic transmission is enhanced in juvenile mice compared to adult mice. In addition, juvenile mice show long-term depression in response to tetanic stimulation, while adult mice exhibit long-term potentiation in response to the same stimulus.

In order to study the behavioral development in cognitive and emotional aspects, we tested the mice in the open-field, the elevated plus maze and the object recognition for temporal order task. We find that juvenile and adult mice do not show differences in their anxiety levels. However, juvenile mice exhibit greater locomotor responses in response to the novel environment compared to adult mice. Experiments in the object recognition for temporal order task are currently underway in our laboratory in order to study the development of object recognition memory across time.

Our goal to unravel the developmental changes in the synaptic and intrinsic mechanisms important for cortical network activity and behavior, will lead us to better understand the cellular substrates on which mental illnesses develop. This will ultimately contribute to the designing of novel therapeutic regimens, pharmaceutical or cognitive, specific to the different developmental stages for the successful treatment of mental illnesses.

7. Loss of function analysis of Geminin's role in the developing and adult mouse brain

Kyrousi C.1, Lalioti M.1, Spella M.1, Lygerou Z.2, Taraviras S.1

1. Department of Physiology,
2. Department of General Biology, Medical School, University of Patras, Greece

Neurons, astrocytes and oligodendrocytes of the adult brain arise from a heterogenic pool of progenitor cells in the neurogenic regions of the developing cortex. At the embryonic mouse cortex there are four well-defined types of progenitor cells, the neuroepithelial cells, the radial glial cells and short neural precursors that reside in the ventricular zone while the forth progenitor population the basal progenitor cells reside in the subventricular zone. These neural progenitors must balance between self-renewal that allows maintenance of their population and differentiation into different cell types.
At the end of embryonic development the neural progenitors that have maintained their undifferentiated state convert into adult neural stem cells, in the adult brain, which are capable of proliferation and differentiation into neurons and glial cells. During adulthood, neurogenesis is occurred in the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus. In the subventricular zone the slow dividing type B cells act as neural stem cells and they can either generate committed neuronal precursors or oligodendrocytes.
We have previously shown that overexpression of Geminin in cortical progenitors cells reduce the number of progenitor cells and resulted in increased cell cycle exit and neuronal differentiation. In order to further elucidate the role of Geminin in the regulation of self-renewal and differentiation decisions of neural stem cells in the embryonic and adult neurogenesis we have generated mice lacking Geminin expression in the central nervous system.
Our data show that in the absence of Geminin embryonic progenitor cells at E12.5dpc show a lengthening of their cell cycle, which lead to an increase of apical and basal embryonic progenitor cells. Additionally deletion of Geminin affects the neuronal output of cortical neurons. During adult neurogenesis the number of type B adult neural stem cells is increased upon Geminin deletion and their transition through different progenitor populations and neuronal differentiation is altered. Our study suggests Geminin as a key regulator factor of self-renewal and differentiation decisions in embryonic and adult neural stem cells.

8. Cend1 knockout mice show decreased proportion of GABAergic cortical neurons and deficits in behavioural tests.

Alexandros A Lavdas[1], Antonis Stamatakis[2], Florentia Papastefanaki[1],Pari Koutsoudaki[1], Fotini Stylianopoulou[2] and Rebecca Matsas[1]

[1]Laboratory of Cellular and Molecular Neurobiology, Hellenic Pasteur Institute, Athens, Greece
[2]Laboratory of Biology-Biochemistry, Faculty of Nursing University of Athens, Greece

Cend1 is a neuronal-lineage specific modulator involved in the coordination of cell cycle exit and differentiation of neuronal precursors. Previous work has demonstrated that Cend1 ablation leads to irregularities in cerebellar lamination which impact on the motor behaviour.
Here, we sought to examine the effects of Cend1 ablation on aspects of cortical development. Our data demonstrate that Cend1 ablation leads to a decrease of the proportion of GABAergic neurons in various cortical areas and the amygdala. To assess possible behavioural effects of increased disinhibition, mice were subjected to behavioural tests. Cend -/- mice showed increased anxiety as assessed in the elevated plus maze task, spending less time in the open arms of the maze and making fewer entries into the open arms than the wild-type animals. Moreover, in the open field, Cend1 -/- had lower mobility both horizontal (number of crossings) and vertical (rearings), indicative of increased anxiety levels. In addition to the emotional dysfunction, Cend1 -/- mice exhibited severe cognitive deficits in the Morris water maze test, both during learning and on the memory trials. Further analysis is in progress to assess the behavioural versus morphological phenotype of the Cend1 mutants.

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9. Cellular prion protein PrPc is required for proper proliferation and differentiation of subventricular zone neural stem/precursor cells while its interaction with NCAM promotes neuronal differentiation

Prodromidou K.[1], Papastefanaki F.[1], Sklaviadis T.[2] and Matsas R.[1]

[1]Laboratory of Cellular and Molecular Neurobiology, Hellenic Pasteur Institute, Greece
[1, 2]Prion Diseases Group, Aristotle University of Thessaloniki, Greece

Cellular prion protein PrPc is a ubiquitous glycoprotein prominently expressed in brain, in differentiated neurons but also in neural stem/precursor cells (NSCs) (Steele, A.D. et.al., 2006, Proc Natl Acad Sci U S A *103*, 3416-3421). The misfolding of PrPc is a central event in prion diseases, yet the physiological function of PrPc has by large remained elusive. PrP-/- mice show no obvious abnormalities, however recent studies have associated PrPc with proliferation and differentiation events in NSCs. In the present work we investigated the role of PrPc in subventricular zone (SVZ) neurogenesis *in vitro* and *in vivo* in wild type and PrP-/- mice. We report that PrPc is required for proper proliferation and differentiation of SVZ precursor cells, both *in vitro* and *in vivo*, *w*hile there is an accumulation of cycling neuronal progenitors in the SVZ of PrP-/- mice. In agreement, fewer newly born neurons reach the RMS of the mutant mice. To asses if this deficit is due to impaired NSC proliferation versus differentiation or to abnormal chain migration of newborn neurons in PrP-/- mice, we analyzed the migratory behaviour of SVZ cells in microexplant cultures. We observed no differences in chain migration between wild type and PrP-/- mice *in vitro*, indicating that the observed accumulation of cycling neuronal progenitors may be attributed to abnormal proliferation/differentiation of neuronal progenitors. Furthermore, as PrPc has been previously reported to directly interact with the neural cell adhesion molecule NCAM in differentiated neuronal cells, we asked if these two proteins interact to influence the proliferation/differentiation properties of neural stem cells. We found that PrPc and NCAM act in concert to promote neuronal differentiation of NSCs in vitro, while disruption of the PrP-NCAM interaction leads again to accumulation of neuronal progenitors at the proliferation stage. Cellular prion protein is, therefore, identified as a hitherto 'unknown' heterophilic partner for NCAM acting to promote neuronal differentiation of NSCs. We postulate that the compromised developmental state of SVZ progenitors in PrP-/- mice may be responsible for their aberrant response to signals and cues that control migration in the RMS.

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10. The orphan nuclear receptor NR5A2 promotes neurogenesis versus astrogliogenesis and inhibits self-renewal of Neural Stem Cells

Athanasios Stergiopoulos and Panagiotis K. Politis

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

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 vertebrate nervous system remains elusive. To this end, we show here that NR5A2 is widely expressed in various regions throughout neuronal lineage in mouse and chick embryos, exhibiting higher levels of expression in neurons than neural stem cells (NSCs), suggesting a correlation to neuronal differentiation. Accordingly, adenovirus 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 is limited to neuronal lineage. Mechanistically, NR5A2 expression via adenoviral transduction leads to a strong induction of Prox1, which directly interacts with 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. Consistently, the over-expression of NR5A2 together with the down-regulation of Prox1 can reduce the potential of NSCs for neurogenesis at basal levels, while the generation of astrocytes is slightly increased. Collectively, these observations indicate an important function of NR5A2 in the balance between NSC self-renewal and differentiation and thus, in nervous system development. These data, in conjunction with the recently discovered pharmacological agonists and antagonists of NR5A2, render it a candidate gene for therapeutic strategies in applications of regenerative medicine.

11. Cerebellar granule cell proliferation is reduced by CNQX in adult zebrafish (Danio rerio).

Themistoklis Tsarouchas , Panayiota Makantasi & Catherine R. Dermon

Laboratory of Human and Animal Physiology, Department of Biology, University of Patras, Patras, Greece

Teleost cerebellum, while sharing common features with birds and mammals, is characterized by extensive adult neurogenesis, resulting the continuous addition of granule cells throughout life, and thus provides an excellent model for the study of adult plasticity mechanisms. Recent studies have implicated glutamate, an excitatory amino acid neurotransmitter, in the regulation of neural stem and progenitor cell proliferation. The present study questioned whether glutamate receptors have a role in such mitotic activity in adult teleost cerebellum, regulating the production of granule cells. For this, adult female zebrafish were injected intraperitoneally with a solution of the competitive AMPA/kainate receptor antagonist, CNQX (6-cyano-7-nitroquinoxaline-2,3-dione, 543μM) and BrdU (a thymidine analogue used to label the active cycling cells, 0,2mg/kg) in 0,9% NaCl solution. Matching control animals were injected with BrdU-saline solution. Behavioral effects of the CNQX injection were monitored, and following 24h post-BrdU survival, zebrafish were perfused under anesthesia, their brains were fixed and stored at -80^{o}C. Single and double BrdU immunohistochemistry was applied to determine the CNQX effects on the proliferative activity of adult zebrafish cerebellum. Detailed observation and microscopic analysis of the BrdU positive cells showed a marked decrease in the number of newborn cells within the corpus cerebelli (CCe) and specifically in the mitotically active cells of the stem cell niche, in comparison to the controls. Our data are consistent with studies in mammals and suggest that blocking of glutamate receptor subtype by the AMPA/kainate antagonist, CNQX, down regulates the cell proliferation and the granule cell production in the adult zebrafish cerebellum. A possible mechanism for this CNQX effect on the proliferating cells might be related to the lengthening of the cell cycle. Whether this mechanism is evolutionary conserved remains to be established.

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12. The role of BMP7 in the development of trigeminal ganglion

Vasiliki Zouvelou1* and Daniel Graf1,2

1Institute of Immunoogy, Biomedical Sciences Research Center 'Al. Fleming', 34 Al. Fleming Street, 166 72 Vari, Hellas/Greece
2Department of Orofacial & Structural Biology, Institute of Oral Biology, ZZMK, Faculty of Medicine, University of Zurich, Plattenstrasse 11, 8032 Zurich, Switzerland

The trigeminal ganglion (ganglion V) originates from the hindbrain (rhombomere 2) and the trigeminal epibranchial placode and gives rise to the trigeminal nerve (sympathetic nerve V). The trigeminal nerve has three branches (i.e. ophthalmic, maxillary, mandibular), which innervate most of the orofacial tissues and organs, teeth included. Palsies associated with trigeminal nerve injuries have major psychological impact in patients because disturb the aesthetic of their faces, influencing thus the quality of their life. Until today little is known on the molecules that regulate the formation of the neuronal branches and the ganglion itself.
We have previously shown that Bmp7 is required for the development of several orofacial structures. Here we investigate neuronal aspects of the facial components affected by Bmp7 deficiency. The development of the trigeminal nerve is severely compromised in mice with a Bmp7 deletion. Given the relationship between neural crest cells and the orofacial formation we have generated mutant mice with a neural crest specific BMP7 deletion (i.e. Wnt-1Cre/BMP7flx) that recapitulate the trigeminal malformation observed in the complete knock-out mice. Using lineage tracing, we show that the migration of neuronal precursors is disrupted in the absence of Bmp7 mainly because of the deficient differentiation of the glia cells which support the growth/orientation of the neuronal fibers.

Discussion & Conclusion

1. Expression of BMP7 during the development of trigerminal ganglion & its neuronal branches
2. Morphology of trigerminal ganglion is affected both in the BMP7ko and Wnt-1 Cre+ BMP7ko/flx mice pointing to neural crest derived malfunction
3. Branching of ophthalmic component of trigeminal nerve is reduced and the neuroaxons are thinner due to the limited number od Schwann cell neuroglia
4. Sox10+ melanocytes which are also neural crest derivatives are reduced in numbers in the transgenic BMP7 mice
5. Rosa Wnt-1 Cre BMP7 ko/flx mice depict altered neural crest migration

13. A new computer program under development for scoring and processing behavioural data

D. Baltas, C. Dalla, V. Kafetzopoulos, F. Theocharis, Z. Papadopoulou-Daifoti and N. Kokras

Department of Pharmacology, Medical School, University of Athens, Greece

Behavioural analysis in preclinical neuropsychopharmacology relies in the accurate measurement of animal behaviour. Advances in computer science allowed the development of elaborate software which records animal behaviour often with a high degree of automatism taking advantage of artificial intelligence and image tracking technologies. However, those commercial software solutions have a high purchasing cost and although they rely on advanced algorithms sometimes they do not provide as detailed and accurate assessment of animal behaviour as human observation does. The objective of this project was to develop a versatile and expandable software package for manual scoring and data processing of behavioural pharmacology experiments.

The program is developed in Visual C# and is compatible with personal computers able to run MS WindowsXP © or later operating system versions with the .NET 4.0 extensions. All data are stored in a relational database compatible with different engines (SQLite, MySql, MS-SQL, Oracle, etc). It is released under the Creative Commons license, freely available to the academic community.

Although the program is in its first alpha versions and under active development, it is used in the Department of Pharmacology for registering and analysing behavioural experiments such as the Forced Swim Test and the Elevated Plus Maze. The graphical interface is organized around user-defined projects, which may contain one or more behavioural tests. The researcher defines experimental groups and subjects within the projects and thus a complete user-manageable record of all behavioural data is stored in the local database. Upon launching the behavioural registration routine, customizable key codes allow the registration of state and instant behaviours at their time of appearance. Following completion of the session, the processing of data allows calculation of several parameters, such as duration of behaviour or its latency to appear as well as time and sequential analyses. Output files can easily be imported to any spreadsheet program for further analysis. In addition, a high-quality representation of the recorded behaviours over time can be exported in a graphical format for further use.

Accurate behavioural analysis remains of paramount importance in preclinical psychopharmacology and the deployment of this new, modern software platform significantly increased productivity without imposing the heavy burden of cost associated with available commercial solutions. Continuous further development is scheduled and suggestions for future improvements are most welcomed.

Web link

http://www.med.uoa.gr/pharmacology/en/research/neuro_psycho/observador/index.html

14. Cannabinoid modulation of the dopaminergic system of the rat after chronic administration of win55,212-2 and abstinence

Eleni Fanarioti[1], Martha Tsarouchi[1], Panagiotis Perdikaris[1], Ada Mitsacos[2], George Panagis[3] and Panagiotis Giompres[1]

[1]University of Patras, Department of Biology, Laboratory of Human and Animal Physiology, 26500 Patras, [2]Department of Physiology, University of Patras, Faculty of Medicine, Patras, Greece, [3]University of Crete, Department of Psychology, Laboratory of Behavioral Neuroscience, 74100 Rethymno, Crete, Greece

The cannabinoid system interacts widely with other neurotransmitter systems at the presynaptic or postsynaptic levels in order to regulate brain function. Several studies have shown that cannabinoids exert complex effects on motor and cognitive processes. The aim of the present study was to examine the protein and mRNA expression levels of dopamine receptors and transporter (DAT), tyrosine hydroxylase (TH) as well as CB1 receptor in rat brain following chronic exposure to WIN55,212-2. For this purpose, rats received systemic injection of WIN55,212-2 (1mg/kg, ip), a CB1 receptor agonist, for 20 days. Another group of rats received repeated WIN55,212-2 treatment followed by a period of abstinence (1 week). Control rats received vehicle injections. Following the last injection, rats were euthanized, brains removed, frozen, sectioned in a cryostat and stored until they were processed for *in situ* hybridization, *in vitro* binding and Western blot. Our results showed a significant increase in D1 and D2 receptor binding in frontal cortex of treated and rats followed an abstinence period. DAT protein expression showed no significant change in striatum in all groups examined. A statistically significant decrease was observed in substantia nigra pars compacta (SNpc) and VTA for DAT binding sites in all groups. DAT mRNA expression was significantly decreased following WIN55,212-2 administration but significantly increased following a period of abstinence. The same effect was also observed for TH mRNA expression in the regions mentioned above. TH protein expression in striatum showed a significant decrease following the cannabinoid exposure. Decrease was also shown in mRNA expression for D2S isoform of D2 receptor in SNpc and VTA, while the mRNA expression for D2L isoform was increased following chronic cannabinoid administration or a period of abstinence in SNpc and VTA. The mRNA expression for CB1 receptor in striatum and nucleus accumbens (NAc) was significantly reduced after WIN55,212-2 exposure and the abstinence period. Reduction was also observed in CB1 receptor in striatum. The present findings indicate significant alterations in dopaminergic system in the rat brain after WIN55,212-2 exposure and the abstinence period, which may underlie possible neuroadaptive changes induced after the cannabinoid exposure.

