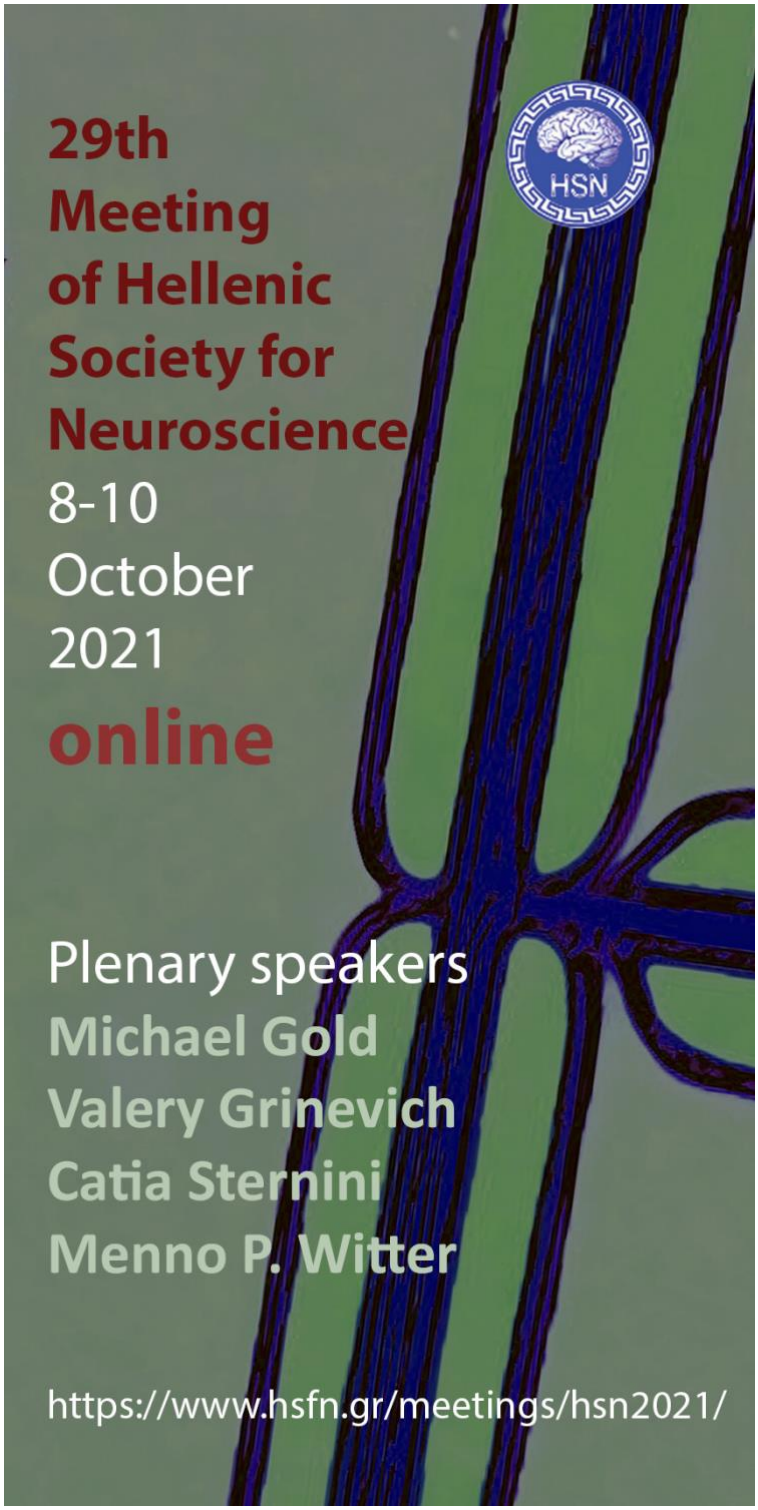
29th Meeting of Hellenic Society for Neuroscience

“Brain in function and dysfunction”



8-10 October 2021, online

welcome letter

Those who dream by day are cognizant of many things
which escape those who dream only by night
Edgar Allan Poe

The 29th meeting of the Hellenic Society for Neuroscience aims to bring together distinguished neuroscientists and promising young investigators. Due to these unprecedented times our national Society decided to hold this years' meeting on line. However, technology helps us consolidate the distance and we can still attend numerous diverse talks on distinctive neuroscientific fields from development to pain and from optogenetics to organoids in basic neuroscience.

Hence, I am welcoming you to our event wishing you to have a fruitful and enjoyable meeting with the hope to meet in person in future instances.

Anastasia Tsingotjidou

Chair of the Organizing Committee

organizing committee

Anastasia Tsingotjidou – chair
Associate Professor, Laboratory of Anatomy, Histology and Embryology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki

Chryssa Bekiari, postdoctoral researcher, Laboratory of Anatomy, Histology and Embryology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki

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Vasilios Kimiskidis, Professor of Neurology & Clinical Neurophysiology, 1st Department of Neurology, AHEPA University Hospital, Aristotle University of Thessaloniki

Efstatios Kosmidis, Associate Professor, Laboratory of Physiology, Medical School, Aristotle University of Thessaloniki

Georgios Pampalakis, Assistant Professor, School of Pharmacy, Aristotle University of Thessaloniki

Nikos Papaioannou, Rector, Professor of Pathological Anatomy, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki

Zoi Polyzopoulou, Professor of Clinical Pathology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki

Evangelia Spandou, Professor, Laboratory of Experimental Physiology, School of Medicine, Aristotle University of Thessaloniki

Despina Tata, Associate Professor, School of Psychology, Faculty of Philosophy, Aristotle University of Thessaloniki

plenary speakers

Michael Gold
Professor of Neurobiology, University of Pittsburgh
“Exploring mechanisms underlying the initiation and maintenance of persistent pain”

Valery Grinevich
MD, PhD, Dr. Med. Sci., Dr. rer. nat. habil., Full Professor,Chair, Department of Neuropeptide Research in Psychiatry, Central Institute of Mental Health, Heidelberg University, Germany
"Optogenetics to interrogate the brain oxytocin system"

Catia Sternini, MD, Professor of Medicine and Neurobiology, Division of Digestive Diseases, David Geffen School of Medicine, UCLA
“Opioid Receptors in the Gut: from Neurons to Immune Cells, from Health to Disease”

Menno P. Witter
Affiliation: Kavli Institute for Systems Neuroscience, NTNU Norwegian University of Science and Technology, Trondheim, Norway

Menno P. Witter is professor of Neuroscience at the Kavli Institute for Systems Neuroscience and Centre for Neural Computation at the Norwegian University of Science and Technology (NTNU) in Trondheim, Norway. He received his Ph.D. in 1985 at the VU University in Amsterdam, The Netherlands, where he subsequently started his independent research on the anatomical organization of the hippocampal region. He became full professor in 1996 and served as director of The Institute for Clinical Neuroscience at the VU University Medical School in Amsterdam (1996-2006). He further served as director of the of the Graduate School of Neurosciences Amsterdam (1996-2006). He joined professors May-Britt and Edvard Moser at the Kavli Institute for Systems Neuroscience in 2007, continuing a productive collaborative period that started in 2000, leading to the discovery of grid cells in the entorhinal cortex in 2005 for which the Mosers were awarded the Nobel prize in Physiology and Medicine in 2014. He is the initiator and current director of the Norwegian Research School in Neuroscience since 2013. He is elected member of the Royal Norwegian Society of Sciences and Letters, and The Norwegian Academy of Science, member of the European Dana Alliance for the Brain and received the Olav Thon Foundation International Research Award (2016).
He holds a visiting position at the Graduate School of Life Sciences and Faculty of Medicine, Tohoku University, Sendai, Japan. He is member of the Advisory Board Center for Behavioral Brain Sciences – CBBS Otto-von-Guericke-Universität Magdeburg, and Chair of Marseille

