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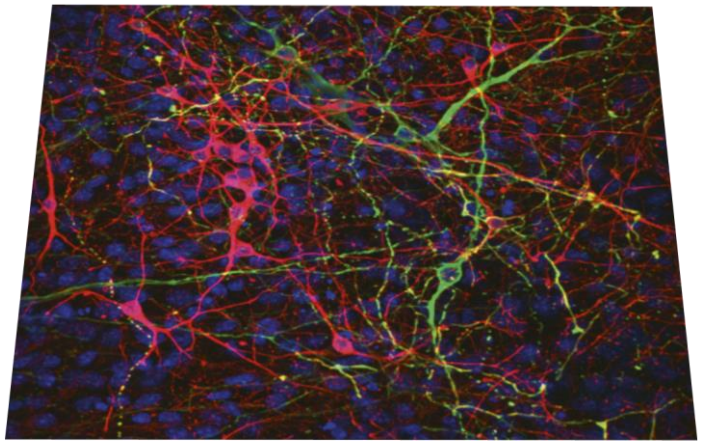

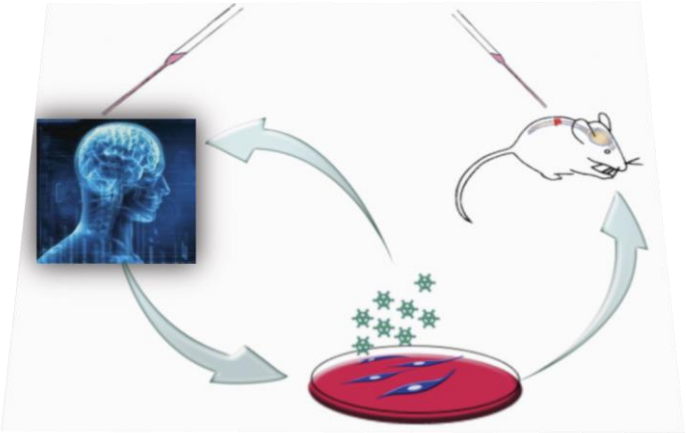

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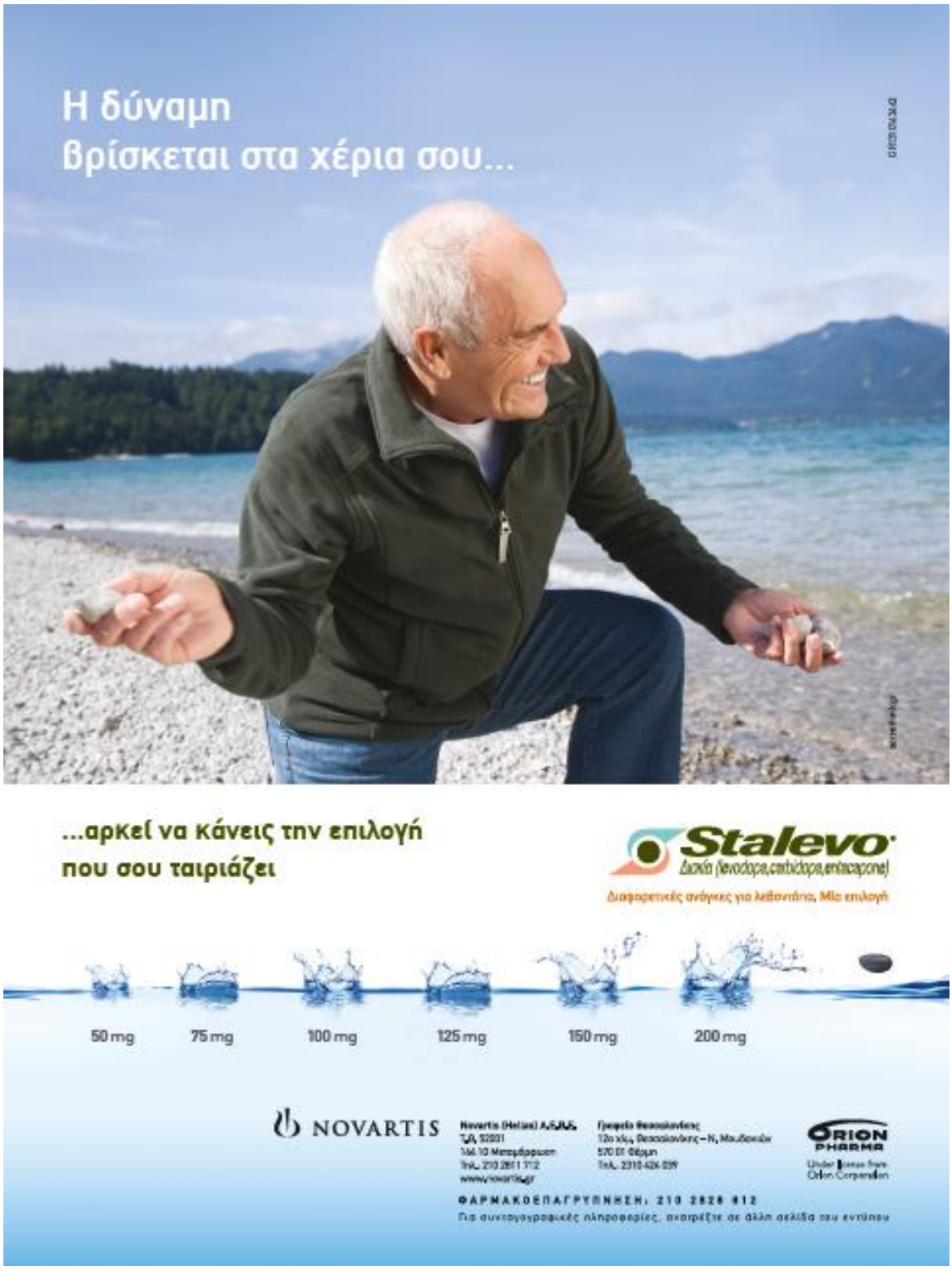
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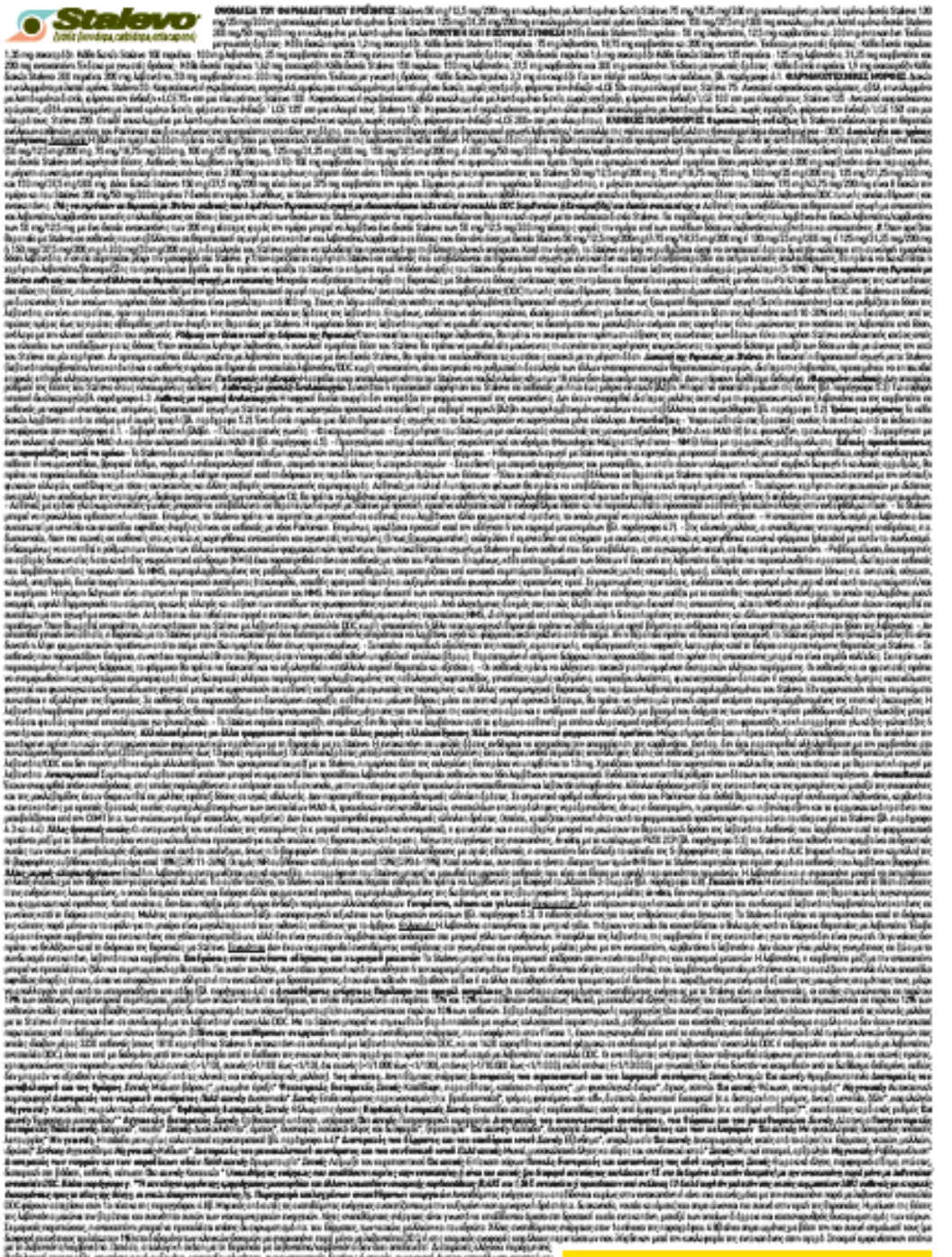
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The site of the conference
The 26th Meeting of the Helenic Society for Neuroscience will take place at the Eugenides Foundation during November 29th –December 1st 2013.
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The registration fee for the congress per category is shown in the table below and can be paid at the conference site or deposited earlier in the following bank account:

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AWARDS

The Hellenic Society for Neuroscience is delighted to announce the availability of 4 Awards for PhD candidates and post-docs to attend the FENS Forum 2014 in Milan:

1. George Paxinos award,

2. Antonia and Spyros Mylonas award

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Two of these Awards will be awarded to the best oral presentations and 2 to the best poster presentations at the 26th Annual Meeting of the Hellenic Society for Neuroscience.

The following criteria apply:

1) PhD students must have completed the second year of their doctoral studies.

2) Post-docs should have been granted their PhD no earlier than November 2009.

3) Each applicant must be principal investigator (first author in the abstract submitted) and present his/her work in person.

4) Only studies conducted in Greek or Cypriot Laboratories qualify.

ORAL PRESENTATIONS

All oral presentations should be delivered as powerpoint files in a CD or a USB flash to the meeting staff at least 15 minutes prior to the relevant session. Speakers may also use their own laptops especially if animation is included in the presentation.

All speakers should take care not to exceed the total duration of their talk, as shown in the program. In addition, they should leave at least 3 minutes of the total duration of their presentation for discussion.

ORAL PRESENTATIONS

SYMPOSIUM I: ION AND MOLECULE CHANNELS: FROM STRUCTURE AND FUNCTION TO DISEASE MECHANISMS

ON PROKARYOTIC ANCESTORS OF ACETYLCHOLINE RECEPTORS AND LIGAND GATED ION CHANNELS

P.J. Corringer

Pasteur Institute, Channel-receptor Unit, CNRS UMR 3571, 25 rue du Docteur Roux, 75015 Paris, France

Pentameric channel-receptors, including nicotinic acetylcholine and $GABA_A$ receptors, play a key role in fast excitatory and inhibitory transmission in the nervous system and are the target of numerous therapeutic and addictive drugs. They carry several neurotransmitter binding sites which govern the opening of a transmembrane ion channel. Extensively expressed in animals, they were recently found in several bacteria, especially the homolog from the cyanobacteria *Gloeobacter violaceus* (GLIC)[1] which functions as a proton-gated ion channel, and the homolog from *Erwinia chrysanthemi* (ELIC). The simplified architecture of these archaic homologues, as well as their prokaryotic origin, allowed solving the first X-ray structures of integral membrane ELIC[4] and GLIC[2,3] in a closed and an open conformation, respectively. Comparative analysis of ELIC and GLIC suggests that channel opening may occurs through a symmetrical quaternary twist and tertiary deformation, according to a global transition that couples channel opening with reorganization of the binding pockets for neurotransmitters and allosteric effectors. In addition, recent co-crystallization of GLIC with allosteric inhibitors[5] that are clinically used as general anesthetics reveals the mechanism of action at the membrane of these amphipatic molecules and will help designing new drugs targeted to pentameric channel-receptors. Recent cross-linking study allowed capturing a "locally-closed" conformation of GLIC, which may constitute an intermediate state occurring in the course of activation or desensitization[6].
Altogether, these combined structural and functional data give insights into the allosteric mechanisms operating in these integral membrane proteins. Future work is yet to be performed to understand the mechanisms of activation and desensitization which mediate synaptic transmission and plasticity within the brain.

1. Bocquet, N. et al, Nature, 457:111-4 (2009), 2. Bocquet, N. et al, Nature, 457:111-4 (2009), 3. Hilf RJ & Dutzler R. Nature. 457:115-8 (2009), 4. Hilf RJ & Dutzler R. Nature. 452:375-9 (2008), 5. Nury et al, *Nature*, 469:428-31 (2011), 6. Prevost, M., et al Nat Struct Mol Biol. 19:642-9 (2012).

HIGH RESOLUTION STRUCTURE OF THE LIGAND-BINDING DOMAIN OF A HUMAN NICOTINIC ACETYLCHOLINE RECEPTOR: TOWARDS RATIONAL DRUG DESIGN FOR RELATED DISEASES

Marios Zouridakis[1], Petros Giastas[1], Eleftherios Zarkadas[1,2] & Socrates J. Tzartos[1,2]
[1]Hellenic Pasteur Institute, 127, Vas. Sofias Ave., GR11521 Athens, Greece
[2]Department of Pharmacy, University of Patras, Rio, GR26500, Patras, Greece

The first atomic-resolution structures of the extracellular domain (ECD) of a neuronal nicotinic acetylcholine receptor (nAChR) and its complex with a small antagonist are presented. The X-ray crystal structures of a modified form of human α9-ECD and its complex with methyllycaconitine (MLA) were solved at the atomic level. α9 nAChR is a unique member of the nAChR family, since it is blocked by the classic nAChR agonist nicotine. α9 subunit forms either homopentameric α9 nAChRs or co-assembles with α10 subunit to form the heteropentameric α9α10 nAChR. α9-containing nAChRs are mainly found at the base of outer hair cells in the inner ear where they mediate synaptic transmission between efferent olivocochlear fibers and cochlea hair cells. They are also reported in the pituitary gland, in sympathetic neurons and other non-neuronal cells, such as skin keratinocytes and lymphocytes. The α9-containing nAChRs are potential targets for the therapy of ear disorders, vertigo, chronic pain, inflammation, human cancers and for the autoimmune disease pemphigus vulgaris, where α9 appears to be one of the auto-antigens. α9 is also important for wound healing. Comparison of the α9-ECD structures revealed the rearrangements taking place in residues and regions upon MLA binding. Interestingly, a membrane-facing interaction network between elements at the lower part of α9-ECD, probably applying to all other nAChRs, was observed for the first time, while a similar to the mouse muscle α1-ECD buried hydration pocket was determined. This novel interaction network probably extends to the ligand-binding site through a pathway ultimately involving a sugar chain. The orientations of specific residues implicated in the unique pharmacology of α9-containing nAChRs and in the selectivity to α-conotoxins were well defined, facilitating rational drug design to treat related human diseases.

Etiological mechanisms of myasthenia gravis and immune dysregulations, a disease caused by anti-acetylcholine receptor antibodies

Perrine Cufi, Angeline Gradolatto, Dani Nazzal, Maria Foti, Frédérique Truffault, Sonia Berrih-Aknin and Rozen Le Panse

UPMC UM76/INSERM U974/CNRS UMR7215/AIM - 105 bd de l'hôpital - 75013 Paris - France

Autoimmune Myasthenia Gravis (MG) is a rare neuromuscular disorder, characterized by a defective transmission of nerve impulses to muscles, leading to muscle weakness and disabling fatigability. MG is a multifactorial disease involving a genetic predisposition, immune dysregulations and the influence of environmental factors.

In MG with autoantibodies against the acetylcholine receptor (AChR), the thymus is clearly involved in the pathogenesis. We recently demonstrated the involvement of the innate immunity in MG development. Poly(I:C), that mimics dsRNA, induced specifically thymic overexpression of α-AChR in vitro and in vivo, and induced an anti-AChR autoimmune response. These effects are clearly linked to the release of IFN-β in response to Poly(I:C), as IFN-β seems to orchestrate all thymic changes observed in MG thymus.

Immune dysregulations observed in the thymus of MG patients also contribute strongly to MG development. Indeed, altered immune regulatory mechanisms are not only due to a functional defect of T regulatory (Treg) cells but also to a resistance to suppression of T conventional (Tconv) cells. Comparing the transcriptome of purified thymic Treg and Tconv cells from MG patients and controls, we observed that T cells from MG patients exhibit a Th1/Th17/Tfh signature. Moreover, an increase in IL-17-related genes was specifically observed in Treg cells, while increases in IFN-γ, and TNF-α were observed in both Treg and Tconv cells.

Altogether, these results indicate that in predisposed individual, a thymic inflammation could play a central role in thymic changes and alter T-cell development leading to the emergence of autoreactive T cells against AChR that are characteristic of MG.

THE ROLE OF GAP JUNCTIONS IN INHERITED AND ACQUIRED DEMYELINATION

Kleopas A. Kleopa

Neuroscience Laboratory and Neurology Clinics, The Cyprus Institute of Neurology and Genetics, Cyprus School of Molecular Medicine, Nicosia, Cyprus

Gap junction (GJ) protein expression allows direct intra- and intercellular coupling that serves signalling, ion buffering and homeostatic functions. Human diseases caused by mutations in GJ proteins and respective animal models have highlighted the crucial roles of GJs in myelinating Schwann cells and oligodendrocytes. Connexin32 (Cx32) mutations cause X-linked Charcot Marie Tooth disease (CMT1X), a peripheral neuropathy often associated with CNS manifestations. Cx32 is expressed by myelinating Schwann cells and oligodendrocytes, forming GJs through the myelin sheath. CMT1X models have demonstrated that most Cx32 mutants result in abnormal trafficking and loss of GJ function, causing demyelination and early axonal pathology. Recessive mutations in Cx47 expressed in oligodendrocytes result in hypomyelinating leukodystrophy known as Pelizaeus-Merzbacher-like disease (PMLD). Similar to Cx32, Cx47 mutants result in loss of function. Ongoing gene therapy studies based on the cellular mechanisms of CMT1X and PMLD focus on delivering GJ proteins to myelinating cells using lentiviral vectors. In addition to inherited myelin disorders, there is emerging evidence that glial connexins are also involved in acquired demyelination. Cx32 and Cx47 GJ plaques and protein levels were reduced in and around multiple sclerosis (MS) lesions, both in white and grey matter, while astrocytic Cx43 and Cx30 are increased as part of astrogliosis. Furthermore, in the normal appearing WM (NAWM) and cortex, Cx32 is significantly reduced along myelinated fibers whereas Cx47 expression is preserved mainly in oligodendrocyte precursor cells (OPCs). However, OPCs showed only limited connectivity to astrocytes. These alterations can be replicated in chronic stages of experimental autoimmune encephalomyelitis (EAE), while in acute EAE loss of astrocytic Cx43 appears to precede the disruption of oligodendrocyte GJs. Disruption of oligodendrocyte GJs in the setting of persistent inflammation and astrogliosis may play a role in MS progression and secondary neurodegeneration, and resembles some aspects of PMLD and CMT1X CNS phenotypes.

PLENARY LECTURE I

ION CHANNELS, ANTIBODIES AND NEUROLOGICAL DISEASE

Angela Vincent

Nuffield Department of Clinical Neurosciences, University of Oxford, OX3 9DU, UK

Over the last 40 years the concept of autoantibodies affecting the number and function of both ligand-gated and voltage-gated ion channels has widened considerably. In the 1970s, myasthenia gravis was shown to be caused by antibodies to acetylcholine receptors (AChRs), and these seminal findings have helped lead the way to the recognition and treatment of other antibody-mediated diseases of the peripheral, autonomic and central nervous systems. In 2001, antibodies to MuSK were found in some of the myasthenia patients negative for AChR antibodies, and more recently antibodies to LRP4 in a small proportion. MuSK and LRP4 are postsynaptic membrane proteins that are involved in AChR localisation and function. The historical aspects are briefly reviewed (1).
In the 1990s, antibodies to voltage-gated calcium channels were identified in the Lambert Eaton syndrome, and antibodies to *shaker* type voltage-gated potassium channels (VGKCs) in acquired neuromyotonia, a condition caused by peripheral nerve hyperexcitability that leads to muscle fasciculations, cramps and pain. Somewhat surprisingly, the VGKC antibodies were also identified in central nervous system disorders, particularly limbic encephalitis (memory loss, sleep disorders and seizures) but this was explained by the fact that the antibodies turned out to be directed at proteins that form part of VGKC complexes in situ (2). The two principal proteins help localise (CASPR2) and modify (LGI1) potassium channel function. Other antibodies bind directly to CNS ligand-gated receptors. Antibodies to NMDA receptors (NR1 principally) are found mainly in younger patients, often women and small children (3), who have a severe encephalopathy with movement disorders. And antibodies to glycine receptors are associated with extreme rigidity and brainstem disturbance which can be life threatening (Carvajal et al in preparation). Importantly, each of these antibodies bind to extracellular epitopes on the target proteins and there is evidence of their pathogenicity. From the bedside perspective, the conditions, although rare, can now be diagnosed regularly by serological tests and the patients treated with immunotherapies which lead to substantial improvement (4,5).
The lecture will review some of these developments and touch on the mechanisms that are being explored in vitro and in vivo.
References: 1. Vincent A (2002) Unravelling the pathogenesis of myasthenia gravis. *Nat Rev Immunol* 2(10):797-804. 2.Irani SR, *et al.* (2010) Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. *Brain* 133(9):2734-2748. 3. Dalmau J, *et al.* (2008) Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol* 7(12):1091-1098. 4. Vincent A, Bien CG, Irani SR, & Waters P (2011) Autoantibodies associated with diseases of the CNS: new developments and future challenges. *Lancet Neurol* 10(8):759-772. 5. Lancaster E & Dalmau J (2012) Neuronal autoantigens--pathogenesis, associated disorders and antibody testing. *Nat Rev Neurol* 8(7):380-390.

PLENARY LECTURE II:

PROGRAMMING STEM CELLS FOR MODELING OF NEUROLOGICAL DISEASE

Oliver Brüstle

Institute of Reconstructive Neurobiology, LIFE & BRAIN Center, University of Bonn, Germany

Experimental access to the pathogenic mechanisms underlying neurodegenerative disorders is restricted by the limited availability of vital human nervous system tissue. This bottleneck has been partially overcome with the availability of human pluripotent stem cells (PSC) and their controlled differentiation into neural cell types. Long-term self-renewing neuroepithelial stem (lt-NES) cells derived from PSC can serve as a standard system for robust and continuous generation of human neurons and glia. Lt-NES cell-derived neurons express the entire array of Alzheimer's disease (AD) associated human proteins and can be used to explore mutants associated with aberrant APP and tau processing – two key pathogenic routes associated with this disorder. Derivation of lt-NES cells from induced pluripotent stem cells (iPSC) extends this approach to a patient-specific level. Using this strategy we detected an unexpectedly low responsiveness of human neurons to gamma-secretase modulators. These data are concordant with the observed failure of these compounds in clinical trials and underline the importance of assessing compound efficacy in the appropriate human target cell type. Interestingly, lt-NES cell-derived neurons recapitulate pathological protein aggregation already a few weeks after in vitro differentiation, which makes them a formidable tool for studying the earliest cytopathological events in proteinopathies such as polyglutamine disorders. While cell reprogramming and iPSC-derived lt-NES cells represent robust tools for studying monogenic diseases in the context of selected patient families, there is a strong interest in extending this methodology to complex disorders. Requiring samples from large numbers of patients, such studies call for even more efficient methods for establishing disease-specific cells. We found that overexpression of two neurogenic transcription factors together with small molecule-based inhibition of GSK-3ß and SMAD signaling permits direct conversion of postnatal human fibroblasts into induced neurons (iNs) with efficiencies suitable for downstream biomedical applications.

SYMPOSIUM II: STEM CELLS AND REPROGRAMMING

INDUCTION OF MULTI-, PLURI- and TOTIPOTENCY

Hans R. Schöler

Department of Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, Münster, 48149, Germany

The pluripotent and multipotent states of stem cells are governed by the expression of few, specific transcription factors that form a highly interconnected regulatory network along with numerous, widely expressed transcription factors. When the set of master transcription factors comprising Oct4, Sox2, Klf4, and Myc is expressed ectopically in somatic cells, this network organizes itself to support a pluripotent cell state. But when Oct4 is replaced by Brn4, another POU transcription factor, the network supports the conversion of fibroblasts into multipotent neural stem cells. Oct4 and Brn4 therefore appear to play distinct yet interdependent roles in remodelling gene expression by influencing the local chromatin status during reprogramming. Furthermore, structural analysis of Oct4 bound to DNA shows that the Oct4 linker—a region connecting the two POU domains of Oct4—is exposed to the surface, and we therefore postulate that it recruits key epigenetic players onto Oct4 target genes during reprogramming.
The role of Oct4 in defining totipotency and inducing pluripotency during embryonic development so far remained unclear, however. We genetically eliminated maternal *Oct4* using a Cre/lox approach and found no effect on the establishment of totipotency, as shown by the generation of live pups. After complete inactivation of both maternal and zygotic *Oct4* expression, the embryos still were capable of forming *Oct4*-GFP– and Nanog–expressing inner cell masses, albeit nonpluripotent ones, indicating that Oct4 is not a determinant for the pluripotent cell lineage separation. Interestingly, *Oct4*-deficient oocytes were capable of reprogramming fibroblasts into pluripotent stem cells. Our results indicate that, in contrast to its crucial role in the maintenance of pluripotency, maternal *Oct4* is crucial for neither the establishment of totipotency in embryos, nor the induction of pluripotency in somatic cells using oocytes.

GENERATION OF INDUCED NEURONS VIA DIRECT CONVERSION IN VIVO AND IN VITRO

Shane Grealish ,Shong Lau, Maria Pereira, Ulrich Pfisterer, Olof Torper, Daniel Wolf, Malin Parmar[1]

Department of Experimental Medical Science, Wallenberg Neuroscience Center and Lund Stem Cell Center, Lund University, 221 84 Lund, Sweden
[1]Author for correspondance

By viral expression of neural fate determinants, it is possible to directly reprogram mouse and human somatic cells into functional neurons, termed induced neurons (iNs). The resulting cells are non-proliferating, and presents an alternative to induced pluripotent stem cells (iPS cells) for obtaining patient and disease specific neurons to be used for disease modeling and for development of cell therapy. We present here a highly efficient protocol for direct neural conversion that results in the formation of a high number of functional dopamine neurons *in vitro*. To facilitate clinical translation, we have developed a new vector system that allows for generation of hiN cells using non-integrative and self-regulating vectors.
We also show that transplanted human fibroblasts and human astrocytes that are engineered to express inducible versions of neural reprogramming genes convert into neurons *in vivo*, when reprogramming genes are activated after transplantation. Using a Cre-LoxP system to specifically direct expression of re-programming genes to endogenous glial cells in the striatum, we show that also parenchymal mouse astrocytes and NG2 glia can be directly converted into NeuN expressing neurons *in situ*. These experiments provides the first proof-of-principle that direct neural conversion can take place *in vivo*, in normally non-neurogenic regions of the adult rat brain.

IPS-DERIVED NEURAL CELLS FROM PATIENTS WITH FAMILIAL PARKINSON'S DISEASE CARRYING THE A53T A-SYNUCLEIN MUTATION

Georgia Kouroupi[1], Era Taoufik[1], Konstantinos Tsioras[1], Delphine Bohl[2], Alexander P. Polyzos[3], Panagiotis K. Politis[4], Dimitris Stellas[5], Maria Xilouri[4], Kanella Prodromidou[1], Nasia Antoniou[1], Dafni Chroni[6], Kostas Vekrellis[4], Piotr Bregestovski[7], Leonidas Stefanis[4,8], 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 Molecular Biology, Biomedical Research Foundation of the Academy of Athens, 4 Soranou Efesiou street, 11527 Athens, Greece*
[4]*Division of Basic Neurosciences, Biomedical Research Foundation of the Academy of Athens, 4 Soranou Efesiou street, 11527 Athens, Greece*
[5]*Department of Cancer Biology, Biomedical Research Foundation of the Academy of Athens, 4 Soranou Efesiou street, 11527 Athens, Greece*
[6]*Department of Biochemistry, Hellenic Pasteur Institute, Athens, Greece*
[7]*Epilepsy and Cognition Brain Dynamics Institute, INSERM U 1106, Aix-Marseille University, 27 Bd Jean Moulin, 13385 Marseille Cedex 05, France*
[8]*Second Department of Neurology, University of Athens Medical School, Athens, Greece*

Parkinson's disease (PD) is a common, progressive neurodegenerative disorder characterized by loss of dopaminergic neurons in the nigrostriatal pathway of the brain, resulting in motor and cognitive deficits. A major drawback in PD research is due to inaccessibility of diseased tissue for study. Human induced pluripotent stem (iPS) cell technology now offers a unique opportunity to study disease pathogenesis and may have important implications in Regenerative Medicine. Here we report the generation of iPS cells from skin fibroblasts of Parkinsonian patients with a familial form of the disease and aged-matched unaffected individuals. The dominantly inherited G209A mutation in the α-synuclein gene SNCA encoding the A53T mutant **α**-synuclein protein (αSYN), was first identified in the Greek population and is associated with the appearance of an early form of PD which is prevalent in Greece (Polymeropoulos et al. Science 1997). Multiple iPS lines were generated and their pluripotency and karyotype integrity was confirmed using a number of in vitro and in vivo assays. As wild-type αSYN and the A53T mutant protein have a central role in PD, we sought to develop an iPS-based cellular model to study disease pathogenesis by directed differentiation of patient- and control-derived iPS cells to dopaminergic precursors and dopaminergic neurons. This model represents a new experimental system to investigate the mechanistic basis of neurodegeneration caused by α-synuclein dysfunction with ultimate aim to develop novel therapeutics for PD.

Supported by EU FP7 264083 Neurosign, the Foundation BNP Paribas and the Greek General Secretariat for Research and Technology grants 2272 ARISTEIA ParkinsonTransMed and 09SYN-21-969 NoisePlus

HOW MIGHT STEM CELLS HELP IN THE TREATMENT OF PARKINSON'S DISEASE

Roger A Barker

John van Geest Centre for Brain Repair, Department of Clinical Neuroscience, Forvie Site, Robinson Way, Cambridge CB2 0PY, UK

Parkinson's Disease (PD) is a common disorder that has as part of its core pathology the loss of the dopaminergic nigrostriatal neurons and the formation of alpha synuclein positive Lewy bodies. However whilst it is now recognised that PD has a much more complex pathology than this, most patients respond well to dopaminergic drug therapies in the early stages of disease. However with time this efficacy wears off and side effects develop and thus there is a need for a better, more biological, way to deliver dopamine to the parkinsonian brain.

One approach has been to use dopamine producing cells grafted into the striatum, of which the most successful have been those derived from the developing human fetal ventral mesencephalon (hfVM). The use of this tissue was the subject of many successful open label trials in the late 1980s and 1990s, but at the turn of the century two "double blind placebo group" trials showed that this therapy was ineffective and produced side effects in the form of graft induced dyskinesias. The outcome of these two trials essentially brought clinical trials of cell based therapies in PD to a halt.