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15. Denial of maternal contact during training in a T-maze on post-natal days 10-13 results in decreased function of the serotonergic system in the adult rat brain and a depressive-like behavioral phenotype following chronic social stress.

Giannis Gampierakis, Giorgos Aspiotis, Anastasia Diamantopoulou, Fotini Stylianopoulou, Antonios Stamatakis

Biology-Biochemistry lab, Dept. Basic Sciences, Faculty of Nursing, School of Health Sciences, University of Athens

Mother-infant interactions constitute the main component of early experiences and play a determinant role in shaping adult emotionality. Early experiences with a negative valence can result in psychopathology such as depression and anxiety in which the function of the serotonergic system is disturbed. In the present work we studied the effects of an early experience in which rat pups are trained in a T-maze in which contact with the mother is either permitted (RER group) or denied (DER group) on the serotonergic system following chronic resident-intruder social stress in adulthood. Previous work from our laboratory has shown that these experiences affect the function of the serotonergic system under basal conditions. Our results showed that serotonergic activity was decreased in the brain of the DER animals. More specifically, serotonin (5-HT, as measured by HPLC) was decreased in the hippocampus and 5-HT1A receptors (as measured immunohistochemically) were decreased in the prefrontal cortex, the hippocampus and the amygdala. These biochemical results could be related to the behavioral phenotype of the DER animals which showed anhedonia, as assessed by no sucrose preference, increased immobility time in the forced swimming test and delayed behavioral adaptation to the chronic stress. All these three parameters are considered as expressions of depressive-like behaviour.

16. Nitric oxide modulates apomorphine-induced recognition memory deficits in rats

I. Gourgiotis, N.G. Kampouri, V. Koulouri, I.G. Lempesis, M.D. Prasinou, G. Georgiadou, N. Pitsikas

Department of Pharmacology, School of Medicine, University of Thessaly, Larissa, Greece

Nitric oxide (NO) is an important intracellular messenger in the brain. The implication of NO in schizophrenia is well documented although it is not yet clear whether net over or underproduction of NO is typical of this disease. In line with this, either NO donors or NO synthase (NOS) inhibitors were found to abolish psychotomimetic effects, including cognition deficits, produced by N-methyl-D-aspartate (NMDA) receptor hypofunction. In addition, there is poor experimental evidence concerning the efficacy of NO to modulate memory deficits produced by dopamine (DA) dysfunction. The present study was designed to investigate the ability of NO modulators (NO donors and NOS inhibitors to reverse recognition memory impairments produced by the DA D_1/D_2 mixed receptor agonist apomorphine in rats. For these studies, the novel object recognition test (NORT) was used as the memory test. Apomorphine (0.05, 0.1, 0.5 and 1.0 mg/kg), dose-dependently, disrupted performance in this recognition memory procedure in rats. The NO donors molsidomine (2.0 and 4.0 mg/kg) and SNP (0.3 and 1.0 mg/kg), reversed the impairing effects of apomorphine (1.0 mg/kg) in the NORT. Administration of the NOS inhibitors L-NAME (1.0 and 3.0 mg/kg) or 7-NI (1.0 and 3.0 mg/kg) produced similar results. The present findings indicate a) that apomorphine dose-dependently impaired recognition memory and b) that a cognitive deficit produced by DA dysfunction is sensitive to NO

17. Long-term voluntary physical exercise overcomes emotional and social deficits induced by early-life stress in rats.

Vicky Katsidoni & George Panagis

University of Crete, Department of Psychology, Laboratory of Behavioral Neuroscience, 74100 Rethymno, Crete, Greece

Human research shows that childhood traumas are associated with an increased risk for mood and anxiety disorders. On the other hand, there is evidence that exercise helps to prevent and to ameliorate anxiety and depression. Likewise, in rats, maternal separation (MS) during early postnatal period, functions as a stressor and has been associated with emotional and social deficits in adulthood. Although several studies have shown that environmental enrichment overcomes behavioral deficits and depressive-like behavior induced by stress, the specific role of physical exercise, commonly used as enrichment stimulus in such studies, is not fully understood. In the present study we tested the effects of MS on emotional and social aspects of behavior in adulthood, combined with the examination of potential reversibility of such deficits by chronic wheel-running (WR). Male Sprague Dawley rats were divided in four groups: MS - free access to WR, MS - no access to WR, non-MS - free access to WR, non-MS - no access to WR. Anxiety-like behavior, depressive-like behavior, social behavior and social memory were tested using the elevated plus maze (EPM), forced-swim test (FST), social interaction (SI) and social recognition (SR) tasks, respectively. MS rats showed increased anxiety- and depressive-like behaviors in adulthood. WR increased the time spent in the open arms and the exploratory behavior in the EPM while it decreased the immobility and increased the climbing in FST, showing anxiolytic and antidepressant actions. Moreover, MS lead to social behavior and recognition deficits in adulthood. WR reversed those effects by decreasing social avoidance and increasing the contact behavior, while significantly improving social discrimination. These results suggest that WR overcomes early life stress-induced emotional and social impairments and provide further support to the hypothesis that exercise may be used as an adjunct to treatment of anxiety and mood disorders.

18. The Influence of the rs1229761G/C *FOXP2* Polymorphism on Gating, Cognition, Language/Thought and Affect in Healthy Males.

Erasmia Koiliari[1], Manolis Pasparakis1, Stella G Giakoumaki2, PanosRoussos1,3, Panos Bitsios1.

1Department of Psychiatry and Behavioral Sciences, University of Crete, Heraklion, Crete, Greece; 2 Department of Psychology, University of Crete, Heraklion, Crete, Greece; 3Department of Psychiatry, Mount Sinai School of Medicine, New York, New York, United States

Introduction: Forkhead box P2 (*FOXP2*) encodes a transcription factor involved in speech and language (1) and in the control of the corticobasal ganglia circuits (1 , 3) . There is also evidence supporting a role for the *FOXP2* locus in schizophrenia (2), autism (1) and ADHD (4). Language impairment, inattention, impulsivity and abnormalities of corticobasal ganglia circuitry are central features of these disorders; however the impact of *FOXP2* risk polymorphisms on relevant intermediate phenotypes has not yet been studied. We selected the rs1229761G/C *FOXP2* non-coding polymorphism which has shown significant association with ADHD (4) .

Methodology: This polymorphism was analyzed in 829 healthy males, phenotyped for prepulse inhibition (PPI), cognition, schizotypy, emotional personality traits and affective startle modulation. Subjects were grouped according to genotype in three groups GG (n=276), GC (n=404) and CC (n=149) and ANOVAs or Kruskal-Wallis tests were used to analyse the phenotypic variables.

Results: The G allele carriers (n=680) performed worse ($p<0.05$) in Spatial Working Memory [Strategy, Total-, Within- and Between- Errors in the difficult 8-box condition). They also scored higher ($p<0.05$) in measures of Schizotypy [STQ_Magical Thinking and STQ_Unusual Experiences ($p<0.01$)] and Impulsivity [BAS_Fun seeking, TCI_Novelty seeking, low scores in EPQ_lie scale]. Finally, they demonstrated a Gating deficit as evidenced by a significant ($p<0.01$) 3-way interaction in the ANOVA [reduced PPI at the short (30ms) interval with the 85dB prepulse].

Conclusions: The rs1229761 G allele, which has been associated with ADHD, impacts on important intermediate phenotypes such as short interval gating, working memory, strategic thinking, schizotypy and impulsivity in healthy males. These results elucidate the function of the *FOXP2* gene in the human brain and suggest that it may be a "hub" for pathological features (gating, cognition, language/thought, impulsivity) common to ADHD, schizophrenia and autism.

Keywords: *FOXP2*, rs1229761, ADHD, Intermediate Phenotypes, Association Study

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19. Effect of an early experience involving reward or denial through maternal contact on NMDA receptor subunits in the adult rat brain.

Manatos Vasileios, Raftogianni Androniki, Stamatakis Antonios, Stylianopoulou Fotini

Laboratory of Biology-Biochemistry, Faculty of Nursing, University of Athens

Early experiences are known to influence adult brain plasticity. A key molecule in plasticity related processes is the NMDA receptor, which is activated by phosphorylation.

Based on the above, we determined by Western blot analysis the levels of phosphorylated and unphosphorylated NR1 and NR2B subunits of NMDA receptor in adult brain areas of the limbic system (prefrontal cortex, PFC, hippocampus, HIP, amygdala, AMY), which is known to be the prime target of early experiences. As an early experience we employed a model developed in our lab. In this model 10 to 13 day-old rat pups are exposed to a T-maze, one arm of which leads to the mother and were either allowed (RER) or denied (DER) the contact with the mother.

Our results showed that the early experience of exposure to the T-maze training affects the levels of the NMDA receptor subunits in the adult brain and in some cases in a sex dependent manner. More specifically, in the PFC, both RER and DER animals had higher levels of the unphosphorylated NR1 and NR2B subunits than the control. Furthermore, among males, the DER animals had higher levels than the RER. In the HIP unphosphorylated NR2B levels were increased as a result of the early experience both in the RER and DER animal groups, but only in the DER animals were the levels of the unphosphorylated NR1 subunit higher. In the AMY, in both sexes, RER and DER animals had less unphosphorylated NR2B subunits than the control.

These results indicate that the early experience of exposure to the T-maze induced long term differences in brain plasticity expressed in adulthood. These early experience-induced changes in NMDA receptor subunit composition could underlie differences in cognitive abilities and emotional copying.

20. Acute but not chronic aromatase inhibition results in antidepressant responses

T. Mavrides[1], T. H. Porto[1,3], N. Kokras[1,2], H.G. Mikail[1], V. Kafetzopoulos[1], C. Dalla[1] and Z. Papadopoulou-Daifoti[1]

1. Department of Pharmacology, Medical School, University of Athens, Greece; 2-First Department of Psychiatry, Eginition Hospital, Medical School, University of Athens, Greece; 3-Psychology Institute, University of São Paulo, Brazil

Aromatase is the rate-limiting enzyme that catalyses the conversion of androgens to estrogens and has a wide distribution in the central nervous system. Aromatase inhibitors are used for treating estrogen-dependent cancers, but there are reports of psychotropic actions, as well. Available preclinical data are scarce (Dalla et al 2004), hence in the present study, we investigated the effect of acute and chronic estrogen depletion by an aromatase inhibitor in the Forced Swim Test (FST) (Dalla et al 2010). Adult female Sprague-Dawley rats received the Serotonin-Selective-Reuptake-Inhibitor (SSRI) antidepressant fluoxetine for 28 days (5 mg/kg, i.p.) or vehicle. During the last week of the fluoxetine treatment, a subgroup of animals received the aromatase inhibitor letrozole in a chronic way (i.p., 1 mg/kg, 7 days), whereas another subgroup received letrozole sub-acutely (i.p., 1 mg/kg, 3 injections in 24h). All rats were subjected to a 15min FST pretest session and twenty-four hours later, they were subjected to a second 5min FST test session. Rats in the sub-acute groups were treated at 0, 19 and 23 hours after the pretest session of the FST with three injections of vehicle or letrozole. All videos (5 min test sessions) were manually scored with the in-house developed software Observador. 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 monitored.

Results were analyzed using a two-way multivariate analysis of variance (MANOVA) and Dunnet's post-hoc pairwise comparisons. Regarding swimming duration, the analysis revealed a significant letrozole - fluoxetine interaction [$F_{(2,50)}$=4.051 p=0.023] and a significant letrozole main effect [$F_{(2,50)}$=10.167 p<0.001]. As expected, fluoxetine enhanced swimming duration (p<0.05), in comparison to vehicle. Acute letrozole treatment also enhanced swimming duration, in comparison to vehicle-treated rats (p<0.001). On the other hand, chronic letrozole treatment had no effect. Regarding immobility duration, there was a letrozole main effect [$F_{(2,50)}$=8.487, p=0.001] and post-hoc comparisons clearly showed that rats treated acutely with letrozole spent less time immobile, in comparison to vehicle-treated rats (p=0.01). However, this was not observed in rats treated chronically with letrozole. Regarding climbing behavior, neither letrozole nor fluoxetine altered this behavioral response.

Present findings indicate that acute estrogen depletion by letrozole results to an antidepressant effect in the FST similar to the one observed following antidepressant SSRI treatment. This is of particular interest because enhanced swimming duration in the FST due to SSRI treatment correlates with serotonergic changes in the prefrontal cortex (Mikail et al 2012). On the other hand, chronic letrozole treatment had no effect in the FST, although it eliminated the presence of estrous cycle and depleted estrogen levels. The antidepressant effect of acute letrozole treatment could be attributed to enhanced testosterone levels, due to inhibition of its metabolism to estrogens by aromatase inhibitors, but interestingly other compensatory mechanism take place when estrogen synthesis is chronically disrupted. Present findings have potential implications for women treated with aromatase inhibitors, as well as for the role of gonadal hormone in the expression of depressive symptoms.

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21. Effects of an early experience involving reward through maternal contact or its denial on the noradrenergic system of the neonatal and adult rat brain.

Pelagia Melissa, Fotini Stylianopoulou, Antonios Stamatakis

Biology-Biochemistry lab, Dept. of Basic Sciences, Faculty of Nursing, University of Athens, Greece

Early life experiences have profound effects on the development of adult behavior and especially stress responses and emotionality. Given that the noradrenergic system is a major regulator of these behaviours, in order to investigate the effects of neonatal experiences on this system we employed an animal model which includes exposure of rat pups during post-natal days (PND) 10 to 13 to a T-maze in which contact with the mother is used as reward (RER group) or denial of this contact as a negative, frustrating event (DER group). We determined the effects of these experiences on β1 and α2 adrenoceptor levels on PND13 and adult male and female brains, using immunohistochemistry and autoradiographic *in vitro* binding. Immediately following exposure to the T-maze, on PND13, levels of β1 receptors were decreased specifically in the amygdala of the DER animals. In adulthood, more extended effects were observed: In the prefrontal cortex of the RER group β1 receptors were increased while α2 were decreased. In contrast, in the hippocampus and the amygdala of the DER β1 receptors were decreased while α2 were increased. These differences in adrenoceptor levels could underlie the differences in stress coping, anxiety and fear responses exhibited by the DER and RER animals.

22. Colocalization of tyrosine hydroxylase with urocortin-1 in Edinger-Westphal nucleus of the human neonate under severe perinatal hypoxia

Marianna A. Pagida[1,2], Anastasia E. Konstantinidou[3], EffrossiniTsekoura[4], Efstratios Patsouris[3] and Maria T. Panayotacopoulou[1,2]

[1]Department of Psychiatry, [2]University Mental Health Research Institute, [3]1st Department of Pathology and [4]3rd Department of Pediatrics, National Kapodistrian University of Athens, Greece

Epidemiological studies indicated that hypoxia during the last trimester or during the intrapartum period could cause long-term damage to the central nervous system, leading to behavioral and/or neurological deficits later in development. Experimental models showed that hypoxia to the fetus –a consequence common to many birth complications in humans- results in selective long-term disturbances of the dopaminergic (DA) systems that persist in adulthood[1]. Our previous study in human autopsy material[2] showed increased expression of tyrosine hydroxylase (TH, first and rate limiting enzyme of catecholamine synthesis) in the mesencephalon of the human neonate under severe/ acute perinatal hypoxia. Surprisingly, under hypoxic conditions, TH was expressed not only in the well-known DA cell groups substantia nigra and ventral tegmental area, but also in Edinger- Westphal nucleus (EW). In humans, EW nucleus -as delineated in Olszewski and Baxter atlas (1982)- contains the non preganglionic urocortin 1/ centrally projecting neurons, that have been implicated in adaptation to stress, anxiety, depression and alcohol consumption[3,4].

Purpose of the present study was to investigate whether TH colocalizes with urocortin 1 -member of the corticotrophin releasing factor (CRF) family- in EW neurons of the human neonate. We studied the EW of 15 infants (aged 34-46.5 weeks) prospectively collected from the Greek Brain Bank (director Prof. Patsouris), after parental written consent for the use of brain material for diagnostic and research purposes. The evaluation of the duration and severity of the hypoxic insult was based on established neuropathological criteria. Two consecutive sections of mesencephalon at the central level of EW[5] were stained immunohistochemically for TH and urocortin 1, respectively. Expression of TH was observed in almost all the neurons of EW in cases suffered severe/ abrupt hypoxic insult, but not in subjects died after prolonged perinatal hypoxia. No correlation between TH expression and the age of the neonates was evident, suggesting that TH expression in EW is not developmentally determined, but reflects a response to the acute hypoxic stress. In neonates suffered severe/ abrupt perinatal hypoxia, the majority of urocortin 1 neurons colocalize TH. TH-positive neurons without urocortin 1, however, were also evident. No differences in the expression of urocortin 1 were observed between neonates suffered perinatal hypoxia of different severity or duration. Since TH is expressed in the urocortin 1 neurons of EW only under acute hypoxic conditions, we suggest that the early shift in catecholamine phenotype of these neurons provides an additional adaptation mechanism to the human neonate to survive under severe hypoxic stress.