Neuroschool Scientific, Educational and Economic Advisory Board. He is also President of the board of the FENS Trust Foundation, The Netherlands.
His current research focusses on the structural neurobiology of the lateral and medial entorhinal cortex and their contributions to learning and memory. His group also works on the mechanisms of Alzheimer's disease, using animal models. He published over 200 papers in international peer reviewed journals. For more information and full publication list, go to http://www.ntnu.edu/employees/witter

Title: "The entorhinal cortex. Key player in the medial temporal lobe memory system"

The medial temporal lobe memory system is crucially involved in conscious memory. It comprises the hippocampal formation and the entorhinal cortex. The quest to understand the system started in the late 1950th and was boosted by the discovery of spatially modulated neurons in the hippocampus in 1971. Subsequently, many spatially modulated neurons were discovered in the entorhinal cortex, and research has focused on the pivotal position of the entorhinal cortex and its two subdivisions, the lateral and medial entorhinal cortex.
The current, generally accepted organizational scheme is that the medial entorhinal complex conveys spatial information to HF, the 'where pathway', whereas the lateral entorhinal complex conveys information concerning objects, the 'what pathway' to HF. In my presentation I aim to brief you on the development of this scheme and show recent connectional data indicating that this concept needs to be revised. I will elaborate on recent findings indicating that the local networks of the lateral and medial entorhinal cortex are remarkably similar and emphasize the difference in extrinsic connectivity as a major defining feature for the known functional differences. The lateral entorhinal cortex is a high-order multimodal cortex appropriately positioned to integrate representations of the external world. In contrast, the medial entorhinal cortex seems to provide information to faithfully map the subjects allocentric position in space.
In my lecture, I will detail some of the groundbreaking findings in rodents that led to this alternative functional view and in the last part emphasize recent findings on the intriguing interplay between the two parts of the entorhinal cortex.

ALBA event

Carmen Sandi, EPFL (École Polytechnique Fédérale de Lausanne), Switzerland

Igor Branchi, Center for Behavioral Sciences and Mental Health, Istituto Superiore di Sanità, Italy

Myrto Denaxa, BSRC Alexander Fleming, Athens, Greece

https://www.alba.network/alba-hsn-2021:

The session will start with an introduction of the ALBA Network activities by Carmen Sandi (ALBA Past-Chair; EPFL, Switzerland).

The panelists Igor Branchi (Sapienza University of Rome and Istituto Superiore di Sanità, Italiy) will discuss DEI issues in Italy; and Myrto Denaxa (Group Leader BSRC "Alexander Fleming") will discuss DEI issues in Greece.

A discussion with the audience will follow the presentations.

symposia

S1: Brain-Gut Axis

Chair: Marirena Grigoriou

Chris Zimmerman

Affiliation: Princeton Neuroscience Institute, Princeton University USA.

Title: “The neurobiology of thirst”

Valeria Silva

Affiliation: Centro Interdisciplinario de Neurociencia de Valparaíso (CINV), Universidad de Valparaíso, Chile.

Title: to be announced

Dafni Hadjieconomou
Affiliation: MRC London Institute of Medical Sciences & Faculty of Medicine, Imperial College London, London, UK.
Title: “Clever guts and pregnant brains: How gut neurons regulate physiology”

The metabolic plasticity of the brain-gut axis

Dafni Hadjieconomou[1,2], George King[1,2], Pedro Gaspar[1,2], Alessandro Mineo[1,2], Laura Blackie[1,2], Tomotsune Ameku[1,2], Chris Studd[1,2], Alex de Mendoza[3,4], Fengqiu Diao[5], Benjamin White[5], Andre Brown[1,2], Pierre-Yves Plaçais[6], Thomas Preat[6] and Irene Miguel-Aliaga[1,2]

[1] MRC London Institute of Medical Sciences, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0NN, UK
[2] Faculty of Medicine, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0NN, UK
[3] Australian Research Council Centre of Excellence in Plant Energy Biology, School of Molecular Sciences, The University of Western Australia, Perth, WA, 6009, Australia
[4] Harry Perkins Institute of Medical Research, Perth, WA, 6009, Australia
[5] Laboratory of Molecular Biology, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA.
[6] Genes and Dynamics of Memory Systems, Brain Plasticity Unit, CNRS, ESPCI Paris, PSL Research University, 10 rue Vauquelin, 75005 Paris, France

A communication network between the brain and the gut, the so called “brain-gut axis”, has emerged as a key player in metabolic regulation. Nevertheless, in depth understanding of the cells and molecular mechanisms involved is still lacking, partly due to the astonishing anatomical complexity of the underlying neural circuits in mammals. Using the simpler *Drosophila* brain-gut axis as model system, we have recently discovered that adult enteric neurons are functionally plastic and this plasticity is physiologically important for adjusting food intake to subserve metabolic demands. I explored this in the context of reproductive needs in females, the effect of which on food intake is evolutionary conserved across multiple species. Specifically, I found that signals from two different organs, an ovarian steroid and a gut enteroendocrine hormone, relay reproductive status directly to gut neurons. These neurons in turn release a neuropeptide that acts on the gut muscle, in a portion equivalent to the mamalian stomach, and changes its mechanichal properties allowing for increased food intake. I now aim to investigate how other homeostatic challenges, such as an obesogenic diet, the levels of physical activity and their combination impact gut-neuron plasticity and function. This integrative approach will uncover novel, and likely evolutionary conserved, mechanisms employed by gut neurons to regulate metabolic adaptation, and may help elucidate their contribution to the development of pathophysiology.

S2: Junior Scientists Symposium

Anastasia Vamvaka-Iakovou, *Institute of Biosciences and Applications, NCSR Demokritos, Athens, Greece and Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal*

A novel method for isolation of spontaneously-released exosomes from mouse and human brain

Anastasia Vamvaka-Iakovou [1,2], Patrícia Gomes[2], Carlos Noguera-Ortiz[3], Martina Samiotaki[4], George Stamatakis[4], George Panayotou[4], Christos Gatsogiannis[5], Nuno Sousa[2], Dimitrios Kapogiannis[3], Bruno Costa-Silva[6], Ioannis Sotiropoulos [1,2]

[1] Institute of Biosciences and Applications, NCSR Demokritos, Athens, Greece.

[2] Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal.

[3] Laboratory of Clinical Investigation, Intramural Research Program, National Institute on Aging, NIH, Baltimore, Maryland, USA.

[4] Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", 16672, Vari, Attica, Greece.

[5] Department of Structural Biochemistry, Max Planck Institute of Molecular Physiology, Dortmund, 44227, Germany.

[6] Systems Oncology Group, Champalimaud Research, Champalimaud Centre for the Unknown, Av. Brasília, 1400-038 Lisbon, Portugal.

Abstract

Extracellular vesicles (EVs), especially exosomes, exhibit great potential for the diagnosis and treatment of brain disorders, representing an advantageous tool for Precision medicine which demands high-quality human biospecimens, especially in complex disorders in which pathological and specimen heterogeneity as well diverse individual clinical profile often complicate the development of precision therapeutic schemes and patient-tailored treatments. Thus, the collection and characterization of physiologically relevant exosomes are of the utmost importance. However, current brain EV and exosome isolation approaches rely on tissue dissociation, which can contaminate EV fractions with intracellular vesicles. Based on multiscale analytical platforms such as cryo-EM, label-free proteomics, advanced flow cytometry, and ExoView analyses, we hereby present an efficient purification method that captures a more physiologically relevant, exosome-enriched EV population spontaneously released by mouse and human brain tissue. The spontaneous release method of EV yield may contribute to the characterization and biomarker profile of physiologically relevant brain-derived exosomes in brain function and pathology.