However a re-evaluation of the data from all these trials suggests that this therapy may work if targeted to a more specific population of patients with PD with greater standardization of the protocols for delivering and supporting the grafted tissue. This has led to a new EU funded trial of hfVM tissue in younger patients with earlier stage PD (TRANSEURO). This trial is seen as creating a template for taking future dopaminergic cells derived from stem cell sources to clinic, as the ethical and logistical problems of using human fetal tissue precludes it from every becoming a main stream treatment for PD. As to which stem cells will act as the source of such neurons remains unresolved but it is likely to be human ES cell lines in the first instance.

In this talk I will confine my discussion to the history of neural grafting in PD and how stem cells could be used in the next generation of trials adopting this strategy, as the use of stem cells to model disease will be covered by other speakers.

My work in PD is supported by an NIHR award of a Biomedical Research Centre to Addenbrooke's Hospital/University of Cambridge; Parkinson's UK; Michael J Fox Foundation; Cure-PD; Rosetrees Trust; EU-FP7; and MRC.

ECNP SPONSORED LECTURE

ROLE OF P11 IN DEPRESSION AND ANTIDEPRESSANT TREATMENTS

Per Svenningsson,

Karolinska Institute, Stochholm

The serotonin system is critical in regulating emotional states. The multifunctional proteinp11 was identified as adaptor protein to 5-HT1B and 5-HT4 receptors and recent studies of p11 are shedding light on the molecular and cellular mechanisms underlying depression and Parkinson´s disease. Data will be presented implicating p11 both in the amplification of serotonergic signaling and regulation of gene transcription. The talk will also review studies demonstrating that the levels of 5-HT1B receptors and p11 are regulated in depression and by antidepressant regimens and, conversely, that 5-HT1B receptors and p11 regulates behavioral responses to these treatments.

ORAL PRESENTATIONS FROM SELECTED ABSTRACTS

IMPAIRED MITRAL CELL MIGRATION DUE TO TAG-1 DEFICIENCY LEADS TO OLFACTORY DYSFUNCTION

Maria Savvaki[1], Georgios G Bastakis[1], Antonis Stamatakis[2], Marina Vidaki[1] and Domna Karagogeos[1]

1. Dpt. of Basic Science, Faculty of Medicine, University of Crete and Institute of Mol. Biol. &Biotech-FoRTH, Vassilika Vouton, 71110, Heraklion, Crete, Greece
2. Laboratory of Biology, Faculty of Nursing, School of Health Sciences, University of Athens, Papadiamantopoulou 123, GR11527, Athens, Greece

The olfactory system constitutes a sensory system of major importance for mammals as well as a valuable tool for studying neuronal connections in the central nervous system. Much knowledge has been obtained in recent years on the organization of the odorant receptor map, formed by the connections of olfactory receptor neurons with the dendrites of projection neurons of the olfactory bulb, in specialized structures, the glomeruli. On the other hand, little is known about the corresponding mitral cell map, located in the mitral cell layer, consisting of the main projection neurons of the olfactory bulb. The present study aims to shed light on the organization and function of the mitral cell layer. We show that the absence of the cell adhesion molecule Transient Axonal Glycoprotein-1 (TAG-1) in mice results in a significant reduction in the number of mitral cells inside the main olfactory bulb as a consequence of impaired migration of a subpopulation born at embryonic day 11.5. We analyze the developmental series of events that occur before the final positioning of projection neurons into the mature mitral cell layer. Furthermore, our study reveals that the detected alterations in the number of mitral cells are reflected in an aberrant neuronal activation profile as well as disturbed olfactory behavior. Our results propose that TAG-1 is crucial for the proper organization of projection neurons in the main olfactory bulb and suggest that disturbing this station of the odorant information route disrupts its integration into the olfactory circuitry.

NUCLEAR RECEPTOR NR5A2 REGULATES PROLIFERATION AND DIFFERENTIATION OF NEURAL STEM CELLS DURING DEVELOPMENT THROUGH DISTINCT MECHANISMS

Athanasios Stergiopoulos[1], Kristina Schoonjans[2], Johan Auwerx[2] and Panagiotis Politis[1]

1 - *Center for Basic Research, Biomedical Research Foundation of the Academy of Athens, Greece*
2 - *Laboratory of Integrative Systems and Physiology (LISP), School of Life Sciences, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland*

Nuclear receptors (NRs) play key roles in central nervous system (CNS) development and function. Among them, NR5A2 (LRH-1), an orphan NR, has been recently reported to be highly expressed in CNS. Despite this finding and its involvement in stem cell pluripotency, embryogenesis, tumorigenesis, metabolism homeostasis, steroidogenesis, development and function of many other tissues and organs, the physiological role of NR5A2 in CNS still remains largely elusive. We have only recently shown that NR5A2 is involved in the regulation of Notch signaling during neuronal differentiation (Kaltezioti et al, 2010, *PLoS Biol*; Stergiopoulos & Politis, 2013, *Arch Biochem Biophys*). Here, we provide functional evidence that NR5A2 is a critical regulator of CNS development by controlling proliferation and differentiation decisions in neural stem cells (NSCs). By expression studies, we showed that NR5A2 is specifically associated with the neuronal lineage in CNS of vertebrates. In agreement, gain- and loss-of-function experiments in primary NSCs and analysis of knock-out mice embryos suggest that NR5A2 strongly arrests proliferation, induces neurogenesis and blocks astrogliogenesis. Mechanistically, NR5A2 induces neuronal differentiation via a direct action on the promoter of *Prox1* gene, and blocks proliferation through a direct binding and transcriptional de-repression of *Cdkn2a* (p16) and *Cdkn2b* (p15) genes of the *INK4/ARF* locus. Collectively, these observations, together with the recently discovered pharmacological agonists/antagonists of NR5A2, render it a candidate target gene for regenerative medicine and treatment of nervous system-related diseases and cancers.

CRANIOFACIAL DEVELOPMENT IS FINE TUNED BY SOX2

Nikolaos Mandalos[1,*], Muriel Rhinn[2], Zoraide Granchi[3], Thimios Mitsiadis[3], Aris N. Economides[4], Pascal Dollé[2] and Eumorphia Remboutsika[1,@]

[1]Stem Cell Biology Laboratory, Biomedical Sciences Research Centre "Alexander Fleming", 34 Fleming Str., 16672-Vari, Greece. [2]Institut de Genetique et de Biologie Moleculaire et Cellulaire, 1 Rue Laurent Fries, 64707,Strasbourg Cedex, France. [3]Institute of Oral Biology, ZZM, University of Zurich, Plattenstrasse 11, 8032 Zurich, Switzerland. [4]Regeneron Pharmaceuticals, Tarrytown, New York, USA

Precise control of self-renewal and differentiation of the neural progenitor cells into the neural crest pool ensures proper head development. Here, we show that Sox2 fine-tunes craniofacial development in mice. We took advantage of a surgical ablation of *Sox2* using a novel Conditional by Inversion *Sox2* allele (*Sox2*^{COIN}), we have created, in order to study the role of sox2 in the anterior epiblast-derived stem cell pool. Epiblast-inversion of the *Sox2*^{COIN} allele generates normal heterozygote adult animals (*Sox2*$^{INV/+}$). *Sox2*$^{INV/+}$ intercrosses generate a true *Sox2* null allele as homozygote embryos are not viable and fail to form the epiblast. Sox2$^{INV/+}$ haploinsufficient and *Sox2*$^{INV/mosaic}$ mutant embryos proceed beyond gastrulation, but die around E11. Moreover, mutant embryos exhibit hydrocephaly and frontonasal truncations. These defects could be attributed to the deregulation of the epithelial to mesenchymal transition of neural progenitor cells during neural crest development resulting in the exacerbated and aberrant migration of Sox10^{+} neural crest cells in the branchial arches and the frontonasal region of mutant embryos. Thus, we unravel a novel role for Sox2 as a rheostat of the epithelial to mesenchymal transitions to control the neural crest flow during craniofacial development.

SUBVENTRICULAR ZONE-DERIVED NEURAL STEM/PRECURSOR CELL GRAFTS RESTORE COGNITIVE DEFICITS IN A MOUSE MODEL OF TRAUMATIC BRAIN INJURY

Paraskevi N. Koutsoudaki[1], Antonios Stamatakis[2], Evangelia Xingi[3], Georgia Kouroupi[1], Florentia Papastefanaki[1], Fotini Stylianopoulou[2], Rebecca Matsas[1]

[1] Laboratory of Cellular and Molecular Neurobiology, Hellenic Pasteur Institute, Athens, Greece.

[2] Biology-Biochemistry Laboratory, Dept. Basic Sciences, Faculty of Nursing, School of Health Sciences, University of Athens, Greece.

[3] Light Microscopy and Flow Cytometry Units, Hellenic Pasteur Institute, Athens, Greece.

Traumatic brain injuries (TBI) constitute an important public health issue due to their high mortality rate and extended clinical and socioeconomic consequences. While repair of injury- or disease-caused damage in the nervous system is crucial for survival, the central nervous system (CNS) has little capacity for self-repair after loss of cellular components due to such events. Transplantation of neural stem/precursor cells (NSC), in their native state or genetically modified to express neuroprotective agents has been suggested as a possible therapeutic strategy. Insulin-like growth factor I (IGF-I) has been shown to have neuroprotective properties following a wide range of experimental insults to the nervous system. Additionally, we have recently shown that *ex vivo* transduction of NSC with a lentiviral vector for expression of IGF-I enhances their ability to give rise to neurons *in vitro*, but also *in vivo* after intrahippocampal transplantation in a mouse model of temporal lobe epilepsy. Here we investigated the therapeutic potential of NSC transduced, or not, with IGF-I in a mouse model of penetrating hippocampal injury. Transplantation of NSC either with or without IGF-I transduction, significantly improved the injury-induced spatial learning deficit and had beneficial effects on the host tissue by reducing regeneration-inhibiting events, such as astroglial activation. Our analysis showed that the grafted NSC differentiated primarily into cells of the oligodendroglial, but not the neuronal or astrocytic lineage *in vivo*, as revealed by their characteristic morphology and expression of cell-type-specific markers. These observations illustrate the remarkable plasticity of transplantable NSC which can acquire injury-dependent phenotypes within the host CNS. The reciprocal interactions between transplantable cells and the injured host tissue are important parameters when designing prospective alternative therapies for CNS injuries. Supported by FP7 REGPOT Project 264083 Neurosign, and by the European Regional Development Fund and national resources (Action: “KRIPIS: Development proposals of Research Institutions”).

DEREGULATION OF CALCIUM HOMEOSTASIS MEDIATES SECRETED α-SYNYCLEIN-INDUCED NEUROTOXICITY

Katerina Melachroinou[1], Maria Xilouri[1], Evangelia Emmanouilidou[1], Roser Masgrau[2], Panagiota Papazafiri[3], Leonidas Stefanis[1,4], Kostas Vekrellis[1]

[1]Division of Basic Neurosciences, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
[2]Department de Bioquímica i Biologia Molecular, and, Institut de Neurociències, Universitat Autònoma de Barcelona, Barcelona, Catalonia, Spain
[3]Division of Animal and Human Physiology, Department of Biology, University of Athens, Athens, Greece
[4]Second Department of Neurology, University of Athens Medical School, Athens, Greece

α-Synuclein (AS) is an abundant presynaptic neuronal protein, that is genetically and biochemically linked to Parkinson's disease (PD). It is now established that AS is normally secreted from neuronal cells and can thus have paracrine effects. To assess the effects of secreted AS on neuronal homeostasis, we have used SH-SY5Y cells inducibly overexpressing human wild-type (WT) AS, as a source of naturally secreted AS. We have previously shown that these cells readily secrete AS species into their culture medium, which can be toxic to recipient cells. In the current study, we show that application of secreted AS alters membrane fluidity and increases Ca^{2+} entry. This influx is reduced upon pharmacological inhibition of voltage operated Ca^{2+} channels (VOCs). Although no change in free cytosolic Ca^{2+} levels is observed, a significantly increased mitochondrial Ca^{2+} sequestration is found in recipient cells. Application of VOCs blockers or Ca^{2+} chelators abolishes AS-mediated toxicity. AS-treated cells exhibit increased calpain activation, while calpain inhibition greatly alleviates the observed toxicity. Collectively, our data suggest that secreted AS exerts toxicity through engagement, at least in part, of the Ca^{2+} homeostatic machinery. Therefore, manipulating Ca^{2+} signaling pathways may represent a potential therapeutic strategy for PD.

IDENTIFICATION OF A NOVEL SLC25 FAMILY MEMBER OF MITOCHONDRIAL CARRIERS CAUSING RECESSIVE NEUROLOGICAL DISEASE IN MICE

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

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

Following a forward genetics approach through random mutagenesis, we 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. Through genetic analysis, we identified a nonsense point mutation in the coding region of a novel gene member of 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, while mutations in SLC25 genes impair mitochondrial functions. This novel member is highly conserved among species, but its function remains completely unknown. Our results confirm its mitochondrial localization by confocal and western blot analysis. Moreover, we identified that the wild-type 46 kDa SLC25 protein is predominantly expressed in the Central Nervous System in contrast to the mutant protein that is undetectable. We have also verified the causal role of this mutation in rescue experiments by expressing the human ortholog in transgenic mice. 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.

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: THALES.
Investing in knowledge society through the European Social Fund.

EFFECT OF LITHIUM AND EBSELEN, A NOVEL INOSITOL MONOPHOSPHATASE INHIBITOR, ON MOLECULAR MARKERS OF NEURONAL PLASTICITY IN THE MOUSE

I Antoniadou[1], Esha Johal[1], Marianna Kouskou[1], Trevor Sharp[1]

[1]Department of Pharmacology, University of Oxford

The mood stabilizer and antidepressant agent lithium inhibits inositol monophosphatase (IMPase) to attenuate phosphoinositide signalling (Berridge et al., 1989). When administered repeatedly lithium also activates genes linked to increased neuronal plasticity (Jacobsen & Mørk, 2004), an effect that is common to antidepressants. In a recent 'reprofiling' study, we identified ebselen as a potent IMPase inhibitor (Singh et al., 2013). Here we examined the effect of repeated treatment with lithium and ebselen on the expression of a panel of neuronal plasticity genes. Adult male C57BL/6 mice (8 per group) were injected (i.p.) twice daily for 2 weeks with vehicle, ebselen (5 mg/kg) or lithium (first dose 10 mmol/kg then 3 mmol/kg). Brains were removed 16 h after the last injection, snap frozen and stored (-80^{o}C). Coronal sections (12 µm) were cryostat-cut, and processed for *in situ* hybridization using ^{35}S-dATP labelled oligonucleotides complimentary to BDNF, Arc, VGluT1 and Shank1B mRNA. Autoradiograms were quantified for mRNA using MCID. Data were analysed statistically using Student's unpaired t-test. Compared to vehicle-injected controls, repeated administration of lithium caused a statistically significant increase in mRNA abundance of each of BDNF, Arc, VGluT1 and Shank1B across a variety of cortical and subcortical regions. Interestingly, repeated administration of ebselen also increased mRNA expression of BDNF, Arc, VGluT1 and Shank1B, although ebselen did not always increase mRNA in the same regions as lithium. For example, whereas lithium increased Arc mRNA in hippocampus alone, ebselen had this effect in hippocampus and other cortical regions. In summary, administration of the novel IMPase inhibitor ebselen increased expression of a panel of neuronal plasticity genes in cortical and hippocampal regions in a manner similar (but not identical) to lithium. These results are further evidence that ebselen has lithium-like neuropharmacological effects, and support the testing of this drug in relevant psychiatric patient populations.

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ADRENALECTOMY AND CORTICOSTERONE REPLACEMENT: SEX DIFFERENCES IN ANTIDEPRESSANT RESPONSE

N. Kokras, I. Sotiropoulos, C. Dalla

Department of Pharmacology, Medical School, University of Athens, 75 Mikras Asias st, 11527 Athens, Greece, nkokras@med.uoa.gr

Several studies identified a sex-specific stress response and exposed significant sex differences in animal models of depression. The hypothalamic-pituitary-adrenal (HPA) axis plays a key role in this stress response and the evidence supports a sex-specific HPA-driven release of glucocorticoids. We recently showed that in models of anxiety and depression, the male behavioral response is affected from the disruption of the HPA axis, whereas females are not affected. We also investigated the effect of repeated citalopram and found that key components of the normal HPA axis drive present sex-differences at baseline and in response to treatment. Thus, in the present study we investigate whether the sex-differentiated response to treatment is affected by the elimination of sex differences in circulating corticosterone. Forty male and female Wistar rats were subjected to a bilateral adrenalectomy or sham-operation. Immediately post-surgery all rats had access to an aqueous drinking solution supplemented with 0.9% NaCl and 0.2% ethanol. Adrenalectomized rats were also receiving 25 ug/ml corticosterone. Such oral replacement produces basal levels of corticosterone and mimics its circadian secretion. Rats were allowed to recover and adjust to corticosterone replacement, then they were randomly assigned to i.p. citalopram 10mg/kg/day or vehicle for 4 weeks. Thereafter, all rats were subjected to the Forced Swim Test during which the duration of floating, swimming and climbing was recorded. Upon completion of the FST, rodents were sacrificed and tissues were sampled for analysis of biogenic monoamines. The statistical analysis revealed significant interactions regarding behavioural indices and monoaminergic neurotransmission assays following citalopram treatment on rats with normal and altered HPA axis. In agreement with previous studies, we found that females appear more vulnerable to the FST. Also, present results further extend our recent findings, suggesting that fluctuations in corticosterone levels associate better with the male behavioral response. Interestingly, in male and female rats with stabilized corticosterone levels, citalopram treatment resulted in an intriguing sex-differentiated response. Therefore, in order to better understand the sex-differentiated response to serotonergic antidepressants, the emerging interaction of sex with peripheral corticosterone and HPA activity in general, warrants further attention.

BRAIN MITOCHONDRIA AS PHARMACOLOGICAL TARGETS FOR ANXIETY DISORDERS

Michaela D. Filiou[1], Markus Nussbaumer[1], Larysa Teplytska[1], Mike P. Murphy[2], Rainer Landgraf[1], Chris W. Turck[1]

[1]Max Planck Institute of Psychiatry, Munich, Germany
[2]MRC, Mitochondrial Biology Unit, Cambridge, United Kingdom

Anxiety disorders are the most common psychiatric conditions affecting up to 20% of the general population. To discover candidate molecular biomarkers for anxiety disorders we compared behavioral extremes of high and low anxiety-related behavior using a mouse model of trait anxiety. By applying a hypothesis-free proteomics, metabolomics and bioinformatics platform we found that mitochondria are the common denominator of the observed molecular changes between high and low anxiety-related behavior. Mitochondria alterations in high anxiety-related behavior included oxidative phosphorylation, mitochondria transport and oxidative stress pathways. To investigate whether selective mitochondrial targeting affects the behavioral phenotype we pharmacologically manipulated those alterations in high anxiety-related behavior mice. Our data show that pharmacological targeting of mitochondrial pathways exerts an anxiolytic effect in these mice. This is the first time that a mechanistic-based rather than a symptom-based approach is used to manipulate anxiety phenotypes and emphasizes the therapeutic potential of mitochondrial targeting for brain disorders.

IDAS AND LYNKEAS, TWO NOVEL PROTEINS RELATED TO GEMININ, CONTROL EPENDYMAL CELLS GENERATION.

Christina. Kyrousi[1], Gregor. Pilz[2], Marialena. Lalioti[1], Pinelopi. Nikolopoulou[1], Magdalena. Götz[2], Zoi. Lygerou[3], Stavros. Taraviras[1]

1. Department of Physiology, Medical School, University of Patras, Greece
2. Institute of Stem Cell Research, Helmholtz Centre Munich, Germany
3. Department of General Biology, Medical School, University of Patras, Greece

During adulthood neural stem cells (aNSCs) are restricted to the subventricular zone (SVZ) and the subgranular zone in the hyppocampal dentate gyrus and organised in specific structures called niche. The SVZ niche is comprised of multiciliated ependymal cells lying on the ventricular surface, responsible for receiving secreted molecules by which they control aNSC function. Ependymal cells are generated from a subpopulation of radial glial cells (RGs) at late embryonic and early postnatal stages while the rest will generate aNSCs. We have recently identified two novel proteins, Idas and Lynkeas that share significant homology with Geminin. Our data show that Idas can heterodimerise with Geminin, while Geminin-Idas complex shows reduced affinity for Cdt1. It has been also shown that in Xenopus skin and kidney, Idas activates the expression of genes required for multiciliated cell formation, including genes mediating centriole assembly and Foxj1. Here we show that Idas has a restricted expression pattern in a subpopulation of cells lying in the periventricular cell layer where Idas+ cells are colocalized with Foxj1+ cells suggesting that Idas is expressed in the RGs that will generate ependymal cells. Overexpression of Idas and Lynkeas in RGs of the developing cortex RGs using in utero electroporation promoted exit from the cell cycle and premature differentiation, while it activates Foxj1 expression and ependymal cell markers. Our work proposes Idas and Lynkeas as key regulators for RGs cell fate acquisition and ependymal cells differentiation.

EEG-fMRI OF THE CYCLIC ALTERNATING PATTERN IN HUMAN NREM SLEEP

Vasileios Kokkinos[1,2], Andreas M. Koupparis[2], George K. Kostopoulos[2], Kyriakos Garganis[1]

[1]Epilepsy Center of Thessaloniki, St. Luke's Hospital, Panorama, Thessaloniki, Greece.
[2]Neurophysiology Unit, Department of Physiology, Medical School, University of Patras, Greece.

The EEG of NREM sleep in humans is characterized by pseudo-periodic phasic events referred to as the cyclic alternating pattern (CAP), formed by two phases: phase-A of EEG synchronization and phase-B of EEG de-synchronization. The A-phases of the CAP have been associated with increased interictal activity in idiopathic generalized epilepsy (IGE) syndromes. By means of EEG-fMRI, this study aimed in determining the brain regions responsible for the accumulation of brain rhythms and waves occurring during every CAP-A phase in NREM sleep. Nine patients were selected, who managed to achieve NREM sleep of considerable duration in the MR scanner in the context of their assessment for epilepsy. A GLM with CAP-A blocks (664 in total) was formed and analyzed by means of SPM for every patient. The fMRI analysis brought out a basic network involving the insula (9/9), the thalamus (7/9) and the cingulate gyrus (8/9), that exhibits maximal BOLD responses during the CAP-A phases, regularly accompanied by cerebellar-pontine signal changes. Secondarily, occipital (8/9) and central-precentral (7/9), as well as diffuse frontal (7/9), parietal (6/9), and temporal (6/9) areas, were found to have BOLD changes correlated to the A phases of the CAPs. Our results support the existence of an insular-cingulate-thalamic network that temporally aggregates rhythms and waves produced over the hemispheres during NREM.

EBBS SPONSORED LECTURE

IDEAS ON LOWERING THE IMPACT OF NEUROPSYCHIATRIC DISORDERS BY EXPLOITING THE BRAIN'S PROGRAMMABILITY AND ADAPTIVE CAPACITY

Osborne Almeida,

Max Planck Institute of Psychiatry, Munich, Germany

The brain is probably the most plastic and adaptive of all organs. Yet, the brain succumbs to serious diseases, a problem amplified during ageing. We are beginning to recognize that the triggers for many of these disorders occur early during life and accumulate over time. Genetics contributes significantly to brain development and ageing, but so do lifetime stressful events. In humans, there is growing awareness that other factors such as socio-economic and educational status are important in determining mental lifespan. While basic research in animals and cells will continue to advance our understanding of the mechanisms of disease and also the development of treatment strategies, translation of those findings for human health must recognize the potentially important modulatory role of factors that cannot be studied in surrogate models of disease.

NICOTINE DEPENDENCE, $GABA_B$ RECEPTORS, REWARD AND IMPULSIVITY: TAKING A STEP BACK INTO THE FUTURE

Styliani Vlachou

Dublin City University, Faculty of Science and Health, School of Nursing and Human Sciences, Glasnevin, Dublin 9, Ireland

Tobacco smoking is partly attributed to the addictive properties of nicotine and constitutes a worldwide drug abuse problem with serious health effects. γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain and is implicated in the modulation of brain reward processes. Acute or chronic administration of γ-aminobutyric acid B ($GABA_B$) receptor positive allosteric modulators (PAMs) decreases self-administration of various drugs of abuse and inhibits cue-induced reinstatement of drug-seeking behavior. Impulsivity, a tendency to pursue rewarding stimuli without consideration for potential harmful/negative consequences, is strongly associated with habitual tobacco smoking. High impulsivity levels may be a risk factor for nicotine dependence, leading to its initiation and maintenance. In different experiments, BHF177, a $GABA_B$ receptor PAM, decreased both the reinforcing and motivational effects of nicotine without affecting motivation for natural reinforcers, such as food, using the nicotine self-administration fixed-ratio 5 and progressive ratio procedures, respectively, both in rats from the general population as well as in high and low impulsive rats in a similar manner. Interestingly, BHF177 had a larger magnitude of effect in low impulsive rats at the highest dose tested. High/low impulsivity animals were selected based on the poor inhibitory control aspect of impulsivity, as assessed in the 5-choice serial reaction time task (5-CSRTT). Further, BHF177 dose-dependently and selectively blocked cue-induced reinstatement of nicotine seeking, a putative animal model of relapse in humans, and not food seeking, while chronic treatment with BHF177 decreased nicotine self-administration with only small tolerance developed in the general population. Thus, BHF177, or other similar $GABA_B$ receptor PAMs, could be useful therapeutics for the treatment of different aspects of nicotine dependence, by assisting both in smoking cessation by decreasing the reinforcing effects of nicotine, as well as in preventing relapse to smoking in the general population and/or in both high and low impulsive individuals.

Work on the effects of $GABA_B$ receptor compounds on nicotine dependence, conducted as part of my post-doctoral training under the supervision of Prof. Athina Markou at the University of California at San Diego, put the basis of my current research focus in Ireland in the field of Behavioural Neuroscience.

YOUNG INVESTIGATORS LECTURES:

NEW ROLES FOR MELANOMA ANTIGEN PROTEINS IN REGULATING NEURONAL FUNCTION AND DYSFUNCTION

Vassiliki Nikoletopoulou, Nikos Charmpilas and Nektarios Tavernarakis

The Melanoma Antigen (MAGE) family of proteins is receiving increasing attention because of the involvement of its members in human pathologies, including in particular cancer and neurogenetic disorders. Two genes, Necdin and MAGE-2 are located within the locus on Chromosome 15 (q11-13) in humans that is invariably deleted in patients of the neurodevelopmental disorder Prader-Willi Syndrome (PWS). PWS has a neurodevelopmental and a metabolic component, manifested by impaired cognitive function, hyperphagia, high body fat mass and obesity that is often morbid and accounts for the reduced lifespan of these individuals. While it is clear that MAGE proteins play important roles in maintaining neuronal function and neuroendocrine outputs, our understanding of their functional repertoire in the cell remains poor and fragmented, mainly due to the large number of related genes with possible redundant functions in mammals. We have cloned the *C. elegans mage* gene homologue, encoded by the F40E3.2 locus on Chromosome I of the nematode, and named it *mage-1*. MAGE-1 is localized to mitochondria and it regulates lifespan and fat accumulation likely through direct interactions with components of the respiratory chain. Notably, key findings in the nematode, such as the mitochondrial localization of MAGE-1, were also verified for the mammalian homologue Necdin, indicating that *C. elegans* is an insightful model that can help us understand basic functions of the mammalian MAGE proteins that remain un-described and therefore benefit our approach to the associated pathologies.

DENTATE GYRUS: A TESTBED CIRCUITRY FOR SPARSE APPROXIMATION TASKS

Panagiotis C. Petrantonakis and Panayiota Poirazi
Foundation for Research and Technology-Hellas (FORTH), Institute of Molecular Biology and Biotechnology, N. Plastira 100 GR-70013, Heraklion, Greece

Sparse representations are the common way of exhibiting memory oriented activity in Dentate Gyrus (DG). It has been shown that sparse neuronal populations of granule cells, the main encoding cells in DG, are concisely activated, not exceeding 2-4% of the total population (Schmidt et al., 2012). It is assumed that sparsity enhances the ability of DG to perform pattern separation; one of the most valuable contributions of DG during memory formation. Pattern separation guarantees that two separate inputs from the Entorhinal Cortex, even if they slightly differ from each other, are coded by two separate activation patterns in the downstream hippocampal area, CA3 (Bakker et al., 2008). In this work we investigate the possibility of using DG circuitry to implement Sparse Approximation (SA), a widely used strategy in Signal Processing field (Blumensath and Davies, 2008). Sparse approximation stands for the algorithmic identification of few components from a basis set (e.g., a wavelet basis), that approximate a certain signal. The ability of DG to achieve pattern separation by sparsifing its representations is exploited here to augment the robustnesss of such a task. Specifically, we investigate the possibility of improving already used SA algorithms (Maleki and Donoho, 2010) by adding new algorithmic features inspired by the DG circuitry. Lateral inhibition of granule cells, either direct or indirect, e.g., via mossy cells, seems to enhance the performance of certain SA algorithms, i.e., Iterative Soft Thresholding algorithms (Maleki and Donoho, 2010), paving the way for further dissecting the relation between DG's circuitry and functionality.

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AGE-DEPENDENT CHANGES IN PREFRONTAL CORTICAL FUNCTION: THE ROLE OF INHIBITION

Kyriaki Sidiropoulou

Department of Biology, University of Crete, Heraklion, Greece

The prefrontal cortex (PFC) is involved in both cognitive functions and emotional processes. Postnatal maturation of PFC circuits is suggested to be delayed compared to other cortical areas. In addition, several neuropsychiatric diseases in which PFC dysfunction plays a critical role are neurodevelopmental and emerge during childhood and adolescence. Although age seems to play a significant role in both PFC normal function and emergence of disease states, very little is known about age-dependent changes of behaviors involving the PFC and the underlying cellular mechanisms.

I will present you data showing that both cognitive functions and emotional processes change during postnatal development. I will focus on two age groups of mice that have been tested: juveniles (postnatal day (PD) 25-35) and adults (PD> 60). Cognitive function was tested using three different types of object recognition tests and the delayed alternation task in the T-maze, while anxiety was assessed with the open-field test and the elevated plus maze.

Evoked field potential recordings revealed an enhanced basal synaptic transmission in juveniles compared to adults within layer II PFC. Furthermore, adults exhibit long-term potentiation (LTP) in response to tetanic stimulation, while juveniles significantly decreased LTP. Decreased number of spines and possibly decreased NMDA receptors might underlie the LTP defect in juveniles.

Due to the role of inhibition in mental disorders, similar experiments were to mice that exhibit decreased number of interneurons by PD15 (Vidaki et al, 2012, Cereb Cortex;22(3):680-92). Both behavior and physiology were altered in these mice, whereby the phenotype of adult transgenic mice resembled that of juvenile controls. Similar results were obtained when mice received picrotoxin injections during the juvenile period.

In summary, we find that an increase in synaptic plasticity in PFC could underlie the observed changes in behavior with age with GABAergic inhibition playing a critical role in shaping the adult ‘control’ phenotype.

SYMPOSIUM III: INFLAMMATION ANDDEGENERATION IN THENERVOUS SYSTEM

GLIAL CELLS IN INFLAMMATORY DISORDERS

Trevor Owens.

[1]Neurobiology, IMM, University of Southern Denmark, Odense, Denmark

Microglia and astrocytes play a major role as contributors to or regulators of the inflammatory milieu in neuroinflammatory disease such as multiple sclerosis (MS). In neuromyelitis optica (NMO) astrocytes are a primary autoimmune target. Microglia are phagocytic, and can present antigen to $CD4^+$ T cells, which places them at the center of many schema for central nervous system inflammation. Microglia recognize pathogen and danger signals via innate receptors and they are a source of pro-inflammatory cytokines such as IL1□ □□□ □□□- □□□ □□□□-i□□□□□□□□□L□□ □□□ □L□□, and chemokines. They are also a source of counter-inflammatory or neuroprotective mediators such as Type I interferons (IFN) and Insulin-like Growth Factor-1, and there may be functional microglial heterogeneity. Astrocytes are also a source of many cytokines, including Type I IFN, as well as chemokines, and factors that are implicated in regeneration and repair. Their response to innate signals is in some cases microglia-dependent. Like microglia, they appear functionally dichotomous, so that transgenic depletion or disabling approaches have shown both protective and pro-inflammatory roles, depending on the experimental system. Astrocytes play a specific role at the blood-brain barrier glia limitans, which is compromised in MS and NMO. It is not clear whether the opposing effects of IFN□ on pathology in NMO versus MS reflects specific aspects of astrocyte biology, or antibody/complement versus T-cell inflammatory mechanisms.