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23. Measuring cerebral lateralisation for language using functional transcranial Doppler ultrasound: the case of hearing impaired students

M. Papadatou-Pastou, N.M. Rantou, A. Koufaki, D.M. Tomprou

Research Center for Psychophysiology and Education, Faculty of Education, National and Kapodistrian University of Athens

Cerebral lateralization for language has been considered a prerequisite for the full realization of an individual's linguistic potential. A large body of research has been indeed dedicated to the study of the relationship between atypical language lateralization (right-hemispheric or symmetrical) with conditions such as hearing impairment, where the oral component of language is missing. Nevertheless, cerebral laterality in hearing impaired populations using the Greek sign language (GSL) has not been studied to date. The present study assesses cerebral lateralization for language in hearing impaired adolescents, using functional transcranial Doppler ultrasound (fTCD), a brain-imaging technique which compares blood flow velocities in the middle cerebral arteries while participants are engaged in a language task. FTCD is cost-effective, non-invasive, reliable, and it has been validated against the Wada technique as well as against laterality assessments performed using functional magnetic resonance imaging. It has been applied for nearly 15 years in adults for the assessment of cerebral laterality of different cognitive functions, mainly language. Recently, children-friendly cognitive tasks appropriate to use with fTCD were developed, tasks that are friendly even to populations with special educational needs such as hearing impaired students (Bishop, Watt, Papadatou-Pastou, 2009, Neuropsychologia, 587-590). Here, the technical characteristics of fTCD will be described, together with the tasks that can be used for the assessment of cerebral laterality for language. Moreover, preliminary data comparing the cerebral lateralisation for language in deaf and hearing adolescent students who use the GSL will be presented.

24. Sex differences in emotional responses: effects of gonadal- and brain- derived estrogens

N. Pastromas[1], C. Dalla[1], V. Kafetzopoulos[1], N. Kokras[1,2], E. Tzouveka[1], J. Balthazart[3], C. A. Cornil[3] and Z. Papadopoulou-Daifoti[1]

1. *Department of Pharmacology, Medical School, University of Athens, Greece*
2. *First Department of Psychiatry, Eginition Hospital, Medical School, University of Athens, Greece*
3. *Behavioral Neuroendocrinology Research Group, GIGA Neurosciences, University of Liège, Belgium*

Depression is an important psychiatric disorder, which affects women twice as much as men. Fluctuations in peripheral estrogen levels in women have been implicated in the etiology of this sex difference (Dalla et al 2010). Recently, local *de novo* estrogen synthesis has also been discovered in the brain and emerging evidence suggests that these neuroestrogens are responsible for the fine-tuning of neuronal circuits in males and females. However, it has not been identified whether neuroestrogens have an effect in behavioral indices of anxiety and depression.

In the present study, we investigated the role of gonadal hormones and neuroestrogens in the open field and the Forced Swim Test (FST). Open field and FST were performed 4 weeks after gonadectomy or sham-operation of adult female and male Wistar rats. Before the behavioral tests, sham-operated and gonadectomized rats were injected, i.p. for one week, with either vehicle or the aromatase inhibitor letrozole (1 mg/kg). Aromatase is the rate-limiting enzyme that catalyses the conversion of androgens to estrogens and is located in both sexes in the gonads and in the brain. Thus, by treating gonadectomized male and female rats with letrozole, we aimed to eliminate gonadal hormone secretion and to further investigate the role of neuroestrogens in behavior. Aromatase activity in the hypothalamus was determined by the production of tritiated water associated with the conversion of [1β-^{3}H]-androstenedione into estrone and verified that letrozole inhibited aromatase in the brain.

Analysis of behavioral data revealed that females were overall more active and explorative than males in the open field test (sex effect: $p<0.05$), while gonadectomy eliminated this sex difference. This was mainly due to the fact that ovariectomy in females decreased ambulatory and vertical counts in the open field test (surgery effect: $p<0.05$). Inhibition of estrogen synthesis with letrozole had no effect in the open field test neither in sham nor in gonadectomized male and female rats. On the other hand, letrozole treatment enhanced immobility and decreased swimming duration (interaction of sex with treatment $p<0.05$) only in ovariectomized females; a finding indicative of enhanced "depressive-like" symptomatology. Notably, letrozole had no effect on sham-operated or gonadectomized males.

These results indicate that estrogens originating from the gonads and the brain significantly affect the behavioral response. Furthermore, present data suggest a role of estrogens depletion in the development of affective disorders in post-menopausal women treated with aromatase inhibitors.

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25. Hormonal and behavioral regulation of adult cell proliferation in the zebrafish (Danio rerio) brain.

Panayiota Makantasi, Diamantina X. Spilioti & Catherine R. Dermon

Laboratory of Human and Animal Physiology, Department of Biology, University of Patras, Patras, Greece

Epigenetic factors, such as steroid hormones and behavior, are known to influence brain neurogenetic events. Adult zebrafish brain proliferation zones provide an excellent model to study such influences. The present study investigated estrogen effects and the potential role of social aggression in the mitotic activity of adult zebrafish brain. For this, groups of male and female zebrafish were treated for 7 days with 17-beta-estradiol (2.5 microgr/L). Additionally, male zebrafish were kept in isolation or in pairs for the development of stable social interactions. Teleosts develop well-established social hierarchies, with dominant fish exhibiting aggression. The combined effects of estrogen treatment and social experience (behavioral data recorded for 7 days) were studied in isolated and pairs of males with established hierarchies. To determine active cycling cells within the adult proliferation zones, animals were allowed to swim in 5mM BrdU solution for 6 hours. For the quantification of proliferating cells, BrdU immunohistochemistry followed by disector methodology was applied. Stereological analysis of BrdU positive cells revealed that estrogen treatment as well as social interaction had a region- and sex-specific impact on mitotic activity. Specifically, statistically significant decreases were observed in the proliferation zones of corpus cerebelli and the ventral telencephalon of estradiol-treated female zebrafish. Within the social groups studied, the dominant fish exhibited higher numbers of BrdU+ cells in the cerebellar and hypothalamic regions in comparison to the subordinates. Our data provide novel important evidence on the significant influences of exogenous estrogens in relation to sex and social status in the proliferative potential of adult brain.

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26. Lack of cannabinoid-induced effects on recognition memory and spatial learning in ERK1-deficient mice.

Alexia Polissidis[1], Katerina Antoniou[2], Sylvia Dimitriadou[1], Vasiliki Kanta-Chantzi[1] and Irini Skaliora[1]

[1]Neurophysiology Laboratory, Biomedical Research Foundation Academy of Athens
[2]Department of Pharmacology, Medical School, University of Ioannina

The endocannabinoid system is intimately involved in synaptic plasticity and thus, in learning and memory processes. One of the main intracellular signaling pathways involved in these processes is the MAPK/ERK pathway and cannabinoid agonists have been shown to induce downstream phosphorylation of ERK1/2 via activation of the CB1 receptor. In addition, both natural and synthetic CB1 cannabinoid receptor agonists have been shown to affect memory and learning, however, their mode of action for the most part is unknown. The goal of the present study was to explore the relationship between cannabinoid CB1 receptor agonists and their effects on ERK signaling in learning and memory. In experiment 1, we examined the pharmacological profile of a potent CB1 receptor agonist, HU210, on general locomotor activity and short-term recognition memory using the novel object recognition task in the rat and in experiment 2, we extended these findings with studies in ERK1 deficient mice to assess both short term recognition memory and spatial learning/memory, using the novel object recognition task and the Morris water maze, respectively. In experiment 1, adult male Sprague Dawley rats were assessed for locomotor activity for 60 min, or novel object recognition to assess short-term (1 h) recognition memory following administration of HU210 (0, 0.003, 0.01, 0.03 mg/kg, i.p.). In experiment 2, ERK1 deficient mice and wildtype littermates received a low dose of HU210 (0.01 mg/kg, i.p.) and two memory tasks were examined: (i) short term (1 h) recognition memory in the novel object recognition task; and (ii) spatial learning and memory in the Morris water maze task consisting of three phases: a 2-day cued version with a visible platform, a 7-day acquisition phase with a hidden platform and a long term memory retention probe trial on the final day. Results from experiment 1 revealed a dose-dependent decrease in locomotor activity following HU210 administration as well as a dose-dependent short-term memory deficit in the novel object recognition task. Similarly, in experiment 2, HU210 produced a discrimination deficit in wildtype mice. In contrast, ERK1 deficient mice were not affected by HU210 administration and displayed the same level of discrimination as wildtype and ERK1 deficient vehicle-treated mice. Spatial learning and memory were undifferentiated between the two genotypes in the Morris water maze. However, HU210 administration had opposite effects on wildtype and ERK1-/- mice, causing improved learning in the acquisition phase in the former, but delayed learning in the latter. Long term memory retention as assessed by a probe trial 24 h following the last acquisition session revealed no differences between genotype or drug treatment. Taken together, our results provide evidence for an ERK1-dependent effect of cannabinoid administration on short-term recognition memory and spatial learning, suggesting that ERK2 is not sufficient or required to mediate this effect.

27. Sex dependent long-lasting effects of an early experience of reward through maternal contact or its denial on the dopaminergic system of rat brain.

Androniki Raftogianni, Anastasia Diamantopoulou, Anna-Maria Kollia, Antonis Stamatakis, Fotini Stylianopoulou

Lab of Biology-Biochemistry, Faculty of Nursing, University of Athens, Greece

It is well accepted that early experiences have long lasting effects, influencing adult brain function and behavior. During the neonatal period the mother is an extremely salient stimulus, and seeking for her a powerful inherent drive. Based on the above we developed an experimental model in which during the neonatal period (PDN 10-13) rat pups were exposed to a T-maze one arm of which led to the mother. One group of animals was allowed contact with the mother (RER) while the other was denied (DER). Since the dopaminergic system is an important component of the reward system, we determined in both the prefrontal cortex (PFC) and the nucleus accumbens (NAc), the levels of dopamine (DA) and its metabolites by HPLC and those of D1 and D2 receptors by autoradiographic *in vitro* binding on postnatal day 13 and in adulthood. Interestingly the early life experience induced changes in the dopaminergic system in a sex-specific manner. In the NAc of adult RER female rats (but not in males) we found that DA and HVA levels were higher than those of the control or DER animals. Notably 13 day old rat RER pups also had higher DA and HVA levels in the NAc than the DER or CTR. These pups also had higher levels of D1 receptors in the NAc. These results indicate a general activation of the dopaminergic system in the NAc of RER pups which could be related to the reward the pups received through the contact with their mother. The activation of the dopaminergic system appeared to be long - lasting in a sex-dependent manner being also evident in the adult female rats. Furthermore, the early experience of denial the expected reward also had long term sex-dependent effects on the dopaminergic system. In the PFC of the adult DER male animals DA levels were reduced compared to the RER or control male animals. It is quite interesting that in this specific brain area of 13 day old rat pups exposed to the denial experience (DER) we found that the levels of DA and its metabolites (DOPAC and HVA) were reduced compared to those of the RER pups or the controls. Our results document that early experiences affect in a sex-dependent and brain area specific manner the dopaminergic system which is the neurobiological substrate for reward processes and its function is disturbed in many psychiatric diseases**.**

28. Pharmacogenetic dissection of the locomotor hyperactivity in dfmr1 heterozygotes.

Semelidou Ourania, Kanellopoulos Alexandros and Skoulakis MC Efthimios

BSRC 'Alexander Fleming', 16672, Vari, Greece

Fragile X Syndrome (FXS) is one of the most common forms of inherited mental retardation and results in a spectrum of disorders including hyperactivity, social anxiety and sleep disorders among others. The syndrome is caused by transcriptional silencing or loss of function of one gene, *fmr1*, which encodes for a RNA-binding protein. In D. melanogaster there is one homologous gene, dfmr1 and in the absence of its protein (dFMRP) flies show phenotypes resembling these of FXS patients. It is known that dFMRP is required for normal circadian behaviour and mutants display arrhythmic circadian activity and erratic locomotor activity patterns.

In this study we use heterozygous mutants to examine the phenotype of partial absence of dFMRP in locomotor activity. Heterozygotes present deficits in learning and long term memory, behaviors affected in FXS. In contrast to the *dfmr1* mutant homozygotes which seem hypokinetic, the 50% dFMRP reduction in heterozygotes yields a hyperactive phenotype, also often observed in FXS patients. This phenotype is rescued by panneuronal overexpresson of wild type *dfmr1* and with particular pharmaceuticals. Interestingly, flies exhibiting hyperactivity present defective habituation as well. More specifically, reduced dFMRP results in habituation resistant flies, a phenotype rescued with drugs that are also effective in ameliorating hyperactivity. This indicates that reversal of hyperactivity may be a good surrogate phenotype to screen pharmaceuticals targeting the defective habituation and perhaps learning and memory as well.

29. Neocortical spontaneous slow-rhythmic activity in mice lacking the β2-subunit of the nicotinic acetylcholine receptor.

Charalambos Sigalas, Pavlos Rigas, Panagiotis Tsakanikas, Eleni Konsolaki and Irini Skaliora

Biomedical Research Foundation of the Academy of Athens, Greece.

During quiescent brain states, such as non-REM sleep and anaesthesia, a spontaneous slow-rhythm activity develops in the cerebral cortex. This activity is largely maintained in vitro, in cortical slice preparations, indicating that it is largely the outcome of intrinsic properties of the local neural networks. The cholinergic system is believed to regulate the cycling between sleep and wakefulness and activation of the cholinergic system has been associated with REM sleep and arousal. In addition, the β2 subunit of the nicotinic acetylcholine receptor (nAChR) has been linked to the high affinity nicotine binding sites of the brain and is thought to be related to cognitive deficits associated with ageing. In the present study we examined the characteristics of spontaneous rhythmic activity in brain slices of adult (3-9 months) and old (>18 months) mice lacking the β2 subunit of the nAChR (b2KO). Field potential recordings were obtained from the somatosensory cortex of wild-type (WT) and b2KO C57BL/6 mice. We found that the duration of individual spontaneous events was longer in the b2KO mice compared to WT, and this did not seem to be affected by mouse age. Additionally, we found differences in the power spectrum of individual events: the relative power of the lower gamma frequency band was significantly increased in adult b2KO mice compared to adult WT and old b2KO mice. Furthermore, preliminary pharmacological experiments using carbachol indicate differences in the mechanisms supporting the spontaneous rhythmic activity of the b2KO compared to WT mice. Overall, the spontaneous network activity exhibits systematic differences between WT and b2KO mice, suggesting that this network phenomenon could serve as an *endophenotype* of cortical physiology.

30. Phobos: A novel software for recording rodents' behavior during the thigmotaxis and the elevated plus-maze tests

Aristeidis G. Telonis*, Marigoula Margarity

Laboratory of Human and Animal Physiology, Department of Biology, School of Natural Sciences, University of Patras
** Current Address: Metabolic Engineering and Systems Biology Laboratory, Institute of Chemical Engineering Sciences, Foundation of Research and Technology Hellas*

Fear and anxiety are physiologically widespread behavioral outcomes under stressful conditions among vertebrates in general and mammals in particular [1, 2]. Moreover, evaluation of fear and anxiety levels offers valuable insight on the impact of experimental conditions. The elevated plus-maze (EPM) test [3] and the open field, also known as thigmotaxis test [4], are two well established experimental procedures for the quantification of fear and anxiety in rodents by providing the ability of estimating multiple relevant parameters. However, a single experimentalist faces difficulties in evaluating all possible ones in terms of parallel assessment.

Here, "Phobos", a novel and efficacious application developed for recording rodents' behavior during the EPM and the thigmotaxis test, is being presented for the first time. Powered by Python 2.7, "Phobos" is able to generate all possible locomotor-related behavioral results at once, immediately after a simple record of the rodent's behavior. The parameters calculated for the EPM test are the total time that the animal spent in each compartment of the apparatus and the number of entries to the closed or open arms. For the thigmotaxis test, the total time that the rodent spent on the peripheral and central area of the open field, the number of central entries and the total squares crossed are measured. These parameters are estimated for each 5-min period of the total duration of the latter test which can be set to run for either 10 or 30 min. Saving the experimental outcome extracts a txt file, containing the raw data and the analytical results underneath, paving the way for direct and panoramic statistical evaluations.

"Phobos" consists of two easily controlled and self-explanatory timer windows and each one corresponds to the respective experiment. On the upper left of each window there is a static box demonstrating the minutes and the seconds passed during the experiment, while a picture on the right illustrates the current animal's position on the experimental apparatus during the test. Further specific characteristics concerning control buttons and graphical representations are appropriately programmed in each timer. Noteworthy is the convenience offered by Phobos in terms of controlling the animals position. Any two and any four keyboard buttons are just required during the EPM and the thigmotaxis test, respectively, to update the animal's position and these are handily changed, along with color preferences, in order to best adapt to the experimentalist's individual preferable style.