Chrysoula Dioli, *Institute of Biosciences and Applications, NCSR Demokritos, Athens, Greece and Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal*

Neurogenic brain plasticity under stress: a novel role for Tau dyshomeostasis

Chrysoula Dioli[1,2], Patricia Patricio[2], Nuno Sousa[1,2], Luisa Pinto[2], Ioannis Sotiropoulos[1,2]

[1] Institute of Biosciences and Applications, NCSR Demokritos, Athens, Greece
[2] Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Chronic stress is implicated in susceptibility to brain pathologies such as depression or Alzheimer's disease (AD), as it promotes neural plasticity damage and glial reactivity, which can lead to dendritic/synaptic loss, reduced neurogenesis, mood deficits, and impaired cognition. While the suggested underlying mechanisms include glutamate release, NMDA signaling and/or reduced levels of neurotrophic factors, little attention has been given to the importance of cytoskeletal dynamics in newborn cells, which allows them to divide, migrate, differentiate and synaptically integrate into the pre- existent brain network(s). Despite the well-known role of Tau in regulating cytoskeletal dynamics and the suggested relationship of hyperphosphorylated Tau with cytoskeletal damage, the involvement of Tau in damage of neurogenic brain plasticity caused by chronic stress remains poorly explored. Herein, we demonstrate that chronic stress triggers Tau hyperphosphorylation and 4R-Tau:3R-Tau imbalance in newborn cells and immature neurons of the adult brain via the PI3K/mTOR/GSK3β/β-catenin signaling, known to regulate cell survival and proliferation. Moreover, deletion of Tau attenuated the stress-driven neurogenic, but not astrogliogenic or oligodendrogenic, damage in the cytogenic niches of the adult brain (hippocampus and subventricular zone-olfactory bulb system) indicating the neuronal- specific involvement of Tau in the stress-driven cytogenic damage of the adult brain. We also monitor the impact of stress on dendritic maturation of immature neurons demonstrating for the first time that chronic stress triggers opposite neuroplastic effect on different dendritic compartments of the same immature neuron. In summary, the above studies suggest one cell-autonomous and one non-cell autonomous mechanism through which chronic stress damages neurogenic plasticity in different areas of the adult brain. Overall, our current findings add novel mechanistic insights about the involvement of Tau dyshomeostasis into the cellular cascades that convey the pathogenic role of chronic stress in adult brain plasticity and open new pathways for strategies designed to mitigate its deleterious effects.

Engie Prifti, *Institute for Fundamental Biomedical Research, and 2Institute for Bio-innovation, Biomedical Sciences Research Centre "Alexander Fleming," Vari, Greece*
The Two Cysteines of Tau Protein Are Functionally Distinct and Contribute Differentially to Its Pathogenicity in Vivo

Engie Prifti,[1] Eleni N. Tsakiri,1 Ergina Vourkou,[1] George Stamatakis,[2] Martina Samiotaki,[2] and Katerina Papanikolopoulou[1]
[1]*Institute for Fundamental Biomedical Research, and*
[2]*Institute for Bio-innovation, Biomedical Sciences Research Centre "Alexander Fleming," Vari, 16672, Greece*

Although Tau accumulation is clearly linked to Tau pathogenesis in Alzheimer's disease and other Tauopathies, the mechanism that initiates the aggregation of a highly soluble protein such as Tau in vivo remains largely unanswered. Interestingly, in vitro Tau can be induced to form fibrillar filaments by oxidation of its two cysteine residues, generating an intermolecular disulfide bond that promotes dimerization and fibrillization. The recently solved structures of Tau filaments revealed that the two cysteine residues are not structurally equivalent since Cys-322 is incorporated into the core of the fibril whereas Cys-219 projects away from the core to form the fuzzy coat. Remarkably, mutation of these Cysteines to Alanines differentially affects Tau-mediated toxicity and dysfunction in the well-established Drosophila Tauopathy model. Replacement of each one of the two Cysteine residues leads to differential effects on Tau's stability, phosphorylation status, aggregation propensity, resistance to oxidative stress, learning and memory performance. Our work clearly shows that the two cysteines are not functionally equivalent and uncovers a critical role of Cys-322 in determining Tau toxicity and dysfunction

Anna Bourouliti, *Institute for Fundamental Biomedical Research, BSRC "Alexander Fleming", Vari, Greece and Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, Greece*

Protein-synthesis-independent memory attenuates spaced training-generated protein synthesis-dependent memory

Anna Bourouliti[1,2], and Efthimios Skoulakis[1]

[1] *Institute for Fundamental Biomedical Research, BSRC "Alexander Fleming", Vari, Greece*
[2]*Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, Greece*

Long lasting memories assume many different forms. Two such memories are apparent n *Drosophila*, Protein Synthesis Independent Memory (PSI-M) and Protein Synthesis Dependent Long Term Memory (PSD-LTM). While PSI-M perdures for two days, PSD-LTM may last for weeks. These memories are also differentiated operationally and molecularly by their dependence on *de novo* protein synthesis. Specifically, PSD-LTM is blocked by administration of the protein synthesis inhibitor cyclohexamide (CXM), contrary to PSI-M which remains unaffected. Suggestive behavioural and molecular evidence indicates distinct and potentially antagonistic formation mechanisms. Importantly however, it remains unknown whether PSD-LTM and PSI-M preserve their putative initial antagonistic traits after consolidation. Memory-type specific mechanisms may operate in parallel, antagonistically or even synergistically, to yield enhanced or attenuated behavioral outputs.

Thus, we investigated the potential transition from consolidated PSI-M to a longer-lasting PSD-LTM using the same CS and US stimuli. We trained flies so that they form PSI-M, then re-trained them, and studied the nature of the newly acquired memory. Our results show that enhanced memory output observed after re-training is CXM-independent. Consequently, PSI-M does not serve as a potential substrate for PSD-LTM formation. Furthermore, to our surprise, we observed that PSD-LTM formation which is normally induced by spaced training, cannot form in the presence of consolidated PSI-M. So, we conclude that consolidated PSI-M of a CS/US contingency leads to attenuation of Protein Synthesis Dependent long-term storage of the same information.

Vasiliki Meletaki, *Cognitive Neuroscience Research Unit, Department of Psychology, City, University of London*
Title: Dance your emotions: Expertise modulates visual and embodied emotion

VasilikiMeletaki ,BettinaForster ,BeatrizCalvo-Merino

[1]*Cognitive Neuroscience Research Unit, Department of Psychology, City, University of London*

Dance expertise modulates visual, sensorimotor and psychophysiological responses to affective body movements. We investigated if this enhanced emotion sensitivity is domain-specific or general to other forms of emotional expression by comparing neural responses to happy, fearful and neutral facial expressions in professional dancers/experts and control participants with no prior dance experience. Visual Evoked Potentials (VEPs) and Somatosensory Evoked Potentials (SEPs) were measured in the somatosensory cortex while participants performed a visual emotion/ gender discrimination task on emotional faces. In half of the trials participants received a tactile stimulation to enhance activity over SCx. Our results showed distinct group differences and group x emotion interactions over the occipital lobe for the VEPs (P1, N170 and P2) and over the somatosensory cortex in the SEPs (P50, N80, P100 and N140) suggesting a differential visual and embodied response to facial expression between experts and non-experts. This data suggests an enhanced general emotion sensitivity in experts that is reflected beyond the observation of their motor acquired skill but onto general and everyday emotional expressions. These results point towards new venues for emotional sensitivity training based on engaging motor and artistic knowledge.