INTERACTIONS BETWEEN NEUROINFLAMMATION, DEMYELINATION AND AXON DAMAGE IN A MOUSE MODEL FOR CHRONIC MULTIPLE SCLEROSIS

Maria Karamita, Lesley Probert

Laboratory of Molecular Genetics, Hellenic Pasteur Institute, Athens, Greece

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) that shows autoimmune features. It usually starts as a relapsing-remitting disease. In most patients the disease evolves into a chronic progressive phase (pattern III) characterized by the continuous accumulation of neurological deficits. While treatment of relapsing-remitting MS has improved dramatically over the last decade, the therapeutic options for chronic progressive MS, either primary or secondary, are still limited and animal models have to be further studied to identify inflammatory and non-inflammatory mechanisms of neurodegeneration and demyelination. One commonly used animal model for pattern III lesions of MS is toxin-induced demyelination induced by the copper chelator cuprizone (CPZ). Treatment with CPZ, administered as a food additive, results in decreased energy production by mitochondria and an increase in the production of oxidants by cells. In C57BL/6 mice, disease is manifested in the brain by neuropathological changes including generalized neuroinflammation and focal oligodendrocyte apoptosis and demyelination mainly in the corpus callosum, which develop behind an intact blood brain barrier. Withdrawal of the toxin allows complete remyelination within a short period of time whereas continued administration of the toxin results in massive, irreversible axon damage [1]. In this model a rapid proliferation and recruitment of microglia/macrophages and astrocytes as well as oligodendrocyte precursor cells (OPCs) precedes the demyelination process [2, 3]. Major mediators of pathology in this model are the pro-inflammatory cytokine TNF, that is produced by activated microglia and astrocytes [4, 5] and neuronal nitric oxide [6]. More recently, CNS-infiltrating CXCR2+ neutophils [7] and $CD3^+$ Th17-cells [8] have also been shown to contribute to disease progression. TNF further contributes to CNS repair by promoting the proliferation of OPC and remyelination through TNFR2 [5]. However, understanding of the precise cellular and molecular mechanisms involved in the pathology of CPZ-induced CNS demyelination is still in its infancy. Recently, the presence of IKKβ, which is essential for the activity of NF-κB, in nestin-positive cells including astrocytes was shown to promote glial cell activation and demyelination [9]. In our laboratory we are using cell-specific IKKβ knockout mice, or TNFR1 and TNFR2 knockout mice in combination with selective TNF inhibitors, to gain new insights into the mechanisms of neuroinflammation, demyelination, axon damage and remyelination in the CPZ-model with the aim of identifying new therapeutic targets for treatment in chronic MS.

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TARGETING INFLAMMATORY PATHWAYS TO PROTECT DOPAMINERGIC NEURONS: RELEVANCE TO PARKINSON'S DISEASE

X. Chen[1],T. N. Martinez[2], S. Bandyopadhyay[3], A. H. Merrill[3], M. G. Tansey[1,2],

[1]Department of Physiology, Emory University School of Medicine, Atlanta, GA 30322; [2]Department of Physiology, The University of Texas Southwestern Medical Center, Dallas, TX 75390; [3]Sch. of Biol. and the Petit Inst. for Bioengineering and Bioscience., Georgia Inst. of Technol., Atlanta, GA 30332 USA

The role of inflammation and immunological processes in the pathogenesis of neurodegenerative diseases is an area of intense investigation. A wealth of new data suggest that neuroinflammatory mechanisms contribute to the pathophysiology of Parkinson's disease (PD). Our group has previously demonstrated that neutralization of soluble tumor necrosis factor (TNF) by dominant-negative TNF (DN-TNF) inhibitors significantly attenuated the loss of DA neurons both *in vitro* and *in vivo* in rat models of nigral cell death and studies are ongoing to extend and confirm these findings in non-human primate models of PD. However, the identity of the signaling pathways downstream of soluble TNF required to mediate neurotoxicity and degeneration remain to be fully elucidated. Using a mouse DA neuron-like cell line (MN9D) and rat primary neuron-glia cultures from ventral mesencephalon (VM), we have identified cellular and signaling mechanisms by which TNF induces DA neuron death. Ceramide is a sphingosine-based lipid signaling molecule downstream of TNF that is involved in the regulation of cellular differentiation, proliferation and apoptosis. Using lipidomics and high-content imaging approaches, we have discovered that soluble TNF-dependent generation of ceramide species and two novel atypical sphingoid bases exert toxic effects on primary DA neurons by triggering ER stress and disrupting mitochondrial respiration leading to their degeneration. Pharmacological inhibitors of sphingomyelinase (SMase), an enzyme that hydrolyzes active ceramide from inactive sphingomyelin pools attenuated solTNF-dependent toxicity in MN9D cells and primary DA neurons from VM. Ongoing studies to understand the links between ceramide metabolism and TNF-dependent death of DA neurons may reveal novel targets for drug discovery and may provide an opportunity to evaluate neuroprotective effects of compounds that prevent formation of toxic ceramide species or aid in their metabolism with the long-term goal of identifying new therapeutic strategies for PD.

TUMOR NECROSIS FACTOR-α (TNF-α) AS A COMMON MEDIATOR IN ALZHEIMER'S DISEASE AND RHEUMATOID ARTHRITIS PATHOGENESIS IN TRANSGENIC MICE.

Evi Paouri, Spiros Georgopoulos

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

Tumor necrosis factor-alpha (TNF-α) is a potent pro-inflammatory cytokine, involved in many infectious, autoimmune and neurodegenerative diseases, including rheumatoid arthritis and Alzheimer's disease [1, 2]. In the central nervous system (CNS), TNF-α can be synthesized by microglia and astrocytes [1]. TNF-α is up-regulated in both CSF (cerebrospinal fluid) and serum of AD patients, and is colocalized with amyloid plaques, as shown in post-mortem analysis of AD brains [1, 2]. TNF-α also mediates the induction and maintenance of chronic inflammation in rheumatoid arthritis (RA), and it has been reported that RA patients are protected from AD [3]. In the present study, we evaluated the effect of TNF-α deletion or over-expression in the amyloid pathology in an AD transgenic mouse model (5xFAD) by mating 5xFAD mice with TNF-α knock-out mice or with a human TNF-α transgenic mouse (Tg197) that develops rheumatoid arthritis, respectively. TNF-α deletion in the 5xFAD mouse model of AD resulted in decreased amyloid deposition and decreased astrocytic and microglial response, thus confirming previous findings where inhibition of TNF-α by an anti-TNF-α neutralizing antibody had a protective effect in AD transgenic mice [4]. 5xFAD/Tg197 arthritic AD mice displayed decreased amyloid deposition and increased microglial and astrocytic response, suggesting that TNF over-expression in the AD arthritic mice confers a protective effect in the amyloid phenotype. These results support the use of anti-TNFα therapy for human treatment of AD, as well as the observation that rheumatoid arthritis patients are protected from AD.

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FUNCTIONALIZED GOLD NANOPARTICLES FOR TREATMENT OF CENTRAL NERVOUS SYSTEM INJURIES

Florentia Papastefanaki[1], Nafsika Poulia[1], Igor Jakovcevski[2], Florian Schulz[3], Gaby Loers[2], Tobias Vossmeyer[3], Neus G. Bastús[3], Horst Weller[3], Melitta Schachner[2], Rebecca Matsas[1]

1. Laboratory of Cellular and Molecular Neurobiology, Hellenic Pasteur Institute, 127 Vas. Sofias Avenue, 11521 Athens, Greece
2. Zentrum für Molekulare Neurobiologie, Universitätsklinikum Hamburg-Eppendorf, Universität Hamburg, Martinistrasse 52, 20246 Hamburg, Germany
3. Institut für Physikalische Chemie, Universität Hamburg, Grindelallee 117, 20146 Hamburg, Germany

The inability of the mammalian central nervous system (CNS) to regenerate after injury causes functional impairment. During the acute phase after spinal cord injury (SCI), disruption of cell membranes leads to excitotoxicity, oxidative stress, Ca^{2+} influx, inflammation and cell death, resulting to the detrimental secondary neurodegenerative events of the chronic phase. Nanotechnology offers the possibility of a superior drug delivery platform that may be adapted to the clinic. In particular, colloidal gold nanoparticles (AuNPs) appear as leading candidates in the field of nanomedicine due to their inert and non-immunogenic properties, good biocompatibility, ease of preparation and modification towards several functionalities. Previous studies have shown that poly-ethylen-glycol (PEG) administration in CNS injury models can restore action potentials and induce functional recovery due to its membrane sealing potential. Yet there are serious limitations, arising mostly from PEG toxicity and limited bioavailability when administered systemically. To overcome this drawback we developed PEG (MW 2000)-functionalized 40-nm-diameter AuNPs for in vivo use as a key treatment to seal disrupted cell membranes at early stages after SCI. Our results show that PEG-functionalized AuNPs delivered in the lesioned spinal cord caused no adverse effects, such as body-weight loss or animal death when compared to control vehicle-injected mice. Behavioral analysis of locomotor function revealed significantly greater recovery in the PEG-AuNP treated group as compared to controls, up to 8 weeks post injury. Additionally, immunohistological analysis indicated attenuated inflammatory response and enhanced motoneuron survival. Our data show prospects of an efficient drug carrier platform applicable to the clinic.

TRANSGENIC RESCUE OF OLIGODENDROCYTE GAP JUNCTIONS IN A MOUSE MODEL OF HYPOMYELINATING LEUKODYSTROPHY

Natasa Schiza[1, 2], Irene Sargiannidou[1], Alexia Kagiava[1], Christos Karaiskos[1], George Lapathitis[1], Kleopas A. Kleopa[1, 3]

[1]The Cyprus Institute of Neurology and Genetics, Neuroscience Lab, [2] University of Cyprus, Nicosia, Cyprus [3]Cyprus School of Molecular Medicine

Glial cells are coupled by gap junctions (GJs) that mediate intra- and intercellular communication and are vital for the integrity of myelinating cells. Recessive *GJC2*/Cx47 mutations cause Pelizeaus-Merzbacher-like disease (PMLD), a hypomyelinating leukodystrophy. *GJB1*/Cx32 mutations, which cause peripheral neuropathy known as X-linked Charcot-Marie-Tooth Disease-1 (CMT1X), may also result in chronic or acute-transient encephalopathy syndromes, often induced by conditions of metabolic stress. The respective mouse models support the crucial role of GJs for CNS myelination: both Cx32 and Cx47 knockout (KO) mice develop mild CNS myelin defects, whereas Cx32/Cx47 double KO mice suffer severe CNS demyelination and oligodendrocyte apoptosis. There is currently no effective treatment for patients with leukodystrophy, such as PMLD, or for patients with CMT1X and other types of CMT.

In this project, we introduced the wild type human *GJB1* gene into the CNS of *Gjb1*-knockout mice (KO), to examine whether human Cx32 (hCx32) derived from the transgene could replace the function of the mouse Cx32 and prevent the manifestations of CNS demyelination in Cx32KO and Cx32/Cx47 double KO mice. Expression of hCx32 is driven by the myelin-specific mouse proteolipid protein (PLP) promoter. By crossing these mice with Cx32KO mice, we obtained transgenic mice expressing the exogenous wild type hCx32 on Cx32KO background. Immunohistochemical and immunoblot analysis confirmed strong expression of hCx32 specifically in oligodendrocytes and Schwann cells and correct localization forming GJs at cell bodies and along the myelin sheath. Further crossing of these mice with Cx47KO mice will offer the opportunity to confirm whether transgenic expression of Cx32 can rescue the severe early phenotype of CNS demyelination in Cx32/Cx47 double KO mice.

This study will provide proof of principle that gene replacement therapy targeting myelinating cells is feasible for CMT1X and PMLD patients.

PLENARY LECTURE III:

MICROGLIAL CLEARANCE FUNCTION IN NEURODEGENERATIVE DISEASES

Linnartz-Gerlach B., Bodea L.G, Claude J., Neumann H.

Institute of Reconstructive Neurobiology, University Hospital of Bonn, University of Bonn, Germany.

Microglia express innate immune receptors for the recognition of intact or lesioned cells of the central nervous system.
Lesioned and/or complement opsonized cells are recognized by the triggering receptor expressed on myeloid cells-2 (TREM2) and the complement receptor-3. Both receptors signal via the transmembrane adaptor protein DAP12/TYROBP containing an immunoreceptor tyrosine-based activation motif (ITAM), thus, leading to microglial migration, phagocytosis and an oxidative burst. Loss-of-function mutations of the adaptor molecule DAP12/TYROBP are known to cause the human Nasu-Hakola disease, which is characterized by inflammatory neurodegeneration. Recently, variants of TREM2 were also causally-linked to Alzheimer's disease and frontotemporal dementia.
Those ITAM-signaling receptors are counter-regulated by immunoreceptor tyrosine-based inhibition motif (ITIM)-signaling receptors such as sialic acid-binding immunoglobulin superfamily lectins (Siglecs). Siglecs like Siglec-E of mouse microglia and Siglec-11 of human microglia recognize the sialic acid cap of healthy neurons leading to an ITIM-signaling in microglia that turns down the microglial proinflammatory responses, phagocytosis and the associated oxidative burst.
Thus, immune function and radical production of microglia are fine tuned by this recognition system of paired ITAM- and ITIM-signaling receptors that sense the neuronal glycocalyx and modulate microglial neurotoxic function.

POSTER PRESENTATIONS

BEHAVIOR AND COGNITION

CELLULAR ACTIVATION UNDERLYING THE AGE-DEPENDENT CHANGES IN ANXIETY LEVELS BETWEEN JUVENILE AND ADULT MICE

Kleanthi Chalkiadaki, Mary Vlassopoulou, Xanthippi Konstantoudaki[1,2] and Kyriaki Sidiropoulou[1,2]

[1]Department of Biology, University of Crete, Heraklion, Greece
[2]Institute of Molecular Biology and Biotechnology/Foundation for Research and Technology- Hellas, Heraklion, Greece

Anxiety is an emotional state and involves the activation of several brain regions. Amygdala activation has a central role in fear expression and can give rise to anxiety. The prefrontal cortex (PFC) is critical for emotional regulation and has dual control of the amygdala, either exciting or inhibiting different amygdala. Pathological anxiety is characterized by hyperactivity of the amygdala and hypoactivity of the prefrontal cortex. Furthermore, recent studies have recognized the central role of hippocampus in modulating anxiety. Recent data from our lab have revealed an age-dependent component in the anxiety levels of mice tested in the open-field and the elevated plus maze. In this study, we focus on the expression of an immediate early gene product, the c-Fos protein, following exposure or not to the open-field. Brain areas examined included PFC, amygdala and hippocampus, in two different age-groups of mice: juveniles (postnatal day (PD) 25-35) and adults (PD> 60). We find that c-fos activation was significantly elevated in amygdala of both juvenile and adult mice. C-fos activation in hippocampus was not elevated in both juveniles and adults compared to their respective non-exposed controls. In PFC, several differences were found between juvenile and adult mice. First, basal levels of c-fos staining were decreased in juvenile mice compared to adult mice. Second, there was a tendency towards decreased c-fos activation in juvenile compared to adult mice. In summary, we find that both basal and open-field induced activation of the PFC is differentially regulated in juvenile compared to adult mice. Current experiments are performed to determine whether neurotransmitter receptors related to cellular activation and anxiety are differentially expressed in the PFC.

EARLY LIFE STRESS HISTORY MODULATES ACUTE STRESS RESPONSES IN ADRENOCEPTORS AND PROLIFERATION PATTERN OF JUVENILE SEA BASS BRAIN.

[1*]Stefanos Fokos, [1*]Themistoklis Tsarouchas, [1]Thomas Yiotis, [3]Nikos Papandroulakis, [2]Michalis Pavlidis, [1]Catherine R. Dermon

[1]Laboratory of Human and Animal Physiology, Department of Biology, University of Patras, Greece
[2]Department of Biology, University of Crete, Heraklion, Crete, Greece
[3] Hellenic Centre for Marine Research, Institute of Aquaculture, Heraklion, Crete, Greece
*indicates equal contributions

Early stressful experiences can be critical, not only for the development of an organism, but also for the function of the psychobiological systems during juvenile and adult life. The present study aimed to determine the effects of early stress experience in brain development and to acute stress responses. For this, the number of newborn cells and adrenoceptors' expression (α_{2A}–ARs, β_2–ARs) were examined in the neurogenic zones of juvenile *D. Labrax (sea bass*) brain with or without early life mild stress experience, by means of bromo-deoxy-uridine (BrdU) methodology and quantitative immunofluorescence, respectively. Specifically, different mild stressors were applied during larvae flexion and formation of all fins developmental stages, including random application of different mild stressors (daily / for 2 weeks), such as, water current increases, different lighting spectrum, chasing with a net for 20 sec, flash lights, confinement (collection in beakers), and air exposure for 5 sec. BrdU immunohistochemistry and double immunofluorescence showed extensive active proliferation in most periventricular areas throughout juvenile sea-bass brain that in most cases coincided with α_{2A}–AR or β_2–AR expression. Quantification of the newborn cells and fluorescence density showed that acute stress exposure (confinement) resulted significant decreases in cell proliferation in telencephalic and cerebellum proliferation zones of non-previously stressed fish. Statistical two-ways ANOVA revealed that although chronic mild stress during the flexion and all fins stages did not change the juvenile proliferation pattern, it significantly reduced the acute stress-induced changes in cell proliferation in ventral and dorsal telencephalon and valvula cerebellum of juvenile animals. Our results further support the hypothesis that chronic mild stress experience during early life can modulate acute stress responses later in life, in juveniles, suggesting that adrenoceptors' expression and cell proliferation are involved in allostatic brain plasticity mechanisms related to effective coping behaviors.
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MAPPING THE LEARNING DEFICIENCY ASSOCIATED WITH NEUROFIBROMATOSIS TYPE-1 IN THE *DROSOPHILA MELANOGASTER* MODEL

Eirini-Maria Georganta, Anastasios Moressis, Jean Y. Gouzi, Efthimios M.C. Skoulakis

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

Neurofibromatosis 1 (NF1) is an autosomal dominant hereditary multisymptomatic disorder occurring in 1 out of 3500 individuals. The NF1 clinical picture includes tumors of the nervous system, short stature and learning disabilities, among others. NF1 results from loss-of-function mutations in the neurofibromin protein (Nf1), which is a negative regulator of Ras. Loss of the highly conserved Drosophila *dNf1* ortholog results in organismal size reduction and deficits in associative learning and memory, thus resembling human NF1 symptoms. Recent work from our lab has reported the functional interaction between the Receptor Tyrosine Kinase, Anaplastic Lymphoma Kinase (Alk) and dNf1. Abrogation of dAlk activity restored the normal size of *dNf1* null mutant homozygotes and ameliorated their learning deficits. Since Alk signals through Ras to activate MAPK, these results implicate the Ras/ERK pathway in both *dNf1* loss phenotypes. More recently, we focused our studies on the phenotypical characterization of a novel point mutation located within the *dNf1* gene. To our surprise, preliminary results suggested that this mutation presents a more severe learning phenotype than the null, but its effects on MAPK are marginal suggesting that dNf1 may engage additional signaling pathways than Ras/ERK to control learning. Therefore, we are currently aiming to: a) define the specific neurons within the brain where Nf1 is required for normal learning and b) elucidate the potential functional differences between the point and null mutations of *dNf1*. Since the cause of the learning disabilities and cognitive defects in NF1 patients remains poorly understood, we expect that our studies will provide essential information on the molecular mechanisms and pathways, as well as the neuronal circuits affected by *NF1* loss and mutations. Better understanding of Nf1 itself at the molecular level and the processes it regulates could lead to the development of specifically targeted therapeutic approaches for the amelioration of cognitive impairments associated with NF1.

EFFECTS OF AN EARLY EXPERIENCE INVOLVING REWARD THROUGH MATERNAL CONTACT OR ITS DENIAL ON THE NORADRENERGIG RECEPTOR OF THE ADULT RAT BRAIN

Theodora Kalpachidou[1], Antonios Stamatakis[1], Pelagia Melissa[1], Fotini Stylianopoulou[1]

[1] University of Athens, Faculty of Nursing, Basic Sciences, 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, we investigated the effects of a neonatal experience involving reward or its denial 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 a reward (RER group), whereas denial of this contact as a negative, frustrating event (DER group). We determined in adult rat male brains, the effects of these experiences on the methylation pattern of the β1 gene promoter, using bisulphite treatment and gene sequencing, as well as on the β1 adrenoceptor levels, using immunohistochemistry and autoradiographic in vitro binding. β1 promoter methylation was markedly decreased in the prefrontal cortex of RER animals while it was increased in the amygdala and the hippocampus of DER males. These epigenetic modifications were accompanied by increased levels of β1 receptors in the prefrontal cortex of the RER group and decreased β1 levels in the amygdala and the hippocampus of the DER. The observed differences in β1 adrenoceptor levels could underlie the differences in stress coping, anxiety and fear responses exhibited by the DER and RER animals.
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INCREASED INTRA-SUBJECT REACTION TIME VARIABILITY IN THE VOLITIONAL CONTROL OF MOVEMENT IN SCHIZOPHRENIA

Thomas Karantinos[1], Evangelos Tsoukas[2], Asimakis Mantas[2], Emmanouil Kattoulas[1] , Nicholas C. Stefanis [1], Ioannis Evdokimidis [2], Nikolaos Smyrnis [1].

1. Psychiatry Department, National and Kapodistrian University of Athens, Eginition Hospital, Athens, Greece.
2. Neurology Department National and Kapodistrian University of Athens, Eginition Hospital, Athens, Greece.

Increased reaction time (RT) intra-subject variability (ISV) is an emerging and consistent finding in RT studies of schizophrenia. A group of 23 patients suffering from DSM-IV schizophrenia and a group of 23 age-matched control subjects performed two RT tasks requiring basic sensorimotor processing and engaging two different motor systems: the Finger Lift Reaction Time task (FLRT) and the Voluntary Saccade Reaction Time task (VSRT). The Ex-Gaussian model was applied to the RT distributions measuring the mean (mu), and standard deviation (sigma) of a Gaussian component thought to reflect sensorimotor processing and an exponential component (tau), thought to reflect an intermediate decision process. In both tasks, a significantly larger RT intra-subject variability (ISV) effectively dissociated patients from controls. RT ISV in the two tasks was highly correlated only for patients. Both sigma and tau were significantly higher in the patient group with tau being the best predictor of schizophrenia. Furthermore, only in the patient group were sigma and tau highly correlated between the two tasks. The results reflect a deficit in information processing that may not be confined to decision processes related to the frontal cortex; rather, they may indicate dysfunction in distributed neural networks modulating adaptive regulation of performance.

ALTERATIONS IN THE PHYSIOLOGY OF PREFRONTAL CORTEX UNDERLIE THE DEVELOPMENT OF EMOTIONAL AND COGNITIVE PROCESSES ACROSS LIFESPAN

Xanthippi Konstantoudaki[1,2] , Kleanthi Chalkiadaki[1,2], Simona Tivodar[2,3], Elisabeth Vassiliou[1],
Stergia Hatzimanoli[1], Domna Karagogeos[2,3], Kyriaki Sidiropoulou[1,2]

[1]Department of Biology, University of Crete, Heraklion, Greece
[2]Institude of Molecular Biology and Biotechnology/Foundation for Research and Technology- Hellas, Heraklion, Greece
[3]Faculty of Medicine, University of Crete, Heraklion, Greece

Cognitive and emotional deficits in neurodevelopmental diseases, as schizophrenia or autism, are associated with disrupted inhibition in prefrontal cortex (PFC) and emerge either in childhood or in adolescence. Cellular changes during the development of PFC throughout these stages last considerably longer compared to primary sensory areas, determining both PFC normal function and emergence of disease states. However, very little is known about the development of PFC-dependent behaviors and their underlying cellular mechanisms across lifespan. Our study aims to identify age-dependent modifications in emotional processes and cognitive functions in two age-groups of mice: juveniles (postnatal day (PD) 25-35) and adults (PD> 60). The open-field and the elevated plus maze test revealed an increased anxiety phenotype in juvenile mice. Distinct aspects of recognition memory were tested, using three types of object recognition tests (ORT): the novel, object-to-place and temporal order ORTs, where juvenile mice performed poorer only in the latter one. Finally, juvenile mice exhibited decreased spatial working memory function, as evaluated with the delayed alternation task in the T-maze. Using local field potential recordings within layer II of PFC brain slices, changes in basal synaptic transmission and in short- and long-term potentiation were observed, while Golgi-cox staining revealed that PFC pyramidal neurons undergo developmental changes in dendritic morphology and dendritic spine number, uncovering a possible explanation for the plasticity alterations. To decipher the contribution of inhibition in the aforementioned changes, we studied two developmentally decreased inhibition experimental models: the Rac1 transgenic mouse model with decreased number of interneurons[1] and mice treated with picrotoxin during the juvenile period. Developmentally decreased inhibition in mice alters anxiety levels and deregulates PFC synaptic plasticity in an age-dependent manner. Therefore, the presence of decreased inhibition during the juvenile period is critical for the proper development of PFC.

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EFFECTS OF ROSEMARY INFUSION ON BEHAVIORAL PARAMETERS OF ADULT MICE AND ON ACETYLCHOLINESTERASE ACTIVITY OF THEIR BRAIN REGIONS

A. Katsikoudi[1], A.V. Ferlemi[1,2], V.G. Kontogianni[3], A.G. Kokkosis[1], G. Iatrou[4], F. Lamari[2], A.G. Tzakos[3], M. Margarity[1]

[1]Laboratory of Human and Animal Physiology, Department of Biology, University of Patras, 26504, Rio, Greece
[2]Laboratory of Pharmacognosy & Chemistry of Natural Products, Department of Pharmacy, University of Patras, 26504, Rio, Greece
[3]Department of Chemistry, Section of Organic Chemistry and Biochemistry, University of Ioannina, 45110, Ioannina, Greece
[4]Division of Plant Biology, Department of Biology, University of Patras, 26504, Rio, Greece

The aromatic herb *Rosmarinus officinalis* L. (Labiatae) is known for its medicinal properties since the ancient times. Its essential oil has been attributed with beneficial effects on rodent behavior and on cholinergic system function. However, its infusion has not been thoroughly studied. The aim of our research was to identify the phytochemical profile of rosemary infusion and study its effects on behavior (memory/learning, anxiety/fear and depression) and acetylcholinesterase (AChE) isoform activity [both salt-soluble (SS) and detergent-soluble (DS) fraction] in whole brain, individual brain regions (cortex, cerebellum, midbrain, striatum and hippocampus) and liver (comparison tissue) of adult male mice. At first, the phytochemical profile of the *R. officinalis* infusion was determined by liquid chromatography-UV diode array coupled to ion-trap mass spectrometry with electrospray ionization interface (LC-ESI-MS). Animals were randomly separated in two groups (n=8): control group and Ro-treated group (2% w/v of *R. officinalis* tea for 4 weeks). Memory/learning was evaluated using the passive-avoidance test, anxiety/fear using thigmotaxis and elevated plus maze test, and depression using the forced swimming test. AChE activity was assessed using the colorimetric method of Ellman. The phytochemical analysis showed that rosemary infusion is rich in phenolic acids and diterpenes. Rosemary tea consumption reduced the time spent on closed arms (35.20 %) in the elevated plus maze test and immobility time (55.71%) in the forced swimming test, whereas learning/memory was unaffected. Moreover, SS-AChE activity was lower ($p<0.05$) in liver (44.71%) and all examined brain regions (19.29 % whole brain, 29.93% cortex, 31.30% cerebellum, 32.25% midbrain, 24.60% striatum), except from hippocampus; whereas DS-AChE activity was reduced in all examined tissues including hippocampus (15.13% - 39.60%) compared to the control group. In conclusion, rosemary infusion consumption inhibits significantly cerebral and liver's acetylcholinesterase activity and exerts anxiolytic and antidepressant effects on adult male mice.

EFFECTA OF NEONATAL T-MAZE LEARNING UNDER CONDITIONS OF DENIAL OF MATERNAL CONTACT ON ADULT RAT BEHAVIORAL FLEXIBILITY

Paraskevi Kogionou*, Lydia Panagopoulou*, Fotini Stylianopoulou, Antonios Stamatakis
* Authors equally contributing to the work

Biology-Biochemistry lab, Faculty of Nursing, School of Health Sciences, University of Athens, Papadiamantopoulou 123, 11527 Athens, Greece.

Early life experiences have major influences in shaping adult behavior and brain function. Adverse events during early post-natal periods affect the development of brain areas, e.g. the prefrontal cortex, and predispose individuals for maladaptive behaviours. We utilized the animal model of neonatal training in a T-maze under denial (DER) or receipt (RER) of expected reward through maternal contact to investigate its long-term effects on prefrontal cortex function in two tests measuring behavioral flexibility: Fear-extinction and a T-maze choice based on food-reward. In these tests animals have to modify their learned behaviour based on alterations in environmental cues/predictors: In fear-extinction, animals learn not to freeze in response to a formerly conditioned stimulus as it is no-longer associated with foot shock and in the T-maze to shift their acquired preference for one arm of the maze based on food availability, as food is no-longer present in this arm but has been moved to the other arm of the T-maze. Most interestingly, adult male DER rats, showed lower extinction performance compared to either control or RER animals. Moreover, in the T-maze test adult male DER rats made more mistakes in their choices before shifting their preference. These behavioral deficits were accompanied by neurochemical alterations in the median prefrontal and orbitofrontal cortex of DER animals, where the number of glutamatergic neurons (vGLUT+) was lower while the number of GABAergic neurons (GAD67+) was not affected. Our findings that the neonatal experience of learning under DER conditions had long-term effects on the prefrontal cortex both at the neurochemical and the behavioral level support the concept that adult brain function and behavior can be programmed by early-life experiences in an experience-specific way.

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THE CORRELATION BETWEEN THE ACETYLCHOLINESTERASE ISOFORMS AND BEHAVIOR AFTER SHORT-TERM LEAD INTOXICATION IS GENDER-DEPENDENT

A.G. Kokkosis[1], A. Kouli[1], E. Flogera[1], M. Aivalioti[3], A. Krontira[1], C. Constantinou[2], M. Margarity[1]

[1]Laboratory of Human and Animal Physiology, Department of Biology, University of Patras, 26500, Rio, Greece
[2]Laboratory of Pharmacology, Department of Medicine, University of Patras, Patras 26500, Greece
[3]Laboratory of Molecular & Cellular Biology, Department of Biology, University of Patras, Patras 26500, Greece

Lead (Pb), a neurotoxic bio-accumulative heavy metal, is associated with cognitive dysfunctions. Our previous studies showed that lead administration to adult mice affected acetylcholinesterase (AChE) activity, an enzyme which is involved in acetylcholine metabolism, in a tissue-specific and dose-dependent way (250 ppm/500ppm). The aim of the present study was to investigate whether a short-term Pb administration affected brain acetylcholinesterase (AChE) activity of specific brain regions (cerebellum, cerebral cortex, midbrain, striatum, hippocampus and diencephalon) and behavioral indices (anxiety/fear, depression-like) in male and female adult mice. Male animals were divided into 2 groups. The first group was given orally 250 ppm $Pb(CH_3CO_2)_2$ dissolved in drinking water for 15 days. Respective control animal group was also prepared. The same procedure was done with the female animals. On the completion of each Pb treatment the animals were sacrificed. Anxiety was assessed by measuring: a) the percentage of time mice spent in the open arms of elevated plus maze apparatus and b) the thigmotaxis time (time spent close to the walls of an open field). Depression-like behavior was evaluated by recording immobility time in the forced swimming test. AChE activity was determined in both salt-soluble (SS-AChE) and detergent-soluble (DS-AChE) fractions of all brain regions tested, by Ellman's colorimetric method. The possible correlations between fear/anxiety or depression-like behaviour with the AChE activity, were also examined. The present results corroborate our previous observations that Pb engendered anxiogenic and depression-like behavior in mice. Moreover, we observed significant decrease in both SS- and DS-AChE activity in all the brain regions studied. Each animal group had, both behavioral indices and AChE isoforms, differentially affected by the metal, suggesting gender-dependence. Spearman correlation analysis revealed that the coefficients between the particular behaviors and AChE activity were brain region- and AChE isoform-specific, and each gender presented different profile.