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***Contact**: atelonis@upatras.gr*

Poster Session II

1. Boosting Chaperone-Mediated Autophagy as a means to mitigate alpha-synuclein-mediated neurotoxicity

Maria Xilouri[1], Oystein Rod Brekk[1], Natalie Landeck[2], Pothitos M. Pitychoutis[3], Themistoklis Papasilekas[1], Zoi Papadopoulou-Daifoti[3] , Kostas Vekrellis[1], Deniz Kirik[2] and Leonidas Stefanis[1,4]

1: Division of Basic Neurosciences, Biomedical Research Foundation of the Academy of Athens (BRFAA), Athens, Greece
2: Brain Repair and Imaging in Neural Systems, Department of Experimental Medical Science, Lund University, Lund, Sweden
3: Department of Pharmacology, Medical School, University of Athens, Athens, Greece
4: Second Department of Neurology, University of Athens Medical School, Athens, Greece

Parkinson´s Disease (PD) is a neurodegenerative disorder of unknown etiology. An increasing wealth of data implicates increased a-synuclein (AS) burden in PD pathogenesis. Previous data suggest that a major pathway for AS clearance is Chaperone-Mediated Autophagy (CMA). CMA inhibition via down-regulation of its rate-limiting step, Lamp2a, leads to accumulation of aberrant AS species in neuronal cells (Vogiatzi et al., 2008), whereas AS mediated neurotoxic effects are partly attributed to CMA inhibition (Xilouri et al., 2009). Moreover, CMA markers are diminished in the parkinsonian brain (Alvarez-Erviti et al., 2010), indicating that CMA may represent a promising target for therapeutic intervention in PD and related synucleinopathies. To adress this *in vivo*, we overexpressed Lamp2a concurrently with AS via rAAVs (recombinant adeno-associated viruses) in the rat substantia nigra (SN), in a well established human rAAV-AS model of synucleinopathy. Animals were either decapitated or intracardially perfused at 8 weeks post-infection, for biochemical or histological analysis, respectively. We observed robust colocalized expression of both transgenes in the dopaminergic neurons of SN, with Lamp2a expression effectively ameliorating AS-induced neurotoxicity observed in GFP+AS injected animals - as measured by an unbiased stereological estimator. This protective effect was also evident at the level of the nigrostriatal terminals, with an almost complete rescue of the dopaminergic phenotype - estimated by measuring dopamine levels by HPLC and tyrosine hydroxylase (TH) immunoreactive intensity by densitometry. Nigral overexpression of human AS resulted in accumulation of phosphorylated (S129) and SDS-soluble high-molecular weight (HMW) AS species in the GFP+AS injected animals. Importantly, both monomeric- and HMW AS species were profoundly reduced in the nigral fractions of AS+Lamp2a animals, with a definitive trend, albeit non-significant, for reduction of phosphorylated AS species. Taken together, we postulate that Lamp2a overexpression and subsequent CMA upregulation is sufficient to protect against AS-induced neurotoxicity in vivo, by preserving dopaminergic system integrity and facilitating AS clearance (both monomeric and HMW aberrant AS forms).

2. Cellular activation following epileptic seizures in mice with decreased inhibition: Effect of enriched environment

Kleanthi Chalkiadaki[1,2], X. Konstantoudaki[1], S. Tivodar[2,3], D. Karagogeos[2,3] and K. Sidiropoulou[1,3]

[1] Department of Biology [2]Department of Basic Sciences, School of Medicine, University of Crete [3]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas

Both genetic and environmental factors are known to affect brain function. The mammalian cortex is composed of both pyramidal neurons and interneurons. Although the vast majority of studies have focused on the pyramidal neurons, interneurons also play a significant role in cortical function and behavior. Here, we use a mouse genetic model that displays reduced number of inhibitory neurons in order to determine the effect of decreased inhibition on epileptic seizures and on cellular activation following the seizures. In addition, we study how enrichment of the cage environment affects the behavioral phenotype of these mice.

The mouse model used has been shown to express about 50% less interneurons in cortical areas, due to the loss of the Rac1 protein from MGE-derived interneurons ($Rac1^{Fl/Fl}/Nkx2.1^{+/cre}$ mice). The 'missing' interneurons are known to express either the parvalbumin or somatostatin proteins. Our results revealed that 30% of $Rac1^{Fl/Fl}/Nkx2.1^{+/cre}$ mice die between 15 and 35 days old, shortly after expression of spontaneous tonic-clonic epileptic seizures. The surviving mice were tested for decreased threshold in pharmacologically-induced seizures. Specifically, mice were injected with different doses of pilocarpine (100, 200 and 300mg/kg) and their behavior was observed for at least 2hrs for seizure induction according to the Racine's scale. Ninety minutes following the highest reported seizure, the brains were collected. Immunohistochemistry was performed in order to determine cellular activation, based on expression of the immediate early gene, c-fos, following the seizure. Brain areas examined included the barrel cortex, the hippocampus and the prefrontal cortex (PFC). Although the first two areas are well known to be activated in epileptic seizures, very little is known with regards to PFC, despite known impairment of its function. We find that $Rac1^{Fl/Fl}/Nkx2.1^{+/cre}$ mice exhibit stage 6 epileptic seizures in a significantly greater percentage when injected with 100mg/kg pilocarpine compared to their wild-type controls, rendering the $Rac1^{Fl/Fl}/Nkx2.1^{+/cre}$ more similar to the control mice that received 200 mg/kg pilocarpine. In addition, c-fos activity is impaired in $Rac1^{Fl/Fl}/Nkx2.1^{+/cre}$ mice, in all brain areas examined.

Our next goal was to determine whether environmental enrichment could improve the deficits of $Rac1^{Fl/Fl}/Nkx2.1^{+/cre}$ mice. Breeding pairs were housed in cages that included a small 'house' where newborn mice grow up. At weaning, mice are transferred to cages that include a running wheel, a straight and a curved tube. We find that only 12% of $Rac1^{Fl/Fl}/Nkx2.1^{+/cre}$ mice reared in enriched environment die until 35 days old, a significantly decreased percentage compared to mice reared in standard housing conditions. In addition, control mice exhibit increased latency for induction of epileptic seizures following pilocarpine injection, suggesting a possible effect of the enriched housing conditions on pilocarpine-induced epileptic seizures. Overall, our results show that decreased inhibition renders the mice either epileptic or more susceptible to epileptic seizures with disrupted neuronal networks activated, while enriching the housing conditions likely improves the pathological phenotype.

3. Amyloid Precursor Protein enhances both calcium storage and the dynamics SOCs activity

Maria Chatzistavraki, Elli Kyratzi, Angeliki Fotinopoulou, Panagiota Papazafiri, Spiros Efthimiopoulos

Department of Biology, Division of Animal and Human Physiology, University of Athens, 157 84 Panepistimiopolis, Ilisia, Greece

The amyloid precursor protein (APP) is a type-1 transmembrane protein involved in Alzheimer's disease (AD). It has become increasingly evident that APP, its protein-protein interactions and its proteolytical fragments may affect calcium homeostasis and vice versa. In addition, there is evidence that calcium dysregulation contributes in AD. To study the role of APP in calcium homeostasis, we downregulated its expression in SH-SY5Y cells using shRNA (SH-SY5Y/APP-) or increased expression of APP695 by transfection (SH-SY5Y/APP+). The levels of cytosolic Ca^{2+} after treatment with thapsigargin, monensin, activation of Capacitative Calcium Entry (CCE) and treatment with SKF, a Store Operated Channel (SOCs) inhibitor, were measured by fura-2AM fluorimetry. SH-SY5Y/APP+ cells show reduced response to thapsigargin, monensin and reduced CCE, although this reduction is not statistically significant. On the other hand silencing of APP potentiates thapsigargin-induced calcium efflux from the ER, and monensin-induced calcium release from acidic stores, and accelerates inhibition of SOCs by their specific inhibitor, SKF. The increase of calcium release from the ER and the acidic stores, when APP is downregulated, could be attributed to elevated Ca^{2+} content or to a dysregulation of Ca^{2+} transfer through their membranes. These data along with already existing evidence regarding the role of APP in calcium homeostasis and the early occurring structural and functional abnormalities of endosomes substantiate further the role of APP in calcium homeostasis and in AD.

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4. Transcriptional regulation of α-synuclein

Dermentzaki Georgia[1], Clough Richard Lee[1], Stefanis Leonidas [1,2]

[1]Division of Basic Neuroscience, Biomedical Research Foundation of the Academy of Athens, Athens
[2]Second Department of Neurology, University of Athens Medical School, Athens, Greece

α-Synuclein (SNCA) is an abundant neuronal protein linked to the development of neurodegenerative diseases, and in particular Parkinson's disease (PD). Genetic overexpression or missense point mutations of SNCA lead to PD in humans, and its overexpression is sufficient to cause PD-like symptoms and neuropathological changes in some animal models. We previously identified elements in the 1st intron of SNCA that are important in its transcriptional regulation in PC12 cells in response to treatment with NGF and bFGF (Clough and Stefanis, 2007). In further experiments, we showed, using a variety of methods, that the transcription factor ZSCAN21 (also known as Zipro 1) is involved in SNCA transcriptional regulation in this cell system. In current experiments, we have gone on to characterize the expression of ZSCAN in the rat brain, as a first step in deciphering the potential role of ZSCAN21 in SNCA transcriptional regulation *in vivo*. Using RT-PCR and Western immunoblotting, we have found that ZSCAN21 is expressed in various regions of the developing and adult rat brain where SNCA was also detected, including the ventral midbrain and the hippocampus although protein expression in the former area was rather low. More specifically, ZSCAN21 protein levels are highly expressed at embryonic stages and gradually decrease from postnatal stage to adulthood (following the mRNA pattern of SNCA). Next, we have produced a specific antibody against ZSCAN21 that efficiently detects endogenous ZSCAN21 in both western blot and immunohistochemistry assays. Double immunohistochemistry with anti-ZSCA21 and anti-NeuN (neuronal marker) verified neuronal expression and nuclear localization of ZSCAN21. We have accordingly constructed lentiviruses expressing shRNAs against ZSCAN21 or against scrambled sequences, with the purpose of expressing these via stereotaxic injection in the rat hippocampal area, an area directly correlated with cognitive impairment and dementia in PD subjects, and, importantly for our purposes, an area where ZSCAN21 is expressed to a considerable degree. We aim to examine the effects of such injections on SNCA protein levels using immunohistochemistry and Western immunoblotting. Such studies may cement ZSCAN21 as an important regulator of SNCA transcription, and may provide potential therapeutic targets not only for PD but also for other synucleinopathies.

5. Lead-induced neurotoxicity effects on behavior, acetylcholinesterase activity and oxidative markers of adult male mouse brain

Ferlemi Anastasia-Varvara[1], Avgoustatos Dionisis, Margarity Marigoula

Laboratory of Human and Animal Physiology, Department of Biology, University of Patras, Patra 26500, Greece
[1]Present Address: Laboratory of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, University of Patras, Patra 26500, Greece

Lead (Pb) is considered as a neurotoxin, affecting various aspects of brain development, function and behavior. The aim of our study was to examine the effects of Pb exposure on adult male mice behavior (learning and memory and fear/anxiety indices). Moreover, we studied the activity of two isoforms of acetylcholinesterase (AChE), the membrane-bound G4-isoform, brain's most significant isoform, and cytosolic G1-isoform. We, also, evaluated the ratio G4/G1 isoform activity which is considered as an index that can rapidly alter in response to stressors in order to maintain homeostasis [1]. Further we examined the effects of Pb consumption on brain's oxidative markers.

Adult male mice were randomly divided into four groups: two vehicle groups (served as controls, one for each examined behavioral task) which consumed sodium acetate solution [500 ppm CH_3COONa] in their drinking water for 28 days and two Pb-treated groups (one for each examined behavioral task) which consumed Pb solution [500 ppm $Pb(CH_3COO)_2$] in their drinking water for 28 days. At the end of the treatment period, the mice were subjected to the behavioral tasks. Learning and memory was tested by step-through passive avoidance test, whereas fear and anxiety behavior was studied using the elevated plus-maze and thigmotaxis test. We further examined the activity of two isoforms of AChE in cortex, midbrain, hippocampus, striatum and cerebellum using the colorimetric method of Ellman. GSH and MDA levels in brain regions were determined fluorometrically.

Pb consumption caused significant deficits on mice learning/memory ability and increased anxiety as indicated by the decreased time spent in the open arms of the apparatus in elevated plus-maze (40.19%) and increased thigmotaxis time (13.45%) compared to the respective controls. The consumption of the Pb solution inhibited the activity of the two AChE isoforms in all brain regions tested (DS fraction: striatum 36.12%, hippocampus 30.11%, midbrain 19.72%, cortex 13.37%, cerebellum 11.1%, SS-fraction: cortex 41.19%, striatum 39.58%, hippocampus 27.71%, cerebellum 23.7%, midbrain 14.92%). However, Pb consumption revealed variations in the ratio of G4/G1 isoform activity among the examined brain regions. Furthermore, Pb exposure increased lipid peroxidation and decreased GSH levels in all brain regions examined. Conclusively, Pb-induced neurotoxicity causes learning/memory deficits and increases fear/anxiety in adult male mice through tissue-depended inhibition of AChE activity, while it disrupts the redox balance of mouse brain.

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6. Luciferase immunoprecipitation assay as a new tool for studying antibodies in autoimmune neurological diseases

Fouka P[1], Alexopoulos H[1], Trohatou O[2], Akrivou S[1], Politis P[2] and Dalakas MC[1]

[1]Neuroimmunology Unit, Department of Pathophysiology, Faculty of Medicine, National and Kapodistrian University of Athens, Greece
[2]Center for Basic research, Biomedical Research Foundation of the Academy of Athens, Greece

Objective: We aim to establish an easy to perform and sensitive assay for the detection of autoantibodies in autoimmune diseases and screen cohorts of neurological patients.

Background: Autoimmune CNS disorders are in the limelight in the last decade. Autoantibodies in demyelinating autoimmune diseases, such as neuromyelitis optica (NMO) have been proven to be pathogenic, while new findings suggest that a few more CNS disorders have an autoantibody association. Certain forms of cerebellar ataxia and limbic encephalitis can be attributed to the rise of autoantibody titers, such as anti-NMDAR, anti-VGKC (Voltage-gated Potassium Channel accessory proteins, anti-LG1, anti-CASPR2) and anti-GAD. Anti-GAD antibodies are also found in a rare disabling autoimmune CNS disorder, Stiff Person Syndrome (SPS). SPS is characterized by progressive muscle rigidity, superimposed spasms and psychogenic symptoms. We have established a new luciferase immunoprecipitation assay (LIPS) for the rapid detection of autoantibodies of neurological interest

Methods: The DNA sequences coding for different autoantigens are cloned next to the Renilla luciferase reporter gene into the pcDNA3.1 mammalian expression vector. The chimeric proteins are then overexpressed in the human cell line HEK293T and extracted. These crude extracts are incubated with patient sera and the IgG fraction is immunoprecipitated with the use of protein A/G beads (either agarose or magnetic) , which recognize and bind the Fc region of human antibodies. Immunoprecipitation pulls down the chimeric proteins-autoantibodies complexes. The quantity of these complexes is determined by measuring the luciferase enzyme activity of the reporter gene using commercially available substrate and instrumentation. First, to determine the sensitivity and reliability of our assay we screened a cohort of SPS patients for anti-GAD antibodies and compared the results with the ELISA method. We are also investigating the existence of new autoantibodies, such as anti-GABARAP.

Results: The comparison of these two methods (LIPS vs ELISA) showed an excellent correlation in the detection of positive high-titer anti-GAD patients. High titers of anti-GAD65 antibodies are typical of SPS, but the previously reported finding of anti-GABARAP needs to be confirmed. We have already established the LIPS assay for GABARAP autoantibodies and we are currently trying to improve the methods' sensitivity. Our preliminary data show that choosing magnetic over agarose protein A/G beads reduces background and increases sensitivity. This can be very helpful when trying to detect antibodies that may be of lower titers.

Conclusions: LIPS is a promising method for the rapid detection of autoantibodies. First, it is easily automated, quantitative and can be used for a panel of autoantibodies. Second, the assay is functional for membrane-bound antigens too, as previous results from our lab indicated, e.g. the detection of anti-AQP4 antibodies in patients suffering from NMO. Third, the chimeric proteins are expressed in mammalian cells and therefore undergo all the post-translational modifications. This is a significant advantage over well-established methods like ELISA, where its sensitivity detecting conformational epitopes is low.

7. Global expression signatures in the hippocampus of a mouse model for Mesio-Temporal Lobe Epilepsy

Georgia Kalozoumi[1], Hélène Bernard[2], Olga Kel-Margoulis[3], Christophe Heinrich[2], Edgar Wingender[3], Antoine Depaulis[2], Despina Sanoudou[1]

[1]Pharmacology Department, Medical School, National and Kapodestrian University of Athens, Athens, Greece
[2]Grenoble Institut des Neurosciences, U836 Inserm, Université Joseph Fourier, Grenoble, France
[3]geneXplain GmbH, Wolfenbuettel, Germany

Introduction: The Mesio-Temporal Lobe Epilepsy (MTLE) syndrome is the most common form of intractable epilepsies. It is characterized by the recurrence of focal seizures occurring in mesio-temporal limbic structures and is often associated with hippocampal sclerosis and drug resistance. The aim of the study was to characterize the pathogenetic mechanisms involved in epileptogenesis of MTLE in order to identify novel potential therapeutic targets.