Thanasis Rogdakis, *Department of Pharmacology, Medical School, University of Crete, Heraklion, Greece*

Identifying novel neurotrophin analogues as putative therapeutics against Alzheimer's Disease

Thanasis Rogdakis[1,2*], Despoina Charou[1,2*], Marianna Papadopoulou[1], Eleni Papadimitriou[1], Dimitris Lipitkas[1], Alessia Latorrata[3], Daniele Narducci[3], Theodora Calogeropoulou[3], Achille Gravanis[1,2], Ioannis Charalampopoulos[1,2]

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** equal contribution*

Neurotrophins, like Nerve Growth Factor (NGF) or Brain Derived Neurotrophic Factor (BDNF), are growth factors that exert neuroprotective effects by preventing neuronal death and promoting neurogenesis. They act by binding to their respective high-affinity, pro-survival receptors TrkA, TrkB or TrkC. Additionally, all mature neurotrophins and their immature pro-isoforms bind to $p75^{NTR}$ death receptor, resulting to the activation of pro-apoptotic signal transduction cascades. While these molecules have been shown to slow or prevent neurodegeneration, their reduced bioavailability and inability to penetrate the blood-brain-barrier limit their use as potential therapeutics. Previous work in our lab has shown that the endogenous neurosteroid Dehydroepiandrosterone binds and activates both NGF receptors, namely TrkA and $p75^{NTR}$ (Lazaridis et al., 2011). Using a library of novel, synthetic DHEA derivatives, we screened for molecules that exert neuroprotective actions and selectivity induce neurotrophin receptor activation. Neurotrophin mimetics were tested on neurotrophin-dependent TrkA/TrkB/$p75^{NTR}$ cell lines, investigating receptors activation and downstream signaling, as well as cell survival. Based on the biological evaluation of the compounds, we selected the most potent activators of TrkA and TrkB to further investigate their action in primary neuronal cells and neural stem cells. We report that we have identified ENTA013, a selective TrkA agonist, ENTA011, a TrkB and $p75^{NTR}$ agonist, and ENTA061, a selective TrkB agonist. ENTA013 can induce survival of primary Dorsal Root Ganglion neurons upon NGF withdrawal, mimicking NGF action. ENTA011 and ENTA061 promote neural stem cell differentiation, proliferation and survival upon Aβ treatment through TrkB receptor activation. Furthermore, all compounds protect primary hippocampal neurons against Amyloid β-induced apoptosis and synapse degeneration. These compounds present favourable pharmacological properties as lead molecules for further preclinical development in animal models of Alzheimer's Disease and human clinical trials.

Reference

Lazaridis I, Charalampopoulos I, et al. ***PLoS Biol.*** 2011 Apr;9(4):e1001051.

S3: Pain

Chairs: Anastasia Tsingotjidou and Alex Binshtok

Igor Spigelman

Affiliation: Chair of the Section of Oral Biology, UCLA School of Dentistry, USA

Title:"Selective targeting of peripheral cannabinoid receptors prevents behavioral symptoms and sensitization of trigeminal neurons in mouse models of migraine and medication overuse headache"

Toru Yamamoto [1], Yatendra Mulpuri [1], Mikhail Izraylev [1], Qianyi Li [1], Menooa Simonian [1], Christian Kramme [1], Brian L. Schmidt [2], Herbert H. Seltzman [3] and Igor Spigelman [1,4]*.

[1] Division of Oral Biology & Medicine, School of Dentistry, University of California, Los Angeles, Los Angeles, CA. [2] Department of Oral & Maxillofacial Surgery and Bluestone Center for Clinical Research, New York University College of Dentistry, New York, NY. [3] Organic and Medicinal Chemistry, Research Triangle Institute, Research Triangle Park, NC. [4] Brain Research Institute, University of California, Los Angeles, Los Angeles, CA.

* Presenting author

Cannabinoids acting on $Gi_{/o}$-coupled cannabinoid 1 and 2 receptors (CB1Rs and CB2Rs) alleviate migraine symptoms in humans and in animal models. However, side effects mediated by CB1Rs in the central nervous system (CNS) limit their widespread use. We developed peripherally restricted cannabinoids (PRCBs), demonstrating their effectiveness in relief of cancer and neuropathic pain symptoms in animal models, without CNS side effects. Here we tested the effects of PRCB treatment in mouse models of migraine induced by repetitive glyceryl trinitrate (GTN, 10 mg/kg), and medication overuse headache induced by repetitive sumatriptan (0.6 mg/kg) injections, respectively. PRCB pretreatment, but not posttreatment, prevented behavioral and biochemical correlates of GTN-induced sensitization. Low pH-activated and allyl isothiocyanate-activated currents in acutely isolated trigeminal neurons were reversibly attenuated by PRCB application. GTN treatment significantly enhanced these currents. PRCB pretreatment also prevented all behavioral and biochemical correlates of sumatriptan-induced allodynia and latent sensitization. Importantly, PRCB treatment alone did not produce any behavioral or biochemical signs of sensitization. These data validate peripheral cannabinoid receptors as potential therapeutic targets in migraine and medication overuse headache.

Alex Binshtok
Affiliation: Alpert Professor of Pain Research, The Hebrew University School of Medicine and ELSC, The Hebrew University of Jerusalem, Israel
Title: " The Gain Changer: Inflammation-induced plasticity of action potential initiation in peripheral nociceptive neurons"

Arkady Khoutorsky
Affiliation: Department of Anesthesia and Faculty of Dentistry, McGill University, Canada
Title: "Microglia-mediated degradation of perineuronal nets in the spinal cord promotes pain"

Istvan Nagy
Affiliation: Faculty of Medicine, Department of Surgery & Cancer, Imperial College London, UK
Title: "Peripheral mediators of pain in burn injury"

S4: "Stress and environmental manipulations: impact on neuroplasticity and behavior"

Chair: Despina Tata

Nafissa Ismail
Affiliation: Department of Psychology, NISE Laboratory (NeuroImmunology, Stress, and Endocrinology), University of Ottawa, Canada
Title: "Understanding the link between stress and our gut microbiome"

Roee Admon
Affiliation: Department of Psychology, Stress and Psychopathology Research Laboratory, University of Haifa, Israel
Title: "Dynamics in personality and limbic reactivity prior to, during and following real-life combat stress"

Aniko Korosi
Affiliation: Center for Neuroscience, Swammerdam Institute for Life Sciences, University of Amsterdam, the Netherlands

S5: Imaging and Optogenetics

Chair: Efstratios K. Kosmidis

Yulong Li
Affiliation: Peking University, China
Title: "Spying on neuromodulation with new genetically encoded fluorescent sensors"

Diverse neuromodulators in the brain, such as acetylcholine, monoamines, lipids and neuropeptides, play important roles in a plethora of physiological processes including reward, movement, attention, sleep, learning and memory. Dysfunction of the neuromodulatory system is associated with a range of diseases, such as epilepsy, addition, neurodegenerative and psychiatric diseases. A longstanding yet largely unmet goal is to measure the dynamics of different neuromodulators reliably and specifically with high spatiotemporal resolution, particularly in behaving animals. To achieve this goal, we develop a series of genetically encoded GPCR-activation-based (GRAB) sensors for the detection of acetylcholine, dopamine, norepinephrine, adenosine, ATP, serotonin, histamine, endocannabinoids and neuropeptides, and validate the performance of these sensors in multiple preparations in vitro and in vivo. The GRAB sensor toolbox provides new insights into the dynamics and mechanism of neuromodulatory signaling both in health and disease. The GRAB strategy is now being applied to develop new sensors for other important extracellular signaling molecules.

Bradley Baker
Affiliation: KIST, S. Korea

Title: “Manipulating charge transfer through a fluorescent protein”

One class of genetically encoded voltage indicators (GEVIs) fuses a pH sensitive fluorescent protein (FP) to a voltage sensing domain consisting of four transmembrane segments. Voltage transients in the plasma membrane elicit a conformational change causing the fused FP residing in the cytoplasm to also move. We have recently reported that there is an electrostatic interaction between two neighboring FPs in the cytoplasm that is altered when the transmembrane segments respond to voltage (Kang et al., 2021). Since the architecture of the FP places the chromophore inside a protective barrel structure consisting of 11 □-sheets, pathways must exist for charge to migrate from the external surface of the □-barrel structure to the internal chromophore thereby changing the optical properties of the FP. By mutating several amino acids on the external surface of the FP, we have found that a combination of polar and hydrophobic resides are required to funnel the charge into the interior of the protein as opposed to dispersion along the surface of the barrel. Further investigation of the internal charge pathway strongly suggests that the voltage-dependent signal is due to a transient rotation of the chromophore in response to an altered electrostatic environment. These insights into how charge migrates through a fluorescent protein should enable the development of better red-shifted GEVIs and other biosensors as well as provide insights into how proteins handle charge fluctuations in general.