SOCIAL STRESS IN MALE ZEBRAFISH AND THE INFLUENCE OF ESTRADIOL TREATMENT

Panayiota Makantasi[1], and Catherine R. Dermon[1]

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

Social hierarchy can influence many aspects of brain physiology and neurochemistry, while several cues, such as estrogens, can alter such interactions. The mechanisms by which estrogens affect aggression and social status have not yet been fully understood. The aim of the present study was to investigate the role of social stress in the mitotic activity and the expression of α_2A and β_2 adrenoceptors in the brain of adult zebrafish. Furthermore, the regulation of social dominance by gonadal hormones was studied. For this purpose, male zebrafish were kept in isolation or in dyads until the formation of stable social hierarchies. Additionally, 6 pairs having formed dominance and 6 isolated animals received 2,5μg/L of 17-β estradiol for 7 days and then all animals were treated with the proliferation marker 5-bromo-2′-deoxyuridine (BrdU) for 6 hours. Immunohistochemistry was used for the observation and quantification of newborn cells and the density of cells expressing α_2A and β_2-ARs. Stereological analysis of BrdU-IR cells revealed that estrogen treatment as well as social interaction had a region- and social status-specific effect on the proliferation rate of male fish. In addition, subordinate zebrafish were found to express reduced levels of β_2-ARs in the dorsal telencephalic regions Dm (homologue to mammalian amygdala) and Dl (homologue to mammalian hippocampus) and in the dorsal zone of periventricular hypothalamus (Hd). Taken together, our data demonstrate the importance of psychosocial stress in aspects of neurogenesis and adrenergic expression and the modulatory effects of estradiol in social interactions in the model organism zebrafish. This research has been co-financed by the European Union (European Social Fund- ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF)-Research Funding Program: Heracleitus II. Investing in knowledge society through the ESF.

INTERACTION OF AN EARLY EXPERIENCE INVOLVING REWARD OR ITS DENIAL THROUGH MATERNAL CONTACT, WITH EXPOSURE TO A NOVEL ENVIRONMENT ON NMDA RECEPTOR SUBUNITS IN THE ADULT RAT BRAIN

Vasileios Manatos[1], Antonios Stamatakis[1], Fotini Stylianopoulou[1]

[1]Biology-Biochemistry Lab, Faculty of Nursing, University of Athens, Greece

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 (pNR1, pNR2B) and unphosphorylated NR1 and NR2B subunits of NMDA receptors 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, before and after exposure to novelty in an open-field. As an early experience we employed a model developed in our lab, in which rat pups are exposed to a T-maze, one arm of which leads to the mother, and are either allowed (RER) or denied (DER) the contact with the mother, during postnatal-days 10-13.
The phosphorylation of NR1 and NR2B subunits was increased in all groups as a result of the exposure to novelty. In the HIP and PFC, DER animals had lower levels of pNR1 subunit after exposure to novelty. Furthermore, in the PFC, DER animals showed less phosphorylation of NR1 than the other groups, also under basal conditions. In the AMY, under basal and novelty conditions both DER and RER had lower levels of pNR1 compared to CTR animals; following novelty pNR2B levels were increased in DER and RER groups.
These results indicate that the early experience of exposure to the T-maze induced long term differences in NMDA receptor subunit composition. These early experience-induced changes could underlie differences in brain plasticity affecting cognitive abilities and emotional copying in adulthood.

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ACTION OBSERVATION RESPONSE PROFILE OF F5 VENTRAL PREMOTOR NEURONS IN THE MACAQUE BRAIN

Vassilis Papadourakis[1,2], Vassilis Raos[1,2]

[1]Institute of Applied and Computational Mathematics, Foundation for Research and Technology - Hellas & [2]Department of Basic Sciences, Faculty of Medicine, University of Crete, Iraklion, Greece

Our aim was to re-investigate the visual and motor properties of F5 mirror neurons (MNs), using a controlled paradigm in order to correlate the neuronal discharge with temporal events of the observed movement. For this purpose, the activity of single neurons was extracellulary recorded while the monkey executed and observed reaching to grasp actions. Initially, the monkey was trained to reach for and grasp, with its own hand, 3D objects with appropriate grips. A LED placed above the object instructed the various phases of the task. Then, it was also trained to observe the experimenter employing two randomly interleaved variations of the above task. In the first, the cuing LED was visible to the monkey (CUEOBS) whereas, in the second, the LED was off and the experimenter was getting instructions on a screen out of the monkey's view (NOCUEOBS). Moreover, a LED fixation condition in which the experimenter was not performing any movement was used as a control. Hierarchical cluster analysis of the response profile temporal characteristics divided the 140 recorded neurons into three classes. The response of the neurons belonging to the 1st class starts after the onset of the movement, displaying an almost identical profile in the two OBS conditions. These neurons are similar to the MNs described by Rizzolatti and coworkers. In contrast, the firing of the majority of neurons comprising the 2nd and 3rd class starts several hundreds of ms before the onset of the movement in both OBS conditions. In addition, the neurons of the 3rd class respond also to the LED fixation condition. This is the first study to demonstrate that the response of F5 MNs is modulated by the presence of cues in the observation scene, providing new elements about their response-triggering features and paving the way to new considerations about their functions.

NEUROCHEMICAL DISSECTION OF CENTRAL AMYGDALA OF ADULT MALE RATS TRAINED AS NEONATES IN A T-MAZE UNDER RECEIPT OR DENIAL OF REWARD THROUGH MATERNAL CONTACT

Petros Psoromytis[1], Andoniki Raftogianni[1],Fotini Stylianopoulou[1], Antonios Stamatakis[1]

[1]Biology and biochemistry lab, Faculty of Nursing, National and Kapodistrian University of Athens

Early-life experiences shape adult emotional reactions and the formation of fear memories. These processes are regulated by a wide brain network with the central nucleus of the amygdala (CeA) having a pivotal role. We have previously shown that neonatal training in a T-maze with either contact with the mother as reward (Receipt of Expected Reward, RER) or denial of this contact as a frustrative experience (Denial of Expected Reward, DER), affect amygdalar function in adulthood during fear-conditioning resulting in a contradictory finding: Although CeA shows higher activation (number of c-Fos immunopositive cells) in DER animals, freezing behaviour during memory recall is more intense is the RER group.
In an effort to explain this discrepancy, we have determined neurochemical characteristics of CeA neurons under basal conditions and following fear-conditioning. CeA contains mainly GABAergic neurons ($GAD67^{+}$), classified into two categories based on the co-expression, or not, of PKC-delta. Thus, we have determined the number of GAD67 and PKC-delta immunopositive neurons in the CeA under basal conditions: Although the number of $GAD67^{+}$ neurons did not differ between the three groups, DER animals had a lower number of PKC-$delta^{+}$ neurons in the CeA compared to either control or RER animals.
Furthermore, we determined the phenotype of activated neurons in the CeA following fear-conditioning memory recall (double immunofluorescence for c-Fos and PKC-delta). Most interestingly, the ratio of PKC-$delta^{+}$ to PKC$delta^{-}$ activated neurons (c-Fos+) did not differ between control and DER animals while it was lower in the RER group. Given that PKC-$delta^{+}$ cells are considered as inhibitors of CeA-mediated freezing, the observed difference in this ratio could explain the discrepancy between CeA activation and freezing behavour observed in DER and RER animals.

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AGE-RELATED DECLINE IN FUNCTIONAL CAPACITY OF SHORT AND LONG LIVED STRAINS OF DROSOPHILA.

Stamatia N. Rakou and Christos Consoulas

Laboratory of Experimental Physiology, Medical School, University of Athens, M. Asias 75, Athens 11527, Greece.

In humans, aging has profound effects on the function of the motor system. The first signs of age-related functional decline are the decreased ability to walk, the decreased motor reactivity to environmental stimuli and the increased difficulty to balance. Improved diagnosis and treatment of age-related motor decay could therefore have an enormous positive impact on the quality of life for elderly individuals. The effect of aging on nervous system function appears to be conserved between vertebrates and invertebrates. Hence, studies on model invertebrate organisms, such as the fruit fly *Drosophila melanogaster*, may allow understanding the fundamental cellular and molecular mechanisms underlying functional senescence.
In this horizontal study, we examined the physiological status of the short-lived (~40 days), wild-type Lausanne-S and the long-lived (~120 days), mutant strain Methuselah (encodes a G-protein coupled receptor). The reflective escape behaviour in the form of climbing (negative geotaxis) as a reaction to mechanical stimuli was studied daily in individual flies until their death. We found that Lausanne-S flies retained a high level of functionality until almost the time of death. In contrast, mth flies exhibit functional decline in motor reactivity and walking/climbing deficits many days prior to their death, suggesting that increased longevity does not result in enhanced reactive motor performance. During the pre-death stage, both strains exhibited all signs and symptoms, observed in our previous work on Oregon-R and W1118 flies. This suggests that the pattern of functional collapse at the final stage of live is independent of the genetic background and thus appears to be a general principle. Our findings on mth flies offer the opportunity to investigate the genetic and molecular players involved in the functional deterioration of the motor system during aging and test whether particular pharmacological interventions in aged individuals can ameliorate, delay or/and reverse functional decay.

DROSOPHILA FRAGILE X MENTAL RETARDATION PROTEIN REGULATES THE TRANSLATION OF PROTEINS CRUCIAL FOR LEARNING AND MEMORY

Ourania Semelidou, Eirini – Maria Georganta, Jean Gouzi, Efthimios MC Skoulakis

BSRC 'Alexander Fleming', 16672, Vari, Greece

Fragile X syndrome (FXS) is the most common form of inherited mental retardation, caused by loss-of-function mutations in *FMR1* gene. This gene encodes an apparent RNA binding protein, which is involved in the regulation of transport, translation and stability of target mRNAs, especially locally at the dendrites. Fragile X mental retardation protein (FMRP) is associated with polyribosomes throughout neurons and it is also present in smaller messenger ribonucleoprotein complexes (mRNP) and dendritic "RNA granules". FMRP binds to many presynaptic and postsynaptic target mRNAs and by stalling ribosomal translocation on target mRNAs, it regulates the synthesis of a set of plasticity-related proteins. Loss of function of the FMRP results in defects in dendritic spine structure and dynamics, synaptic plasticity and cognition in many models of the disease. The Drosophila melanogaster homolog of FMRP has a high degree of amino acid sequence identity/similarity compared to the human protein. In our lab we use flies heterozygous for *dfmr1* gene, which present learning and memory phenotypes resembling those of FXS patients. In this study we are trying to identify potential targets regulated by dFMRP locally in the dendrites. We focus on two different kinases, MAPK and ALK. MAPK is known to be downregulated in FMRP mutants, while our results show that ALK is upregulated in dFMRP mutants. Using genetic and pharmacological tools to modify MAPK and ALK levels, we aim to rescue the long term memory (LTM) deficts of dFMRP mutants. This will identify possible interaction between FMRP and these molecules, linking the aberrant levels of these proteins in FXS mutants to the absence of regulation by dFMRP.

SHORT TERM EXPOSURE TO FLUOXETINE AFFECTS DOMINANT SUBORDINATE BEHAVIOUR IN ZEBRAFISH, *DANIO RERIO*

Antonia Theodoridi, Alexandra Tsalafouta, Michalis Pavlidis

Department of Biology, University of Crete

Zebrafish, *Danio rerio*, is an emerging model organism in stress and neurobehavioral studies.In nature the species forms shoals, yet when kept in pairs it exhibits an agonistic and anxiety-like behavior that leads to the establishment of dominant-subordinate relationships. Fluoxetine, a selective serotonin reuptake inhibitor, is used as an antidepressant in humans and rodents, as it has been proved that it can reduce anxiety. Pairs of male zebrafish were held for 2 hours in small aquaria to develop dominant – subordinate behavior. Individuals were then exposed to a fluoxetine immersion for an hourandthen,the same pairs were reformedand allowed to interact.Their behavior was recordedprior to fluoxetine administration and at the end of the experiment.Trunk and brain samples were taken for cortisol determination and mRNA expression studies, respectively.Post treatment,fish spent an increasedamount of time in the upper part of the tank when compared to their behavior prior to the treatment, indicating a decreased anxiety-like behavior. There was no statistically significant difference in whole-trunk cortisol concentrations between dominant and subordinate fish, while fluoxetine treatment resulted in higher ($P< 0.01$) cortisol concentrations in both groups. Both coping style (dominant/subordinate fish) and treatment affected statistically significantly mRNA expression levels of genes relevant to stress axis (glucocorticoid, *gr* and mineralocorticoid, *mr* receptors) and neurogenesis (brain derived neurotrophic factor, *bdnf*). Administration of selective serotonin reuptake inhibitors may provide a useful tool towards a better understanding of the molecular mechanisms underlying social behavior in zebrafish.

CEREBRAL LATERALITY FOR LANGUAGE IN INTELLECTUALLY GIFTED ADOLESCENTS

Tomprou, D. M.,[1] Papadatou-Pastou, M.,[1] Koufaki, A., [1] & Vlachos, F. [2]

[1]Research Centre for Psychophysiology and Education, Faculty of Primary Education, University of Athens, Deinokratous Street 27, 10675 Kolonaki, +30 2103642712
[2]Special Education Department, University of Thessaly, Argonafton & Filellinon Street, 382 21 Volos, +30 24210-74789 & 74787

Cerebral laterality 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 laterality (right-hemispheric or symmetrical) with learning difficulties (e.g., dyslexia) and conditions such as mental retardation. Nevertheless, the language laterality in healthy populations with higher cognitive abilities, such as intellectually gifted students, has not been adequately studied to date. The present study assesses cerebral laterality for language using functional transcranial Doppler ultrasound (fTCD), a brain-imaging technique which compares blood flow velocities in the middle cerebral arteries while a participant is 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. Complementary measures of laterality included behavioral assessment and self-report questionnaires. Thirty intellectually gifted adolescents (18 males; mean age 185 months, *SD* = 12.39 months; IQ measured by Wechsler Intelligence Scale for Children, WISC-III>120) and 42 adolescents of normal intelligence (21 males; mean age 187 months, *SD* = 13.48 months; 80<IQ<120) were administered the Peg-Moving test and the Hemispheric Mode Indicator Questionnaire. Nineteen of the intellectually gifted adolescents (9 males; mean age 184 months, *SD* = 13.95 months) and 16 of the normal intelligence adolescents (7 males; mean age 176 months, *SD* = 15.41 months) were further administered the Animation Description task through fTCD. Analysis of data revealed that intellectually gifted adolescents present with a higher degree of typical behavioral, but not cerebral, laterality. Overall, the current findings add to our limited understanding of the neuropsychological profile of the intellectually gifted population.

Keywords: laterality, intelligence, functional transcranial Doppler ultrasound, fTCD

THE ASSOCIATION OF SCHIZOTYPAL TRAITS WITH EXECUTIVE FUNCTIONS AND PREPULSE INHIBITION IN UNAFFECTED RELATIVES OF SCHIZOPHRENIA PATIENTS

Chrysoula Zouraraki[1,2], Eva-Maria Tsapakis[3], Ismini Kopsahili[3], Eugenia Chourdaki[4], Panos Bitsios[2], Stella G. Giakoumaki[1,2]

[1]Department of Psychology, Faculty of Social Sciences, University of Crete, Rethymno Crete, Greece
[2]Department of Psychiatry & Behavioural Sciences, Faculty of Medicine, University of Crete, Heraklion Crete, Greece
[3]Aghios Charalampos Mental Health Center, Heraklion, Crete, Greece
[4]Department of Psychiatry, Venizeleio General Hospital, Heraklion, Crete, Greece

"Negative" schizotypy, executive functions and Prepulse Inhibition (PPI) at long lead-intervals are associated with prefrontal function while "positive" schizotypy and PPI at short lead-intervals are associated with subcortical structures. The aim of this preliminary study was to examine the association between positive/negative schizotypy, executive functions and PPI at short and long lead-intervals in unaffected first-degree relatives of schizophrenia patients. Sixteen unaffected relatives were assessed for schizotypal traits (Schizotypal Personality Questionnaire), executive functions (tasks from the Cambridge Neuropsychological Test Automated Battery) and PPI (75- and 85-dB prepulses; 30, 60, 120ms lead-intervals). We examined associations between schizotypy, executive functions and PPI with univariate regressions [dependent variable: PPI at each trial type or executive function measure; independent variables: a) age, cigarettes smoked/day, schizotypy scores; b) age, cigarettes smoked/day, schizotypy scores, age at illness onset and Clinical Global Impression-Severity score of the affected relative]. High "positive" schizotypy predicted lower PPI with the 75dB-prepulse at the 30ms (PPI 75dB_30ms) interval (R^2=0.610, $p<0.05$) and high "negative" schizotypy predicted lower PPI with the 75dB-prepulse at the 120ms (PPI 75dB_120ms) interval (R^2=0.677, $p<0.01$). When the clinical characteristics of the affected relative were included in the predictors, the significances increased and larger percentages of the variance were explained ("positive" schizotypy and PPI 75dB_30ms: R^2=0.752, $p<0.01$; "negative" schizotypy and PPI 75dB_120ms: R^2=0.814, $p<0.005$). The remaining models were not significant (all p values >0.05). "Positive" schizotypy predicted lower levels of subcortically-mediated PPI, while "negative" schizotypy predicted lower levels of prefrontally-modulated PPI. These effects were strengthened when indexes of illness severity of the affected relative were taken into consideration, suggesting that the "endophenotypic risk", as assessed with PPI and schizotypal traits, depends on the clinical characteristics of the affected relative. The lack of significant associations between positive/negative schizotypy and executive functions might be due to the limited sample size of the study.

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NERVOUS SYSTEM DEVELOPMENT

THE ROLE OF CHAPERONE MEDIATED AUTOPHAGY IN NERVOUS SYSTEM DEVELOPMENT

Daphne Antoniou[1], Maria Xilouri[2], Oysten Rod Brekk[2], Leonidas Stefanis[2,3] *, Panagiotis Politis[1] *
1) Center for Basic Research, Biomedical Research Foundation of the Academy of Athens, 115 27, Athens, Greece
2) Division of Basic Neurosciences, Biomedical Research Foundation of the Academy of Athens (BRFAA), 4 Soranou Efesiou Street, Athens, 11527, Greece
3) Second Department of Neurology, University of Athens Medical School, Athens, Greece
*equal contribution

Proteolytic mechanisms that remove unwanted or damaged proteins are of paramount importance for cellular physiology. Among them, chaperone mediated autophagy (CMA) plays a critical role for the clearance of select cellular proteins and has been associated with the degradation of disease-related proteins, such as alpha-synuclein, in the context of neurodegenerative diseases. Despite its potential importance for adult brain, almost nothing is known about the role of CMA in nervous system development. In particular, the aim of this study was to examine the possible involvement of CMA in the early stages of neural differentiation during development. Accordingly, here we present functional data indicating that CMA is critically involved in the differentiation decisions of neural stem cells (NSCs). Specifically, we show that CMA is highly active in NSCs and that Lamp2a and Hsc70, basic components of the CMA machinery, are strongly expressed in *ex vivo* cultured NSCs. Interestingly, Lamp2a, which is the lysosomal receptor and rate limiting step for CMA, is preferentially expressed in early post mitotic neurons as compared to nascent astroglial cells. Most importantly, Lamp2a reduction, by lentiviral-mediated shRNA, strongly impairs the ability of NSCs to produce neurons and induces their potential to generate astrocytes. These data suggest a key role for Lamp2a and consequently of CMA in the regulation of neuronal fate acquisition. To further understand the *in vivo* role of CMA in brain development, we are currently undertaking gain-and-loss of function studies for Lamp2a in the rodent brain. Moreover, we are engaged in detailed proteomic analysis to identify CMA protein-targets that mediate these effects in NSCs.

ENVIRONMENTAL ENRICHMENT AFFECTS THE VISUAL SYSTEM IN A SEX-RELATED WAY

Dimitrios P. Bessinis[a], Christina Dalla[a], Nikolaos Kokras[a,b], D. Papassava[a], Pothitos M. Pitychoutis[a,c] and Zeta Papadopoulou-Daifoti[a]

A. Department of Pharmacology, Medical School, University of Athens, Greece
B. First Department of Psychiatry, Eginition Hospital, Medical School, University of Athens, Greece
C. Department of Biology & Center for Tissue Regeneration and Engineering (TREND), University of Dayton, 300 College Park, Dayton, OH, 45469-2320, USA

Sex differences in the visual system have been reported in aspects of human vision, such as color perception, peripheral vision and even in the activation of the primary visual cortex. Similarly sex differences have been identified in the visual system of laboratory animals such as monkeys and rats. On the other hand, environmental enrichment (EE) has long been known to affect visual tissues. Taking into consideration the variation in the experimental approaches concerning EE and the sex differences in the visual system, we investigated in male and female rats the serotonergic and dopaminergic effects of EE in the retina and the visual cortex at different timepoints (ie. P0-25, P0-P90 and P90-P150). Early EE in adulthood increased the serotonergic activity of the male visual cortex and the female retina (P0-P90). In addition early EE (P0-P90) increased dopaminergic activity in the female retina and in the visual cortex of both sexes. Late enrichment increased the serotonergic activity in the retina and visual cortex of both sexes (P90-P150), but increased the dopaminergic activity in the visual cortex only in male animals. In the present study we expose marked sex differences in the neurochemistry of visual tissues and we demonstrate for the first time that environmental enrichment can in fact modify the serotonergic and dopaminergic neurotransmission in the retina and visual cortex. Overall, the present study underpinned the sex-dependent neurochemical status of the visual system and provided insights into the different mechanisms underlying visual processing in the two sexes.

PYRAMIDAL CELL LOSS AND GFAP INCREASE IN THE CA4 REGION OF HIPPOCAMPUS AFTER *IN UTERO* EXPOSURE OF RATS TO CORDLESS PHONE RADIATION

Adamantia F. Fragopoulou[1], Marianna Stasinopoulou[1,2], Antonis Stamatakis[3], Aikaterini S. Skouroliakou[4], Fotini Stylianopoulou[3], Nikolaos Kostomitsopoulos[2], Lukas H. Margaritis[1]

[1]*Dept of Cell Biology and Biophysics, Faculty of Biology, UoA, Panepistimiopolis 15784, Athens, Greece.*
[2]*Laboratory Animal Facilities, Centre of Experimental Surgery, BRFAA, 11527, Athens, Greece.*
[3]*Biology – Biochemistry Lab, Faculty of Nursing, School of Health Sciences, UoA, 11527 Athens, Greece.*
[4]*Dept of Energy Technology Engineering, TEI of Athens, Egaleo 12210, Athens, Greece*

Non-ionizing electromagnetic radiation (EMR) emitted from daily used wireless devices has been studied the last three decades in a variety of model systems for potential health hazards but the results are still inconclusive. So far studies have revealed cell loss in hippocampus and GFAP expression increase following exposure to mobile phone radiation in rats, but no attempt has been made to study the impact of Digital Enhanced Cordless Telecommunications (DECT) phones radiation on these parameters. To pursue this issue we determined pyramidal cells number and GFAP levels in the hippocampus following exposure to an EMR emission source during embryonic and post-natal life in a rat model. Three groups of rats were used: a sham exposed group (Group A) and two experimental groups, exposed to DECT base radiation (operating intermittently every half an hour for 24 h/day) until birth (Group B) or until weaning (Group C). Randomly selected offspring were sacrificed on postnatal day 22 and pyramidal cell number was estimated in CA areas following cresyl violet staining as well as GFAP levels by immunohistochemistry. DECT base exposure significantly reduced the pyramidal cell number specifically in the CA4 region and increased the GFAP expression in the same area in both experimental groups. These data can possibly explain the memory deficits and protein expression changes that have been reported previously by our group following DECT exposure. Work is in progress to study spatial memory performance in the adult male offspring.

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INTER-REGULATION OF PKC ISOFORMS DURING NEURONAL DIFFERENTIATION IN THE PHEOCHROMOCYTOMA PC12 PARADIGM

Michalis Kodounis, Dimitra Mangoura

BRFAA, Neurosciences

Neuritic outgrowth is a pivotal feature in the establishment of neuronal differentiation and the basis for synaptogenesis. Pheochromocytoma cells (PC12) have been widely used as a model system for studying the mechanisms associated with these specific events. Sustained activation of ERK is an established requirement for the ability of growth factors like NGF and the FGFs to promote differentiation of PC12 into sympathetic neurons, whereas transient activation of ERK by factors like EGF promote proliferation. Multiple PKC isoforms, in particular of novel PKCs have been identified as the downstream targets of such growth factor receptors to regulate the Ras/MAPK pathway, yet PKC inter-regulation has not been addressed. To investigate this, we employed treatments with ψεRACK, a PKCε-selective activator peptide and εV1-2, a PKCε-selective inhibitor peptide, and the known to increase the incidence of neurite outgrowth phorbol ester TPA. The time-course of ERK activation induced by ψεRACK (1μM) revealed a sustained temporal and low-magnitude pattern, similar to TPA, which directly binds to and activates PKCs. In the long term (48h), both induced expression of drebrin that binds to and organizes F-actin in dendritic spines, of neurofibromin, involved in dendritic arborization, and of PKCζ that is activated in response to NGF, all possibly through activation of c-Jun. The two agents had, however, opposing effects on the expression of PKCι, an isoform associated with NGF-dependent differentiation, while TPA also down-regulated PKCα, a PKC shown by us and others to mediate proliferation in tumor cells; interestingly this event was partially ameliorated by εV1-2. These differential effects correlated well with the fact that, like with NGF, ψεRACK-treated cells first underwent one cell cycle and then proliferation ceased as differentiation prevailed. Thus our data provide evidence for inter-regulation of PKC isoforms during the early steps of the transition to differentation in neural cells.

VIMENTIN FILAMENTS IN CHICKEN CEREBELLUM AND BRAINSTEM EMBRYOGENESIS

Vasiliki Kommata, Antonis Pittaras, Catherine R Dermon

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

Intermediate filaments consist general cytoskeleton components that in contrast to microfilaments and microtubules, are not directly involved in cell movements, but play a structural role providing mechanical support to cells and tissues. The present study questioned the role of vimentin, a type III intermediate filament protein expressed in glial cells, in chicken cerebellum and brainstem morphogenesis during cerebellar lobule formation. For this, we examined the embryonic days 10 to 19 (E10-E19) of chicken embryos using vimentin immunohistochemistry and double immunofluorescence to determine co-expression with glial fibrillary acidic protein (GFAP) and possible associations with proliferating external granule cells, labeled with 5-bromo-2-deoxy-urudine (BrdU). From day E10, a bundle of long vimentin+ fibers run through the brainstem, following a ventro-lateral to medio-dorsal pattern, with vimentin filaments distributed centrally in a perpendicular direction. At E12 day, vimentin fibers remained in brainstem and extended in the most lateral cerebellar lobules (I, X). Vimentin expression within cerebellum gradually increased, with E13 showing strong staining specifically in the fissure floors of external granule layer (EGL). At later stages (E15-E17 days), vimentin expression extended to all cerebellar fissure compartments (folia floors, walls and apexes) covering most of EGL proliferating zone, whereas scattered filaments were also observed throughout the inner granular layer and white matter. In contrast, vimentin expressing fibers were gradually reduced within the brainstem, with minimum expression at embryonic days E18-E19. At day E19 vimentin expressing fibers were confined only to cerebellar folia apexes. Interestingly, double immunofluorescence with GFAP showed only partial co-expression of vimentin+ fibers depending on the embryonic stage. In addition, vimentin filaments were found in close association to proliferating cells, suggesting their role as scaffold for the migration of the newborn granule cells (BrdU+), further supporting the fundamental role of transient vimentin expression in the formation and cytoarchitecture of cerebellum foliation.

ENTERIC NERVOUS SYSTEM PROGENITOR CELLS SHOW ABNORMAL CELL CYCLE PROFILE AND DELAYED DEFFERENTIATION INTO NEURONS AND GLIAL CELLS

Pinelopi Nikolopoulou[1], Athanasia Stathopoulou[1], Marialena Lalioti[1], Zoi Lygerou[2], Stavros Taraviras[1]

[1] Laboratory of Physiology, and [2] Laboratory of General Biology, Department of Medicine, University of Patras, Greece

Neural Crest cells (NCCs) comprise a multipotent, migratory cell population that generates a diverse array of cell and tissue types during vertebrate development, including neurons and glial cells of Peripheral Nervous System (PNS). Enteric Nervous System (ENS), is a subdivision of the PNS that controls the function of the gastrointestinal (GI) tract and is derived from the Vagal and Sacral NCCs. The formation of a fully functional ENS depends on proliferation and differentiation decisions of NCCs. Our goal is to elucidate the mechanisms that control the self-renewal and differentiation decisions of the NCCs. Towards this direction we are studying the in vivo role of Geminin in the developing ENS. Geminin is a molecule that has been shown to affect the balance between self-renewal and differentiation by inhibiting re-replication of DNA and interacting with transcription factors and chromatin remodeling proteins. Mice that lack Geminin specifically in NCCs, have been generated and analyzed. We have shown that upon Geminin deletion, the population of NCCs is decreased possibly due to increased cell death of NCCs. Moreover, decreased number of committed enteric nervous system progenitor cells (ENCCs) was observed resulting in an aganglionic gut. ENCCs that lack Geminin expression show delayed differentiation into neurons and glial cells and an abnormal cell cycle profile. Our findings show that Geminin has an important role in self-renewal and differentiation of enteric nervous system progenitor cells.

PAX6 IS EXPRESSED IN SUBSETS OF V0 AND V2 INTERNEURONS IN THE VENTRAL SPINAL CORD IN MICE

Elena Panayiotou[1,2], Elena Panayi[1], George Lapathitis[1], Cedric Francius[3], Frederic Clotman[3], Nicoletta Kessaris[4], William D. Richardson[4], Stavros Malas[1,2]

[1]The Cyprus Institute of Neurology and Genetics, P.O. Box 23462, 1683 Nicosia, Cyprus
[2]Department of Biological Sciences, University of Cyprus, P.O. Box 20537, 1678 Nicosia, Cyprus
[3] Université Catholique de Louvain, Institute of Neuroscience, Laboratory of Neural Differentiation, Av. Hippocrate 55, B-1200 Brussels, Belgium
[4]Wolfson Institute for Biomedical Research and Research Department of Cell and Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK

The embryonic spinal cord in mice is organized into eleven progenitor domains. Cells in each domain first produce neurons and then switch to specifying glia. Five of these domains known as p3, pMN, p2, p1 and p0 are located in the ventral spinal cord and each expresses a unique code of transcription factors (TFs) that define the molecular profile of progenitor cells. This code is largely responsible for determining the subtype specification of neurons generated from each domain. Pax6 codes for a homedomain-containing TF that plays a central role in defining the molecular boundaries between the two ventral-most domains, p3 and pMN. Using fate mapping and gene expression studies we show that PAX6, in addition to each patterning function, is expressed in a group of late born interneurons that derive from the p2 and p0 domains. The p2-derived neurons represent a subset of late born V2b interneurons and their specification depends on Notch signalling. The V0 neurons represent V0v ventral neurons expressing Pax2. Our data demonstrate that interneuron diversity in the ventral spinal cord is more complex than originally appreciated and point to the existence of additional mechanisms that determine interneuron diversity, particularly in the p2 domain.

POSTNATAL MORPHOMETRIC CHANGES IN THE SWINE MAXILLARY NERVE.

Konstantinos Papageorgiou[1], Ioannis Grivas[2], Maria Chiotelli[2], Nikolaos Papaioannou[3], Hans Nauwynck[4], Spyridon Kritas[1]

[1] Department of Microbiology and Infectious Diseases, [2] Department of Anatomy Histology, and Embryology, [3] Department of Pathology, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki.
[4] Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University.