Methods: The mouse model for MTLE obtained by intrahippocampal microinjection of kainate (KA; 1 nmol in 50 nL) was used {Riban:2002wi} in parallel with saline-injected animals as controls. The animals were decapitated at 12 hours post injection and the injected hippocampi dissected. Total RNA was extracted, labeled and hybridized to GeneChip ® whole Mouse Genome 430 2.0 Arrays (Affymetrix) (34,000 genes). Three pools of RNA, from the hippocampi of 3 different mice each, were prepared for each treatment (kainate or saline injection), and each pool was hybridized to a different array. Significant gene expression changes were identified using the Significance Analysis of Microarrays (SAM) software with 2-fold and <0.05 false discovery rate thresholds, while the transcription factors likely orchestrating the observed changes were predicted by the ExPlain^{TM} software.

Results: The focal *status epilepticus* that resulted from intrahippocampal KA treatment was associated with significant changes in 929 probe sets, representative of a range of molecular mechanisms. Significant over-expression was noted in activity-dependent genes regulating proliferation and survival (Fosb↑, Junb↑), negative regulators of apoptosis (Cflar↑, Bcl2a1↑, Mcl1↑, Birc3↑, Bag3↑), and genes related to synaptic plasticity (Homer1↑, Arc↑, Egr2↑) including glutamate signaling (Gria3↓, Grm5↓, Grin1↓). A significant induction of genes involved in inflammatory response (Il6↑,Tlr2↑, Ptgs2↑, Ccl2↑, Icam1↑) as well as astrocyte (Vim↑, Serpina3n↑) and microglia activation (Cd68↑, Cd14↑, Lgals3↑) was also observed, along with stress response mechanisms (Hmox1↑, Hspa1a↑,Hspa1b↑,Hspb1↑). A large number of the genes involved in these functional categories are predicted to be regulated by transcription factors which are also significantly changed themselves, namely Fosb, Junb, Crem, Fosl1, Myc, Nr4a2, Rora, Rorb, Bach1 and Hmga1.

Conclusion: Our data suggest that during MTLE epileptogenesis synaptic plasticity is highly modulated, along with activation of a range of stress and inflammatory processes including reactive astrocytes and microglia, and in parallel to cell survival mechanisms. These mechanisms emerge as moderators of the neurotoxic effects, following the initial insult, in the early stages of epileptogenesis.

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8. Aβ-mediated cell toxicity via Lysosomal Membrane Permeabilization in Alzheimer's Disease

Archontia Kaminari and Line de Kimpe

University of Amsterdam, the Netherlands, Academic Medical Center, the Netherlands

One of the major pathological hallmarks of Alzheimer's disease (AD) is the extracellular plaques that are formed by progressive accumulation of the amyloid beta (Aβ) peptide. The most common Aβ isoform that has been associated with disease stages is the $A\beta_{1-42}$ variant. $A\beta_{1-42}$ can also be N-truncated and form the more hydrophobic $A\beta_{3(pE)-42}$ variant. The latter is more prone to aggregation and potentially more pathogenic, than the former variant. Unfolded Aβ variants have been found to aggregate in lysosomes and possibly result in neurotoxicity.

We investigated whether the treatment of oligomeric $A\beta_{1-42}$ and $A\beta_{3(pE)-42}$ variants would result in lysosomal membrane permeabilization (LMP) on a neuronal cell model (SK-N-SH). LMP is characterized by the disruption of the lysosomal membrane and the diffusion into the cytosol of lysosomal hydrolases and proteases that lead to cell apoptosis.

In order to study LMP, we performed subcellular fractionation to separate the membrane (lysosomes) from the cytosolic fraction and subsequently we performed Western Blot analysis, targeting the lysosomal enzyme Cathepsin D and measured its change in localization, before and after LMP. Additionally, we performed immunostaining, targeting the Cathepsin D enzyme as well as Lamp1, a lysosomal membrane protein, in order to visualize LMP and confirm our Western Blot results.

Our results suggest that treatment with oligomeric $A\beta_{3(pE)-42}$ in concentrations of 1-2uM results in LMP in SK-N-SH cells, whereas treatment with oligomeric $A\beta_{1-42}$ in concentrations of 1-2uM does not result in LMP in the same cell line. Further investigation of the Aβ aggregation properties and Aβ-mediated toxicity via LMP will provide the basis for designing therapeutic strategies that will potentially delay the progression of AD.

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9. Understanding the possible neuroprotective role of progranulin: lessons from Parkinson's disease

Karampetsou A[1], Junichi Shioi [2] Nikolaos K. Robakis [2]and Vekrellis K.[1]

[1]Division of Basic Neuroscience, Biomedical Research Foundation of the Academy of Athens [2] Center for Molecular Biology and Genetics of Neurodegeneration, Departments of Psychiatry and Neuroscience, Mount Sinai School of Medicine, New York, NY, USA

Progranulin (PGRN), a pluripotent secreted polypeptide with widespread expression in many different mitotically active tissues in the periphery, has been recently genetically linked to Frontotemporal Lobar Degeneration (FTLD). The discovery emerged the interest to elucidate the function of this protein in the central nervous system (CNS). Although the mechanism by which reduced levels of PGRN lead to neuronal loss in the CNS is poorly understood, recent reports suggest a neuroprotective role of PGRN against various noxious insults. We have recently shown that extracellular PGRN stimulates phosphorylation/activation of the neuronal MEK/ERK/p90RSK and PI3K/Akt cell survival pathways and rescues cortical neurons from cell death induced by oxidative stresses. Because of its apparent role in neuronal survival, we argued that PGRN might also contribute to Parkinson disease (PD) pathogenesis and in particular to alpha-synuclein (ASYN), a key factor underlying PD pathogenesis, induced neuronal toxicity. We have generated a cellular model-system conditionally expressing human WT ASYN in which we have previously shown that not only overexpression of ASYN may act as a toxic insult for the cell but also secretion of ASYN impacts neuronal survival. Using this inducible system as a source of secreted ASYN we are interested in investigating the protective effects of PGRN on ASYN- induced toxicity. Moreover, we have obtained preliminary data to suggest that addition of exogenous PGRN to growth media of cortical neurons, in concentrations found to be neuroprotective in our previous study, alters the levels of secreted ASYN. Since secreted ASYN it is thought lately to play a central role in the propagation of the disease, it is of our great interest to investigate the effects of PGRN on ASYN levels and gain insight into the functional relevance of PGRN in the context of PD.

10. Investigation of the role of the membrane adhesion molecule TAG-1 during de- and remyelination processes in a toxic model of demyelination

Kastriti Maria-Eleni[1,2], Zoupi Lida[1,2] and Domna Karagogeos[1,2]

[1]Department of Basic Science, Faculty of Medicine, University of Crete and
[2]IMBB-FORTH, Heraklion, Crete

TAG-1 (Transient Axonal Glycoprotein-1) is a protein belonging to the immunoglobulin superfamily (IgSF) expressed by both neurons and glia at juxtaparanodes of myelinated fibers and is necessary for the formation and stability of the molecular juxtaparanodal complex of Caspr2 - K_v - TAG-1 (1,2, 3, 4, 5). In the absence of TAG-1, Caspr2 and voltage-gated potassium channels (VGKCs) do not cluster at juxtaparanodes and are found diffused into internodes, accompanied by behavioral defects in the homozygous mutants (*Tag-1*$^{-/-}$) (1, 2, 3). However, the glial expression of the protein (*Tag-1*$^{-/-}$*; plp*$^{Tg(rTag-1)}$) alone is sufficient to rescue the homozygous mutant phenotype (4).

Recently, TAG-1 was identified as a potential autoantigen in a subset of MS patients and was implicated both in white and gray matter pathology (5). In addition, nodes of Ranvier, along with the paranodal and juxtaparanodal domains, have been found to be structurally reorganized in remyelinating plaques appearing in tissue samples from MS patients (6). The exact mechanisms of the implication of TAG-1 in MS pathology are yet to be elucidated.

The goal of the present study was to test whether wild type, *Tag-1*$^{-/-}$ and *Tag-1*$^{-/-}$*; plp*$^{Tg(rTag-1)}$ animals (expressing TAG-1 only by oligodendrocytes) would be differentially affected under demyelinating or remyelinating conditions, using the cuprizone toxic demyelination model (7). This demyelination model resembles the type III and IV MS lesions, where oligodendrocyte death occurs excluding altogether the involvement of the immune system.

Experimentally, the animals were subjected to a 4.5- or 6-week-long period of cuprizone treatment in order to induce demyelination. Following the 6-week-long treatment, a 3-week-long period of normal chow ingestion was adopted to induce remyelination. Cryosections of the corpus callosum (cc) and the cerebellum (crb) were analyzed through immunohistochemistry using specific markers for astrogliosis, myelin and mature oligodendrocytes (GFAP, MBP and CC-1 respectively). In the crb, although the levels of mature oligodendrocytes during demyelination are comparable between the distinct genotypes, during remyelination the *Tag-1*$^{-/-}$ and *Tag-1*$^{-/-}$*; plp*$^{Tg(rTag-1)}$ exhibit increased recruitment and/or differentiation of this cell population. In contrast, in the cc, *Tag-1*$^{-/-}$ mice show a decreased number of CC-1^{+} oligodendrocytes after a 6-week treatment compared to controls and *Tag-1*$^{-/-}$*; plp*$^{Tg(rTag-1)}$ while during remyelination the numbers are comparable. A working hypothesis that is under investigation is that the role of TAG-1 in the two brain areas may be distinct. In the cc (white matter) it may affect oligodendrocyte survival while in the crb (gray matter) it may affect their recruitment in the affected areas. Further analysis is under progress, in order to elucidate the precise role of TAG-1 in the de- and remyelination processes.

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11. Investigation of the mechanisms of α-synuclein secretion in primary cortical neurons and in mouse brain.

Evangelia Emmanouilidou, Maria Keramioti, Kyriaki Gerozissis, and Kostas Vekrellis

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

α-Synuclein is central in the pathogenesis of Parkinson's Disease (PD). Recent discoveries have suggested that α-synuclein can be directly transmitted from pathologically affected to healthy neurons, thereby supporting a role of extracellular α-synuclein in the pathological progression of PD. Using α-synuclein overexpressing SHSY5Y cells, we have shown that α-synuclein is secreted by an unconventional, calcium-sensitive pathway, that involves at least in part, exosomes. Importantly, using an *in vivo* microdialysis approach in conjuction with a novel, ultra-sensitive ELISA, we have recently shown that α-synuclein is normally present in human and mouse brain parenchyma. This secretable form of α-synuclein may be biologically important, since it may act in a paracrine manner affecting neighboring cells. To elucidate the mechanism(s) that regulate the secretion of α-synuclein *in vivo*, we have pharmacologically manipulated α-synuclein release in mouse brain by locally applying reagents that target intracellular calcium concentration, ATP-binding cassette (ABC) transporter operation, and lysosome function. Compounds were locally administered by reverse microdialysis in the striatum of freely moving A53T-synuclein expressing mice. The effects of these reagents were assessed by quantification of α-synuclein concentration in microdialysis fractions from the interstitial fluid (ISF) before and after the administration of the compound. In a parallel, *in vitro* approach, we are trying to investigate the secretory pathway of α-synuclein in primary cortical neurons by direct addition of these compounds in the culture medium. In this case, changes in the levels of released α-synuclein were assessed in the conditioned medium (CM) from control and compound-treated cortical cultures. α-Synuclein concentration in all samples was measured by our in house ELISA. Collectively, our data so far has shown that α-synuclein secretion is a process that can be stimulated by elevation of intracellular calcium concentration since KCl-induced membrane depolarization resulted in a sharp and significant increase in α-synuclein levels. In agreement with a calcium-dependent mechanism of α-synuclein release, application of thapsigargin, a potent inhibitor of the SERCA pump, or the calcium-ionophore, A23187, also induced α-synuclein release. We have also investigated whether the ATP-sensitive K^+ channel (K_{ATP} channel), an important member of the ABC superfamily, is involved in α-synuclein release. To this end, we have locally administered in the striatum and in primary cortical neurons the selective sulfonylurea receptor 1 (SUR1)-blocker, glyburide. Our results indicated that glyburide caused a dramatic decrease in ISF α-synuclein concentration suggesting that a type of SUR1-regulated channels, but not K_{ATP} channels, could be implicated in α-synuclein export. In line with this finding, application of diazoxide, a SUR1-sensitive opener, resulted in an increase of the released α-synuclein both *in vitro* and *in vivo*. To verify these results, we are currently using compounds that induce ATP depletion such as oligomycin and 3-nitropropionic acid. Finally, we are currently investigating the role of compounds that either disturb lysosomal function or interfere with exosome production in a-synuclein release. Our data so far adds to the suggestion that mechanisms involved in the regulation of α-synuclein release may underlie a common pathway in the disease pathogenesis and as such may be targets to modify PD progression

12. PKCε phosphorylation induces nuclear localization of the tumor suppressor neurofiromin in post-mitotic neurons and prevents its proteasomal degradation.

Xenia Koliou[1], George Leondaritis[1], Nikos Sakellaridis[2], and Dimitra Mangoura[1]

1: Biomedical Research Foundation, Academy of Athens, Basic Neurosciences Division, Athens, Greece
2: University of Thessaly, School of Medicine, Larissa, Greece

Neurofibromatosis 1 (NF-1), is an autosomal dominant, progressive disorder, caused by mutations of the *NF1* tumor suppressor gene and its main clinical features include dermal pigmentary changes, learning difficulties, and development of benign or malignant tumors of the nervous system. The lack of genotype-phenotype correlation reflects the fact that the current collective knowledge on the function of the *NF1* gene product neurofibromin, the main neuronal RasGAP, remains very limited. Neurofibromin, an *in vivo* PKC substrate, contains a functional NLS and we have recently shown that it actively shuttles to the nucleus in a PKC-phosphorylation specific manner, for functions related to neuroblastoma differentiation. To delineate the regulatory mechanisms of neurofibromin's nucleocytoplasmic shuttling in a post-mitotic environment, we used primary cultures of chick embryo cortical neurons, where neurofibromin is highly expressed in the nucleus. Subcellular fractionation and confocal microscopy studies after incubation with Leptomycin B, a specific inhibitor of CRM1-dependent nuclear export pathway, showed decreased nuclear levels of neurofibromin indicating possible nuclear degradation of the protein. Further treatments with the proteasomal inhibitor MG-132, significantly increased nuclear neurofibromin levels and verified our hypothesis. Moreover, we found that phosphorylation of neurofibromin on Ser2808, a PKC-specific site, inhibited its nuclear degradation and that PKCε is the specific isoform responsible for this phosphorylation. More importantly, we found that PKCε-induced phosphorylation not only stabilized neurofibromin both in the nucleus and the cytoplasm but also promoted its nuclear accumulation. Our results provide evidence that PKCε-dependent phosphorylation of neurofibromin on Ser2808 is a major regulatory mechanism for its subcellular localization as well as its degradation by the nuclear proteasome.

13. Transplantation of neural stem/precursor cells secreting IGF-I in a mouse model of traumatic hippocampal injury ameliorates cognitive decline.

Koutsoudaki P.N.[1], Stamatakis A.[2], Kouroupi G.[1], Lavdas A.A.[1], Ntolkos D.[1], Stylianopoulou F.[2], Matsas R.[1]

[1] Laboratory of Cellular and Molecular Neurobiology, Hellenic Pasteur Institute, Athens, Greece.
[2] Biology-Biochemistry Lab, Dept. Basic Sciences, Faculty of Nursing, School of Health Sciences, University of Athens, Greece.

Traumatic brain injuries (TBI) are an important public health issue, due to their high mortality rate, as well as their extended clinical, social, and financial impact over time after the initial incident. The inability of the central nervous system to efficiently repair itself can result in severe functional impairment after such a trauma or after neurodegenerative diseases. Transplantation of neural stem/precursor cells (NSC), in their native state or genetically modified to express molecules with neuroprotective potential has emerged as a possible therapeutic strategy for these conditions. Insulin-like growth factor I (IGF-I) has been shown to be neuroprotective following a number of experimental insults to the nervous system, and in a variety of animal models for neurodegenerative diseases. Here, we investigate the therapeutic potential of SVZ-derived NSC transduced, or not, with IGF-I in a model of mechanically-induced, penetrating hippocampal injury. Animal performance in the Morris Water Maze task revealed that intrahippocampal transplantation of NSC, with or without IGF-I transduction, significantly improves the hippocampal injury-induced cognitive decline 38 days after transplantation. Additionally, NSC transplantation acts in a protective way towards the host tissue as it significantly reduces astrogliosis in the hippocampus in both time points examined, at 38 and 60 days post injury. Analysis of the fate of the transplanted cells *in vivo* showed that they are able to survive and migrate in the host tissue, and that they differentiate primarily into myelin-forming oligodendrocytes. The potential effect of the grafted NSC on endogenous hippocampal neurogenesis as well as their ability to mobilize endogenous precursor cells towards repair mechanisms are being analyzed. The findings of these studies should indicate whether NSC transplantation may constitute a prospective future therapy for CNS injuries.