Marco Canepari

Affiliation: ***Univ. Grenoble Alpes, CNRS, LIPhy, Grenoble, France.*** *Laboratories of Excellence "Ion Channel Science and Therapeutics", France. Institut National de la Santé et Recherche Médicale, France*

Title: "Optical analysis of neuronal ionic currents in their native system"

In spatially-complex neurons, the conventional patch clamp technique does not allow the measurement of native ionic currents that underlie physiological excitation. Here, I will present the basic principles of the optical measurement of native Ca^{2+} [1,2] and Na^{+} [3] currents from neuronal compartments in brain slices, that overcome the limitations of conventional electrode techniques. Ultrafast fluorescence imaging techniques are exploited to provide faithful measurements of native currents at the site of origin. This optical analysis of native ionic currents, combined with selective pharmacology using peptides derived by animal toxins, can reveal the physiological kinetics of ion channels. I will show how localised photo-release of these peptides can unravel the physiological role and function of individual ion channel isoforms.

[1] Jaafari N, De Waard M, Canepari M (2014) Imaging Fast Calcium Currents beyond the Limitations of Electrode Techniques. *Biophys J*, 107: 1280-8. doi: 10.1016/j.bpj.2014.07.059

[2] Jaafari N, Marret E, Canepari M (2015) Using simultaneous voltage and calcium imaging to study fast Ca2+ channels. *Neurophotonics*, 2: 021010. doi: 10.1117/1.NPh.2.2.021010

[3] Filipis L, Canepari M (2021) Optical measurement of physiological sodium currents in the axon initial segment. *J Physiol*, 599: 49-66. doi: 10.1113/JP280554

S6: Junior Scientists Symposium

Ioannis Gkekas, *Centre for Research and Technology Hellas, Institute of Applied Biosciences, Thessaloniki, Greece*

Pharmacological inhibition of the aggregation-promoting ATXN1-MED15 protein interaction

Ioannis Gkekas[1], Stelios Mylonas[2], Sotiris Katsamakas[3], Apostolos Axenopoulos[2], Petros Daras[2] and Spyros Petrakis[1]

[1] *Centre for Research and Technology Hellas, Institute of Applied Biosciences, Thessaloniki, Greece*
[2] *Centre for Research and Technology Hellas, Information Technologies Institute, Thessaloniki, Greece*
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Polyglutamine (polyQ) diseases are a group of neurodegenerative disorders including Huntington's disease and spinocerebellar ataxias. They are caused by trinucleotide (CAG) repeat expansions within the coding region of causative genes. In the case of spinocerebellar ataxia type 1 (SCA1), CAG expansions in ATXN1 gene results in longer polyglutamine chains in the mutant protein. A striking feature of polyQ-expanded ataxin-1 is its ability to misfold into toxic oligomers that slowly aggregate into larger insoluble inclusions within the nucleus. Despite the fact that the polyQ tract is the main determinant of protein aggregation, previous studies also indicate human proteins that influence the misfolding and proteotoxicity of mutant ataxin-1. MED15, a key component of Mediator Complex, interacts with ataxin-1 and strongly enhances its aggregation, suggesting that this protein-protein interaction (PPI) may affect the progression of SCA1 (1).

Here, we present a computational and experimental workflow enabling the discovery of compounds that would block ATXN1-MED15 PPI. First, we predicted the structures of target proteins using the I-TASSER software and computationally simulated their interaction. Predicted interaction sites between ATXN1 and MED15 were validated using LuTHy, a mammalian cell-based assay which enables the detection and quantification of PPIs. Next, we performed an *in silico* screening and identified 30 compounds that bind to the ATXN1 interaction site. These compounds will be tested whether they inhibit ATXN1-MED15 PPI in high-throughput LuTHy assays. Hit compounds will be further tested whether they reduce MED15-induced ataxin-1 protein aggregation in a novel inducible cell-based model. Our work may lead to the discovery of novel compounds that would suppress SCA1 disease progression.

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Investigation of p.A53T-αSynuclein Mediated Synaptic Dysfunction

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Parkinson's disease (PD) is the second most common neurodegenerative disorder. Intracellular protein inclusions consisting mainly of α-synuclein aggregates called Lewy bodies, are the neuroanatomical hallmark of the disease, however the exact cause of PD remains unidentified. Most PD cases are sporadic, yet 5-10% of cases are caused by mutations in specific genes. The *SNCA* gene that encodes α-synuclein is highly associated with sporadic PD, while point mutations and multiplications of the locus cause familial forms of the disease. The best-characterized mutation is p.A53T (G209A *SNCA*), first identified in families of Italian and Greek ancestry. Pathological p.A53T α-synuclein (αSyn) causes axonal abnormalities and distortions in synaptic connectivity. Our goal is to investigate the mechanisms underlying synaptic impairment in PD using a) a transgenic mouse model that expresses the human p.A53T-αSyn in brain neurons (Prnp-SNCA*A53T) and b) a human induced pluripotent stem cell (iPSC)-based model that bears the p.A53T mutation and displays PD-associated phenotypes (Kouroupi et al., PNAS 2017). Of relevance, this model suggests defects in synapse formation and function and presents dysregulation of genes involved in synaptic signaling. By applying artificial synapse formation assay, using members of NLGN and SLITRK families, we have studied synaptogenesis defects in murine and human derived p.A53T neurons. Additionally, complementary analysis of synaptic contacts has been performed to define how the p.A53T-αSyn mutation affects excitatory and/or inhibitory synapses and whether this imbalance can be reversed using small molecules that target pathological αSyn. Altogether, this work aims to gain a better insight in the events leading to synaptopathy caused by p.A53T-αSyn mutation.

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Modeling the variability of neurofibromatosis Type 1 behavioral deficits

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Neurofibromatosis Type 1 (NF1) is an autosomal dominant, multisymptomatic and characteristically clinically variable disorder. It affects 1:3000 individuals and results from mutations in the neurofibromin (Nf1) gene. 50%-80% of children with NF1 manifest impairments in executive and higher-order functions, motor coordination and mainly learning and attention deficits. However, the molecular and cellular circuitries whose perturbation underlies these cognitive dysfunctions remain poorly understood.

Neurofibromin is expressed in almost all tissues but most highly in the brain, spinal cord, and the peripheral nervous system. Despite its large size, the only well understood evolutionary conserved function of Nf1 is its role as a GTPase-activating protein (GAP) for Ras, through its GAP-related domain (GRD).

Previous and current work from our and other labs in the Drosophila NF1 model have shown that 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. We reveal a novel GABAergic neuronal circuit, presynaptic to the higher brain region essential for learning and memory in Drosophila (Mushroom Bodies), where dNf1 is acutely required to support normal associative learning. The deficient learning of dNf1 null flies depends on dAlk (Anaplastic lymphoma kinase) activation and Ras1 engagement within these neurons, strongly suggesting its assignment to loss of GRD function. On the other hand, point mutations outside the GRD domain show distinct spatiotemporal effects and implicate impairment of different molecular mechanisms than those upon total dNf1 loss. These results posit that distinct Nf1 mutations affect differentially known and novel functions of the protein, possibly in a cell-type-specific manner, thus contributing to the variability of NF1 pathologies.

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The implication of furin, a schizophrenia-linked gene, in Drosophila habituation

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Habituation devalues the importance of a stimulus, enabling animals to focus on other, more significant stimuli. It plays a significant role in stimulus filtering and has been reported to be defective in a variety of disorders, including schizophrenia. Antipsychotics are prescribed to ameliorate schizophrenic symptoms and manifestations. Taking into consideration the extensive range of antipsychotics' targets, including serotonin, dopamine, muscarinic, adrenergic, glutamatergic and histamine receptors, it is evident that a number of genes are likely affected in this disorder, as also suggested by GWAS studies, which however need experimental validation. Besides GPCR receptors, other molecules have also been associated with schizophrenia in these studies, including the cellular endoprotease Furin. Furin encodes for a calcium-dependent serine endoprotease which activates a variety of proprotein substrates through proteolysis. Some of its substrates include growth factors, receptors, extracellular-matrix proteins and other protease systems. In this study we provide experimental proof that Furin is essential for Drosophila shock habituation and that effects of its attenuation are ameliorated by antipsychotics. Therefore, we provide experimental validation of the GWAS prediction on the *furin* implication in schizophrenia via habituation experiments in Drosophila, which lead to further ongoing experimental investigations of additional loci implicated in the disease.