Myelination, the ensheathing of neuronal axons by myelin, is important for the proper function of both central and peripheral nervous systems. Various studies had investigated the quantitative parameters of myelination in certain species. However, there are no data concerning the myelination of maxillary nerve in pigs. As a preliminary step for the investigation of the neuropathogenesis of Aujeszky's disease (pseudorabies) virus in pigs, a quantitative analysis of various myelination parameters of the maxillary nerve has been attempted, during the first 5 weeks of porcine postnatal development. The evaluation was conducted in three groups of uninfected pigs at the age of 1 week (Group A), 3 weeks (Group B) and 5 weeks (group C), using toluidine blue staining and immunofluoresence techniques. The thickness of axon, myelin sheath, and total nerve fiber at cross sections of maxillary nerves of these pigs were measured. In addition, the ratio of total object to total region area, and the ratio of the axon to fiber diameter (g-ratio) have been calculated. The thickness of myelin sheath was 1.001 (±0.247)μm for group A, 0.889 (±0.214)μm for group B and 0.984 (±0.169)μm for group C. The g-ratio was 0.526 (±0.074) for group A, 0.579 (±0.066) for group B and 0.562 (±0.065) for group C. The results of this study will be used in the future for comparison with infected pigs.

DIVERGENT ORIGIN AND FUNCTION OF SHOX2 INTERNEURON SUBPOPULATIONS IN THE MOUSE SPINAL CORD

Ismini Rozani[1], Kimberly J. Dougherty[2], Thomas M. Jessell[3], Ole Kiehn[2], Laskaro Zagoraiou[1]

[1]Division of Basic Neurosciences, Biomedical Research Foundation of the Academy of Athens
[2]Dept of Neuroscience, Karolinska Institute, Stockholm, Sweden
[3]HHMI and Dept. of Neuroscience, Columbia University, NY, USA.

Molecularly distinct classes of ventral spinal interneurons, termed V0-V3, have been shown to contribute to different aspects of locomotion – rhythm generation, left-right alternation, flexor-extensor coordination. However, each of the four populations of neurons is heterogeneous and therefore may play several roles in locomotion. Inactivation of these broad classes perturbs locomotion, but because of class heterogeneity, the circuitry resists interrogation. Indeed, physiological and anatomical studies point to two dozen smaller, more specialized interneuron populations. Aiming to identify such small populations, a screen for genes preferentially expressed in the ventral spinal cord was performed. Shox2, a homeodomain transcriptor factor expressed by interneurons present along the entire rostrocaudal axis of the spinal cord, was detected. In cross-sections Shox2 neurons are located in the intermediate zone. Experiments at various embryonic ages revealed three glutamatergic subpopulations of Shox2 neurons. The more abundant one is located in the ventral spinal cord. The two smaller subpopulations are located more dorsally, with the one holding a dorsolateral and the other a dorsomedial position. The main Shox2 subpopulation co-expresses Chx10 (93% at e12.5), a marker of V2a interneurons, indicating a p2 origin. P2 domain progenitors give rise to excitatory Chx10+/Lhx3+ V2a interneurons, as well as to inhibitory GATA3-derived V2b and V2c interneurons. In a Shox2::Cre; Fluorescent Protein (FP) reporter mice, we identified a distinct set of FP+Lhx3+Chx10- interneurons (the rest 7% of the main subpopulation), which are likely p2-derived. We termed them V2d interneurons, to distinguish them from FP+Lhx3+Chx10+ V2a interneurons. The dorsomedial Shox2 subpopulation expresses mainly Isl1, which is indicative of dI3 (dorsal) origin, while the dorsolateral expresses Lmx1b and Lbx1suggesting a dI5 descent. Inactivation of the output of Shox2 interneurons reduces the frequency of locomotor-like activity, whereas ablation of Shox2 V2a interneurons had only a modest effect on locomotion, demonstrating divergent functions for the different Shox2 populations.

RAC-GTPases REGULATE MICROTUBULE STABILITY AND AXON GROWTH OF CORTICAL GABAergic INTERNEURONS.

Simona Tivodar[1,2], Katerina Kalemaki[1,2*], Zouzana Kounoupa[1,2*], Marina Vidaki[1,2,#], Kostas Theodorakis[1,2], Myrto Denaxa[3], Nicoletta Kessaris[4], Ivan DeCurtis[5], Vassilis Pachnis [3], and Domna Karagogeos[1,2]

1: Institute of Molecular Biology & Biotechnology (IMBB, FORTH), Heraklion, Greece,
2: Department of Basic Science, Faculty of Medicine, University of Crete, Heraklion, Greece, 3: Division of Molecular Neurobiology, 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: Cell Adhesion Unit, Dibit, San Raffaele Scientific Institute, 20132 Milano, Italy,

**These authors contributed equally to this work*
 # Current address: Koch Institute for Integrative Cancer Research at MIT, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.

Cortical interneurons are characterized by extraordinary functional and morphological diversity. Although tremendous progress has been made in uncovering molecular and cellular mechanisms implicated in interneuron generation and function, several questions still remain open. RhoGTPases have been implicated as intracellular mediators of numerous developmental processes such as cytoskeleton organization, vesicle trafficking, transcription, cell cycle progression and apoptosis. Specifically in cortical interneurons, we have recently shown a cell-autonomous and stage-specific requirement for Rac1 activity within proliferating interneuron precursors. Conditional ablation of Rac1 in the medial ganglionic eminence leads to a 50% reduction of GABAergic interneurons in the postnatal cortex. Here we examine the additional role of Rac3 by analyzing Rac1/Rac3 double mutant mice. We show that in the absence of both Rac proteins, the embryonic migration of MGE-derived interneurons is further impaired. Postnatally, double mutant mice display a dramatic loss of cortical interneurons. In addition, Rac1/Rac3-deficient interneurons show gross cytoskeletal defects *in vivo* and *in vitro*, with the length of their leading processes significantly reduced and a clear multipolar instead of bipolar, morphology. We propose that in the absence of Rac1/Rac3 cortical interneurons fail to tangentially migrate towards the pallium due to defects in actin and microtubule cytoskeletal dynamics.

ONTOGENY OF STRESS RESPONSES IN PRE-LARVAE AND LARVAE SEA BASS ADRENOCEPTORS.

[1*]Themistoklis Tsarouchas, [1*]Stefanos Fokos, [3]Nikos Papandroulakis, [2]Michalis Pavlidis, [1]Catherine R. Dermon

[1]Laboratory of Human and Animal Physiology, Department of Biology, University of Patras, Greece
[2]Department of Biology, University of Crete, Heraklion, Crete, Greece
[3]Hellenic Centre for Marine Research, Institute of Aquaculture, Heraklion, Crete, Greece
[*]indicates equal contributions

Stress experience is known to modulate neuro-endocrine function leading to multi-system allostatic regulation, including the remodeling of neural circuits. The present study questioned the possible modulation of adrenergic system after stress exposure of sea bass (D. Labrax) pre-larval and larval stages. Stressors applied included at pre-larvae (mouth opening/first feeding) exposure to chasing with a net for 20 sec and high aeration (1,000 - 1,500 ml min^{-1} vs. 150-200 ml min^{-1}) for 90 sec, while at larvae (flexion) exposure to high aeration (as above), chasing with a net for 20 sec, confinement (collection in beakers), and air exposure for 5 sec before being transferred to baskets within a 500 L tank. Samples were taken at 1 and 24 hours post-stress exposure, in order to study α_{2A} and β_2 adrenoceptors' (ARs) expression, using whole mount double immunofluorescence. The quantification of adrenoceptor expression was performed using ImageJ, in stressed and non-stressed pre-larvae and larvae brain area, eye and body. Both types of ARs were determined by means of fluorescence measurements, according to corrected total tissue fluorescence (CTTF) method, and density counts of AR-expressing cells, in selected areas. The results showed that stress exposure during the flexion stage led to a significant decrease of α_{2A} adrenoceptor expression 24 hours after stress, while β_2-AR expression showed no significant changes following stress. In agreement, increased adrenaline and noradrenaline levels consist a well-known early stress response mechanism. Our data suggest that these increases in adrenaline levels are mediated by decreases of Gi protein-coupled (GiPCRs) presynaptic α_{2A}-ARs, that inhibit norepinephrine and adrenaline release. Therefore the observed stress-induced decreases in receptor density possibly consist a preserved allostatic mechanism of a reverse negative feedback, which is α_{2A} adrenoreceptor-mediated. Supported by EU, FP7 grant COPEWELL.

NERVOUS SYSTEM DISORDERS AND NEUROPSYCHOPHARMACOLOGY

EFFECTS OF ANESTHETIC KETAMINE ON ANXIETY-LIKE BEHAVIOUR IN RATS

Katerina Antoniou[1] Foteini Delis[1] and Nikolaos Pitsikas[2]

[1]*Department of Pharmacology, School of Medicine, University of Ioannina, Ioannina, Greece*
[2]*Department of Pharmacology, School of Medicine, University of Thessaly, Larissa, Greece*

There is poor experimental evidence concerning the effects of anesthetic doses of the non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist ketamine on anxiety. The present study investigated the effects of anesthetic ketamine (100 mg/kg, i.p.) on anxiety-like behaviour in rats evaluated in the light/dark procedure and the exposure to a novel open field apparatus. The effects of anesthetic ketamine on motility were assessed using a motor activity chamber.
Anesthetic ketamine tested 24 h after treatment induced decreases in the number of transitions between the light and dark chambers and time spent in the light chamber compared to the vehicle-treated animals in the light/dark test. In the open field test, rats that received anesthetic ketamine spent less time in the periphery and the center of the apparatus compared to their control cohorts. Motor activity of ketamine-treated animals was lower to that displayed by the vehicle-treated rats.
Interestingly, the anesthetic ketamine induced effects on anxiety indices were differentiated following an interval of 48 h post-treatment. The rats displayed an anxiogenic-like profile in both light/dark procedure and open field exposure while ketamine did not influence rats' motor activity.
The present results indicate that an anesthetic dose of ketamine induced a decrease in motor activity 24 h post-treatment while produced an anxiogenic-like profile that cannot be attributed to changes in motor activity 48 h post-treatment.

PYRAMIDAL CELL LOSS AND GFAP INCREASE IN THE CA4 REGION OF HIPPOCAMPUS AFTER *IN UTERO* EXPOSURE OF RATS TO CORDLESS PHONE RADIATION

Adamantia F. Fragopoulou[1], Marianna Stasinopoulou[1,2], Antonis Stamatakis[3], Aikaterini S. Skouroliakou[4], Fotini Stylianopoulou[3], Nikolaos Kostomitsopoulos[2], Lukas H. Margaritis[1]

[1]*Dept of Cell Biology and Biophysics, Faculty of Biology, UoA, Panepistimiopolis 15784, Athens, Greece.*
[2]*Laboratory Animal Facilities, Centre of Experimental Surgery, BRFAA, 11527, Athens, Greece.*
[3]*Biology – Biochemistry Lab, Faculty of Nursing, School of Health Sciences, UoA, 11527 Athens, Greece.*
[4]*Dept of Energy Technology Engineering, TEI of Athens, Egaleo 12210, Athens, Greece*

Non-ionizing electromagnetic radiation (EMR) emitted from daily used wireless devices has been studied the last three decades in a variety of model systems for potential health hazards but the results are still inconclusive. So far studies have revealed cell loss in hippocampus and GFAP expression increase following exposure to mobile phone radiation in rats, but no attempt has been made to study the impact of Digital Enhanced Cordless Telecommunications (DECT) phones radiation on these parameters. To pursue this issue we determined pyramidal cells number and GFAP levels in the hippocampus following exposure to an EMR emission source during embryonic and post-natal life in a rat model. Three groups of rats were used: a sham exposed group (Group A) and two experimental groups, exposed to DECT base radiation (operating intermittently every half an hour for 24 h/day) until birth (Group B) or until weaning (Group C). Randomly selected offspring were sacrificed on postnatal day 22 and pyramidal cell number was estimated in CA areas following cresyl violet staining as well as GFAP levels by immunohistochemistry. DECT base exposure significantly reduced the pyramidal cell number specifically in the CA4 region and increased the GFAP expression in the same area in both experimental groups. These data can possibly explain the memory deficits and protein expression changes that have been reported previously by our group following DECT exposure. Work is in progress to study spatial memory performance in the adult male offspring.

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THE EXPRESSION OF HIF1-α AND NF-κB IN DRG NEURONS DURING NEUROPATHIC PAIN

Christos Galanis, Charalampos Labrakakis

Dept. of Biological Applications and Technology, University of Ioannina

Neuropathic pain is a chronic pain disorder that has a serious impact on the quality of life. Because of its complex nature it is difficult to treat. Despite the efforts in understanding pain states, the exact mechanisms involved in the pathogenesis of neuropathic pain are not yet well understood. Damage or disease of peripheral nerves is a main cause for the development of neuropathic pain. Following the damage, the cellular components of peripheral nerves might respond to it by activating chain of signaling cascades involving cell stress and transcription factors. The resulting changes in gene expression could underlie the sensitization of sensory neurons that leads to increased pain sensitivity. In this study we used rat model of neuropathic pain to investigate the timeline of Hypoxia Inducible Factor-1 alpha (HIF1-α) and Nuclear Factor kappa-B (NF-κB) expression in dorsal root ganglion neurons during the pathogenesis of neuropathic pain. Our results might unfold the molecular mechanisms involved and contribute to our understanding of chronic pain syndromes.

HIPPOCAMPUS AND PREFRONTAL CORTEX COMMUNICATION IS REQUIRED FOR DEPRESSIVE-LIKE BEHAVIOR IN RATS

V. Kafetzopoulos[1], N. Kokras[1,2], A. Vassalou[1], I. Sotiropoulos[3], H. Leite-Almeida[3], J.F. Oliveira[3], K. Antoniou[4], N. Sousa[3], Z. Papadopoulou-Daifoti[1] and C. Dalla[1]

1. Department of Pharmacology, University of Athens, Athens, Greece.
2. First Department of Psychiatry, Eginition Hospital, University of Athens, Athens, Greece
3. Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal
4. Department of Pharmacology, University of Ioannina, Ioannina, Greece.

Prefrontal cortex (PFC) and hippocampus are well known to be involved in the pathophysiology of depression and in antidepressant response. However, the contribution of the circuit formed by their connection is not clear. Hippocampus has a direct connection to PFC, whereas the reverse connection relies mainly on *nucleus reuniens* (RE). This study aims to investigate the properties of the prefrontal cortex – hippocampal circuit and its function in models of depression and behavioral antidepressant screening tests. Adult male rats received an excitotoxic RE lesion, or a sham-operation. After one week of recovery, rats were tested in the modified forced swim test (FST). Rats received either a standard subacute treatment of sertraline (an SSRI, 10mg/kg) or vehicle. Other rats were subjected to 8 weeks of chronic mild stress (CMS) or left undisturbed serving as controls, receiving a chronic treatment of sertraline (10mg/kg/day) or vehicle in the last 3 weeks. Sucrose preference was measured throughout CMS. Following sacrifice, immunohistochemistry for cFOS expression and Golgi/Cox morphological analysis for dendritic length in PFC, hippocampus and RE was performed. Lastly, electrophysiological attributes of PFC to hippocampus connection were measured in anesthetized lesion- or sham-operated rats. RE-lesioned rats had lower immobility duration than sham controls in the FST. SSRI treatment reduced immobility duration in sham-operated rats. Both sertraline treatment and RE-lesion increased the number of cFOS positive cells in the infralimbic PFC. Lesioned/CMS animals exhibited increased sucrose preference and dendritic length of PFC and ventral CA1 hippocampal neurons compared to sham-operated, CMS animals. SSRI treatment successfully reversed behavioral and morphological changes induced by stress. RE-lesioned rats had reduced PFC/hippocampus synchronization, without an effect on overall region activity or long-term potentiation. Our results uncover that the two-way communication of PFC and hippocampus, depending on RE, is required for typical stress processing and depressive-like behavioral response.

EFFECTS OF CURCUMIN ON BRAIN STIMULATION AND THE REWARD-FACILITATING EFFECT OF MORPHINE

Vicky Katsidoni[1], Marilena Fotiadou[2], Marina Sagnou[3] and George Panagis[1]

[1]University of Crete, Department of Psychology, Laboratory of Behavioral Neuroscience, 74100 Rethymno, Crete, Greece
[2]City Unity College, Department of Psychology, 15-17Thiseos str,
Syntagma, Athens, T.K. 10562
[3]National Centre for Scientific Research Demokritos, Institute of Biology, 15310 Ag. Paraskevi, Attiki, Athens, Greece.

Curcumin is a multimodal polyphenol natural product isolated from the plant *Curcuma longa*. Recent animal studies reported that curcumin has several central actions, among which are an antidepressant and a neuroprotective effect. In the present study we utilized the intracranial self-stimulation (ICSS) paradigm to examine the effects of the commercially available curcuminoid mixtrure, pharmaceutically used as food supplement, on brain reward and on the reward-facilitating effect of morphine. To the best of our knowledge, this is the first study on the effects of curcumin on brain stimulation reward and the reward-facilitating effect of opioids. Male Sprague-Dawley rats were implanted with a monopolar electrode into the medial forebrain bundle (MFB) and were trained to respond for electrical stimulation using a rate-frequency paradigm. In the first experiment, rats were injected with curcumin (5, 10 and 20mg/kg, i.p.). The highest doses of curcumin tested significantly increased the threshold frequency required for MFB ICSS. In a second experiment, we examined whether curcumin (5mg/kg, i.p.) could counteract the reward-facilitating effect of morphine (10mg/kg, s.c.). Curcumin inhibited the reward-facilitating effect of morphine, at a dose that did not by itself affect brain reward function. The present results indicate that curcumin does not exhibit reinforcing properties in the ICSS paradigm over a range of doses tested, but rather has an inhibitory influence on reward mechanisms at high doses and even reduces the reward-facilitating effect of morphine. The present data indicate that curcumin interferes with brain reward mechanisms responsible for the expression of the acute reinforcing properties of opioids and provide evidence that curcumin may be used as a food supplement in attenuating the rewarding effects of opioids.

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THE NMDA RECEPTOR ANTAGONIST KETAMINE DISRUPTED STORAGE BUT NOT RETRIEVAL ABILITIES IN RATS EVALUATED IN A RECOGNITION MEMORY TASK. THE D1/D2 AGONIST APOMORPHINE COUNTERACTED THIS EFFECT

Nikolaos Pitsikas[1], and Mirjana Carli[2]

[1]Department of Pharmacology, School of Medicine, University of Thessaly, Larissa, Greece
[2]Department of Neurosciences, Institute of Pharmacological Research "Mario Negri", Milan, Italy

Ketamine, a non-competitive NMDA receptor antagonist has been reported to produce cognitive deficits in rodents that are of relevance to schizophrenia such as impairments of visual learning and memory. However, the effects of ketamine on specific memory components such as storage and retrieval of information have been much less investigated. To this aim, we investigated the effects of 8 and 20 mg/kg ketamine given i.p. immediately after the acquisition trial (storage component) or before (60 min) the retention trial (retrieval component) in the novel object recognition test (NORT). The data show that control rats successfully discriminated between familiar and novel object whereas ketamine injected after the acquisition trial dose-dependently impaired the storage of information as shown by decreased discrimination between the familiar and novel object. In contrast, given before the retention trial no dose of ketamine had any effect on discrimination between the novel and familiar object.
Evidence suggests that dopamine receptors may be important in regulating cognitive functions in schizophrenic patients and in cognitive deficits caused by NMDA receptor antagonists in animal models. In the present study we evaluated whether low doses of the D1/D2 dopamine receptor agonist apomorphine (0.05 and 0.1 mg/kg, i.p.) was able to counteract the ketamine-induced memory storage impairments in the NORT. We report that ketamine-induced deficit was abolished by apomorphine at both doses.
The findings suggest a functional interaction between dopamine and NMDA receptors in the control of memory storage, which may be of relevance to cognitive deficits in schizophrenia.

SEX DIFFERENCES IN BRAIN PLASTICITY: LESSONS FROM ENRICHED ENVIRONMENT

E. Tzouveka[1], D. Bessinis[1], N. Kokras[1,2], I. Sotiropoulos[1,3], Z. Papadopoulou-Daifoti[1], C. Dalla[1]

1. Department of Pharmacology, University of Athens, Greece.
2. First Department of Psychiatry, Eginition Hospital, University of Athens, Greece
3. Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal

Accumulating evidence suggest that early life experiences have an essential role in brain development and lifespan exhibiting long-lasting effects that alter neuronal structure, function and connectivity. Many studies demonstrate the beneficial effect of environmental enrichment (EE) on brain plasticity and performance with therapeutic implications against brain aging and pathologies. Although brain structure, connectivity and function exhibit marked sex differences in response to risk environmental factors (e.g. stress), little is known about sex-related responses to beneficial environmental stimuli facilitated by EE. Thus, this study focuses on the investigation of the differential effects of early EE (i.e. P0-P90) on cognitive performance, neuronal structure and neurotransmission in male and female Wistar rats. In the novel object recognition test, rearing in an enriched environment increased attention only in male rats, whereas the preference index for the novel object was higher in animals of both sexes. Neurochemical analysis revealed that EE females exhibited reduced glutamate levels and increased serotonergic activity in the hippocampus. These findings were accompanied by alterations in cytoskeletal and synaptic proteins essential for neuronal and synaptic function detected by Western blot analysis. Our findings provide further evidence about the importance of sex on the impact of beneficial environmental stimuli, which promote brain plasticity and enhance cognitive function.

NEURODEGENERATIVE DISEASES

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF APOLIPOPROTEIN E4 MUTANT (LEU28PRO) ASSOCIATED WITH INCREASED RISK OF LATE-ONSET ALZHEIMER'S DISEASE

Letta Argyri[1], Ioannis Dafnis[1], Efstratios Stratikos[1] and Angeliki Chroni[1]

[1]National Center for Scientific Research "Demokritos", Athens, Greece

Apolipoprotein (apo) E4 isoform has consistently emerged as a susceptibility factor for late onset Alzheimer's disease (AD). An apoE4 mutant, namely apoE4[L28P] (Pittsburgh), has been linked with increased risk of late onset developing AD and this risk remained significant even after adjusting for the known effect of apoE4. ApoE is characterized by structural plasticity and thermodynamic instability and can undergo significant structural rearrangements as part of its biological function. In an effort to gain insight how a single amino acid mutation in apoE4 may affect the pathogenesis of AD, we evaluated the effect of this apoE4 variant on the structural and functional properties of the protein. Circular dichroism (CD) spectroscopy revealed that the mutation significantly reduces the helical content of the protein. In addition, thermal and chemical unfolding analysis showed that apoE4[Leu28Pro] is significantly thermodynamically destabilized lacking a functionally relevant unfolding intermediate. Finally, 1-anilinonaphthalene-8-sulfonic acid (ANS) binding suggested that this apoE4 variant exposes a larger portion of hydrophobic surface to the solvent. ApoE4[L28P] was able to remodel multillamelar phospholipid vesicles, but it did so with slower kinetics compared to wild-type apoE4. In addition, apoE4[L28P] formed populations of lipoprotein particles with structural defects. The lipoprotein particles-associated apoE4[L28P] was found to reduce human neuroblastoma SK-N-SH cell viability and promote reactive oxygen species formation. Overall, our findings suggest that the L28P mutation leads to significant structural and conformational perturbations in apoE4, and can induce functional defects that can be associated with cellular toxicity. These structural and functional changes could be associated with the observed increased risk of apoE4[L28P] for AD development.

NEUROPROTECTION OF THE NIGROSTRIATAL DOPAMINERGIC NEURONS BY ADMINISTRATION OF BNN-50 IN THE DOPAMINE DENERVATED "WEAVER" MICE.

Botsakis K.[1], Mourtzi T.,[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 (wv/wv) mouse, a genetic model of Parkinson's disease (PD), exhibiting progressive dopaminergic neurodegeneration in substantia nigra (SN), consists an ideal animal model for neuroprotection studies. In this study, we administrated the neurosteroid dehydroepiandrosterone-sulphat (DHEA-S), its analogue 17-beta-spiro-[5-androsten-17,2'-oxiran]-3beta-ol(BNN-50)* that is not metabolized to estrogens, as well as BNN-50 combined with N-acetylcysteine (NAC) in wv/wv mice from P1 to P22 and investigated: a) the survival of nigrostriatal dopaminergic neurons, b) the expression of the apoptosis-regulating proteins Bcl-2 and Bax, and c) the mechanism underlying the "in vivo" neuroprotective action. Using immunohistochemical experiments, and a specific-antibody against tyrosine-hydroxylase(TH) we found that DHEA-S and BNN50 had a similar, great neuroprotection on the dopaminergic neurons in the wv/wv SN. The combination of BNN-50/NAC had an even more robust neuroprotective effect on the dopaminergic neurons, restoring completely also the dopaminergic terminals, in striatum. Using western blotting and specific-antibodies against Bcl-2 and Bax proteins, we revealed a significant restoration of the reduced Bcl-2/Bax ratio seen in the SN of the wv/wv mice following administration of BNN-50 and DHEA-S, indicating that the induced neuroprotection is, at least partially due, to an antiapoptotic effect of these agents. Double labeling experiments, using antibodies against TrkA and TH, showed no expresion of TrkA receptor on the dopaminergic neurons in SN neither of the normal or the "weaver" mice and its expression was not induced after chronic administration of the neurosteroids. Thus, the molecular mechanism underlying the dopaminergic neuroprotective effect of DHEA-S and BNN-50 "in vivo", on the dopaminergic neurons of SN must be further investigated. These results could be of clinical relevance, as they suggest that BNN-50 as well as BNN-50/NAC represent very effective neuroprotective agents for the nigrostriatal dopaminergic neurons and their axons. Since BNN50 is not metabolized to estrogens, it could be proposed for treatment of PD.

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TRANSCRIPTIONAL REGULATION OF A-SYNUCLEIN

Georgia Dermentzaki [1], Lee R. Clough [1], Leonidas Stefanis [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 a neuronal protein that has been strongly linked to 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 syndromes in some animal models. Focusing on the transcriptional regulation of SNCA, we previously identified a transcription factor (TF), the ZSCAN21 TF, with a binding site in the 1st intron of SNCA that is important in SNCA transcriptional regulation in PC12 cells. In current experiments, we have gone on to characterize the role of ZSCAN21 in the regulation of SNCA in the rat brain. We have found that ZSCAN21 is expressed in various regions of the developing and adult rat brain where SNCA was also detected. 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). Double immunohistochemistry with anti-ZSCAN21 and anti-NeuN verified neuronal expression and nuclear localization of ZSCAN21. In addition, ZSCAN21 colocalizes in the same neuronal somata with the SNCA protein in rat early postnatal brain sections. Lentiviral-mediated silencing of ZSCAN21 (50%) revealed a 50% increase of SNCA RNA levels in rat embryonic cortical cultures, using Real-Time PCR. Future work includes the use of stereotactic injections of lentivirus or adeno-associated virus expressing shRNA against ZSCAN21 in the hippocampus, an area affected in PD and associated with dementia. 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.

PROTEASOMAL ACTIVATION PROTECTS AGAINST PROTEOTOXICITY IN *CAENORHABDITIS ELEGANS* MODELS OF NEURODEGENERATIVE DISEASES

Konstantina Georgila[1*], Niki Chondrogianni [1*], Nikos Kourtis[2], Nektarios Tavernarakis[2] and Efstathios S. Gonos[1]
*equal contribution

[1] *Institute of Biology, Medicinal Chemistry & Biotechnology, National Hellenic Research Foundation, Athens Greece*
[2] *Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece*

Perturbation of proteostasis, leading to the accumulation of protein aggregates, is associated with various age-related neurodegenerative diseases, including Huntington's and Alzheimer's. The proteasome represents the predominant machinery for degradation of unwanted normal and damaged proteins and is thus critical for the maintenance of several aspects of cellular homeostasis. Indeed, impaired proteasomal function leading to formation of abnormal ubiquitin-protein aggregates has been implicated in the pathogenesis of numerous progressive neurodegenerative disorders. Herein, we have investigated the impact of enhanced proteasomal function on proteotoxicity underlying neurodegenerative disorders by exploiting *Caenorhabditis elegans* as a model organism. We have ectopically expressed a core rate-limiting proteasomal subunit, which was found to increase proteasomal degradation, in different nematode models of proteotoxic diseases associated with aberrant protein aggregation. Genetic activation of the proteasome protected against the detrimental effect of polyglutamine toxicity, whereas knock-down of a key component of the proteasome exaggerated the disease phenotypes. Similar results were obtained by using a temperature inducible model of Amyloid beta (Aβ) –induced toxicity, mimicking Alzheimer's disease. The suppression of Aβ toxicity, upon proteasomal activation was also associated with alterations of toxic Aβ protein species. The impact of enhanced proteasome degradation in age-related pathologies is further corroborated by the documented lifespan extension and oxidative stress resistance of transgenic animals. Collectively, these findings demonstrate that enhanced proteasomal degradation confers a general protective effect against proteotoxicity and promotes longevity. Therefore, proteasomal activation could serve as a therapeutic target to minimize deficiencies in neurodegenerative disorders associated with deregulated protein homeostasis.

A QUICK METHOD FOR TARGETED GENE DELIVERY TO TREAT LEUKODYSTROPHY AND INHERITED NEUROPATHIES

Alexia Kagiava[1], Irene Sargiannidou[1], Stavros Bashardes[2], Jan Richter[2], Natasa Schiza[1], Christina Christodoulou[2], Angela Gritti[3], Kleopas A. Kleopa[1,4]

[1]Neuroscience Laboratory and [4]Neurology Clinics, and [2]Department of Molecular Virology, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus
[3]Fondazione Centro San Raffaele del Monte Tabor, Milan, Italy

Pelizeaus-Merzbacher-like disease affecting CNS myelin and X-linked Charcot Marie Tooth disease affecting mainly the PNS but also CNS myelin are both associated with mutations in genes encoding two main gap junction (GJ) proteins, connexin 47 (Cx47) and Cx32, respectively. The aim of this study was to generate lentiviral vectors to allow gene delivery specifically to oligodendrocytes and Schwann cells in order to establish a method for targeted gene therapy.
For this purpose we generated a lentiviral vector containing the Cx47 or Cx32 gene along with IRES-EGFP as a reporter gene under the control of CNP promoter specific for both Schwann cells and oligodendrocytes or P0 promoter specific for Schwann cells. CNP-Cx47 vectors were delivered into the brain of wild type and Cx47KO mice at postnatal day 1, while P0-Cx32 vectors were delivered by intrathecal injection in 2 month-old mice. EGFP and connexin expression was assessed using immunochemistry and immunoblot analysis at different time points post injection.
In the CNS, a widespread expression of EGFP was detected from P15 until 3 months post-injection in different brain regions colocalizing with oligodendrocyte markers. On average 20.36±2.56% of oligodendrocytes were EGFP positive, with highest rates in the subventricular zone and olfactory bulb and lower rates in the cortex and corpus callosum. Expression of Cx47 was shown in Cx47KO brain with formation of GJ plaques in oligodendrocytes. Intrathecal injection of the P0-Cx32 vector resulted in EGFP expression in roots and distally along to the sciatic nerve. Cx32 expression was shown in non-compact myelin areas on a Cx32 KO background.
Thus, lentiviral gene delivery may result in stable and widespread targeted expression using cell specific promoters in PNS and CNS providing a possibility for future therapeutic approaches in leukodystrophies and neuropathies that should be further studied.

EFFECTS OF MATRIX METALLOPROTEINASE-9 ON INSULIN SURVIVAL PATHWAYS IN ALZHEIMER'S DISEASE

Archontia Kaminari[1], Athina Tzinia[1], Nikolas Giannakas[1] and Effie Tsilibari[1]

[1]NSCR "Demokritos", Institute of Biosciences and Applications, Athens.