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14. Investigation of the neuroprotective action of saffron and its crocetin constituent against behavioral and neurochemical disturbances induced by dietary/environmental toxins in adult mice

Linardaki Z.I.[1], Orkoula M.G.[2], Kokkosis A.G.[1], Koliadima A.[3], Lamari F.N.[2], Margarity M.[1]

[1]Department of Biology, University of Patras, [2]Department of Pharmacy, University of Patras, [3]Department of Chemistry, University of Patras, Patra 26500, Greece

Aim of the present study was to investigate the neuroprotective potential of saffron (*Crocus sativus* styles) and crocetin (its crocin metabolite) against learning/memory deficits and cerebral cholinergic and oxidative disturbances induced by either of two prevalent dietary/environmental toxins, aluminium (Al) and aflatoxin B1 (AFB1, mycotoxin), in adult male mice.

Step-through passive avoidance test was performed for learning/memory assessment and the activity of acetylcholinesterase [AChE, salt-(SS)/detergent-soluble (DS) isoforms], butyrylcholinesterase (BuChE, SS/DS isoforms), the levels of malondialdehyde (MDA, lipid peroxidation index) and reduced glutathione (GSH), were determined in whole brain (-cerebellum) and cerebellum. Animals received either Al (50 mg/kg/day in drinking water for 5 weeks) or Al plus saffron extract (60 mg/kg/day i.p. for the last 6 days of Al treatment) or AFB1 (0.6 mg/kg/day i.p. for 4 days) or saffron infusion (*ad libitum* access to 0.45 mg/ml infusion for 2 weeks) plus AFB1 i.p. for the last 4 days of saffron treatment or crocetin (4 mg/kg/day i.p. for 3 days) before or after AFB1 treatment. Saffron infusion or crocetin alone were also administered. Cerebral Al levels were determined by atomic absorption spectrometry, while, for the first time, crocetin was determined in brain after i.p. saffron extract administration by HPLC.

Al or AFB1 administration caused memory impairment, significant differential decrease of AChE and BuChE isoforms activity, significant elevation of brain (in both toxic conditions) and cerebellar (only under AFB1 toxicity) MDA levels and significant reduction of GSH content in both cerebral tissues. Mice receiving saffron infusion or crocetin alone displayed significantly decreased AChE activity and brain MDA and GSH levels, compared to the controls, while their learning/memory ability was not affected. Saffron extract co-administration reversed significantly the Al-induced changes in the levels of MDA and GSH and caused further significant reduction in cerebral AChE activity; however no effect on cognitive performance of mice was observed. Pre-treatment of mice with saffron infusion significantly enhanced their learning/memory ability, decreased cerebral DS-AChE activity, GSH content and MDA levels, compared to AFB1-treated mice. Mice treated with crocetin prior to AFB1 displayed increased cerebellar DS-AChE and brain SS-BuChE activity, decreased brain MDA levels and increased brain GSH content, compared to AFB1-treated mice. Administration of crocetin subsequent to AFB1 caused further reduction of brain AChE (SS/DS) and cerebellar DS-AChE activity, increase of brain SS-BuChE activity and decrease of brain MDA levels. However, pre- or post-treatment with crocetin had no beneficial effect on memory performance of mice. Conclusively, our findings indicate strong neuroprotective action of saffron infusion, as it prevented AFB1-induced memory decline and cholinergic and oxidative disturbances, while saffron extract and crocetin possessed moderate efficacy against Al- and AFB1-induced neurotoxicity.

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15. Post-operative complications in Idiopathic Normal Pressure Hydrocephalus (INPH) predicted by neuropsychological examination in the absence of clinical deterioration: A case report.

Evangelia Liouta[1,2], Theodosis Kalamatianos[1,2],Faidon Liakos[1,2] Damianos Sakas[1,2], George Stranjalis[1,2]

[1]Dept of Neurosurgery, University of Athens, Evangelismos Hospital, Athens, Greece
[2]Hellenic Center for Neurosurgical Research "Prof. Petros Kokkalis", Athens, Greece

Indroduction

Idiopathic normal pressure hydrocephalus (INPH) the clinical syndrome characterised by dementia, gait disturbance, urinary incontinence, ventriculomegaly on CT or MRI and normal cerebrospinal fluid (CSF) pressure on lumbar tap (LT), typically manifests in the elderly [1]. While the pathophysiological mechanisms of INPH remain imperfectly understood, surgical treatment of CSF shunting remains the most widely practised treatment option, presenting an acceptable risk-to-benefit ratio [2]. Among the most common postoperative complications in INPH are subdural effusions that include hygromas and hematomas; these can be clinically silent [3].

Aim

To present the case of a shunted INPH patient harboring clinically silent post operative bilateral hygromas that were detected on brain CT scan following recommendation for further investigation due to abnormal neuropsychological findings. This case highlights the value of neuropsychological assessment in the routine postsurgical assessment of INPH.

Patient and Methods

The patient was a 65-years-old male who complained of all three clinical INPH symptoms. MRI revealed the typical enlargement of ventricles. According to our clinic's protocol gait disturbance and memory deficits and their putative post-lumbar tap (LT) alleviation, were investigated with a battery of neuropsychological and gait tests. Tests were repeated one month post-surgery as part of routine examination.

Results

One hour after LT the patient showed an average of 20% improvement in all neuropsychological tests administered. In addition, his gait improved 25% according to the 10 m walking test. These results indicated a positive response to CSF shunting and thus the eligibility of the patient for surgical treatment. Postoperatively, despite significant improvement in the patient's gait performance (100%), neuropsychological tests revealed a significant deterioration (50%) of his frontal lobe-executive functions (language, attention and working memory). On the basis of these neuropsychological findings a CT scan was recommended. The scan revealed large bilateral frontal hygromas.

Conclusions

Neuropsychological evaluation as part of routine clinical examination in INPH presents a valuable diagnostic/prognostic tool. Thus, in combination with neuroimaging, it can contribute to the differential diagnosis of INPH from other types of dementias (e.g. Alzheimer's type). Moreover, it can be used prior to and following diagnostic LT to assess improvement in cognition, a positive marker for shunting. Finally, it can contribute to the detection of clinically silent postoperative complications as highlighted by the present findings.

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16. Dysregulation of Ca^{2+} homeostasis contributes to extracellular α-synuclein-mediated neurotoxicity

Melachroinou K.[1], Xilouri M.[1], Emmanouilidou E.[1], Papazafiri P. [2], Stefanis L.[1,3], and Vekrellis K.[1]

[1]Division of Basic Neuroscience, Biomedical Research Foundation of the Academy of Athens
[2]Dept. of Animal and Human Physiology, National and Kapodistrian University of Athens
[3]Second Department of Neurology, University of Athens Medical School, Athens, Greece

α-Synuclein (AS) is an abundant presynaptic neuronal protein genetically and biochemically linked to Parkinson's disease (PD) pathogenesis. It has become common notion that AS is normally secreted from neuronal cells and neurons. To assess the effects of secreted AS on neuronal homeostasis, we have used SH-SY5Y cells inducibly expressing human wild-type AS**,** as a source of naturally secreted AS. We have shown that these cells readily secrete a wide range of AS species into their culture medium, partly via an exosome-mediated manner. Cell-produced secreted AS was toxic to recipient cells. To investigate the possible mechanisms involved in the extracellular AS-mediated toxicity, we applied naturally secreted AS to differentiated SH-SY5Y cells. According to our results, such treatment alters Ca^{2+} homeostasis in recipient neuronal cells, manifested by increased capacitative Ca^{2+} entry (CCE). This effect was AS-dependent, since immunodepletion of the protein from the conditioned medium (CM) abolished Ca^{2+} influx. The observed Ca^{2+} rise was attributed mainly to exosome-associated AS, given the fact that cells pretreated with exosomes isolated from AS-containing CM exhibited higher Ca^{2+} influx compared to cells recipient to exosome-depleted CM. Moreover, inhibition of Voltage Operated Ca^{2+} Channels (VOCs), by use of specific inhibitors, ameliorated AS-induced Ca^{2+} influx, engaging these channels in the observed phenomenon. No change in Ca^{2+} steady state levels of the secreted-AS treated cells was observed, yet a significantly increased mitochondrial Ca^{2+} sequestration was found. Incubation of differentiated naïve neuroblastoma cells either with VOC blockers or with extracellular or intracellular Ca^{2+} chelators was protective against secreted AS-conferred toxicity. Importantly, we found that calpains are implicated in the secreted-AS-induced neurotoxicity as increased calpain activity was detected in extracts from cells recipient to cell-produced AS, whilst application of specific calpain inhibitors on neuronal cultures greatly alleviated secreted-AS-mediated toxicity. Finally, we found that secreted AS alters membrane fluidity of the recipient cells. Collectively, our data suggest that secreted AS, and particularly its exosomal component, is toxic to recipient neuronal cells through engagement, at least partly, of the intracellular Ca^{2+} homeostatic machinery. Manipulating Ca^{2+} signaling pathways mitigates extracellular AS toxicity and may therefore represent a potential therapeutic target for PD and related synucleinopathies.

17. Insights into the mechanisms of secreted α-synuclein clearance

M. Ximerakis[1], G. Pampalakis[2], L. Stefanis[1], SD. Garbis[3], G. Sotiropoulou[2] and K. Vekrellis[1]

[1]Division of Basic Neurosciences, Biomedical Research Foundation, Academy of Athens, Athens, Greece
[2]Department of Pharmacy, University of Patras, Rion-Patras, Greece
[3]Cancer Sciences Unit, Faculty of Medicine, University of Southampton, Southampton, United Kingdom

There is a plethora of evidence supporting that α-synuclein plays a central role in the pathogenesis of Parkinson's disease (PD); the second most common neurodegenerative disorder affecting mostly the elderly population. Although α-synuclein is considered to be a cytoplasmic protein, it was shown recently that it is also secreted and can be detected in conditioned media (CM) of cultured neuronal cells and in biological fluids, including interstitial fluid of mouse brains and cerebrospinal fluid and blood plasma of both healthy and diseased individuals. Thus, it has been proposed that secreted α-synuclein might have paracrine roles in brain homeostasis that could contribute to the cascade of events leading to neuronal degeneration in PD. Considering that the levels of α-synuclein seem to be critical in the etiopathology of PD, we sought to investigate factors and mechanisms that regulate the steady-state protein levels of secreted α-synuclein. As a source of naturally secreted α-synuclein we used CM from SH-SY5Y cells that were modified to overexpress and secrete the wild-type form of the protein, under biologically relevant conditions and concentrations. In order to ascertain extracellular proteolytic degradation, we applied secreted α-synuclein directly on neuronal and non-neuronal cells, or mixed it with corresponding CM for various periods of time. Alterations in protein levels were analyzed by immunoprecipitation and autoradiography, while enzymatic activity was assessed by zymography. Our preliminary data show that high nanomolar levels of the recombinant serine protease kallikrein-related peptidase-6 (KLK6), which has been implicated in the degradation of recombinant α-synuclein, cleaves secreted α-synuclein. Surprisingly, we noticed that secreted α-synuclein is more resistant to KLK6 proteolysis than the recombinant form of the protein. Hence, in order to identify potential post-translational modifications that could explain this resistance we are currently investigating the KLK6 cleavage sites within the α-synuclein amino acid sequence by immunoblotting and liquid chromatography-tandem mass spectrometry (LC/MS/MS). Our first results suggest that KLK6 cleaves mainly the N-terminal region of α-synuclein. Given that all the mutations in PD-associated forms of α-synuclein are localized in the N-terminus, we are further investigating their KLK6 proteolysis profile in comparison to that of the wild-type form of α-synuclein. In parallel with this work, we are searching for other extracellular proteases (cell-associated intracellular & secreted) that may play a role in the metabolism of secreted α-synuclein.

18. Investigation of exosome-associated α-synuclein effects on cellular homeostasis using compartmentalized primary cortical neurons.

Georgia Minakaki, Evangelia Emmanouilidou, Kostas Vekrellis

Department of Neurosciences, Biomedical Research Foundation of the Academy of Athens, Greece

α-synuclein (α-Syn) is an abundant cytosolic protein which is genetically and biochemically linked to Parkinson's disease. Its intracellular levels are important since distinct α-Syn concentration thresholds appear to be permissive for synapse function whereas excessive α-Syn induces a series of pathologic changes. α-Syn can be readily detected in human cerebrospinal, interstitial fluid but also in the conditioned medium of primary neurons and neuronal cell lines. Key studies have demonstrated transmission and propagation of cell-produced α-Syn to neural precursor cells in tissue culture and in transgenic animals [(1)] as well as a host-to-graft propagation of pathology to fetal dopaminergic implanted into PD patients [(2)].Our laboratory has previously shown that α-Syn is secreted, at least in part, via exosomes [(3)]; cell-secreted vesicles of endocytic origin with a diameter of 30-100nm and unique protein and lipid composition [(4)]. The aim of our study is to examine neuronal α-Syn secretion as a physiological phenomenon and to quantitatively validate the biological contribution of exosomes in its release and paracrine effects.

We have examined the neuronal sites of α-Syn secretion using novel, compartmentalized microfluidic culture chambers which allow the separation of neuronal axons from soma. Primary cortical neurons were cultured within these devices, in which axons progressively grow from one chamber, towards a separate, fluidically isolated chamber of the same device. We assessed α-Syn concentration in the conditioned medium obtained from the two compartments by an ultra-sensitive, in house ELISA [(5)] and found that α-Syn is secreted by both the soma and axons. This suggests that its secretion occurs physiologically by neuronal populations in the brain, possibly in the context of intercellular communication.

In addition, we have labeled exosomes isolated from mouse and rat primary cortical neurons with fluorescent ceramide (BODIPY-TR-CERAMIDE) and visualized their uptake by confocal microscopy. Primary neurons were found to readily uptake exosomes in a time-dependent manner, which indicates that exosomes may also be mediators of α-Syn paracrine effects. In order to further assess this hypothesis, we isolated exosomes from KO, WT and A53T ASYN mouse cortical neurons and applied them on KO and WT ASYN neurons cultured in microfluidic devices. We are currently in the process of estimating the effects of exosome-contained α-Syn on neuronal morphology and homeostasis. In addition, we used SH-SY5Y cell lines, inducibly over-expressing WT but also the PD-linked A53T ASYN in order to investigate whether this mutation alters release and/or packaging of α-Syn in exosomes. We examined α-Syn concentration extracellularly as well as in the exosomal fraction using an ultra-sensitive ELISA. Our data so far support the notion that exosome carriers are a physiological means of releasing α-Syn. Increased intracellular levels of either WT or A53T mutant α-Syn do not seem to affect the released exosome number but the vesicular α-Syn load is in both cases dynamically changing and can be correlated also to extracellular α-Syn levels.

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19. Neuroprotection of nigrostriatal dopaminergic neurons by DHEA-S and its analogue BNN50 in the weaver mouse, a genetic model of Parkinson's disease

Mourtzi T.[1], Botsakis K.[1], Panagopoulos N.[1] , Angelatou F.[2], Charalampopoulos I.[3], Gravanis A.[3], Matsokis N.[1]

[1]Laboratory of Human and Animal Physiology, Department of Biology, University of Patras, Patras 26 500, Greece
[2]Department of Physiology, School of Medicine, University of Patras, Patras 26 500, Greece
[3]Department of Pharmacology, School of Medicine, University of Crete, Heraklion 71110, Greece

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

In this study, we administrated the neurosteroid dehydroepiandrosterone sulphate (DHEA-S) and its analogue 17.beta-spiro-[5-androsten-17,2'-oxiran]-3beta-ol (BNN-50), that is not metabolized to estrogens as well as caffeine in wv/wv mice from P1 to P22 and then we measured neuronal survival in SN.

Using western blotting and a specific antibody against TH our results:

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

ii. showed 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 and BNN50 had a similar, great neuroprotective effect (increase of the dopaminergic cell survival by 90%) in the wv/wv SN compared to wv/wv NaCl mice, bringing the dopaminergic neuron number almost to control levels. Caffeine has also shown a neuroprotective effect, increasing the dopaminergic cells in SN by 60%.

These results could be of clinical relevance, as they suggest that DHEA-S and BNN50 represent very effective neuroprotective agents for the nigrostriatal dopaminergic neurons. Since BNN50 is not metabolized to estrogens it could be proposed for treatment Parkinson's Disease.