Christos A. Frantzidis, *Lab of Medical Physics, Medical School, Aristotle University of Thessaloniki, Greece*

The FLAME Project: Towards a Greek Language Mapping Protocol

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The FLAME project focuses on multimodal language mapping with applications on preoperative language mapping. It employs a series of tasks such as noun naming, verb naming, word reading, and sentence repetition and comprehension through visual and auditory language stimuli in Greek. These were implemented through Psychopy [1], which is an open-source tool based on Python programming language. We also employed CMUSphinx [2], which is an open-source system for speech recognition. It is used to utilize the benefits of automated response classification as correct/wrong and response latency extraction. The protocol validation involved the recruitment of 18 (7 males) healthy volunteers. Their mean age was 28 ± 3 years and their education years were 18 ± 1.5. Most of them (16) were right-handed.

Our analysis focused on the data from the noun naming task. According to the automatic latency detection of the participants' correct naming responses, there were 162/231 word stimuli with normal response (less than one standard deviation from the mean response time), 35 stimuli with slow, and 34 with fast responses. Then, we separated word stimuli into infrequent and frequent words based on their lemma frequency. The analysis showed that participants responded to infrequent stimuli significantly slower than the frequent ones ($t(229)=5.695$, $p<0.001$). Ordinary least squares regression analysis also showed that the latency of the response time was robustly predicted by the imageability and the age of acquisition of the words ($R^2=0.965$, $p<0.0001$). We then performed a subject analysis (N=18) of the data through dependent-samples t-test. The difference in the response latencies between frequent and infrequent words remained significant ($t(34)=8.783$, $p<0.001$).

The aforementioned analysis provides preliminary results towards the validation of the proposed protocol in a healthy population cohort. Its fusion with different neuroimaging modalities is expected to provide further insight into the individual language mapping in clinical settings.

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S7: In vivo Brain functional imaging

Chair: Nicolaos Foroglou

Nick Ramsey
Affiliation: University Medical Center Utrecht & CEO of Braincarta BV
Title: "Task fMRI"

Alexandra Touroutoglou
Affiliation: Harvard Medical School, Director of Imaging Operations at Massachusetts General Hospital
Title: "Resting state fMRI"

Vasileios Christopoulos
Affiliation: Department of Bioengineering, Neuroscience Graduate Program, University of California, Riverside
Adjunct Clinical Assistant Professor of Neurological Surgery| USC
Visiting Associate in Biology & Biological Engineering| Caltech

Title: "Functional ultrasound imaging: A revolutionary technology to study the central nervous system"

Recent advances in neuroimaging technology have significantly contributed to a better understanding of human brain organization, and the development and application of more efficient clinical programs. However, the limitations and tradeoffs inherent to the existing techniques, prevent them from providing large-scale imaging of neural activity with high spatiotemporal resolution, deep penetration, and specificity in awake and behaving participants. Recently, functional ultrasound imaging (fUSI) was introduced as a revolutionary technology that provides a unique combination of spatial coverage, unprecedented spatiotemporal resolution (~100 µm and up to 10 ms) and compatibility with freely moving animals. While fUSI is a hemodynamic technique, its superior spatiotemporal performance and single-trial sensitivity offer a substantially closer connection to the underlying neuronal signals than achievable with other hemodynamic methods such as fMRI. In addition, the relative simplicity and portability of ultrasound have allowed fUSI to be performed in awake and behaving animals, providing minimally invasive neural imaging in species ranging from mice to humans. In vivo fUSI was first reported in 2011 by imaging cerebral blood volume (CBV) changes in the micro- vascularization of the rat brain during whisker stimulation. Since then, this technique has been applied to brain activity imaging during olfactory stimuli, resting state connectivity, behavioral tasks on freely moving rats, non-human primates (NHPs) and other animals. However, one of the great advantages of fUSI is the ability to detect hemodynamic changes of only 2% without averaging over multiple trials. The ability to rely on the accuracy of a single-trial is necessary if one intended on using functional ultrasound signal to detect moment-to-moment variations of the blood flow. By taking advantage of the excellent sensitivity of fUSI, our team performed single-trial motor experiments in awake and behaving non-human primates (NHPs). We recorded from outside the dura and above the posterior parietal cortex (PPC), while animals performed memory-delayed reach and eye (saccade) movements. We then used fUSI signal from the delay period before movement to decode the animal's intended direction and effector. We showed for the first time that fUSI is capable of capturing the preparatory motor activity in NHPs that precedes movement responses – a prerequisite to brain-machine interfaces (BMIs), a key application that could benefit from this technology. These results are a critical step in the development of neuro-recording and brain interface tools that are less invasive, high resolution, and scalable. Recently, we took the next major leap in fUSI by extending this technology to study the pathophysiology of neuropsychiatric diseases in pre-clinical (pharmaco-fUSI, mouse model of schizophrenia) and clinical trials (i.e., patients with traumatic brain injury, chronic back pain and others) and to develop modern neuromodulation strategies. Overall, fUSI provides researchers with truly revolutionary capabilities to study the central nervous system in a wide range of species, opening new avenues to understanding basic mechanisms of neuropsychiatric diseases and developing new treatments.

Emmanouil Froudarakis
Affiliation: Institute of Molecular Biology and Biotechnology, Foundation Of Research and Technology-Hellas
Title: "Dissecting cortical computations with large-field-of-view two-photon calcium microscopy"

Despite variations in appearance, we robustly recognize objects. Neuronal populations responding to objects presented under varying conditions form object manifolds and hierarchically organized visual areas untangle pixel intensities into linearly decodable object representations. To systematically study how objects are encoded in the mouse visual system, we used large-field-of-view two-photon calcium imaging to simultaneously record the activity of thousands of neurons across all cortical visual areas of the mouse. These large-scale recordings provide the opportunity to study how object manifolds along the cortex associated with invariant object coding, as well as how population dynamics differ across the visual hierarchy.

S8: Neurodegenerative Disorders

Chairs: George Pampalakis, Zoe Polizopoulou

Dafou Dimitra
Affiliation: Department of Biology, Aristotle University of Thessaloniki, Greece
Title: "The role of epitranscriptomic modifications in neurodegenerative disorders"

Vekrellis Kostas
Affiliation: Biomedical Research Foundation of the Academy of Athens, Greece
Title: "Hitching a ride to the next cell: the role of exosomes in neurodegeneration"

Proukakis Christos

Affiliation: University College London, UK

Title: "Somatic mutations in the brain: so they have a role in synucleinopathies and other neurodegenerative disorders?"

The aetiology of synucleinopathies and other neurodegenerative disorders is incompletely understood. Although there are rare Parkinson's disease (PD) families with autosomal dominant transmission, the heritability is only ~30%, and for multiple system atrophy (MSA) it is <7%. The remaining risk could be partly due to somatic (post-zygotic) mutations leading to mosaicism, with several types now recognised in the brain. We reported somatic copy number variants (CNVs) of alpha-synuclein (SNCA) in PD and MSA brain, correlated with the presence of aggregates in the same cells, in a cell-type restricted manner. Single cell whole genome sequencing revealed genome-wide large CNVs in 30% of MSA brain cells, with distinct profiles in neurons and other cells. Further work will characterise single cell genomes in the PD brain.