Defective brain insulin signaling has been suggested to contribute to the cognitive deficits in patients with Alzheimer's disease (AD). Although a connection between AD and diabetes has been suggested, the mechanism(s) by which insulin resistance in the brain arises in individuals with AD remains to be elucidated. One of the hallmarks of AD is the abnormal accumulation of amyloid-β (Aβ) peptide, the oligomeric form of which is believed to be primarily responsible for cell toxicity and neuronal dysfunction in various experimental models of AD, as well as in AD patients. Interestingly, insulin signaling provides a physiological defense mechanism against oligomer-induced synapse loss. We have previously reported that matrix metalloproteinase 9 (MMP9), an enzyme critically involved in neuronal plasticity, appears to have a neuroprotective role by acting as α-secretase and by decreasing the formation of Aβ. To elucidate the role of MMP-9 on insulin survival pathways in AD, we will examine possible alterations in the proteins involved in the insulin pathway, such as nephrin and insulin receptor substrate -1 and -2 (IRS1, IRS2). Our preliminary experiments suggest that there is no difference in the expression levels of IRS1/2 in mice overexpressing MMP9 in the CNS (TgMMP9), mouse models of AD (5XFAD) and double transgenic animals 5XFAD/TgMMP9, compared to controls. However, since differences are expected in the phosphorylation of IRS1, IRS2 we investigated possible changes in primary hippocampal cultures; overall, we observed an increase in IRS1 expression concomitantly with a downstream increase in AKT phosphorylation, in transgenic mice compared to controls. Moreover, we examined by RT-PCR and confocal imaging the expression levels of survival-associated nephrin in primary cultures; we also observed an increase in nephrin expression in transgenic animals, compared to control mice. Further investigation is required to elucidate the role of MMP9 with regards to the insulin survival pathway.

STUDY OF PERINODAL PROTEINS IN MYELINATED FIBERS IN MULTIPLE SCLEROSIS

M.-E. Kastriti[1,2], I. Sargiannidou[3], K.A. Kleopa[3], D. Karagogeos[1,2]

(1)Department of Basic Science, Faculty of Medicine, University of Crete, Heraklion, Greece; (2)Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Vassilika Vouton, Heraklion, Crete, Greece;
(3)Neuroscience Laboratory and Neurology Clinics, The Cyprus Institute of Neurology and Genetics (CING), Nicosia, Cyprus.

Multiple Sclerosis (MS) is a chronic relapsing inflammatory disease of the CNS characterized by destruction of the myelin sheath and axonal degeneration. Several studies have focused on identifying potential autoantigens in MS. Amongst candidate molecules is TAG-1 (Transient Axonal Glycoprotein-1), found at the juxtaparanodal (JXPs) domains in myelinated fibers, adjacent to paranodes (PNs). Previous studies have analyzed the disruption of nodes and paranodes in MS brain. A recent study by our group using mouse models of demyelination showed that during the onset of demyelination paranodes are firstly affected followed by diffusion of the juxtaparanodes later on. We aim to study the changes in juxtaparanodal components in comparison with other perinodal regions in human MS samples. We performed analysis of the levels and localization of paranodal and juxtaparanodal components in postmortem human brain samples collected from MS patients in comparison to non-MS control cases. We examined samples including demyelinated lesions, periplaques and normal appearing white matter (NAWM) using immunohistochemical, immunoblot and mRNA analysis techniques. Our preliminary data show a diffusion of TAG-1 away from the juxtaparanodes in the periplaques compared to NAWM and control white matter even in the absence of paranodal diffusion. In combination with the diffusion of Kv1.1/1.2 potassium channels (found at juxtaparanodes) into nodal areas prior to paranodal disorganization, our observation suggests that TAG-1 diffusion might be a separate or a consequent event from the Kv mislocalization. We expect this study to provide new insights into the course of events that lead to the disorganization of perinodal proteins during demyelination.

POST-TRANSCRIPTIONAL REGULATION OF ALPHA-SYNUCLEIN EXPRESSION

Pelagia Koukouraki[1], Epaminondas Doxakis[1]

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

Clinical, genetic and experimental evidences have linked alpha-synuclein (asyn) expression to Lewy body diseases, including Parkinson's disease and dementia with Lewy bodies. The aberrant function of asyn remains obscure but it is likely to stem from the excess accumulation of asyn species that form toxic aggregates in presynaptic terminals affecting neurotransmitter release. Several physiological and pathophysiological conditions that lead to lower levels of cap-dependent translation in cells such as differentiation, oxidative stress, hypoxia or nutrient limitation, often drive an alternative mode of translation which is dependent on Internal Ribosome Entry Site (IRES) sequences in the 5′UTR of specific mRNAs. IRESs form hairpin structures that attract eukaryotic ribosomal translation initiation complexes promoting translation initiation independent of the presence of the commonly utilized 5'-m7G cap structure. Here, sequence analysis of asyn mRNA revealed the presence of such an IRES sequence in the proximal 5'UTR. Experimentally, we provide evidence that asyn can be translated in cap-independent manner and we propose that this is maybe a mechanism that contributes to excess accumulation of asyn in pathological conditions.

PROGRESSIVE DOPAMINE DEFICIENCY AFFECTS STRIATAL GLUTAMATERGIC SIGNALING IN A MOUSE MODEL OF PARKINSONISM

Maria Koutsokera[1], Panagiotis Kafkalias[1], Panagiotis Giompres[2], Elias D Kouvelas[1], Ada Mitsacos[1]

[1]Laboratory of Physiology, Faculty of Medicine; [2]Laboratory of Human and Animal Physiology, Department of Biology, University of Patras, 26500 Patras, Greece

Weaver mutant mouse is a valuable tool to further our understanding of Parkinson's disease (PD) pathogenesis since dopaminergic neurons of the nigro-striatal pathway undergo spontaneous and progressive cell death. Abnormalities in striatal glutamate transmission as a response to dopaminergic degenaration have been associated with the pathophysiology of PD. The physiological properties of glutamate receptors depend on their subunit composition and phosphorylation along with the composition of the protein complex formed downstream of receptor activation, where α-subunit of calcium–calmodulin-dependent protein kinase II (αCaMKII), a molecule important to synaptic plasticity, participates. In the present study, using immuoblotting in total striatal homogenate, we investigated the changes in protein expression and phosphorylation of glutamate receptor subunits and αCaMKII at the end of the third and sixth postnatal month. We found increased expression levels of GluN2A and GluN2B subunits of NMDA receptors by 74% and 92% and GluA1 subunit of AMPA receptors by 108% in the 3-month-old weaver striatum and statistically significant increase of GluN2B by 21% in the 6-month-old weaver striatum compared to control. Our results also indicated increased phosphorylations of GluN2B at serine 1303 by 40% and GluA1 at serines 831 and 845 by 40% and 38% in the 3-month-old and increased GluN2B phosphorylation by 22% in the 6-month-old weaver striatum compared to control. Furthermore, our results showed increased pCaMKIIThr286 phosphorylation by 176% in the 6 month-old weaver striatum, while total CaMKII protein levels were not altered at any age. Our results indicate that distinct degrees of DA neuron degeneration differentially affect expression and phosphorylation of striatal glutamate receptors and αCaMKII. Findings on this genetic parkinsonian model suggest that striatal glutamatergic signaling may play an important role in synaptic plasticity and motor behavior that follow progressive and chronic dopamine depletion in PD with biochemical consequences beyond those seen in acute toxic models

COMPLEMENT C1Q ABLATION ACCELERATES AMYLOID FORMATION IN A MOUSE MODEL OF TTRMET30 AMYLOIDOTIC NEUROPATHY

T. Kyriakides[1] , R. Papacharalambous[1] , S. Malas[1] , M.J. Saraiva[2] , E. Panayiotou[1]

[1]The Cyprus Institute of Neurology and Genetics, Nicosia Cyprus
[2]Instituto de Biologia Molecular e Cellular (IBMC), Porto, Portugal

Penetrance and age of onset of TTRMet30 amyloidotic neuropathy varies significantly among different populations. Penetrance in Sweden, Cyprus and Portugal are 2%, 28% and 80% while age of onset is 52, 46 and 32 years respectively. Genetic and epigenetic factors are speculated to play a role. There is good pathological data to implicate the participation of complement in the pathogenic cascades in peripheral nerve tissue. We have recently demonstrated a correlation within between age of onset and C1Q polymorphisms in Cypriot cohort of patients with TTRMet30 amyloidotic neuropathy suggesting that this is a genetic modifier. The object of the current study was to evaluate the role of complement C1Q in the available mouse model of the disease, the TTRKO/Met30$^{+/+}$.

STUDY OF THE EFFECT OF GLUCOSE AND OXYGEN DEPRIVATION ON TAU PHOSPHORYLATION

Myrto Panopoulou, Elpiniki Pappa, Panagiota Mavroeidi, Olga Mavrofrydi, Spiros Efthimiopoulos

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

Alzheimer's Disease (AD) is nowadays the most common form of dementia, characterized by progressive loss of memory, which finally leads to elimination of cognitive functions. The neuropathological features of AD include the presence of extracellular amyloid plaques, that consist of amyloid β peptides (Aβ), and intracellular neurofibrillary tangles (NFTs) consisting mainly of hyper-phosphorylated tau protein. Tau is a member of the MAP (Microtubule-Associated Proteins) family, which under physiological conditions promotes the polymerization and stabilization of microtubules. Reduction of its affinity for the microtubules, due to pathological hyper-phosphorylation, results in accumulation in the axons and disruption of the normal morphology and physiology of neurons. Among the various factors involved in the development of sporadic AD (about 95% of AD cases) is cerebral blood hypoperfusion and subsequent reduced delivery of oxygen supply and nutrients, such as glucose. Recent studies suggest that, at the early stages of AD**,** biosynthesis and energy metabolism are down-regulated, as a protective response to the reduced levels of nutrients and oxygen. On the current study, we investigated the effect of glucose and oxygen deprivation on tau phosphorylation at specific sites. Our results showed low levels of phosphorylated tau protein under both stress conditions, probably due to low energy supplies of the cells, supporting the hypothesis that, at the early stage of AD, the energy metabolism of the neurons is limited as a survival strategy against the glucose and oxygen deprivation. *This research has been co-financed by the European Union and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF)-Research Funding Program: ΑΡΙΣΤΕΙΑ–UOA-The effect of glucose deprivation on the development of Alzheimer's disease neuropathology.*

DIFFERENTIAL PROTEOLYTIC PROCESSING OF RECOMBINANT VERSUS SECRETED A-SYNUCLEIN

Methodios Ximerakis[1], Georgios Pampalakis[2], Theodoros I. Roumeliotis[3], Vasia-Samantha Sykioti[1], Spiros D. Garbis[3], Leonidas Stefanis[1, 4], Georgia Sotiropoulou[2] and Kostas Vekrellis[1]

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

Recent evidence supports that extracellular α-synuclein has an important role in the pathogenesis and progression of Parkinson's disease (PD). Deregulation in the normal degradation and clearance of secreted α-synuclein could underlie some or many cases of the disease. However, the degradation mechanisms involved have received very little attention. Here, we sought to investigate factors and mechanisms that regulate the extracellular levels of α-synuclein. Using kallikrein-related peptidase 6 (KLK6), an enzyme known to cleave recombinant α-synuclein, we demonstrate that unlike recombinant α-synuclein, naturally secreted forms are resistant to direct KLK6 proteolysis. This differential susceptibility appears to be partially due to the non-covalent association of secreted α-synuclein with lipids. We further show that secreted α-synuclein can be cleaved by KLK6 indirectly through activation of a secreted metalloprotease. Our results clearly suggest that physiologic modifications present in secreted α-synuclein confer different biochemical characteristics to the protein and significantly alter its proteolytic behavior.

IMPLICATION OF TAG-1 IN THE DEMYELINATION AND REMYELINATION PROCESS

Lida Zoupi[1], Giulia Bonetto[1] and Domna Karagogeos [1]

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

TAG-1/Contactin-2 is a cell adhesion molecule expressed by axons and glia at juxtaparanodes of myelinated fibers in the adult nervous system. At the juxtaparanode, TAG-1 is fundamental to the formation and stability of the juxtaparanodal complex by interacting with Caspr2 and voltage gated K^+ channels (VGKCs). Loss of TAG-1 from both the axon and glia leads to the disruption of the juxtaparanodal complex, significant hypomyelination of the optic nerve and behavioral deficits. Expression of TAG-1 by oligodendrocytes is sufficient to rescue the mutant phenotype. TAG-1 has also been identified as an autoantigen in MS patients and has been implicated in white and grey matter pathology. We aim to study the putative involvement of TAG-1 in the process of de- and remyelination. We used the cuprizone model of toxic demyelination, an established animal model of CNS demyelination in both wild type and TAG-1 deficient animals (*Tag-1*$^{-/-}$). Cuprizone-induced demyelination resulted initially in paranodal domain elongation with subsequent diffusion of the juxtaparanodal components. During remyelination, the reorganization of the myelinated fiber begun with the formation of the paranodal area while juxtaparanodal reorganization was evident only upon extensive remyelination. Surprisingly, in the *Tag-1*$^{-/-}$ animals, VGKC reclustering was evident during remyelination, indicating a TAG-1 independent mechanism of VGKC accumulation in the juxtaparanodal area upon remyelination. In addition, our work in progress indicates differences in recruitment/maturation of the oligodendrocyte population. More specifically, we noticed a retarded recruitment of oligodendrocyte precursors (OPCs) in the demyelinated area and a significant delay in mature oligodendrocyte reduction upon demyelination in *Tag-1*$^{-/-}$ animals.

NEUROENDOCRINOLOGY-NEUROIMMUNOLLOGY

MAINTENANCE OF WEIGHT LOSS IS ASSOCIATED WITH REPROGRAMMING OF ENERGY HOMEOSTASIS RECEPTORS IN THE HYPOTHALAMUS AND AMYGDALA

Constantinos Fedonidis, Nicholas Alexakis, Xenia Koliou, Dimitra Mangoura

Center for Neuroscience, BRFAA, Athens

The gut-brain axis maintains energy homeostasis through signaling of the membrane receptors for Growth Hormone Secretagogue (ghrelin)-GHSR, Leptin-LEPRb, Melanocortin-4 (a-MSH)-MC4R, and Cannabinoids-CB1R. The inter-regulation of ligand-receptor along this axis, primarily controlled by the hypothalamus, is crucial for the maintenance of physiological body weight as imbalances may lead to positive energy metabolism and then obesity. The appetite-regulating ghrelin acting through the endocannabinoid system promotes food intake and fat storage, whereas leptin inhibits appetite through its actions on a-MSH to reduce body weight. During diet-induced weight loss, long-term alterations such as increased ghrelin and decreased leptin, impose compensatory mechanisms encouraging weight regain. However, the hypothesis that weight loss may alter central sensitivity to circulating hormones has not been examined. Therefore, we investigated possible reprogramming in the central expression of energy homeostasis-related receptors during weight loss induced by diet with or without sleeve gastrectomy (SG), a bariatric surgery where both lower body weight and circulating ghrelin levels are maintained in the long-term. Rats were induced to obesity with high-fat diet (HFD) and then onto weight loss via removal of HFD with or without SG and the expression of these receptors was assessed by RT-PCR and Western blotting in distinct brain areas. We detected significant changes in the expression of CB1R, GHSR and MC4R in the hypothalamus and the reward center amygdala during HFD withdrawal, which depict an “addictive” molecular phenotype. In sleeve-gastrectomized animals short- and long-term changes of the acyl-ghrelin-CB1R axis primarily affected the maintenance of low endocannabinoid signaling to sustain the weight loss, while the impact of leptin signaling remained stable yet low over time. Thus, these results show that SG was necessary to reprogram the hypothalamus and amygdala to maintain lower body weight and suggest that peripheral intervention into the ghrelin-CB1R axis should be considered as a therapeutic target for human obesity.

THE EXPRESSION OF CORTICOTROPIN-RELEASING HORMONE IN THE HYPOTHALAMUS OF THE HUMAN NEONATE UNDER PERINATAL HYPOXIA: AN IMMUNOHISTOCHEMICAL STUDY

Efstathios Poulakis[1,2], Marianna A. Pagida[1,2], Anastasia E. Konstantinidou[3], Yiannis Malidelis[1,2], Effrosini Tsekoura[4], Efstratios Patsouris[3], Maria T. Panayotacopoulou[1,2]

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

Grwing evidence shows close link between adverse in utero environment and increased risk of neurological, psychological and psychiatric disorders in later life. Perinatal hypoxic/ischemic injury may directly or indirectly act at cellular and molecular levels to alter the brain development and to reprogram the stress system set-points. Corticotropin-releasing hormone (CRH) is the critical regulator of the hypothalamus-pituitary-adrenal (HPA) axis involved in stress responses. Experimental studies indicate that perinatal hypoxia augments the basal CRH mRNA levels in paraventricular nucleus (PVN) and the corticosterone response to stress in adult rats (1). Gestational hypoxia alone or combined with restraint stress sensitizes the HPA axis inducing anxiety in adult rat offsprings (2). Reduction of CRH-positive neurons is observed in adult rats exposed as newborns to hypoxia (3). The purpose of our study was to immunohistochemically investigate the expression of CRH in the PVN of 10 human neonates, in relation to their age and the severity/duration of hypoxic injury as estimated by neuropathological criteria. The intensity of CRH staining and the cell/ nucleus size were measured in 3 sections at the central level of PVN, using computerized image analysis system. Our preliminary results showed an interindividual variation in the number and intensity of CRH staining that could not be attributed to the degree of hypoxia grade. The number of CRH neurons was positively correlated with the total (prenatal + postnatal) age of the neonates, as previously reported (4). The variability in CRH expression in subjects of the same age could potentially be attributed to other pathophysiological factors (e.g. inflammation) that may activate the HPA axis alone or in combination with perinatal hypoxia.

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LOW DOSE BISPHENOL A AFFECTS THE NEUROENDOCRINE STRESS RESPONSE OF PERINATALY EXPOSED RATS

Emily Panagiotidou[1], Sophia Zerva[2], Dimitra Mitsiou[2], Michael Alexis[2], Efthymia Kitraki[1]
1 National and Kapodistrian University of Athens, School of Dentistry, Lab of Basic Sciences
2 National Hellenic Research Foundation, Institute of Biology, Medicinal Chemistry & Biotechnology

Bisphenol A (BPA) is an estrogen-mimicking endocrine disruptor, abundant in plastics and other everyday-used items. Perinatal exposure to human relevant concentrations affects the brain sexual differentiation of rodents[1]. We have previously shown that early-life BPA exposure impacts corticosterone-related functions and behavior in the rat brain[2]. In the current study, we present data of perinatal, low dose, BPA exposure on the neuroendocrine stress response of the exposed rats in adolescence. Female breeders were administered 40μg BPA/kg bw/day or vehicle throughout pregnancy and lactation. Adrenal histology, plasma corticosterone, hypothalamic glucocorticoid receptors (GR), pituitary proopiomelanocortin (POMC) and CRH-receptor1 (CRHR1) were determined in the offspring at rest and following acute swimming stress. The impact on the hypothalamic- pituitary- adrenal axis of adolescent offspring was apparent in both peripheral and central components of the axis and was sexually dimorphic. BPA led to reduced zona reticularis especially in males, hyperplasia of zona fasciculata in both sexes and increased adrenal weight in female offspring. Female BPA offspring exhibited increased resting corticosterone and reduced GRs, whereas following stress they had a lower corticosterone response and did not down-regulate GR in the hypothalamus, compared to control females. In contrast to females, treated males avoided desensitization of stress responsiveness and had higher post stress POMC and CRHR1 levels, compared to control males. Collectively these data demonstrate that early-life exposure to BPA levels below the ‘safe’ dose can induce long-lasting and sexually dimorphic deregulations in the rat stress response system, implicating perinatal exposure to this disruptor in the appearance of stress-related disorders in late life.

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STEM CELLS AND NERVOUS SYSTEM REGENERATION

TWO NEUROGENIC FACTORS, CEND1 AND NEUROGENIN-2, DRIVE ASTROCYTIC AND MEFS' REPROGRAMMING TOWARDS MULTIPOTENCY AND NEUROGENESIS

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

1-Cellular & Molecular Neurobiology, Hellenic Pasteur Institute, Athens, Greece
2-Mainz University, Physiological Chemistry Department, Germany

Recent studies demonstrate that astroglia isolated from non-neurogenic brain regions has the potential to be reprogrammed into functional neurons through forced expression of factors instructing neurogenesis. Based on our previous studies on the potential of the neurogenic gene Cend1 in directing NSCs to exit the cell-cycle and acquire a neuronal phenotype, in parallel with evidence demonstrating Cend1 activation by genes of the neurogenin family, we explored the reprogramming potential of Cend1 and Neurogenin-2 on postnatal cortical astrocytes. To this end, forced expression of either Cend1, Neurogenin-2 or both, resulted in trans-differentiation of astrocytes towards the neuronal lineage, as they were exhibiting differentiated neuronal morphology and expressed β-III-tubulin and neuronal subtype-specific markers, GABA and TH.Only in double-transduced cultures, $Cend1^{+}/Ngn2^{+}$ astrocytes formed high proliferating spheres (astrospheres) of $Glast^{+}/Nestin^{+}$ cells, which, in the absence of growth factors, differentiated into neurons, astrocytes and oligodendrocytes, implying their neural stem cell-like properties. In order to investigate whether Cend1 and Ngn2 have a broader neurogenic potential and are capable of trans-differentiating more distant in lineage cell types, we transduced mouse embryonic fibroblasts (MEFs) with lentiviral vectors expressing the two molecules. Our results indicate that forced expression of Cend1, Ngn2 or both resulted in MEFs reprogramming, initially towards neural progenitors and at later stages towards neurons. This finding demonstrates that common reprogramming mechanisms exist instructing neuronal trans-differentiation of different cell types. It also highlights the existence of a group of common factors that inactivates the differentiated cell program and activates genes associated with NSCs proliferation and differentiation state.

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BIRTH, DIFFERENTIATION AND SURVIVAL PATTERNS OF NEW GRANULE CELLS IN THE SEPTAL AND TEMPORAL PART OF THE ADULT RAT DENTATE GYRUS

C. Bekiari*, A. Giannakopoulou, N. Siskos, I. Grivas, A.S. Tsingotjidou, H. Michaloudi, G.C. Papadopoulos

Laboratory of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Aristotle University, 54124 Thessaloniki, Greece

Knowledge of possible differentiations in all parameters of the ongoing neurogenesis in the septal (SH) and temporal (TH) parts of the adult dentate gyrus, in conjunction with their already established structural and functional differentiations, are expected to shed light to hippocampal region specific involvement in the pathogenesis and prospective therapies of severe neurological diseases.
The present study examines comparatively the proliferation and the progressive differentiation, survival, migration and functional maturation of birth-dated neurons in the SH and TH of the adult rat brain. Following stereological estimation of the total number of granule cells of both hippocampal regions, the combination of 5-Bromo-2'-deoxyuridine (Brdu) admistration and the use of selected immunohistochemical markers (GFAP, Doublecortin, Calretinin and Calbindin) permitted identification of dividing neural stem cells (NSCs), migrating neuroblasts and post-mitotic immature amd mature neurons 2d, 5d, 7d, 2w, 3w and 4w after their genesis. Their functional maturation was confirmed by examining the expression of NMDAR1 and GluR2 receptors. Glial or neuronal commitment of newborn cells was evaluated through expression of S100 and NeuN antigens. Migration from the neurogenic subgranular zone to the inner granular cell layer was also followed.
Comparative quantification of the subpopulations found in successive time-frames of adult neurogenesis, demonstrates that dynamic changes in the number of dividing NSCs and newborn Brdu incorporating cells are almost identical in SH and TH. However, this common pattern begins to fade as soon as two weeks after Brdu administration, resulting in significant differences in migration, maturation process and survival of newborn neurons.
Combined findings of the present study question the widely held notion for comparatively higher levels of neurogenesis in the SH and should be taken into account when facing existing and future hippocampus related challenges.

ADIPOSE STEM CELL-TRANSPLANTATION IN A RAT MODEL OF TRAUMATIC BRAIN INJURY

A. Giannakopoulou[1], I. Dori[1], C. Bekiari[1], S. Petrakis[2], I. Grivas[1], A. Tsingotjidou[1], G. Koliakos[2,3] and G.C. Papadopoulos[1]

[1] Laboratory of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Aristotle University, 54124 Thessaloniki, Greece
[2] Biohellenika Biotechnology company, 57001, Thessaloniki, Greece.
[3]Department of Biological Chemistry, Medical School, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece

Pluripotent mesenchymal stem cells (MSCs) are thought to participate in tissue repair through trophic support and immunomodulation when transplanted into damaged tissue. Adipose tissue is a potentially attractive source of mesenchymal stromal/stem cells (ASCs) for regenerative therapeutic applications. The present study examines host brain responses, induced by the intracerebroventricular (ICV) transplantation of ASCs in an animal model of traumatic brain injury (TBI). TBI was induced and subsequently ICV transplantation of Venus+ ASCs was performed on adult Wistar rats. Normal untreated rats, normal ASCs-transplanted and TBI-saline transplanted rats were used as controls. The survival, spatial distribution and integration of ASCs in the host parenchyma were examined at one and six weeks post transplantation. The proliferative activity of both transplanted ASCs and endogenous reactive cells, focusing on hippocampal neural stem cells (NSCs), was investigated using antibodies for Ki67 and BrdU. The effect of transplantation on the lesion-induced recruitment of innate brain inflammatory cells, i.e. microglia and astroglia, was assessed with the markers Iba-1 and GFAP, respectively. The results demonstrate that one week after transplantation ASCs exhibit proliferative activity, integrate into brain parenchyma, migrating mainly to the periventricular area, and often acquire a mature morphology with ramified processes. ASCs migration is more profound six weeks after transplantation, as they reach the lesion-site through the corpus callosum and accumulate around pre- existing or newly formed blood vessels. Both the induction of TBI and the ASCs transplantation increase hippocampal NSCs proliferation, mainly ipsilaterally. In addition, ASCs enhance brain inflammatory responses with no evidence of their phagocytocis or destruction. Considering that inflammation may have both beneficial and detrimental outcomes, we suggest that ASCs transplantation after injury may contribute to the acceleration of host brain repair mechanisms.

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Quantitative evaluation of adult neurogenesis in the dentate gyrus of the sheep

I. Grivas, C. Bekiari, A. Giannakopoulou, A. S. Tsingotjidou, I. Dori, H. Michaloudi, G. C. Papadopoulos

Laboratory of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Aristotle University, 54124 Thessaloniki, Greece.

Adult hippocampal neurogenesis is widely investigated. Current knowledge on this exceptional neural phenomenon in rodents and the need to elucidate the mechanisms of the adult human neurogenesis highlight the importance of extending comparable studies in gyrencephalic animals. The present study evaluates quantitatively radial glia like neural stem cells (NSCs) and their progenies, newborn granule cells (NGs), in the dentate gyrus of the adult sheep (ovis aries), a species that has been proposed to exhibit certain advantages as an experimental animal compared to other gyrencephalic animals. Nine sheep divided equally in 3 groups were administered i.v. the cell proliferation marker 5- Bromo-2´-deoxyuridine (Brdu) and sacrificed 2 (Group A), 5 (Group B) and 15 (Group C) days later. Brdu and glial fibrilary acidic protein (GFAP) were detected immunohistochemically in distinguished subregions of the dentate gyrus on sequential sections of septal (SH) and temporal hippocampus (TH). The quantitative analysis showed that NGs and NSCs are unevenly distributed in septal and temporal part, granular and subagranular layer, suprapyramidal and infrapyramidal blade of the dentate gyrus. Therefore, we can anticipate that further step by step unfolding of hippocampal neurogenesis in the adult sheep may provide additional information, particularly useful for future translational conclusions.

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A NOVEL MODE OF PLURIPOTENCY

Theodoros Karnavas[1], Anastasia Kotsoni[1, 2], Aida DeGrigorio[3], Panagiota Stamou[2], Dimitris L. Kontoyiannis[2], Tristan A. Rodriguez[3] and Eumorphia Remboutsika[1, *]

[1]Stem Cell Biology Laboratory, Institute of Molecular Biology and Genetics and [2]Institute of Immunology, BSRC Alexander Fleming, 34 Fleming Str., 16672 Vari-Attica, Greece.
[3]MRC-Clinical Sciences Centre, Faculty of Medicine, Imperial College London, Hammersmith Hospital Campus, Du Cane Rd., London W12 0NN, UK.

During early mouse development, pluripotent cells exist in the naïve epiblast (primitive ectoderm) in waiting to get primed for the generation of the three germ layers during gastrulation. The importance of mouse Embryonic Stem Cell (ESC)-derived Primitive Ectoderm like cells (EPL) and Epiblast Stem Cells (EpiSCs) lies with the discovery that they could resemble human ESCs. However, the mechanism of primitive ectoderm induction and maintenance is still unclear. Here, we present a SOX2-instructed conversion of ESC to large numbers of primitive ectoderm bodies (Epispheres) by lineage selection. The ESC to Episphere transition is accompanied by down-regulation of oct4 and the up-regulation of fgf5, brachyury and nanog involved in primitive ectoderm formation. Epispheres do not express any other early mesodermal and endodermal genes; however, they retain their ability to the ability to respond to extrinsic signals guiding the formation of endodermal and mesendodermal derivatives. In contrast, Epispheres acquire an ectodermal character over time, respond to all-trans retinoic acid to generate RC2-positive radial glia cells that in turn differentiate primarily into neurons and oligodendrocytes. Once injected to kidney capsules of immunocompromised $RAG^{-/-}$ mice, Epispheres fail to generate tumours when compared to the parental ESC line. Thus, Epispheres provide for a novel Sox2-instructed population of pluripotent stem cells.

CORTICOTROPIN-RELEASING HORMONE (CRH) DEFICIENCY DECREASES NEUROGENIC ACTIVITY IN ADULT HIPPOCAMPUS CAUSING MEMORY DEFICITS

Y. Koutmani[1], I. A. Gampierakis[1], A. Polissidis [2], P. Politis[3] and K.P. Karalis[1], [4]

[1] Developmental Biology Section, Biomedical Research Foundation of the Academy of Athens, Greece
[2] Division of Basic Neuroscience, Biomedical Research Foundation of the Academy of Athens, Greece
[3] Histology Section, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
[4] Division of Endocrinology, Children's Hospital, Boston MA, USA

Stress exerts differential effects on adult neurogenesis primarily via its stimulation of glucocorticoid release. Despite the negative effects of stress on progenitor cell proliferation in the hippocampus, challenges leading to robust increase in glucocorticoid levels have been reported to promote neuronal growth. We have previously shown that Corticotropin-Releasing Hormone (CRH), a main hormonal mediator of stress response, may overcome the negative effects of glucocorticoid and induce proliferation of neural progenitor cells in the embryonic brain. In order to elucidate the role of CRH in adult neurogenesis, we examined the proliferating activity of neural stem cells in the adult hippocampus of CRH-deficient (*Crh-/-)* and wild type mice. Our results showed that the number of BrdU- positive cells in the dentate gyrus of the *Crh-/-* mice were significantly reduced compared to the wild type group. Since hippocampal neurogenesis is linked to memory, we subjected *Crh-/-* and wild type mice to novel object recognition and novel object location tasks that assess short-term memory. Although *Crh-/-* mice possessed normal object recognition memory, their object location memory was impaired, indicating a hippocampal-dependent cognitive deficit. CRH receptors were detected in adult hippocampal neural stem cells in support of the possibility of direct effects of CRH on this neurogenic population. These findings suggest new physiological roles for CRH in adult brain adaptive responses to stress stimuli.