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20. Correlation between GBA gene encoding glucocerebrosidase enzyme and intracellular and extracellular a-synuclein

Nikolopoulou Georgia[1], Xilouri Maria[1], Emmanouilidou Evangelia[1], Sardi Pablo[2], Stefanis Leonidas[1,3] and Vekrellis Kostas[1]

1. *Division of Basic Neurosciences, Biomedical Research Foundation of the Academy of Athens, Athens, Greece*
2. *Genzyme Cambridge USA*
3. *Second Department of Neurology, University of Athens Medical School, Athens, Greece*

Gaucher Disease (GD) is the most common lysosomal storage disorder which is caused by the GBA (glucosidase-beta-acid) gene. GBA encodes for the enzyme beta-glucocerebrosidase, leading to accumulation of its substrate glucocerebroside. To date, over 300 GBA mutations have been identified, the most common being N370S (consider to give a mild phenotype), L444P and D409H (consider to give a more severe phenotype). Clinical data demonstrate an association between Gaucher Disease and Parkinson Disease (PD). Gaucher Disease (GD) patients and carriers of glucocerebrosidase mutations are at an increased risk for PD. In this respect, Lewy Bodies, the neuropathological hallmark of PD, have been detected in postmortem brain of patients with Gaucher Disease. A pathologic mechanism between GD and PD has yet to be identified. The presynaptic protein a-synuclein has been genetically and biochemically linked to PD. The lysosome represents a major pathway for a-synuclein degradation. We have previously shown that a-synuclein is physiologically secreted in to the extracellular space. In this study we aimed to investigate whether manipulation of GCase function through its mutations alters extracellular a-synuclein levels. We used HEK 293T cells and transiently transfected them using calcium phosphate with plasmids to human a-synuclein together with either wild-type or mutant (N370S, L444P) GCase. Levels of extracellular a-synuclein were assessed using an in house ELISA at 3 and 5 days following transfection. Intracellular asyn levels were also examined with ELISA and western blotting. We show that the mutated forms of GBA seem to modulate the levels of a-synuclein when compared to control transfected cells.

21. Perinatal exposure to a low dose of Bisphenol A affects the behavioural and neuroendocrine stress response of rodents.

Panagiotidou E., Kitraki E.

Lab of Basic and Biomedical Sciences, School of Dentistry, University of Athens, Greece.

Environmental disrupters, such as xenoestrogens, can significantly affect organisms' development, even at concentrations below the daily safe limit. Bisphenol A (BPA) is a known estrogen disrupter, used widely in the plastics and resins industry. BPA has been shown to act as both an estrogen agonist and androgen antagonist and can bind *in silico* to glucocorticoid receptor (Prasanth et al 2010). Exposure to 'safe' doses of this disrupter has been reported to affect the sexual differentiation of the brain and behavior of the exposed rodent offspring (Vandenberg et al 2012). In a previous study (Poimenova et al 2010), we showed for the first time that perinatal treatment of rats with a 'safe' BPA dose can also impact glucocorticoid – related actions within the brain of exposed offspring, such as spatial recognition memory and hippocampal glucocorticoid receptor levels.

The aim of the present study was to further elucidate the impact of BPA treatment on hypothalamic – pituitary – adrenal (HPA) axis response to an acute stress. Female breeders received orally 40 μg/kg bw BPA, or the vehicle, daily throughout pregnancy and lactation. At mid-adolescence, BPA- and vehicle-treated offspring of both sexes were exposed to 15min of swimming stress and their behavioural coping was evaluated. Blood samples were collected through tail vein at t0, t30, t60 and t120min intervals from stress onset for determination of plasma corticosterone levels. Glucocorticoid receptor (GR) and corticotrophin releasing hormone (CRH) levels were determined in animals' hypothalami by western blot analysis at t0 and t120min post stress.

BPA treatment significantly modified basal and stress-induced HPA axis function, as well as behavioural coping, especially in the female offspring. Female BPA - treated animals exhibited significantly higher 'escape' and concomitantly lower 'floating' behaviors during the swimming stress, which is suggestive of a more stressed emotional state. This is in compliance with their hormonal status at the onset of stress, showing higher basal corticosterone levels, combined with lower levels of hypothalamic GRs and CRH, vs. control females. Following stress, BPA - treated females exhibited a reduced efficacy to terminate HPA axis activation, compared to controls, in terms of lowering hypothalamic GR and CRH levels. BPA – treated male offspring did not show significant changes in behaviour or in the HPA axis parameters studied, compared to same sex controls. Finally, BPA exposure abolished sexual dimorphisms existing in the hypothalamic GRs of non-exposed offspring, and revealed new ones in corticosterone levels.

Overall, the present data show that exposure to a 'safe' BPA dose during brain development can affect the behavioural and neuroendocrine stress response of adolescent rats in a gender – dependent manner. These findings support the -broader than expected- impact of low doses of endocrine disrupters in the brain and underline the necessity to clarify the mechanism(s) of exerted actions.

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22. MicroRNA regulation of calcium channels

Panteleli Efstratia and Epaminondas Doxakis

Basic Neuroscience Division, Biomedical Research Foundation, Academy of Athens

Calcium homeostasis is very important for normal brain function since through calcium signalling neurons control a broad spectrum of processes including membrane excitability, neurotransmitter release, differentiation, gene expression, cellular growth and cell death (1,2). It has been noted that cellular calcium regulating systems are, also, compromised in several neurodegenerative disorders, including Alzheimer's disease (2). It is, therefore, very important to identify the molecular mechanisms that control neuronal calcium levels that would allows to later manipulate for therapeutic purposes.

MicroRNAs (miRNAs) are small non-coding RNA sequences of 19-23 nucleotides that regulate expression of related proteins via RNA interference (3). To find putative roles of miRNAs in Ca^{2+} homeostasis, we have performed bioinformatics analysis of Ca^{2+} channels and identified a number of neuronal miRNAs that are likely to regulate these channels.

Experimentally, we have, initially, cloned the 3' untranslated regions (3'UTRs) of Ca^{2+} channels at the 3' end of a luciferase reporter gene and transfected these constructs in the presence or not of the predicted miRNAs in HEK293 cells. Interaction between miRNAs and the mRNA of Ca^{2+} channels was, then, assessed by measuring luciferase activity. We found that most of bioinformatics predictions were valid and that neuronally enriched miRNAs regulated most of these channels. In order to confirm the interactions through the predicted miRNA binding sites we mutagenized the seed sequences of the luciferase hybrid constructs and repeated experiments. We found that miRNA regulation of the hybrid luciferase constructs was, significantly, impaired indicating that miRNAs were exerting their effect by, primarily, binding to the predicted sequences. Currently, in order to investigate whether these miRNAs are able to regulate the levels of endogenous Ca^{2+} channels as well, we are in the process of infecting HEK293 cells and neurons with lentiviral vectors expressing these miRNAs and assess mRNA and protein levels by RT-PCR and Western blot, respectively.

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23. Regulation of tau phosphorylation and the role of Amyloid Precursor protein

Elpiniki Pappa, Spiros Efthimiopoulos

Department of Biology, Division of Human and Animal Physiology, University of Athens, Panepistimiopolis, Ilisia

Alzheimer's disease (AD) is a neurodegenerative disease that neuropathologically is characterized by the presence of extracellular amyloid plaques that consist mainly of amyloid β peptides (Aβ) and intraneuronal neurofibrillary tangles (NFTs) consisting of hyper- phosphorylated tau protein. Tau is a microtubule-associated protein (MAP) that under physiological conditions stimulates the polymerization of microtubules and regulates their stabilization. Furthermore, it should be mentioned that tau protein has a great variety of post-translation modifications, but the most important of these is the phosphorylation. Its pathological hyper-phosphorylation reduces the affinity of tau for microtubles and consequently provokes the accumulation of tau protein into neurons and the disruption of their physiological function.

Thus, it is crucial to study the pathways of phosphorylation of tau protein, as well as treatments which potentially could provoke an abnormal phoshorylation of tau. In this direction, we investigated in SH-SY5Y the effect of the activation of signaling pathways such as PLC/PKC, AC and PI3K on tau phosphorylation. To date, there is no convincing correlation between the amyloid β precursor protein (APP) and the regulation of Tau phosphorylation. Thus, we also investigated the possible regulative role of APP protein in Tau phosphorylation in SH-SY5Y cells where APP expression was downregulated by shRNA.

Accordance with our results, the activation of PLC/PKC or AC signaling pathway has no effect in the phosphorylation of Tau residue S262, while in other residues, such as S199, we observed increased levels of phosphorylation. Moreover, independently of the activating pathway, downregulation of APP resulted in a significant decrease in levels of phosphorylation in all residues, except for S262, in which the levels of phosphorylation were increased.

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: ARISTEIA –UOA

24. Neurofibromin 1 is a miRNA target in neurons

Maria Paschou and Epaminondas Doxakis

Molecular and Cellular Neurobiology Lab, Basic Neurosciences Division, Biomedical Research Foundation, Academy of Athens

Neurofibromatosis type 1 (NF-1) is a common human inherited disorder that results in nervous system dysfunction that includes mental retardation and benign and malignant tumors of the peripheral and central nervous system [1]. NF-1 is characterized by learning and behavioral abnormalities and is completely penetrant but shows variable phenotypic expression in patients [2]. This disease is caused by mutations of the neurofibromin 1 gene (*Nf1*), which encodes for a large multifunctional protein, named neurofibromin 1, that among others exhibits Ras GTPase activity [3].

MicroRNAs (miRNAs) are an emerging class of gene regulators implicated in a wide range of pathways during development and disease. They are short (17-24 nucleotides long), non-coding RNA molecules that are directly involved in negative post-transcriptional regulation of gene expression by hybridizing to complementary sequences in the 3' un-translated region (3'-UTR) of closely related target mRNAs to induce cleavage of the message or inhibit translation [4].

The repertoire of regulatory interactions utilized by neurons to control neurofibromin 1 expression is poorly understood. With this in mind, we sought to characterize the physiological regulation of neurofibromin 1 expression by miRNAs. We have tested a number of predicted conserved miRNAs for neurofibromin 1 regulation and identified miR-128, miR-137 and miR-103 as new regulators. Using reporter assays, we provided evidence that miR-128 and to a lesser extent miR-137 and miR-103 reduced neurofibromin 1 reporter levels through specific binding to *Nf1* 3'-UTR. Mutations in all three predicted binding sites eliminated the reporter response. MiR-128 and miR-137, unlike miR-103 that showed a more ubiquitous expression, were predominantly expressed in brain with a distribution that resembled neurofibromin 1 expression in different tissues as well as during the course of neuronal development. In the nervous system, all three microRNAs showed highest expression in neurons and least in Schwann cells and astrocytes. Overexpression of miR-128 alone or with miR-103 and miR-137 significantly reduced endogenous neurofibromin 1 protein levels, while *Nf1* mRNA levels remained unaltered, in both cell lines and primary cultures of hippocampal neurons. Furthermore, antisense inhibition of these microRNAs enhanced translation of endogenous neurofibromin 1 and reporter in both types of cultures.

In conclusion, these findings revealed a significant additional mechanism by which neurofibromin 1 is regulated in neurons and implicated new candidates for the treatment of multifarious NF-1 cognitive symptoms. Three miRNAs, miR-103, miR-128, and miR-137 have been found to bind directly to the 3'-UTR of *Nf1* mRNA and significantly reduce neurofibromin 1 protein levels in both cell lines and primary cultures of neurons. This data set the stage for further evaluation of the role of miRNAs in the NF-1 pathophysiological process.

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25. Post-transcriptional regulation of α-synuclein expression by ELAV proteins

Koukouraki Pelagia, Fragkouli Apostolia, Doxakis Epaminondas

Molecular and Cellular Neurobiology Lab, Basic Neurosciences Division, Biomedical Research Foundation, Academy of Athens

Alpha-synuclein (SNCA) -a natively unfolded protein which plays a central role in the control of dopaminergic neuronal functions- is thought to be critically involved in Parkinson's disease (PD) pathophysiology. SCNA is the major component of Lewy bodies in sporadic PD and point mutations or gene amplification events in the SNCA locus have been identified in a number of families with autosomal-dominant early-onset (PD). Along the same lines, accumulation of SNCA has been demonstrated to be neurotoxic and associated with increased rate of neuronal cell death (1).

Embryonic lethal abnormal visual (ELAV) proteins are RNA-binding proteins that post-transcriptionally regulate mRNA levels. They are characterized by the presence of three RNA-recognition motifs through which they bind to target labile mRNAs bearing AU- and U-rich sequences and modify their stability and/or translation (2). The family of ELAV proteins consists of four members, HuR, HuB, HuC, and HuD. HuR is ubiquitously expressed, whereas HuB, HuC and HuD are expressed preferentially in terminally differentiated neurons. ELAV proteins appear to be the most recognized post-transcriptional regulators in the nervous system and have been implicated in neurogenesis, axonal regrowth after injury and motor control (3).

In the present study, we investigated whether ELAV proteins could post-transcriptionally regulate SNCA levels. Our sequence analysis revealed the presence of four evolutionary conserved ELAV RNA-binding motifs on SNCA mRNA. Using RNA immunoprecipitation analysis of murine brain extracts and human cell lines with an antibody that recognizes all ELAV proteins, we confirmed that ELAV proteins bind to SNCA mRNA *in vivo*. To identify which of the three main domains of SNCA mRNA - 5'UTR, coding region and/or 3'UTR- interact with ELAV proteins, we cloned each of these regions after the coding sequence of Renilla luciferase. Transfection of each of these chimeric constructs with HuR, HuB, HuC or HuD expressing vectors in HEK 293 cells revealed that ELAV proteins alter the expression of luciferase, suggesting that ELAV proteins not only bind to SNCA mRNA, but also change its expression.

To further explore the latter hypothesis, we next examined the effect of HuR, HuB, HuC or HuD overexpression on SNCA protein levels. Preliminary work, in HEK293 cells and primary cultures of hippocampal neurons has shown that only HuR and HuB change protein levels of SNCA without affecting mRNA levels. To better characterize the physiological interaction of ELAV proteins and SNCA, the levels of all ELAV and SNCA mRNAs were determined in different tissues of embryonic day 18 mice by RT-PCR. Furthermore, it was determined whether ELAV expression correlates with SNCA expression in the cortex, hippocampus and substantia nigra throughout development. It was found that the levels of the ELAV proteins were negatively correlated with SNCA mRNA levels.

Taken together our observations suggest that SNCA levels are likely to be regulated by ELAV proteins. This sets the stage for further evaluations of the role of ELAV proteins in synucleinopathies, such as Parkinson's disease.

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26. STUDY OF THE ROLE OF APOLIPOPROTEIN J IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE IN TRANSGENIC MICE

Katerina Sarantari and Spiros Georgopoulos

Laboratory of Cellular Neurobiology, Centre of Basic Research I, Biomedical Research Foundation of the Academy of Athens.

Alzheimer's disease (AD) is a progressive neurodegenerative disease that attacks the basic cognitive functions of the brain. Amyloid plaques and neurofibrillary tangles are established as the hallmarks of AD. Genomic association studies have identified polymorphisms at the ApoE and ApoJ genes as major risk factors in the pathogenesis of AD.

Apolipoprotein J (ApoJ) is a multifunctional disulfide linked heterodimeric glycoprotein, encoded by a gene located on chromosome 8. It is present in almost all mammalian tissues and physiological fluids, such as plasma, milk, urine, CSF and semen and it is primarily distributed within High Density Lipoproteins. ApoJ has been identified as nuclear protein as well as a dimmer in serum produced by cleavage of a secreted precursor protein. ApoJ has been shown to be involved in spermatogenesis, development, complement inhibition, lipid transport, maintenance of AD's Aβ peptides solubility and apoptosis. Recent studies have implicated ApoJ in the pathogenesis of Alzheimer's disease and have further elucidated its role in amyloid plaque formation and its additive effect with apoE in this process. ApoJ has been found in AD cortex in both diffuse and compact plaques and it may act intracellularly as a protective agent against the formation of neurofibrillary tangles.

We have generated and analyzed transgenic mice where the human nuclear ApoJ has been expressed in the brain under the PDGF promoter. In order to further evaluate the role of the ApoJ in the pathogenesis of Alzheimer's disease, we will generate transgenic mouse lines that express the secreted form of ApoJ. These mice models are currently under evaluation to explore a more detailed role for ApoJ in neurodegeneration and Alzheimer's disease.