Konstantinos I. Tsamis

Affiliation: Assistant Professor of Physiology, Faculty of Medicine, Univ. of Ioannina

Title: “Antidiabetic treatments against Alzheimer's disease”

Research data of the last decades, highlight the important role of metabolic factors in the development and progression of neurodegenerative disorders. Type 2 diabetes mellites (T2DM) has been associated with an increased risk of developing dementia and extensive research has focused on determining the potential role of antidiabetic therapies in the treatment of Alzheimer's disease (AD). Common pathogenetic mechanisms between T2DM and AD provide a great advantage for several antidiabetic drugs to be examined as possible candidates in AD. The newest categories of antidiabetic drugs, SGLT2i and GLP-1R agonists, have shown high efficacy against AD in preclinical studies. The neuroprotection shown by antidiabetic drugs is promising not only for AD but also for other neurodegenerative diseases. However, further investigation is crucial to reveal the best pharmacological agents and their optimal combinations, to maximize their beneficial effects on neurons, and to find ways of increasing their availability in the CNS.

S9: Development

Chairs: Domna Karagogeos and Panagiotis Politis

Theofanis Karayannis
Affiliation: Brain Research Institute, University of Zurich, Switzerland
Title: Somatosensory cortical circuit assembly.

Ilias Kazanis
Affiliation: Department of Biology, University of Patras, Greece
and visiting Senior Research Associate, Wellcome Trust-MRC Cambridge Stem Cell Institute, Cambridge, UK
Title: Neurogenic and oligodendrogenic progenitors in the stem cell niche and the parenchyma. Divergent cell fates, divergent properties.

Kazanis Ilias, Anesti Maria, Dimitriou Christina, Thenia Prantikou, Theodora Mourtzi
Lab of Developmental Biology, Department of Biology, University of Patras, Greece.
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In the postnatal mammalian brain two major pools of neural stem and progenitor cells survive and remain active. Multipotent Neural Stem Cells (NSCs) that cluster in niches and uni- or bi-potent Oligodendrocyte Progenitor Cells (OPCs) that are dispersed in the parenchyma. Both populations retain the cardinal property of self-renewal, serve homeostatic functions and can become activated in response to degeneration. However, the numbers of NSCs are significantly reduced with ageing, with their oligodendrogenic function showing signs of higher resistance in rodents. In contrast, OPC numbers and remyelination potential remain more stable over time, the latter significantly declining mainly in the context of myelin disorders such as multiple sclerosis. Here, I will present data from recent experimental work of the lab addressing the link between cell fate choices of progenitors and their differential key properties. We have modified the way we look at NSC neurosphere cultures in order to investigate, directly, the behavior of cells in respect to the cytoarchitecture of their microenvironment. Our results confirm the empirical view that NSCs are more dependent on a niche microenvironment, while OPCs have adapted to a niche-independent life in the parenchyma. Further experiments, looking at the interaction between neurogenic or oligodendrogenic progenitor cells and platelets also support this notion and provide interesting insight into the clinical relevance of these properties. The gradual elucidation of the molecular mechanisms that control the divergent properties of NSCs and OPCs will allow the design of strategies aiming in strengthening specific aspects of the behavior of these progenitor pools at will.

Alexandros Kanellopoulos
Affiliation: Department of Fundamental Neurosciences, University of Lausanne, Lausanne, Switzerland
Title: The Mitochondrial Transporter Aralar: A Target for Treatment of Social

S10: Organoids in basic neuroscience

Chairs: Myrto Denaxa and Christina Kyrousi

Mina Gouti

Affiliation: Group leader at the Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany

Title: "Building advanced human neuromuscular organoids to study development and disease"

Locomotion results from the interaction between muscles and the nervous system. Dysfunction of such cells results in deadly diseases such as spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). Neuromuscular diseases often show regional selectivity but the underlying reasons remain obscure due to the lack of a suitable human model system. We have recently used human pluripotent stem cell derived axial stem cells, the building blocks of the posterior body, to simultaneously generate spinal cord neurons and skeletal muscle cells that self-organize in 3D to generate neuromuscular organoids (NMOs). NMOs contain functional neuromuscular junctions supported by terminal Schwann cells. They contract and develop central pattern generator-like neuronal circuits. We are currently applying NMOs to study the early development of the human neuromuscular system and to model neuromuscular diseases. This approach promises to uncover the sequence of events and provide greater insight into the mechanisms that lead to specific diseases by tackling previously inaccessible features of neuromuscular junction biology.

Rossella Di Giaimo
Affiliation: Assistant professor at the Department of Biology, University of Naples Federico II, Naples, Italy and Visiting scientist at the Max Planck Institute of Psychiatry, Munich, Germany
Title: "Pathological insufficiency of functional Cystatin B in the dorso-ventral patterning"

Andrea Forero[1], Fabrizia Pipicelli1, Zagorka Bekjarova1, Francesco Di Matteo[1], , Giuseppina Maccarone[1], Pavel Kielkowski[3], Silvia Cappello[2*] and **Rossella Di Giaimo**[1,2*]

1 Max Planck Institute of Psychiatry, Munich, Germany
2 Department of Biology, University Federico II, Naples, Italy
3 Ludwig-Maximilians University, Munich, Germany

Abstract
Progressive myoclonus epilepsy of Unverricht-Lundburg-type (EPM1) is an autosomal recessive neurodegenerative disorder that has the highest incidence among the PME worldwide. Loss-of-function mutations in the gene encoding CYSTATIN B (CSTB) are the primary genetic cause of EPM1. We showed that CSTB levels are critical for cortical development both, *in vivo,* in mouse embryonic brain cortex and, *in vitro*, in a 3D model of human brain development, human cerebral organoids (hCO) and patient-derived cerebral organoids (Di Matteo et al., 2020). Increased amount of CSTB lead to accumulation of proliferating cells and affects the recruitment of interneurons. On the contrary, overexpression of the pathological CSTB mutant as well as low amount of functional CSTB in EPM1 organoids, results in a general decreased proliferation and significant reduction of interneurons.
We decided to further inquire about the role of CSTB during neurogenesis by studying the impact of low amount of the protein in the cortical dorso-ventral patterning. To this aim, we generated patterned spheroids and we found out that ventral-patterned spheroids lost some of their identity in terms of progenitor cells and neurons, providing new important hints for the onset of the epileptic disorder.
Moreover, proteomic analysis of EPM1 organoids suggested a defect in extracellular matrix organization and vesicle secretions. Thereby, we characterized the extracellular vesicles that are secreted by EPM1 hCO during their development in culture and compared the results with extracellular vesicles secreted by hCO generated by 2 different control cell lines.
The dysregulated pathways in EPM1 patient derived vesicles are in line with the epileptic disease and strongly indicate that EPM1 phenotype depends at least in part by an altered mechanism of cell-cell communication mediated by extracellular vesicles.

Christos G. Gkogkas

Affiliation: Group leader at the Biomedical Research Division, Foundation for Research and Technology and Institute of Molecular Biology and Biotechnology, University Campus, 45110, Ioannina, Greece

Title: "The role of translational control in neurodevelopmental disorders"

Neurodevelopmental Disorders (NDDs) are a group of conditions affecting physical, learning, language or behavior areas and are among the most common chronic disorders in children worldwide, affecting ~1% of the world population. These conditions emerge during the developmental period and are strongly linked to the cortex. The causes of NDDs are multifactorial, involving a complex polygenetic and environmental etiology, affecting normal brain development. We do not know the precise causes of NDDs and there are no effective therapies. Gene-expression in brain cells goes awry in both familial and sporadic forms of NDDs, especially at the level of regulation of protein synthesis (translational control). The mammalian target of rapamycin (mTOR) is a master regulator of translational control and is hyperactivated in genetic and non-genetic forms of NDDs. Because previous research focused on transcriptional changes in the developing human forebrain in its different cell-types, we yet do not understand how translational control via cardinal convergence pathways (such as mTOR) is altered in different cell-types of the brain in NDDs. Cortical organoids derived from human induced pluripotent stem cells constitute a new model to study post-transcriptional regulation in cell-types of the brain and a promising platform for the discovery of mechanism-based therapeutics and the development of Precision Medicine applications for NDDs.