NEUROSTEROIDAL AGONISTS OF NGF RECEPTORS: NEUROPROTECTIVE PROPERTIES AND NEUROGENIC ACTIONS

Pediaditakis, I.[1,2], Efstathopoulos, P.[1,2], Kourgiantaki, A.[1,2] Tzeranis D.[3], Yannas I.[3], Arévalo, JC[4], Calogeropoulou T.[5], Charalampopoulos, I.[1] and Gravanis, A.[1,2]

[1]*Dept. of Pharmacology, School of Medicine, University of Crete, Heraklion Greece*
[2]*Foundation of Research & Technology-Hellas (IESL-FORTH), Heraklion, Greece*
3 Dept. of Biological Engineering, Massachusetts Institute of Technology (MIT), Boston MA
4 Department of Cell Biology and Pathology, Instituto de Neurociencias de Castilla y León (INCyL), Universidad de Salamanca, Salamanca,Spain
5 Institute of Biology, Med. Chemistry, Biotechnology, National Hellenic Research Foundation, Athens Greece

Neurotrophins control neuronal cell fate and function during development and adulthood. They act through tyrosine kinase Trk and pan-neurotrophin $p75^{NTR}$ receptors, exerting potent neuroprotective and neurogenic effects. Despite the demonstrated beneficial effects, the therapeutic usefulness of neurotrophins is compromised by their polypeptide nature and their restricted penetrance to the blood-brain barrier (BBB). We have recently shown that neurosteroid dehydroepiandrosterone (DHEA) prevents neuronal apoptosis (*Charalampopoulos et al, PNAS 2004*), through binding to TrkA and $p75^{NTR}$ receptors (*Lazaridis et al, PLoS Biol 2011*), activating prosurvival kinases and anti-apoptotic Bcl-2 proteins, preventing thus the apoptotic loss of NGF receptor positive sensory in NGF null mice. However, DHEA is metabolized *in vivo* to sex steroids, affecting the endocrine system. We have recently synthesized 17-spiro analogs of DHEA with anti-apoptotic, neuroprotective properties (IC_{50} at nanomolar levels), deprived of androgenic-estrogenic actions (*Calogeropoulou et al, J Med Chem 2009*). In the present study, we report that synthetic DHEA derivative BNN27 specifically interacts with NGF receptors, TrkA and $p75^{NTR}$ at nanomolar concentrations. BNN27 induced TrkA tyrosine phosphorylation, affecting downstream signaling of Akt and MAPKs in sympathetic neurons and regulated TrkA internalization. Moreover, BNN27 was shown to promote the interaction of $p75^{NTR}$ receptors with its effector factors RhoGDI, RIP2 and TRAF6. It also reduced apoptosis of NGF-dependent embryonic sensory neurons of NGF null mice while it was capable to reverse neurogenic deficits of 5XFAD mice, an animal model of alzeimer's disease.The neurogenic properties of BNN27 are also tested in 2D and 3D-collagen cultures of embryonic and adult neural stem cells. BNN derivatives may serve as lead molecules to develop BBB permeable, neurotrophin-like small molecules (microneurotrophins) with potential applications in the treatment of neurodegenerative diseases and brain trauma (*Gravanis et al, Science Signaling 2012*).

Keywords: Neurotrophins, neurosteroids, neurodegeneration, neural stem cells,
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FUNCTIONAL CROSS-TALK BETWEEN THE CELLULAR PRION PROTEIN AND THE NEURAL CELL ADHESION MOLECULE NCAM IS CRITICAL FOR NEURONAL DIFFERENTIATION OF NEURAL STEM/PRECURSOR CELLS

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

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

Cellular prion protein PrP is prominently expressed in brain, in differentiated neurons but also in neural stem/precursor cells (NPCs). The misfolding of PrP is a central event in prion diseases, yet the physiological function of PrP is insufficiently understood. Although PrP has been reported to associate with the neural cell adhesion molecule NCAM, the consequences of concerted PrP-NCAM action in NPC physiology are unknown. Here we generated NPCs from the subventricular zone (SVZ) of postnatal day 5 wild type and PrP null (-/-) mice and observed that PrP is essential for proper NPC proliferation and neuronal differentiation. Moreover, we found that PrP is required for the NPC response to NCAM-induced neuronal differentiation. In the absence of PrP, NCAM not only fails to promote neuronal differentiation, but also induces an accumulation of doublecortin-positive neuronal progenitors at the proliferation stage. In agreement, we noted an increase in cycling neuronal progenitors in the SVZ of PrP-/- mice compared with PrP+/+ mice, as evidenced by double labeling for the proliferation marker Ki67 and doublecortin as well as by BrdU incorporation experiments. Additionally, fewer newly born neurons were detected in the rostral migratory stream of PrP-/- mice. Analysis of the migration of SVZ cells in microexplant cultures from wild type and PrP-/- mice revealed no differences between genotypes or a role for NCAM in this process. Our data demonstrate that PrP plays a critical role in neuronal differentiation of NPCs and suggest that this function is, at least in part, NCAM-dependent.

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SYNAPTIC TRANSMISSION / SIGNALING / ION AND MOLECULE CHANNELS

"PITX2+ INTERNEURON SUBSETS IN THE SPINAL CORD: FUNCTION AND CONNECTIVITY"

Panagiota Apostolou[1], Alexandros Polyzos[2], Laskaro Zagoraiou [1]

[1]Division of Basic Neuroscience, Biomedical Research Foundation of the Academy of Athens
[2]Division of Molecular Biology, Biomedical Research Foundation of the Academy of Athens

The executive component of movement- the task of determining which muscles to activate, how intensely and for how long- depends on neural circuits located in the spinal cord. At the core of these circuits are local interneurons that regulate the pattern and frequency of motor neuron firing through a combination of direct excitation, inhibition and neuromodulation. The transcription factor Pitx2 defines a set of spinal interneurons that further fractionates into V0c cholinergic and V0g glutamatergic subsets. These subsets derive from the same progenitor domain and are the smallest identified so far. These characteristics point to the possibility that they participate in the same circuit. Analysis of their connectivity performed in our lab revealed that V0c form synapses on V0g somata and proximal dendrites and vice versa providing the first evidence of their communication. V0c represents the sole source of C boutons, the first identified spinal modulatory input to motor neurons. Behavioral analysis of mice, in which the output of V0c neurons had been genetically inactivated, demonstrated impairment in a locomotor task-dependent increase in motor neuron firing and muscle activation. Because of the abundance of the C boutons on motor neurons, a more severe phenotype was expected. Furthermore, the synapse persisted after the inactivation. This led to the hypothesis that a second neurotransmitter exists. Indeed, our recent data indicate that the neurotransmitter Cart (cocaine amphetamine regulated transcript) is present in somata and terminals of V0c neurons. Confocal microscopy and the use of volocity software confirmed Cart presence in the presynaptic space.

PHOTOTAXIS AND SYNAPTIC FUNCTION DEREGULATION AFTER EXPOSURE OF DROSOPHILA TO WIRELESS DECT AND MOBILE PHONE ELECTROMAGNETIC FIELDS (EMF).

A. Dimitriadoy[1], A. F. Fragopoulou[2], A. S. Skouroliakou[3], L.H. Margaritis[2] and C. Consoulas[1]

1. Laboratory of Experimental Physiology, Medical School, University of Athens, M. Asias 75, Athens 11527, Greece.
2. Department of Cell Biology and Biophysics, Faculty of Biology, University of Athens, Panepistimiopolis 15784, Zografou, Athens, Greece.
3. Department of Energy Technology Engineering, TEI of Athens, Egaleo 12210, Athens, Greece

The extensive use of DECT and mobile phones demands thorough investigation of possible biological effects caused by these devices. Our previous study in the genetic-model organism *Drosophila melanogaster* has revealed defects in phototaxis (innate tendency of flies to move towards a light-source) caused by these devices during metamorphosis [Krikoni, Consoulas and Margaritis, 2011, unpublished]. To ensure that defects are independent of the genetic background, we explored the EMF effects on two Drosophila strains (Oregon-R and W^{1118}). Flies were exposed to DECT base electromagnetic radiation at an average E-field value of 1,4 V/m - 2,6 V/m under the allocated band of 1.88-1.90 GHz in tandem to mobile phone radiation, working at the 1800 DCS system and at an average E-field value of 20,7 V/m. We found that EMF exposure, during the larval or/and adult stage caused statistically significant, decrease in the percentage of animals moving towards the light. Indeed, adult flies exposed for as little as 40 minutes resulted in defective phototaxis. The permanent nature of the phenotype was revealed by testing the same animal groups one week later. Radiation seems to cause disorientation and weak response to light, suggesting a visual or/and signal processing deregulation in central neuronal circuits. Retinograms from flies exposed to radiation did not reveal a light perception defect, suggesting that photoreceptors and first order retina interneurons were not affected. To explore the possibility of a synaptic defect we tested the Giant Fiber circuit. Our initial efforts indicate that a part of the GFS circuit fails to be electrically activated after EMF exposure, suggesting that a rise in the activation threshold is responsible for the signal processing failure during the execution of phototactic behavior.

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VIBRATIONAL PERCEPTION OF ODORS IN DROSOPHILA MELANOGASTER

A. Gaitanidis[(1)] , M. Anezaki[(1)] , L. Turin[(1)] & E. M. C. Skoulakis[(1)]

(1) Institute of Cellular and Developmental Biology, Biomedical Sciences Research Centre "Alexander Fleming," Vari 16672, Greece

From insects to humans the ability to detect volatile molecules can literally mean the difference between survival and demise. The olfactory system is capable of detecting an extremely large number of volatile chemical stimuli. The capability to recognize and discriminate such a vast number of odorous ligands is thought to be due to the special properties of the olfactory receptors (ORs), the large family of trans-membrane proteins selectively expressed in olfactory sensory neurons (OSNs). However, the fundamental mechanism of olfaction is still debated. Understanding how the olfactory system perceives odors via OSNs and translates them into appropriate behaviors is an important goal in contemporary sensory neuroscience. The mainstream theory of olfaction is based on the lock-and-key model (1). According to this, size, shape and functional groups of odorant compounds determine activation of olfactory receptors. Once an associated odorant molecule binds to an olfactory receptor, the receptor is activated and triggers a neural signal. An alternative theory that was presented firstly by G.Dyson and later by L.Turin is the vibrational theory where the differences in key vibrational frequencies of odorant compounds contribute to odor perception (2,3). Recently, it was demonstrated behaviorally that *Drosophila melanogaster* can distinguish between deuterated and regular odorant compounds, despite the fact that the shapes of such compounds are identical, while the only difference arises in the vibrational spectrum (4). In this study, we used electrophysiological methods to test the response of *Drosophila melanogaster* OSNs to normal and deuterated odors. We provide evidence that the OSNs can discriminate molecular vibrations and they respond differently to odorants that have same shape but different vibrational spectra.

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DIFFERENTIAL EFFECTS OF GLUCOSE DEPRIVATION ON PI3K/AKT AND HIF-1a IN SH-SY5Y CELLS. ROLE OF CALCIUM HOMEOSTASIS.

Ioanna Yiaglissi, Maria Kourti, Katerina Melachroinou*, Olga Mavrofrydi, Maria Paschalidi, Panagiota Papazafiri

Division of Animal and Human Physiology, School of Biology, National and Kapodistrian University of Athens, 15784 Athens, *Division of Basic Neurosciences, Biomedical Research Foundation of the Academy of Athens, 4 Soranou Efesiou street,11527 Athens, Greece

Loss of oxygen and glucose during cerebral ischemia activates a cascade of events ultimately leading to neuronal death and, as a rule, calcium overload contributes to this effect. Accordingly, the involvement of calcium, a likely cell death cause, during transient and prolonged ischemia in human neuroblastoma cell line (SH-SY5Y) was considered, together with its crosstalk with activation of PI3K/Akt survival pathway and hypoxia-inducible factor HIF-1a. Decreased Ca^{2+} concentrations in the endoplasmic reticulum and, increased ion influx from the plasma membrane were detected in ischemic cells while glucose restoration resulted in rapid re-establishment of normal calcium mobilization. Survival was significantly decreased only in the case of prolonged ischemia but, 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, re-addition of glucose in the culture medium leads to re-establishment of p-Akt in control levels as soon as after 5 min. On the contrary, HIF-1a protein levels decreased rapidly and remained markedly lower in the absence of glucose. Endoplasmic reticulum depletion by thapsigargin (Tg), a specific calcium pump inhibitor, caused an immediate abolishment of HIF-1a protein levels but, was able to induce PI3K/Akt activation even though cell death was, as expected, inevitable. Notably, PI3K/Akt activation was detected to accompany specifically 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 ischemic conditions provoke cell death through depletion of intracellular Ca^{2+} stores. The pursuing calcium influx could explain the sustained stimulation of PI3K/Akt, which, however, cannot overmaster the apoptotic signals arising from the depleted endoplasmic reticulum.

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PHARMACOLOGICAL STUDIES AND CRYSTALLIZATION OF THE EXTRACELLULAR DOMAIN OF THE α9 NICOTINIC ACETYLCHOLINE RECEPTOR.

Eleftherios Zarkadas[1, 2], Marios Zouridakis[1], Petros Giastas[1], and Socrates Tzartos[1, 2]

[1]Department of Biochemistry, Hellenic Pasteur Institute, Athens, Greece;
[2]Department of Pharmacy, University of Patras, Patras, Greece

The neuronal α9 subunit of the nicotinic acetylcholine receptor (nAChR) is known to form either homopentamers or heteropentamers in association with the α10 subunit. Elucidation of their crystal structure in atomic detail is essential in order to design highly specific drugs for treatment of several neurological and autoimmune diseases related to them and will serve as the prototype for understanding the structure of all other members of the pentameric ligand-gated ion channels (pLGIC) superfamily. Crystallisation of the intact receptors is a difficult task to fulfil, probably due to their large, hydrophobic transmembrane regions. Therefore, we aimed at the expression of crystallisable water soluble α9 extracellular domain (ECD), where the principal side of the cholinergic ligand-binding site of the α9-containing nAChRs lies. Expression of the α9-ECD in yeast *Pichia pastoris* led to the formation of monomers, oligomers (probably dimers) and a few aggregates. The purified and modified monomeric α9-ECD was suitable for crystallisation trials since it showed significant monodispersity, solubility and stability. Crystal growth optimisation yielded single crystals which diffracted X-rays in high resolution. In addition and despite its monomeric state, the crystallisable α9-ECD exhibited moderate to high binding affinity of various ligands, typical for α9-containing nAChRs. These characteristics taken together render the crystallised α9-ECD a suitable material to study the relation of structure and function in the ligand-binding domain of a neuronal nAChR.

ACTIVATION AND MECHANISTIC DETAILS OF CANNABINOID RECEPTOR 1 COUPLING TO EXTRACELLULAR SIGNAL-REGULATED KINASES 1/2 ACTIVATION IN PRIMARY MICROGLIA CELLS

Aikaterini A. Kalargyrou and Dimitra Mangoura

Development Neurobiology and Neurochemistry Group, Biomedical Research Foundation of the Academy of Athens

Cannabinoids, whether plant-derived, synthetic, or endocannabinoids, are neuroprotective against excitotoxicity and acute brain damage, both in vivo and in culture. Exerting their action through activation of cannabinoid receptor 1 and 2 (CB1, CB2), may afford neuroprotection by blocking excitotoxicity, enhancing trophic factor support, or by suppressing neuroinflammation. The latter is mostly due to their ability to contain excessive activation of migroglia cells, the resident immune cells in the CNS that express constitutively CB1 and CB2 when activated. The dependency of microglia activation on MAPK kinase has been in general recognized, yet whether the heptahelical transmembrane receptor CB1 triggers ERK activation has not been addressed. To investigate this coupling as a possible mechanism of action of CB1, we used primary microglia cultures derived from P0-P1 mouse pups and first established that the CB1R agonist R(+)-Methanandamide specifically stimulated tyrosine phosphorylation of several cellular proteins, including ERK1/2. The time-course of R(+)-MA-induced ERK activation revealed a significant increase by 2min that lasted up to 30min and gradually declined thereafter. Similar time courses were established for the tyrosine kinase Fyn and then Raf, while pharmacological analysis with appropriate inhibitors showed that this Fyn/Raf/ERK pathway was $G_{q/11}$(PLC), $G_{i/o}$, and PKCε-dependent and that it emanated from the lipid rafts. Interestingly we could not establish transactivation of FGFR, a receptor that is recruited by an activated PKCε to provide positive feedback on CB1R-dependent ERK activation. Therefore, while our data provide evidence for the first time for the coupling of CB1 to ERK1/2 in primary microglia and define the intermediate steps, they also indicate that CB1 signaling in these cells is modified as to not engage activation of the pro-inflammatory FGFR, possibly by the diversion of the activities of the involved signaling kinases onto proteins involved in increasing cell adhesion in the expense of decelerating immunosurveillance.

EXPRESSION, PURIFICATION AND CRYSTALLIZATION OF A NEURONAL SUBUNIT OF NICOTINIC ACETYLCHOLINE RECEPTOR

Nikolaos Kouvatsos, Petros Giastas, Socrates Tzartos

Hellenic Pasteur Institute, Athens, Greece

Nicotinic acetylcholine receptors (nAChR) are pentameric membrane receptors located in the central nervous system and involved in numerous brain functions such as learning, memory, mood etc. Furthermore, dysfunction of these receptors has been implicated in a variety of diseases and disorders including Alzheimer disease, Parkinson disease, epilepsy, depression etc. Structural studies are rather limited due to difficulties expressing heterologously the intact receptor. Thus, many studies have been focused on the soluble extracellular domain (ECD) of the receptors where the ligand binding domain lies. In this study, the ECD of a neuronal subunit of nAChR has been expressed and purified successfully in *Pichia pastoris*. The purification process involves a two-step procedure, a NiNTA column and a size exclusion chromatography, resulting in a final yield of 6.2 mg per litre of culture. The domain is expressed glycosylated and presents two populations, one oligomeric and one monomeric. The monomeric form have been further purified and subjected to crystallization trials, leading to the formation of multi-crystals. Optimisation of the crystallisation process gave small but single crystals. Nevertheless, diffraction pattern of the crystals was rather poor. Currently, numerous efforts are performed in order to optimise the diffraction pattern and the crystallization process, as well.

ODOR GENERALIZATION ACCORDING TO VIBRATIONAL SPECTRA

Klio Maniati[1,2], Efthimios M.C. Skoulakis[1], Luca Turin[1]

[1]Biomedical Sciences Research Centre "Alexander Fleming", Vari, Greece
[2]School of Chemical Engineering, National Technical University of Athens, Greece

Much has been speculated about the potential activation mechanism of olfactory receptors by an odorant molecule but the answer remains unknown to date. As shown by Franco et al. 2011, the ability of Drosophila melanogaster to avoid a deuterated compound when trained to avoid a nitrile and vice versa provides strong evidence for a vibrational component in olfaction. We proposed that this phenomenon occurs because the C-D bond stretch vibration is at the same energy as the C≡N bond. The flies sensing the vibrations "confuse" one molecule for the other. It is shown here that Drosophila flies generalize between the S-H and the B-H bond, both in the 2550cm^{-1} region of the Infra Red spectrum. The pair of odorants used to test the resemblance in smell between these two bonds is decaborane and β-mercaptoethanol, two molecules totally dissimilar in shape and chemistry. The flies are shocked against one of the two odorants in order to associate the odor with the shock. Then, in the testing step, they are asked to chose between the other compound and air. If the odors "smell" similarly the flies will confuse one for the other. A second way the topic is approached here is with the use of two isomers, which have different spectra. It is known that 2-hydroxynitriles [cyanohydrins] do not smell of nitriles. Remarkably, this fact has a ready vibrational explanation: the C≡N stretch peak in the IR spectrum of cyanohydrins is absent or very small. We suggest that the new data once again show that a) two odorants with totally different shapes but peaks of comparable absorbance in the IR spectrum smell alike and b) two isomers that have identical shape but dissimilar spectra smell differently. The results seem to clearly show that flies use vibrational assessment to smell

THE EFFECT OF INFLAMMATION ON CNS GLIAL GAP JUNCTIONS

Margarita M. Olympiou, Alexia Kagiava, Irene Sargiannidou, Natasa Schiza, Christos Karaiskos, Kleopas A. Kleopa

Neuroscience Laboratory and Neurology Clinic E, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus

X-linked Charcot-Marie-Tooth (CMT1X) disease is an inherited progressive peripheral neuropathy which may also cause transient CNS manifestations under conditions of metabolic stress and systemic inflammation. CMT1X is caused by mutations affecting the gap junction protein connexin32 (Cx32), which is expressed in both Schwann cells and oligodendrocytes forming gap junctions in myelinated fibers. The mechanisms leading to CNS manifestations in CMT1X patients remain unclear and the effects of inflammation in CNS glia and their gap junctional connectivity have not been systematically studied. In this project we use the lipopolysaccharide (LPS) model of systemic inflammation to study CNS glia gap junctions in wild type and Cx32 mutant mice. In order to model the metabolic stress that causes encephalopathy in CMT1X injected intraperitoneally 30μl of 100μg *E. coli* LPS in wild-type (WT) (n=4) and Cx32 knockout (KOCx32) (n=4) (C57BL/6 x129) mice at the age of P40-P60. The systemic inflammatory response caused by the injection was evaluated by detecting TNF-α and IL-6 levels measured by ELIZA in peripheral blood samples collected at different time-points after the injection. Mice were sacrificed one week after injection and examined by immunohistochemistry of CNS tissues. Staining with microglia marker Iba1 confirmed widespread CNS inflammation with diffusely activated microglia in different areas including the brainstem, cerebellum, brain, and spinal cord in both KOCx32 and WT mice. Immunohistochemistry for the major oligodendrocyte and astrocyte connexins expressed in the white matter revealed that there was reduced expression of astrocytic Cx43 along with a loss of Cx47 gap junctions in both KOCx32 and WT mice. Thus, systemic and CNS inflammation induced by LPS injection appears to cause disruption of the main astrocytic/oligodendrocytic (A/O) gap junctions in the CNS providing a possible explanation for the development of encephalopathy in patients with Cx32 mutations, in whom oligodendrocyte homeostasis depends on Cx43/Cx47 A/O gap junctions.

INTERACTIONS OF OPIOID RECEPTORS WITH REGULATORS OF G PROTEIN SIGNALLING

Maria P. Papakonstantinou, Leonidas J. Leontiadis, Zafiroula Georgoussi

Laboratory of Cellular Signaling and Molecular Pharmacology, Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", Ag. Paraskevi, 15310, Athens

Opioid receptors (OR) μ, δ, and κ couple to Gi/Go proteins to modulate a variety of physiological responses in the nervous system through activation of a diverse array of effector systems. Apart from G proteins, opioid receptor activity is also controlled by interactions with other proteins which contribute to the intricate fine tuning opioid receptor signalling (Georgoussi et al. 2012, Leontiadis et al., 2009). Regulators of G protein Signalling (RGS) comprise a large multifunctional protein family that accelerate GTP hydrolysis of Gα subunits and modulate G protein coupled receptor (GPCR) signalling. Pulldown experiments using GST fusion peptides encompassing intracellular portions of opioid receptors demonstrate the ability of two members of the B/R4-RGS family, such as RGS4 and RGS2, to interact directly with all three OR subtypes. Co-immunoprecipitation studies showed that both RGS proteins confer selectivity to the opioid receptors for coupling with a specific subset of G proteins. On the other hand, using a series of functional assays we demonstrate that although both RGS members co-localize with the membrane bound receptor upon agonist stimulation, they differentially alter OR mediated adenylyl cyclase activity while displaying a similar effect on ERK1,2 phosphorylation. Measurements of cell surface receptors in HEK293 cells co-expressing the ORs along with RGS members have shown that RGS4 and not RGS2 influence the internalization fate of δ-OR. Collectively, our results demonstrate that RGS2 and RGS4 are novel interacting partners and pharmacological targets that negatively modulate opioid receptor signaling.

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GLUTAMATE MGLU5 RECEPTORS PROMOTE NR2B TYROSINE PHOSPHORYLATION AT TYR-1472 IN RAT HIPPOCAMPUS: MODULATION BY ADENOSINEA2A RECEPTORS

Sarantis, K.[1], Tsiamaki, E.[1], Badouna, M.[1], Matsokis, N.[2], Angelatou, F.[1]

[1]Physiology Department, Medical School, University of Patras, Patras, Greece
[2]Laboratory of Animal And Human Physiology, Department of Biology, University of Patras, Patras, Greece

In hippocampus, metabotropic glutamate receptors 5 (mGluR5) have been shown to interact with adenosine A2A receptors in regulating NMDA receptor currents.
In order to further understand the molecular basis of mGluR5/NMDA interactions, we have investigated in the present study the effect of the "in vitro" mGluR5 and adenosine A2A activation on NMDA receptor phosphorylation, as well as on ERK1/2 kinases activation in hippocampal slices. Our experimental approach used the western blotting analysis and specific antibodies against pNR2B at ser1303, pNR2B at tyr1472 and pERK1/2. Our results showed that "in vitro" incubation of rat hippocampal slices with the mGluR5 agonist CHPG:
a) Significantly increased, in a dose-dependent manner, the phosphorylation state of NR2B subunit (tyr-1472) of NMDARs compared to control levels, while CHPG had no effect on the phosphorylation level of NR2B subunit (ser-1303) of NMDARs,
b) Increased significantly, in a dose-dependent manner, the phosphorylation state of ERK1/2 kinases, compared to control.
c) Concomitant stimulation of mGlu5 and NMDA receptors (by ineffective doses of their specific agonists- CHPG and NMDA respectively) synergistically upregulated of the phosphorylation levels of both NR2B at Tyr-1472 and ERK1/2.
Interestingly, when ZM 241385, a selective antagonist of A2A receptors, was co-administrated, abolished the CHPG evoked phosphorylation of NR2B subunit (tyr-1472).
In conclusion, the mGluR5 mediated phosphorylation of NR2B subunit at tyr1472, might underlie the enhancement of mGluR5 receptor evoked currents of NMDARs, shown by electrophysiological studies. Furthermore, the fact that ZM 241385 totally abolished the CHPG-induced phosphorylation of NR2B (tyr 1472) subunit, indicates that in hippocampus, adenosine A2A receptors regulate mGluR5 function by permitting them to interact with NMDA receptor. The significance of the CHPG evoked activation of ERK1/2 signal transduction pathway could be related to synaptic plasticity phenomena in hippocampus which must be further investigated.

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TOWARDS THE DEVELOPMENT OF AN ANTIGEN-SPECIFIC THERAPY FOR MUSCLE-SPECIFIC KINASE MYASTHENIA GRAVIS

Lamprini Skriapa[1,2], Paraskevi Zisimopoulou[1], Nikolaos Trakas[1], Eirini Grapsa[3] and Socrates Tzartos[1,2]

1 Hellenic Pasteur Institute, Athens, Greece,
2 Department of Pharmacy, University of Patras, Rio, Greece
3 Department of Nephrology, Aretaieion, University Hospital, Athens, Greece

The formation and maintenance of the neuromuscular junction (NMJ) and especially the post-synaptic muscle nicotinic acetylcholine receptor (AChR) clustering is orchestrated by the muscle specific kinase (MuSK). The agrin-dependent activation of MuSK is important for the formation of NMJs during embryogenesis and for their post-natal maintenance. Failure of AChR clustering is associated with disorders in NMJ such as myasthenia gravis (MG). MG is an antibody-mediated autoimmune disease in which approximately 85% of MG patients have autoantibodies (autoAbs) against the AChR, while approximately 5-7% of patients have autoAbs against the MuSK. We are developing an antigen-specific therapy in which only anti-MuSK autoAbs will be removed from patients' sera using the immobilized MuSK extracellular domain (ECD) as immunoadsorbent. We have expressed the MuSK-ECD in *Pichia Pastoris* and we used it as immunoadsorbent, after its immobilization on CNBr activated sepharose beads. This MuSK-ECD column was used very efficiently for the depletion of anti-MuSK autoAbs from MuSK-MG patients' sera. With MuSK-ECD protein we immunized rabbits and after 5 injections we could observe typical MG symptoms. Following the same protocol we repeated the immunizations and then we depleted the anti-MuSK auto-Abs from the rabbit's plasma with medium scale experiments. The procedure was successful regarding column's toxicity and effectiveness so we are now proceeding with the rabbit's therapeutic immunoadsorption using the appropriate device.

THE FORMATION AND REGULATION OF HOMO- AND HETERO-DIMERS OF THE HOMER FAMILY PROTEINS MEMBERS

M. Tsampoula[1], S. Thomaidou, G. Papadogiannaki, E. Kyratzi, S. Efthimiopoulos

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

Dysregulation of calcium signaling and oligomerization of amyloid β-peptide (Aβ) that forms the amyloid plaques observed in the brains are considered among the factors that contribute in Alzheimer's disease development (AD). Aβ peptides derive from the processing of amyloid precursor protein (APP), a type I transmebrane protein. Mutations in APP are linked with early-onset familiar AD (FAD). There is evidence that APP, as well as other proteins involved in AD, plays a role in calcium homeostasis. In support, there is evidence that APP interacts with Homer proteins that link plasma membrane calcium channels or receptors with ER calcium channels. This property of Homers which attributes them a function in calcium homeostasis is based on the presence of two functional motifs. The N-terminal EVH1 domain which interacts with Homer partners and the coiled coil (CC) domain that allows them to homodimerize and heterodimerize. Both domains may be important for the formation of large molecular complexes that given the differential expression of Homers in brain may contain various isoforms of Homers. In this study we performed a thorough analysis of all the possible interactions between Homer proteins and the regulation of those interactions. We found that Homer2 and Homer3 homodimerize and that Homer2 heterodimerizes with Homer3. In addition, our data indicates that Homer1 lacking the CC domain does not form homo- and heterodimers. We also confirmed that Homer1 homodimerizes and forms heterodimers with Homer2 and Homer3. The formation of Homer complexes was not found to be affected by increased intracellular calcium concentration or phosphorylation of Homer proteins.

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: THALIS –UOA-Study mechanisms of neurodegeneration in Alzheimer's disease.

CANNABINOID MODULATION OF THE DOPAMINERGIC SYSTEM IN THE RAT BASAL GANGLIA AFTER CHRONIC ADMINISTRATION OF WIN55,212-2 FOLLOWED BY A PERIOD OF ABSTINENCE.

Martha Tsarouchi[1], Panagiotis Perdikaris[1], Eleni Fanarioti[1], Ada Mitsacos[2] 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

Cannabinoids, the principal psychoactive constituents of marijuana, have a wide range of effects on the CNS, including disruption of psychomotor activity. The powerful effects caused by cannabinoids on motor activity probably originate from the fact that the endocannabinoid system can act as a modulator of dopaminergic neurotransmission in the basal ganglia. The central cannabinoid system exerts its physiological actions mainly through type 1 cannabinoid (CB1) receptors. In the present study, we examined protein as well as mRNA expression levels of D2 dopamine receptors (D2DR), Tyrosine Hydroxylase (TH) and Cannabinoid Receptor type 1 (CB1R) in regions of the basal ganglia. For this purpose, male Sprague-Dawley rats received systemic injection of WIN 55,212-2 (1mg/kg, intraperitoneally), a CB1R agonist, once daily for 20 days. Rats were sacrificed by decapitation at 2 hours, 7 and 20 days after the final injection of drug. Brains were removed, frozen, sectioned in a cryostat and stored at -80 ^{o}C until they were processed for *in situ* hybridization. For Western blot analysis, striatum was dissected on ice and homogenized in lysis buffer. According to our results, D2DR as well as TH protein levels were reduced in striatum of chronically treated rats. A significant reduction was also observed in the mRNA expression of TH in Substantia Nigra pars compacta (SNpc). Furthermore, CB1R protein and mRNA expression levels were decreased after long-term WIN55,212-2 treatment. Additionally, our results indicate that there is a partial recovery of mRNA and protein levels following a 7 day cessation of chronic WIN55,212-2 administration. The present findings indicate significant interactions between the endocannabinoid and dopaminergic systems.

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SYSTEMS AND COMPUTATIONAL NEUROSCIENCE

EEG SLEEP SCORING WITH A SEMI-AUTOMATED TOOL BASED ON K-MEANS CLUSTERING OF THE HYPNOSPECTROGRAM

Andreas M Koupparis, Vasileios Kokkinos, George K Kostopoulos
Neurophysiology Unit, Laboratory of Physiology, Medical School, University of Patras

Sleep organization is revealed by sleep scoring, a time-consuming process based on strictly defined visual criteria using EEG, EMG and EOG signals. Our aim was to explore the possibility of sleep scoring using the whole-night time frequency analysis of EEG, termed hypnospectrogram, with a computer-assisted K means clustering method. We present a semi-automated tool that groups sleep periods into similar clusters based on their spectral contents. The scorer assigns these clusters into sleep stages and then manually performs corrections, aided by the information-rich graphic representation provided by the hypnospectrogram. To evaluate the method, 10 whole-night sleep EEG recordings were analyzed and hypnograms were derived using either standard visual scoring under the Rechtshaffen and Kales criteria or semi-automated scoring of the hypnospectrogram derived from a single EEG electrode. Substantial agreement was reached between the two approaches with mean Cohen's kappa at 0.61 when 7 stages were used. This work suggests that the hypnospectrogram can be used as an objective graphical representation of sleep architecture upon which sleep scoring can be performed with computer-assisted methods. This research has been co-financed by the European Union (European Social Fund-ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF)-Research Funding Program: Heracleitus II. Investing in knowledge society through the ESF.