27. A nonsense mutation in a novel SLC25 family gene of mitochondrial carriers causes severe recessive neurological disease in mice

Terzenidou Maria-Eirini[1,2], Kano Toshimi[1,2], Karakostas Alexis[1,2], Ioakeimidis Fotis[1,2], Kollias George[2], Douni Eleni[1,2,*]

[1] AUA, Department of Agricultural Biotechnology, Laboratory of Genetics, Iera Odos 75, 11855, Athens
[2] B.S.R.C. "Alexander Fleming", Division of Immunology, Fleming 34, 16672, Vari

Following a forward genetics approach through random mutagenesis with N-ethyl-N-nitrosourea (ENU), we have recently identified a novel mouse model of severe autosomal recessive neurological disease. The symptoms start at 3 weeks of age characterized by ataxia, unsteady locomotion, episodic crises, and growth retardation with severe disease progression that leads to lethality in the majority of the mice by the age of 3 months. Using genome-wide linkage analysis we identified a nonsense point mutation (C to T) in the coding region of a novel gene member of the mitochondrial carrier family or the Solute Carrier Family 25 (SLC25) that introduces a premature stop codon and results in a loss-of-function protein. All SLC25 members localize into the inner mitochondrial membrane where they shuttle a variety of metabolites across it. Mutations in SLC25 genes impair mitochondria functions by affecting either the synthesis of ATP, or the selective transport of solutes in and out of the mitochondrial matrix.
This novel SLC25 member is highly conserved among various species, but its function remains completely unknown. Our results confirm the mitochondrial localization of this SLC25 member by confocal and western blot analysis. Moreover, we identified that the wild-type SLC25 protein is predominantly expressed in neuromuscular tissues in contrast to the mutant protein that is undetectable. To genetically confirm the causal role of identified mutation in the ataxic phenotype we have recently generated transgenic mice carrying the human SLC25 genomic region for performing rescue experiments. Our ongoing studies are focused on a) the identification of the primary site of lesion in order to align the brain pathology of the mouse model with that of similar human neurodegenerative diseases, b) the identification of mitochondrial dysfunctions, and c) the functional analysis of this novel SLC25 protein which constitutes a novel pathogenic target in neurological diseases such as ataxia.

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28. MENBERS OF THE HOMER FAMILY OF PROTEINS FORM HOMO- AND HETERO-DIMERS

Thomaidou S., Papadogiannaki G., Kyratzi E., Liakos A., Markou D., Efthimiopoulos S.

Department of Biology, Division of Human and Animal Physiology, University of Athens, Panepistimiopolis, Ilisia

The neurodegeneration in Alzheimer's disease (AD) has been linked to deposition of amyloid β-peptide (Aβ), derived from the processing of the amyloid precursor protein (APP), in amyloid plaques in brain tissue. The APP is a type 1 transmembrane protein highly expressed in brain. Mutations in APP have been associated with early-onset familiar AD (FAD). We have previously shown that APP interacts with Homer 2 and Homer 3 and that this interaction reduces processing of APP towards the production of Aβ peptide. The interaction is mediated via the EVH1 domain. In addition Homer proteins possess a coiled coil domain that is considered to allow them to form in-trans homodimers and/or heterodimers. In this study we investigated whether indeed Homer proteins homo- and heterodimerize. We found that Homer 1, Homer 2 and Homer 3 form homodimers and heterodimers. We also found that Homer 3 lacking the coil-coiled domain, shows no dimerization capacity. We currently investigate the role of certain sequences and aminoacids in the homodimerization and heterodimerization of Homer proteins.

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29. Juxtaparanodal protein alterations upon EAE onset and throughout the different stages of the disease

Lida Zoupi,[1] Kyriaki Markoullis,[2] Kleopas A. Kleopa[2,3] and Domna Karagogeos [1]

[1]Department of Basic Science, Faculty of medicine, University of Crete and IMBB-FORTH, Heraklion
[2]Neuroscience Laboratory, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus
[3]Neurology Clinics, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus

Multiple Sclerosis (MS), a progressive autoimmune disease of the CNS, affects a large number of young adults worldwide. During the course of the disease, the myelin sheath – the axonal insulator and responsible for the rapid propagation of axon potentials - is destroyed, while the axon progressively degenerates. Numerous studies have indicated both myelin and axonal signals that contribute to axonal demyelination in MS, but the exact cause and details of the pathophysiology of MS remain unknown.

The myelin sheath is attached to the axon through multiprotein complexes that form the so-called axon-glial interactions. These complexes consist of cell adhesion molecules, voltage gated ion channels and members of the Neurexin family. They divide the axon into distinct molecular and functional domains: the node of Ranvier, the paranode, the juxtaparanode and the internode. The nodes are periodic disruptions of the myelin sheath that are characterized by the clustering of voltage gated sodium channels responsible for the generation of axon potentials. Paranodes are adjacent to the nodes and form a physical barrier to the lateral diffusion of the nodal components towards the internode, the area of compact myelin. Finally, the juxtaparanodes that reside next to the paranodes, are characterized by the clustering of voltage gated potassium channels (VGKCs) that are critical for resting potential formation followed by membrane repolarization.

This molecular organization of the myelinated fiber is crucial during the onset and the progression of MS since recent studies identified distinct perinodal proteins as autoantigens in MS patients: nodal and paranodal Neurofascin isoforms and juxtaparanodal TAG-1/Contactin-2. TAG-1 is a cell adhesion molecule expressed both by axons and glial cells. In the adult nervous system, TAG-1 is responsible for the molecular organization of the juxtaparanodal domain of the myelinated fiber, where it interacts with Caspr2 and the potassium channels.

In this study we have analyzed the alterations of the juxtaparanodal proteins Caspr2, TAG-1 and VGKCs in relation to paranodal Caspr and nodal sodium channels upon the onset and progression of EAE. We have performed immunohistochemical, biochemical and quantitative real time PCR experiments in spinal cord samples from naive, complete Freund adjuvant (CFA)-only controls and recombinant MOG-induced EAE animal groups. We have also characterized the differences of the juxtaparanodal protein cohort in a second rodent model of MS, the Cuprizone model of toxic demyelination upon de- and re- myelination. In this way we have studied the trancriptional, protein and localization differences of perinodal proteins during different EAE stages as well as the effects of immune response on the perinodal protein localization. Our results show a higher paranodal susceptibility to disruption compared to juxtaparanodes in EAE and subsequent compensatory efforts for myelinated fiber restoration that include paranodal protein re-clustering and increased heminodal formation, as EAE progresses.

30. How *Drosophila* olfactory receptors sense odorant molecular vibrations?

Drimyli E., Turin L. and Skoulakis EMC.

Biomedical Sciences Research Center "Alexander Fleming", Vari 16672, Greece.

A common explanation of molecular recognition from the olfactory system posits that receptors recognize the structure or shape of the odorant molecule. Shape- based recognition cannot predict an odorant's smell. An alternative hypothesis suggests that molecular vibrations of all atoms, or of particular functional groups of odorant molecules, contribute to odor recognition and odorants with similar vibrational spectra should elicit similar olfactory responses. Vibrational theory could be tested by a rigorous test of shape recognition by replacing hydrogen with deuterium in odorants and asking whether *Drosophila melanogaster* can distinguish these identically shaped isotopes in behavioral assays. We previously showed that wild type flies do smell vibrations [1]. It is known that there are 64 Odorant Receptors (ORs) in *Drosophila*. The holoreceptor is a dimer comprised of a constant (Orco) and a variable subunit. ORs are broadly tuned to alcohols and esters. Moreover, individual olfactory sensory neurons express a single OR. The question arises as to whether an OR receptor that responds to a single odorant also responds to its isotope. Can a single receptor differentiate between C-H and C-D?

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31. Association of spinophilin with the δ- and μ-opioid receptors differentially modifies their signalling

Maria-Pagona Papakostandinou, Danai-Dionysia Fourla, Stavroula-Maria Vrana and Zafiroula Georgoussi

Laboratory of Cellular Signalling and Molecular Pharmacology, Institute of Biology, National Centre for Scientific Research «Demokritos», 15310 Athens, Greece

Previous studies have shown that the intracellular domains of opioid receptors serve as platforms for the formation of a multicomponent signalling complex (signalosome) consisting of various signalling intermediates ranging form members of G protein cycle to transcription factors (Mazarakou and Georgoussi 2005; Georgoussi et al., 2006; Leontiadis et al., 2009; Georganta et al., 2010). In the present study we demonstrate that spinophilin a dendritic-spine enriched scaffold protein associates with δ- and μ-opioid receptors (δ-OR, μ-OR) in HEK293 and neuronal cells. Association of spinophilin is altered upon opioid receptor activation and is enhanced upon forskolin administration for both opioid receptors. Spinophilin interaction is mediated via the third intracellular loop and a conserved region of the C-terminal tails of δ-OR and μ-OR. On the other hand, the portion of spinophilin responsible for interaction with the opioid receptors is narrowed to the receptor binding domain encompassing amino acids 151–444. In vitro pull downs using specific regions of spinophilin together with purified RGS4, Gα and Gβγ subunits of G proteins have shown that spinophilin forms a multi-protein complex with these proteins using as a platform the C-terminal tails of δ- μ-ORs forming the predicted helix VIII. Expression of spinophilin in HEK293 cells altered agonist-mediated adenylyl-cyclase inhibition of δ-OR leaving unaffected the levels of cAMP accumulation mediated by the μ-OR. Moreover, measurements of extracellular signal regulated kinase (ERK1,2) phosphorylation indicated that the presence of spinophilin modified agonist-driven ERK1,2 phosphorylation mediated upon activation of the opioid receptors. Collectively, these findings suggest that spinophilin associates with both δ- and μ-OR in neuronal cells participating in a multimeric signalling complex that displays a differential regulatory role in opioid receptor signalling.

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32. Duration dependent effects of glucose deprivation on SH-SY5Y cells are mediated by endoplasmic reticulum dysfunction

Maria Paschalidi, Olga Mavrofrydi, Maria Kourti, Panagiota Papazafiri

Division of Animal and Human Physiology, School of Biology, National and Kapodistrian University of Athens

The two crucial cellular insults that take place during cerebral ischemia are the loss of oxygen and of glucose, which can activate a cascade of events leading to neuronal death. In addition, toxic overactivation of neuronal excitatory receptors, leading to calcium overload, may contribute to ischemic neuronal injury. In the present study, the toxicity of transient and prolonged glucose deprivation to neurons was tested in a neuronal cell line, the human neuroblastoma SH-SY5Y. The potential role of calcium and PI3K/Akt survival signalling pathways was also evaluated. Viability was assessed at 24 hours, after incubation of cells for 1, 2, 4, 6 and 24 hours in glucose-free medium or in the presence of thapsigargin (Tg), a specific non-competitive inhibitor of the sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA). Survival was significantly decreased only in the case of prolonged absence of glucose, whereas Tg-induced cell death could not be reversed. In fact, the cytotoxicity observed after short time exposure to Tg was nearly equal to continuous exposure. This difference relies on the irreversible nature of Tg interaction with SERCA and indicates the possibility to intervene following an ischemic incident to attenuate cell damage. Despite cell death, the master pro-survival pathway, PI3K/Akt, was clearly activated under all these conditions; a finding that was also confirmed by the increased phosphorylation of GSK3, a direct target of p-Akt. Interestingly, readdition of glucose in the culture medium resulted in restoration of p-Akt in control levels as soon as after 5 min. It is to notice that, none of the above effects was prevented by neurosteroids. Since the activation of PI3K/Akt survival pathway could not support the decreased cell survival after long term glucose deprivation, the possibility of calcium involvement in cell death was examined. Calcium measurements with fura-2 revealed that glucose deprivation caused a significant decrease in thapsigargin-releasable endoplasmic reticulum stores and subsequently, increased calcium influx from plasma membrane. PI3K/Akt activation was detected to accompany the calcium influx induced in cells previously depleted with Tg. This result indicates that calcium movement through the plasma membrane may account for the sustained PI3K/Akt activation recorded. In summary, we have shown that prolonged deprivation of glucose provokes cell death through depletion of intracellular Ca^{2+} stores. The pursuing calcium influx could explain the sustained stimulation of PI3K/Akt, which, however, can not overmaster the apoptotic signals arising from the depleted endoplasmic reticulum.

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33. miRNA regulons associated with synaptic function

Maria Paschou[1], Maria D Paraskevopoulou[2], Ioannis S Vlachos[2], Pelagia Koukouraki[1], Artemis G Hatzigeorgiou[2, 3] and Epaminondas Doxakis[1*]

[1]Basic Neurosciences Division, Biomedical Research Foundation of the Academy of Athens, [2]Institute of Molecular Oncology, Biomedical Sciences Research Center "Alexander Fleming", [3]Department of Computer and Communication Engineering, University of Thessaly

The synapse is a highly regulated specialized asymmetric structure comprised of a presynaptic terminal having the molecular machinery for neurotransmitter release, and a postsynaptic compartment containing the proteins required for neurotransmitter uptake and signal transduction. Synaptic contact is maintained through structural and functional coupling of a repertoire of proteins in both of these compartments, where differential RNA localization and local protein synthesis regulate synapse function and plasticity in neurons [1,2]. Synaptic plasticity, occurs at synaptic terminal, in part, as a result of rapid translation of these localized mRNAs [3]. Consequently, dynamic regulatory mechanisms for both quantitative and qualitative translation of these mRNAs are required. These mechanisms are currently under intense investigation and may involve RNA binding regulators such as RNA binding proteins and microRNAs (miRNAs) [4]. MiRNAs are a conserved class of regulatory RNAs that control mRNA stability and translation in tissues. They are approximately 22-nucleotide in length endogenous non-coding RNA molecules that base pair to complementary sequences on the 3' un-translated region (3'UTR) of mRNAs repressing their translation [5]. They are abundant in brain but the extent into which they are involved in synaptic mRNA regulation is poorly known. Here, an excess of 500 transcripts, representing the different synaptic molecular categories at pre- and post- synaptic terminals were analyzed for their 3'UTR length, miRNA binding sites distribution and density. A computational analysis of the coding and 3'UTR regions of 242 presynaptic and 304 postsynaptic proteins revealed that 91 % of them are predicted to be miRNA targets. Analysis of the longest 3'UTR isoform of synaptic transcripts showed that presynaptic mRNAs have significantly longer 3'UTR than control and postsynaptic mRNAs, indicating that presynaptic proteins maintained a relative long 3'UTR for enhanced miRNA regulation. In contrast, the shortest 3'UTR isoform of postsynaptic mRNAs is significantly shorter than control and presynaptic mRNAs indicating they avert microRNA regulation under specific conditions. Examination of microRNA binding site density of synaptic 3'UTRs revealed that they are twice as dense as the rest of protein-coding transcripts and that approximately 50 % of synaptic transcripts are predicted to have more than five different miRNA sites confirming the higher propensity of synaptic transcripts to be miRNA targets and indicating that multiple miRNAs ensure tight control of synaptic mRNA expression. Furthermore, an interaction map exploring the association of miRNAs and their targets revealed that a set of ten microRNAs is predicted to regulate 77 % and 80 % of presynaptic and postsynaptic transcripts, respectively. Importantly, these miRNAs have been previously associated with psychotic disorders characterized by neural circuitry dysfunction, such as schizophrenia. Finally, to better understand the biological function of the identified miRNAs, the enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway categories of all their predicted target mRNA transcripts were assessed and showed enrichment for neuronal and synaptic processes. These data revealed the extent of miRNA regulation at the synapse and predicted critical miRNAs that would aid future research on the control of neuronal plasticity and etiology of psychiatric diseases.

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34. Activation of the δ-opioid receptor leads to differentiation and neurite outgrowth via a STAT5B-Gαi/o signalling pathway

Eirini-Maria Georganta, Lamprini Tsoutsi, Maria Gaitanou* and Zafiroula Georgoussi

Laboratory of Cellular Signalling and Molecular Pharmacology, Institute of Biosciences and Applications, National Centre for Scientific Research «Demokritos», 15310 Ag. Paraskevi, Athens, *Laboratory of Cellular and Molecular Neurobiology, Hellenic Pasteur Institute, Athens, Greece

Observations have shown that the endogenous opioid system modulates neurogenesis in the adult hippocampus (1), whereas recent findings support the concept that opioid receptors play an important role in cell proliferation, gliogenesis and neurogenesis (2). These studies indicate that a variety of signalling mechanisms, including soluble or membrane bound guidance cues can signal to effector molecules to mediate intracellular rearrangements. Opioid receptors are coupled to Gi/Go proteins and our recent findings have shown that using their C-terminal domains as a platform they can form a multicomponent signalling complex, a "signalosme", consisting of members of G protein cycle such as Gα, Gβγ, RGS4, c-Src kinase and the transcription factors STAT5B and STAT5A (3-6). We thus wondered whether activation of the opioid receptors in neuronal cells can trigger differentiation and neurite outgrowth through a molecular pathway involving STAT5B and other signalling intermediates. In the present study we demonstrate that prolonged δ-opioid receptor (δ-OR) activation with various opioid agonists induces STAT5B phosphorylation in Neuro-2A cells. Moreover, DSLET-activation of δ-OR leads to increased neurite outgrowth; this effect can be blocked by the receptor selective antagonist naltrindole, after pertussis toxin pre-treatment and the expression of a dominant negative mutant of STAT5B (DN-STAT5B), suggesting that the signalling pathway participating in this mechanism involves inhibitory G proteins and p-STAT5B, which are activated upon opioid administration in Neuro-2A cells. Additional studies have shown that while DSLET exposure of neuroblastoma cells induces a marked increase of two differentiation proteins tested, such as synaptophysin and NCAM (7), overexpression of the DN-STAT5B resulted in a dramatic decrease in the expression levels of both proteins. Taken together, our findings demonstrate that δ-OR activation triggers differentiation and neurite outgrowth via a signalling pathway involving Gαi/o proteins and phosphorylated STAT5B. *This work was supported by the GSRT and the EU grant "Normolife" (LSHC-CT2006-037733) to ZG.*

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TRANSMED FP7 PROGRAM

TransMed translational medicine COOPERATION