COMPUTATIONAL SYSTEM ANALYSIS OF TYROSINE METABOLIC PATHWAY IN FIBROBLAST CELLS AS A TOOL FOR STUDYING THE BIOCHEMICAL BACKGROUND OF MENTAL DISEASES

Marianthi Logotheti[1,2,3], Eleftherios Pilalis[2], Mahnaz I .Shemirani[1], Aristotelis Chatziioannou[2], Nikolaos Venizelos[1], Fragiskos Kolisis[3]

1. Neuropsychiatric Research Laboratory, Dept. of Clinical Medicine, Örebro University, 701 82 Örebro, Sweden
2. Metabolic Engineering and Bioinformatics Group, Institute of Biology, Medicinal Chemistry & Biotechnology, National Hellenic Research Foundation, 48 Vassileos Constantinou ave., 11635, Athens, Greece
3. Laboratory of Biotechnology, School of Chemical Engineering, National Technical University of Athens, 15780 Athens, Greece

It is established that amino acids involved in neurotransmitter biosynthesis, as for instance tyrosine, a precursor of the neurotransmitter dopamine, play a central role in mental health diseases as Schizophrenia, Bipolar Disorder, Autism and ADHD. In this direction, metabolic network simulations are emerging as valuable tools for the description of complex biochemical systems, such as the metabolic processes that take place in brain cells. In this work we describe an approach to construct such a model, which is based on the Biochemical Systems Theory (BST). This mathematical and computational framework employs power-law functions for the representation of the metabolic pathways. It has been shown that biological systems, characterized by high variability and nonlinearity, are often well modeled by power laws. The greatest advantage of BST models over traditional kinetic models is that in BST models the effect of any given system component on any given process is uniquely described by one kinetic order plus one rate constant for the overall turn-over rate of the process. Therefore, model design is greatly simplified, as a precise estimation of the complexity scale that renders the model robust, is possible. In this work we present the construction of a model of tyrosine metabolism in fibroblasts, consisting of a number of important selected reactions. The choice of this specific cell type has been done for two main reasons: on one hand, tyrosine transport mechanisms in fibroblasts and in the blood-brain barrier are similar and on the other hand, transport kinetics from these cells are available in healthy and in disease conditions.

THE FORCED SWIM TEST INDUCES DIVERGENT GLOBAL TRANSCRIPTOMIC ALTERATIONS IN THE HIPPOCAMPUS OF HIGH *VERSUS* LOW NOVELTY-SEEKER RATS

Dimitris Nasias1, Marianna Kouskou1, Craig R. Tomlinson3, Panagiotis A. Tsonis2, Despina Sanoudou1, Pothitos M. Pitychoutis2, Zeta Papadopoulou-Daifoti1

1 Department of Pharmacology, Medical School, National & Kapodistrian University of Athens, 75 Mikras Asias Street, Goudi, 11527, Athens, Greece
2 Department of Biology & Center for Tissue Regeneration and Engineering (TREND), University of Dayton, 300 College Park, Dayton, OH 45469, USA
3 Department of Medicine and Department of Pharmacology and Toxicology, Dartmouth Hitchcock Medical Center, Dartmouth College, Lebanon, New Hampshire, USA

Susceptibility to stress and depression is individually different. The best animal model of individual differences that can be used to study the neurobiology of affect regards spontaneous reactions to novelty. When naive rats are exposed to the stress of a novel environment they display a highly variable exploratory activity and are classified as high or low responders (HR or LR, respectively). Importantly, HR and LR rats do not exhibit a substantial differentiation in relation to their "depressivelike" *status* in the forced swim test (FST), but may exhibit distinct active behavioral responses during
either FST session. Herein, we hypothesized that a distinct behavioural pattern in the FST would possibly be accompanied by phenotype-dependent alterations in hippocampal global gene expression. At 24 h following the test FST session, HR and LR rats (stressed and unstressed controls) were sacrificed by decapitation and hippocampal samples were independently analyzed on whole rat genome Illumina arrays (RatRef-12 Expression BeadChip) investigating approximately 22.260 coding
transcripts. Significantly changed genes were annotated according to Gene Ontology Classification. Functional analysis into pathways and networks was performed using Ingenuity pathway analysis software. Interestingly, HR and LR rats present distinct behavioral pattern in the pre-test session but comparable "depressive-like" status in the test FST session. Notably, a markedly higher number of genes (i.e. 2.28 fold) was statistically significantly changed following FST in LR rats, as compared to their HR counterparts. Intriguingly, genes associated with neurogenesis (*Ephb2,Nog,Ntf3 Tgfb1,Smad7,Sox2,Srr*) and synaptic plasticity (*Ephb2,Epha5,Ddn,Stx4,Pfn,Syt12*) were induced in the hippocampus of LR rats in response to FST, whereas in HR rats, FST induced pathways associated with induction of apoptotic mechanisms (*Eif2ak3,Sgpp2,Mbtps2,Perk,Gsk3b,Mbtps2,Atf-6,Sgpp2,Rip1*). Overall, these data imply that the hippocampus of HR and LR rats responds to the same stress regimen in a different way at the transcriptional level, despite their seemingly similar "depressive-like" phenotype in FST test.

IDENTIFICATION OF AN INTRALAMINAR BIPOLAR NEURON THAT RESPONDS TO VISUAL STIMULI IN THE RAT SUPERIOR COLLICULUS

Mariam-Eleni Oraiopoulou[1,3], Irene Theodorou[1,2,3], Athanasia Tzanou[1,2], Yannis Dalezios[1,2,3]
[1]Laboratory of Cellular Physiology, Faculty of Medicine, University of Crete, Heraklion, Greece; [2]Institute of Applied and Computational Mathematics, Foundation for Research and Technology-Hellas, Heraklion, Greece; [3]Interdisciplinary Graduate Programme in the Brain and Mind Sciences, Greece.

Superior colliculus (SC) is a multimodal brainstem structure that encodes for rapid eye movements (saccades). The morphology and physiology of the various cell types that comprise the SC network is insufficiently understood. After a series of *in vivo* electrophysiological recordings and juxtacellular labeling of single SC neurons, the brain is perfused with fixative and cut into 70μm serial sections. The sections are developed for the visualization of labeled structures and some of them are also immunoreacted to test for the expression of certain molecular markers in the labeled structures using an electron microscope. Subsequently, they are flat embedded on slides and labeled structures are drawn using a light microscope equipped with a drawing tube. The full reconstruction of the labeled neurons emerges from the combination of serial drawings that are digitized using graphics software. Analysis of the electrophysiological data of labeled neurons is performed offline. We present a bipolar interneuron, which is confined in *stratum griseum superficiale* (SGS) of the SC. The soma lies 1.7mm in the mediolateral plane and 2.6mm in the interaural one. Its dendrites are spiny, run mostly parallel to the SC surface and extend approximately 1.5mm in the mediolateral axis. Its axon branches extensively around the soma and laterally. The rostrocaudal extent of both dendrites and axon is approximately 1mm. Its spontaneous activity was irregular and the neuron increased its firing rate in response to light stimulation restricted to 5 degrees in the horizontal meridian of the contralateral visual field. This pattern of activity suggests that the neuron is involved in the detection of visual targets around the fovea.

CONNECTIVITY PATTERNS OF SLEEP MICROSTRUCTURAL ELEMENTS

Dimitris Sakellariou, Andreas M. Koupparis, Vasileios Kokkinos, George K. Kostopoulos

Department of Physiology, Medical School, University of Patras

During Non-Rapid Eye Movement (NREM) sleep brain is considered to be relatively disconnected from the environment. Also connectedness between brain areas has been found decreased, although we do not know the role played in this by specific elements of sleep microstructure. We developed a method with millisecond time resolution appropriate for assessing brain connectivity during NREM sleep spindles, the φ-coherence. It is based on the observation by Nolte (2008) that when the phase between two signals is zero, the coherence value can be attributed to volume conduction rather than functional neuronal connection. So φ-coherence excludes this value. The new method counts among the effective connectivity measures as advantageous in (a) its superb time resolution (b) ability to study events clustered from different time periods or subjects (c) simultaneous study of any choice from all possible combinations of EEG electrodes and display their φ-coherence in time-frequency topological maps and (d) parameterization of all the plots included in the maps regarding frequency, time and φ-coherence threshold. Preliminary results from 360 fast spindles recorded in whole night sleep of two healthy volunteers the use of φ-coherence indicated a prevailing connectivity pattern of causal interactions mostly from centroparietal regions (C3, Cz, C4, Pz, P3, P4) to right frontotemporal regions (F8, T4). The study aims to help our understanding of the role played by spindles not only in sleep maintenance but also in memory consolidation and in several neuropsychiatric disorders.

IPSILATERALLY ASCENDING PROJECTION NEURONS IN THE RAT SUPERIOR COLLICULUS

Athanasia Tzanou[1,2], Irene Theodorou[1,2,3], Adonis K. Moschovakis[1,2,3], Yannis Dalezios[1,2,3]

[1]Laboratory of Cellular Physiology, Faculty of Medicine, University of Crete, Heraklion, Greece; [2]Institute of Applied and Computational Mathematics, Foundation for Research and Technology-Hellas, Heraklion, Greece; [3]Interdisciplinary Graduate Programme in the Brain and Mind Sciences, Greece.

The superior colliculus (SC) is a laminar midbrain structure with a distinct role in sensory driven orienting behavior, such as rapid eye movements. Its neuronal projections involve oculomotor related targets, reached via ipsilateral ascending, contralateral and ipsilateral descending as well as tectotectal commissural pathways. The aim of this study is to characterize the somatodendritic and axonal profiles of projection neurons, their ultrastructural morphology, neurochemical identity and synaptic connections. We employed the juxtacellular method to record and label single neurons *in vivo*, reconstruct the labeled neuronal structures in detail and identify their neurochemical features with immunocytochemistry and their synaptic connections using electron microscopy. Here, we present a class of neurons characterized by a wide dendritic field with ipsilateral ascending axonal projections to the nucleus of optic tract, a nucleus involved in smooth pursuit eye movements (retinal error signals). Their somata lie in the superficial layers of SC and their elaborate and widespread (~1mm in rostrocaudal and mediolateral extent) dendritic fields run dorsally reaching the surface of the SC. Axons stem ventrally from the soma or a primary dendrite, give local terminals and run laterally through *stratum opticum*, to reach the nucleus of the optic tract, where they form numerous *boutons*. These, anatomically, identical neurons have different physiological properties. The first neuron (*PS18B*) exhibits a low spontaneous activity characterized by burst firing and does not respond to visual or auditory stimuli. The second neuron (*PS19B*) shows a low irregular spontaneous activity. After the onset of a light stimulus in the contralateral upper visual field, its firing increases transiently, pauses for the rest of the stimulus, and increases, again transiently, after the stimulus offset. It is the first time that wide dendritic field neurons with different responses to light stimuli are shown to project to the nucleus of the optic tract.

NEURONS INVOLVED IN THE INTRINSIC COMMUNICATION OF THE RAT SUPERIOR COLLICULUS

Irene Theodorou [1,2,3], Athanasia Tzanou [1,2], Yannis Dalezios [1,2,3]

[1]Laboratory of Cellular Physiology, Faculty of Medicine, University of Crete, Heraklion, Greece; [2]Institute of Applied and Computational Mathematics, Foundation for Research and Technology-Hellas, Heraklion, Greece; [3]Interdisciplinary Graduate Programme in the Brain and Mind Sciences.

The superior colliculus (SC) is a layered brainstem structure involved in the generation of rapid eye movements (saccades). We employed *in vivo* extracellular recording and juxtracellular labelling of single neurons to classify SC neurons in distinct classes, providing information about their detailed morphological and ultrastructural features, electrophysiological properties and specific molecular markers expression. Labelled neuronal structures in consecutive brain sections were thoroughly reconstructed and areas of interest were further studied with electron microscopy. We present three types of interneurons in the superficial layers *(stratum griseum superficiale*, SGS. *stratum opticum*, SO) of the SC. a) Horizontal GABAergic: The soma lies in SGS, giving rise to two primary dendrites running parallel to the SC surface that emit long spines throughout their extent and form numerous spine appendages at their distal part. Their firing activity was irregular, with one of them increasing its activity in the presence of a light stimulus in the contralateral visual field. b) Neurogliaform-like GABAergic. The soma lies in SGS, giving rise to three primary moderately spiny dendrites, extending medially, superficially and ventrally. The axon of the GABAergic neurons, stems either from the soma or a primary dendrite, branches extensively and is restricted to the superficial layers. Numerous *boutons* form Type II (presumably GABAergic) synapses on dendritic shafts. c) Piriform interneurons: Their soma lies in SGS and two primary dendrites branch dorsally, forming a narrow dendritic field and emit dendritic spines that increase in number to the distal dendrites. The axon originates from the soma, remains within the superficial layers, and forms Type I (presumably excitatory) synapses, mainly, on dendritic shafts. These piriform neurons exhibited irregular firing activity with no identifiable pattern. This is the first time that collicular interneurons are characterized in detail in terms of their morphology and function, by employing the *in vivo* juxtacellular method.

AGE-DEPENDENT CHANGES IN MOTOR FUNCTION OF DROSOPHILA.

Sophia V. Xirou and Christos Consoulas

Laboratory of Experimental Physiology, Medical School, University of Athens, M. Asias 75, Athens 11527, Greece.

Most of the multicellular organisms undergo a form of functional decay which differs among species and among individuals of the same species. The genetic-model organism *Drosophila melanogaster* (fruit fly) offers the opportunity to analyze age-related physiological motor functions which would be used as markers for understanding age-related motor changes in mammals including humans.
In this study, the reflective escape behaviour of W^{1118} flies in the form of climbing (negative geotaxis) as a reaction to a mechanical stimulus was studied during aging. It was found that the torn individuals without stimulation use to occupy position at the lower parts of the vial, do not react to stimuli or lose interest very quickly. In contrast to the decreased levels of reactivity (shorter climbing duration), motor ability remained at high levels of functionality until almost the time of death. It is common sense that particular elder human individuals retained at good level of physical motor ability until the very old age.
In addition, we examined electrophysiologically the GFS, the neural circuit underlying escape behaviour, during aging. It was found that signal generation or/and transmission was blocked in aged animals that were exhibiting very low reactivity levels. In contrast, age-matched animals that responded normally to mechanical stimulation had perfectly functional GFS. Therefore, bearing in mind that during aging of W^{1118} flies there is not a functional decay of GFS, we conclude that the system is subjected to a sudden functional dissemination of the signal, an event that is correlated to the physiological rather than to the chronological age of the individual. Both, the reactivity decay and collapse of GFS circuit function can be used as biomarkers of aging to investigate the genetic-molecular basis of age-dependent signal silencing and its behavioural consequences.

OTHER

ANTI-TUMOR EFFECT OF *CROCUS L. SATIVUS* EXTRACT AND SYNERGISTIC ACTION WITH TEMOZOLIMIDE IN A C6 RAT GLIOMA EXPERIMENTAL MODEL

Δημήτριος Γιακουμεττής[1], Αριστείδης Κριτής[2], Αναστασία Τσιγκοτζίδου[3], Αναστασία Κυριακούδη[4], Μαρία Ζ. Τσιμίδου[4], Μαρίνα Μποζίκη[5], Νικόλαος Φόρογλου[1]

[1] Α' Πανεπιστημιακή Νευροχειρουργική Κλινική, ΑΧΕΠΑ, Τμήμα Ιατρικής Α.Π.Θ.
[2] Εργαστήριο Φυσιολογίας, Τμήμα Ιατρικής Α.Π.Θ.
[3] Εργαστήριο Ανατομικής και Ιστολογίας, Τμήμα Κτηνιατρικής Α.Π.Θ
[4] Εργαστήριο Χημείας και Τεχνολογίας Τροφίμων, Τμήμα Χημείας Α.Π.Θ.
[5] Β' Πανεπιστημιακή Νευρολογική Κλινική, ΑΧΕΠΑ, Τμήμα Ιατρικής Α.Π.Θ.

In this study an investigation of the extract from the plant *Crocus Sativus Linneaus* alone or in combination with temozolomide was conducted in an *in vitro* rat C6 glioma model in order to confirm its antitumor activity and determine the mechanism of its action. The extract was derived from red dried stigmas of the plant and was qualitatively and quantitatively analysed by high performance liquid chromatography and mass spectrometry. Cellular viability of rat glial C6 cells was examined after 48 hour-exposure in various concentrations of the extract, temozolomide or their combination. The tetrazolium (MTT) based colorimetric assay was complemented with trypan blue exclusion assay producing thus an index to assess cellular viability. Flow cytometry and TUNEL assay were used to define apoptotic cells. Finally a clonogenic assay was carried out in order to designate impairment of cells' ability to create clones. MTT assay determined half maximal inhibitory concentration to be *3 mg/ml* and *1700 μM* for the extract of *Crocus* and for temozolomide respectively. Trypan blue exclusion test confirmed the aforementioned. Flow cytometry and TUNEL assay suggested that the extract does not induce apoptosis to the cells. The isobolograms derived showed the potential synergistic action of the extract and temozolomide. Finally, clonogenic assay proved that clonogenic ability of C6 cells after exposure to the extract, temozolomide and their combination was impaired. The extract of *Crocus* induces cell death in the C6 rat glioma cell line in a concentration depended manner after 48 hour-exposure. Evidence strongly suggests that apoptosis is not the mechanism of action. Furthermore the combination of the extract with temozolomide proved to be more effective than for each substance alone suggesting synergistic action. Although the molecular paths of action for the extract remain to be unveiled, the promising in vitro results justify the ongoing in vivo study.

SMELLING VIBRATIONS THROUGH Drosophila EVOLUTION.

E. Drimyli, A. Gaitanidis, K. Maniati, M. Anezaki, EMC Skoulakis.

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

A common explanation of molecular recognition by the olfactory system posits that receptors recognize the structure or shape of the odorant molecule. However, Franco *et al.*, previously performed a rigorous test of shape recognition by replacing hydrogen with deuterium in odorants and showed that flies not only differentiate between isotopic odorants, but can be conditioned to selectively avoid the common or the deuterated isotope. Furthermore, flies trained to discriminate against the normal or deuterated isotopes of a compound, selectively avoid the corresponding isotope of a different odorant. Flies trained to avoid a deuterated compound exhibit selective aversion to an unrelated molecule with a vibrational mode in the energy range of the carbon-deuterium stretch. These findings are inconsistent with a shape-only model for smell, and instead support the existence of a molecular-vibration sensing component to olfactory reception. However, the question arises whether this is a common phenomenon among Drosophila species using a broader spectrum of odorants. To answer that we test responses of wild type Drosophila melanogaster to aldehydes, alcohols, ketones, acetates, nitriles and their deuterated isotopes and determine whether the responses are conserved in closely related D. simulans and the more distantly related D.willistoni, D. pseudoobscura, and D.virilis, which span over 40 million years of separation. These behavioural experiments will be supplemented with electrophysiological assessment of the sum total of antennal responses to the odorants using electroantenograms (EAGs).

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THE RS10503253 CSMD1 GENE MAY MEDIATE RISK FOR SCHIZOPHRENIA THROUGH REDUCTION OF COGNITIVE ABILITY.

Erasmia Koiliari [a], Panos Roussos [b,c,d], Emmanouil Pasparakis [a], Todd Lencz [e, f], Anil Malhotra [e, f], Stella G. Giakoumaki [g], Panos Bitsios [a]

[a] Department of Psychiatry, Faculty of Medicine, University of Crete, Heraklion, Crete, Greece
[b] Department of Psychiatry and [c] Department of Genetics and Genomic Science and Institute for Multiscale Biology, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[d] Mental Illness Research, Education, and Clinical Center (VISN 3), James J. Peters VA Medical Center, Bronx, NY, USA
[e] Division of Psychiatry Research, The Zucker Hillside Hospital, Glen Oaks, NY, USA
[f] Feinstein Institute for Medical Research, Manhasset, NY, USA
[g] Department of Psychology, University of Crete, Rethymno, Crete, Greece

The single-nucleotide polymorphism (SNP) rs10503253, located within the CUB and Sushi multiple domains-1 (CSMD1) gene on 8p23.2, has reached genome-wide support as a risk factor for schizophrenia. There is initial but inconclusive evidence for a role of this variant in aspects of cognition. We investigated the neurocognitive effects of the CSMD1 rs10503253 (C/A) polymorphism in a large, demographically homogeneous sample of young, healthy Greek Caucasian males (n=1149) phenotyped for a wide range of neuropsychological measures, which have been shown to be reliable endophenotypes for schizophrenia. The risk 'A' allele was associated with poorer performance on measures of general cognitive ability, strategy formation, spatial and visual working memory, set shifting, target detection and planning for problem solving but not for emotional decision making. Most of these effects were dependent on risk "A" allele dose, with AA and CC homozygotes being the worse and the best respectively, while CA individuals were intermediate. Potential genotype effects in Stroop and verbal memory performance were also suggested by our dataset. These results underline the relevance of the risk "A" allele to neurocognitive functioning and suggest that its detrimental effects on cognition, may be part of the mechanism by which the CSMD1 mediates risk for schizophrenia.

Keywords: cognition; executive function; endophenotypes, CSMD1 gene, schizophrenia

GENETIC OVERLAP BETWEEN METABOLIC AND PSYCHIATRIC DISEASES: THE GOMAP STUDY

E. Marouli[1,2], V. Mamakou[3,4], I. Serafetinidis[4,5], Eleni Zeggini[3], L. Southam[6,7], W. Rayner[6,7], I. Tachmazidou[6], K. Hatzikotoulas[6], A. Matchan[6], F. Gonidakis[8], P. Kontaxakis[9], N. Tentolouris[10], A. Thanopoulou[11], K. Lamnissou[1], GV. Dedoussis[2], Eleftheria Zeggini [6]

[1]Department of Genetics and Biotechnology, Faculty of Biology, University of Athens, 15701 Panepistimiopolis, Ilisia, Greece, [2]Department of Dietetics and Nutritional Science, Harokopio University of Athens, 17671 Athens, Greece, [3]Department of Internal Medicine, Dromokaiteio Hospital, Iera odos 343, 12461 Chaidari, Athens, Greece, [4]University of Athens, Faculty of Medicine, 75 Mikras Asias str., 11527 Goudi, Athens, Greece, [5]Gastroenterology Department, G. Gennimatas General Hospital, Mesogeion 154, 11527 Cholargos, Athens, Greece, [6]Wellcome Trust Sanger Institute, The Morgan Building, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1HH, UK, [7]Wellcome Trust Centre for Human Genetics, University of Oxford, Headington, Oxford, UK, [8]1st Department of Psychiatry, Aiginiteio hospital Athens, Greece, [9]2nd Department of Psychiatry, National and Kapodistrian University of Athens, Attikon General Hospital, Rimini 1, 12462 Chaidari, Athens, Greece, [10]1st Department of Propaedeutic Medicine, University of Athens Medical School, Laiko General Hospital, 17 Ag. Thoma Street, 11527 Athens, Greece, [11]Diabetes Center, 2nd Medical Department, Athens University Medical School, Hippokration Hospital, Athens, Greece

The prevalence of psychiatric diseases (PD), such as schizophrenia and mood disorders (depression or bipolar disorder) is higher in individuals with type 2 diabetes mellitus (T2DM). The co-occurrence of PD and T2DM may be, at least in part, driven by shared genetic factors. Previous genetic studies of T2D and PD give evidence of overlapped risk loci. Although an increased risk of diabetes has been attributed to environmental factors such as diet, lifestyle and psychiatric medication, the association between these two disorders was noticed well before the advent of current lifestyles and pharmacological interventions, enhancing the possibility of a shared genetic basis. So, common environmental, metabolic and genetic mechanisms may be implicated in the pathogenesis of psychiatric diseases and T2DM. The study GOMAP (Genetic Overlap of Metabolic and Psychiatric Diseases) aims to collect 3,500 patients with T2D, psychiatric disease, and both T2D and psychiatric disease. All participants consent in a written form to participate in the study. Volunteers so far have been recruited from the Dromokaiteio and Aiginiteio hospitals, the Hippokrateio and Laiko Diabetes centres. The study population consists of 1,300 individuals with PD without T2DM, 1,100 individuals with T2DM without PD and 1,100 individuals with PD and T2DM. We have so far collected 643 samples of individuals with T2DM without PD, 1220 samples of individuals with PD (1051 of these with schizophrenia, 114 with bipolar disease, 55 with depression) and 355 samples of individuals with PD and T2DM (293 with schizophrenia and T2DM, 35 with Bipolar disease and T2DM and 27 with depression and T2DM). We have obtained data from the epidemiological, clinical and lifestyle parameters of the individuals with PD and their correlation with the presence of T2DM. DNA has been extracted from 2400 patients and we are generating genome-wide data from the Illumina HumanCoreExome array in the first set of 850 samples. Finally, our goal is to identify genetic loci connecting PD with T2DM and replicate our results with other datasets.

AFFECTIVE STARTLE MODULATION, VERBAL AND WORKING MEMORY AND THEIR RELATION TO CACNA1C GENOTYPE.

Emmanouil Pasparakis [a], Erasmia Koiliari [a], Chrysoula Zouraraki[b], Eva-Maria Tsapakis [a,c], Panos Roussos[a,d], Stella G. Giakoumaki [a,b], Panos Bitsios [a]

[a] Department of Psychiatry and Behavioral Sciences, Faculty of Medicine, University of Crete, Heraklion, Crete, Greece
[b] Department of Psychology, University of Crete, Rethymno, Crete, Greece
[c] Aghios Charalambos Mental Health Clinic, Heraklion, Crete, Greece
[d] Department of Psychiatry, The Mount Sinai School of Medicine, New York, NY, USA

The CACNA1C gene codes for the pore-forming a1C subunit of the L-type voltage-gated calcium channel, playing an important role in synaptic plasticity, memory formation, learning and behavior. The CACNA1C rs1006737 risk 'A' allele is associated with bipolar disorder (BD), major depression (MD) and schizophrenia but clarification of its effects on relevant endophenotypes is required, in order to understand how it affects brain function and identify a mechanism of risk. The non-emotional verbal memory (VM) and working memory (WM) tasks and the affective startle modulation (ASM) (targeting hippocampal, prefrontal and amygdalo-hippocampal-limbic circuitry respectively) are such relevant endophenotypes related to genetic risk for BD and MD (VM, ASM) and schizophrenia (WM). 194 healthy males (GG: 111, GA: 67, AA: 16) were phenotyped for VM, WM, ASM and state mood. Genotypes did not differ for demographic variables, IQ, WM and state mood on arrival. The risk A allele homozygotes had poorer performance in the VM recognition phase, consistent with encoding difficulties compared to the other genotypes. They became more anxious prior to startle testing, suggesting higher contextual sensitivity, with an exaggerated pattern of high startle reactivity in the unpleasant pictures, while the normal startle attenuation during pleasant pictures viewing was less marked. These abnormalities of ASM in the risk individuals are consistent with exaggerated and attenuated activation of their defense and appetitive systems respectively as in anxious/depressed patients, and were predicted by poor VM but not WM performance. The lack of effect of the risk allele on a WM task targeting emotionally neutral cognitive processing related to prefrontal cortex and the absence of association between WM and ASM, suggest that this gene has primary effects on amygdala/hippocampal emotional circuitry and thus may be associated more strongly with mood disorders than with schizophrenia.

GABA IMMUNOHISTOCHEMISTRY IN THE RAT HIPPOCAMPUS – COMPARISON BETWEEN PERFUSION AND IMMERSION FIXATION

N. Pipidou[1], E. Kouvaras[1,2], O. Sinani[1], I.-D. Papageorgiou[1], A. Kantikou[1], G. Koukoulis[2], E. Asprodini[1]

[1]Laboratory of Pharmacology, School of Medicine, University of Thessaly, Larissa.
[2]Laboratory of Phathology, School of Medicine, University of Thessaly, Larissa.

The hippocampal formation is differentiated along its longitudinal axis in terms of its anatomy, biochemistry and function. Electrophysiological evidence, obtained from *in vitro* slice preparations, shows that the ventral hippocampus, compared to the dorsal, exhibits enhanced susceptibility to epileptiform discharges and reduced GABAergic synaptic transmission. Paradoxically, the ventral hippocampus exhibits higher GABAergic neuronal densities and GABA concentration compared to dorsal hippocampus. The controversy between functional and anatomical data could be elucidated if one could study both function and anatomy within the same tissue. The purpose of the present study was, therefore, to evaluate GABA immunoreactivity in the dorsal and ventral hippocampus using immersion-perfused slices and compare it to perfused tissue.
Male Wistar rats (n=15) weighing 250-300gr were used. Tissue was fixed (4% PFA/0,1M PB): **(A)** by whole animal trascardial perfusion and **(B)** by hippocampal slice (500μm) immersion either **(I)** immediately after brain excision or **(II)** after 1h incubation in oxygenated cerebro-spinal fluid. Fixed tissue (4 μm) was stained histologically using Eosin/Hematoxylin or immunohistochemically for GABA using primary polyclonal rabbit antibody against GABA (1:500) and fluoroscein-conjugated secondary goat anti-rabbit IgG (H+L) (1:400).
Tissue specimens, fixed by either perfusion or immersion, retained their histomorphological features and showed intense immunohistochemical staining. Histological sections cut from samples of groups **(I)** and **(II)**, were stained with Haematoxylin and Eosin stain and under microscopic evaluation showed complete tissue viability. In accordance to previously published results, the numeric density of GABA-immunoreactive neurons was higher in the ventral compared to the dorsal hippocampus using either fixation method.
In conclusion, immersion-fixation of hippocampal tissue shows comparable histological and immunohistochemical staining to that obtained following whole-animal perfusion. This fixation method may, therefore, be used for the histological/immuhistochemical study of hippocampal slices which have been previously studied electrophysiologically *in vitro*.
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LONG-TERM EFFECTS OF EARLY-LIFE SEIZURES ON CORTICAL EXCITABILITY

Rigas P[1], Nikita M[1], Sigalas C[1], Tsakanikas P[1,2], Skaliora I[1]

[1]Biomedical Research Foundation of the Academy of Athens, Athens, Greece
[2]Agricultural University of Athens, Athens, Greece

Purpose: The aim of our study is to test the long-term effects of single early life seizures (ELSs) on mouse cortical function and excitability. In humans ELSs can have long lasting detrimental effects on behaviour and cognition, along with a higher propensity for epilepsy. However, clinical studies of ELSs are challenged by the many factors that affect their outcome. In this respect, animal models allow the study of these factors in isolation and hence provide an insight to the underlying mechanisms.
Method: In vitro electrophysiological studies were combined with behavioural tests to assess the long-term effects of single PTZ-induced ELSs on cortical excitability within the context of overall animal behaviour. In order to address the issue of a critical period for the severity of single ELSs, seizures were induced at two distinct developmental periods: P10-15 and P20-25 and mice were left to reach adulthood (>3mo) for behavioural tests and electrophysiology. Cortical excitability was assessed by recording:
(a) spontaneous network activity (Up states) in brain slices,
(b) the induction (i.e. delay to appear after transition from normal to 0 Mg2+ slice buffer) and
(c) the expression (i.e. spectral content) of spike and wave discharges (SWDs) in cortical slices in the 0 Mg2+ model of epilepsy.
Results: Our preliminary results show that single ELSs have a minimal effect on the behaviour of mice treated at P10-15 but not at P20-25, with no effect however on up states and SWDs. On the contrary, the onset of epileptiform activity tends to occur faster in mice with ELSs as opposed to untreated mice.
Conclusion: Single ELSs affect neither normal nor paroxysmal intrinsic cortical activity, however they cause the cortex to be less resistant to the induction of seizures.

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