S11: Junior Scientists Symposium

Melpomeni Galani, *Laboratory of Experimental Neurophysiology, First Department of Neurology, National and Kapodistrian University of Athens, Greece*

Aberrant expression of neuroligin 1 and 3 in subjects of the Autistic Spectrum Disorders

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Autism Spectrum Disorders (ASD) encompasses a range of neurodevelopmental disorders characterized by deficits in social interaction, language, communication and stereotypical behaviors. Many genes linked to ASD are known to contribute to the formation and function of synapses, a fact that supports the view that such disorders are due to dysfunction of synaptic connections. Such genes involve various synaptic proteins, neurotransmitters, voltage-gated potassium channels and metabotropic glutamate receptors. Combining our finding that human T lymphocytes express neuroligins (1, 3, 4X, 4Y) with the fact that potassium channels are expressed in these cells, we studied the expression of neuroligins as well as the activity of Kv1.3 channels in T lymphocytes of healthy subjects and individuals with ASD. We assessed the mRNA levels of neuroligin 1 and 3 in freshly-isolated T lymphocytes of subjects with ASD and healthy controls performing semi-quantitative PCR. We also studied the activity of Kv1.3 channels in both ASD subjects and healthy individuals using patch clamp technique in whole-cell configuration with specific voltage protocols.

The present study not only demonstrates for the first time that neuroligins are expressed in immune cells and specifically in human T lymphocytes from peripheral blood but also shows that subjects with ASD have aberrant neuroligin 1 and 3 mRNA expression. Furthermore, we show that the biophysical properties of the voltage-gated potassium channels (Kv1.3) in these subjects are altered as compared to healthy controls. Given the important role of Kv1.3 channels in T lymphocytes, this finding may at least partly explain the reported immune alterations in ASD. On the other hand, the changes found in the expression of neuroligins (that their role in the immune system remains unknown) provides a potential peripheral biomarker for these CNS disorders.

Zouzana Kounoupa, *IMBB-FORTH and Department of Basic Science, University of Crete Medical School, Heraklion, Crete, Greece*

Defects in cytoskeletal dynamics in cortical interneurons missing Rac1 and Rac3

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GABAergic interneurons provide the main source of inhibition in cortical microcircuits. Impaired interneuronal function results in neurodevelopmental disorders such as schizophrenia, epilepsy, autism. The migration of cortical interneurons (CINs) from their birthplace to the neocortex is determined by extracellular factors which modify CIN cytoskeletal dynamics through activation of many intracellular pathways, such as the family of RhoGTPases. We have previously demonstrated the unique and diverse roles of Rac1 and 3 RhoGTPases in progenitors of the majority of CINs, the population originating from the medial ganglionic eminence (MGE). In transgenic animals where Rac1 is ablated from the MGE, progenitors delay their cell cycle exit resulting in a 50% decrease in CINs (Vidaki et al, 2012). In the Rac1/3 mutant, there are additional cytoskeletal defects resulting in an 80% decrease in CINs (Tivodar et al, 2015). When grown *in vitro,* Rac1/Rac3-deficient interneurons show splitting of the leading process, abnormal growth cone, reduction of axon length, delay in axon outgrowth and reduction of microtubule stability. Centrosome and Golgi complex positioning are also defective. Subsequently, their migratory behavior in *ex vivo* time-lapse imaging assays is severely perturbed as several motility parameters are significantly decreased. The defects in microtubules also affect the lysosomal transport in still extending axons and the lysosomal localization in migrating CINs. RNA seq analysis indicated putative downstream effectors, among them, the two-pore protein TPC2, a lysosomal channel which is implicated in metastatic cell migration (Nguyen et al, 2017). TPC2 levels in Rac1/Rac3-deficient interneurons are reduced and the protein distribution is altered. Pharmacological inhibition of the channel affects negatively the axon outgrowth of wild type CINs and also results in defective migration in *ex vivo* time-lapse imaging experiments, thus supporting a novel role for TCP2 in CIN development.

Anthi C. Krontira, *Department of Translational Research in Psychiatry, Max Planck Institute of Psychiatry, Germany; International Max Planck Research School for Translational Psychiatry, Max Planck Institute of Psychiatry, Germany*

Gene by stress-hormones interaction effects on brain development and implications for psychiatric phenotypes

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Abstract Body (300 words limit)

The brain undergoes rapid growth and maturation during the prenatal period, thus it is highly susceptible to environmental stimuli. During this period increased glucocorticoid exposure of the embryo has been associated with adverse neurobehavioral and physiological outcomes later in life. Glucocorticoids are one of the main factors mediating stress effects prenatally and one of the main systems found dysregulated in stress-related psychiatric disorders. To investigate these processes in a human specific and reactive system, we used induced Pluripotent Stem Cells-derived 3-dimensional cerebral organoids and combined them with *in vivo* mouse models of neurodevelopment. Chronic exposure (7 days) to 100nM Dexamethasone, a synthetic glucocorticoid, leads to increased numbers of apical radial glial cells (aRG- PAX6+) as seen by flow cytometry and immunofluorescence. We focused on *ZBTB16*, a glucocorticoids-responsive transcription factor that has been implicated with psychiatric disorders such as depression and autism. We studied the genetic landscape of ZBTB16 in humans and showed an interaction effect of rs648044 with maternal pregnancy cortisol levels on the size of the insular cortex of the offspring after birth. To study the molecular pathway mediating this effect we used *in vitro* in organoids and *in utero* in mice electroporations of a plasmid overexpressing ZBTB16. These showed a similar effect on the aRG population as seen with dexamethasone. The PAX6+ cells were enriched which led to a significant increase of neuronal production as seen by TBR1 and CTIP2 stainings. In fact, PAX6 expression was not downregulated even after 7 days of the overexpression in cells located in the cortical-like plate of organoids and mice, contributing to increased proliferation capacity. The molecular mechanisms and pathways we shed light on in this work could have profound implications for our understanding of the risk of stress exposure during early brain development, and consequently psychopathology vulnerability.

Maria Anesti, *Laboratory of Developmental Biology, Department of Biology, University of Patras, Patras, 26500, Greece*

Neurogenic and oligodendrogenic cell fate decisions of postnatal brain neural stem cells of the subependymal zone are differentially dependent on their microenvironment

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Two major populations of stem/progenitor cells co-exist in the postnatal mammalian brain: multipotent Neural Stem Cells (NSCs) and the more lineage-restricted oligodendrocyte progenitor cells, which exhibit distinct spatial preferences, with the former being located only within specialized microenvironments (niches), while the latter being broadly dispersed in the brain parenchyma. Here, we employ a cell culture assay in which postnatal brain NSCs were cultured as neurospheres and subsequently were plated on coverslips to create a range of microenvironments, that we classified either as “niche-like” 3D areas, or as “parenchyma-like” 2D areas. Cells were, then, induced to differentiate and the acquisition of a neurogenic or oligodendrogenic cell fate was correlated with the cytoarchitecture of their microenvironment. Our results demonstrated that neurogenesis is much more dependent on the architecture of surrounding cells, observed at significantly higher levels in the “parenchyma-like” areas, although often at the periphery of the “niche-like” structures. However, oligodendrogenesis was found to be independent of the cytoarchitecture of the microenvironment. In the presence of added laminin (a major extracellular component of the niche) cell cultures became more homogeneously two-dimensional, but neurogenesis was switched to a more “niche-like” behaviour, while oligodendrogenesis was not affected. Notably, the inhibition of β1 integrin -aiming at disrupting laminin-dependent signaling- further enhanced the diversion between the two cell fates, almost eliminating neurogenesis but increasing oligodendrogenesis. Finally, the administration of BNN-20, a microneurotrophin previously shown to increase NSC differentiation, was the only factor that affected both cell fates in the same direction, leading to significant increases. In conclusion, our novel method of analysis revealed that neurogenesis exhibits higher microenvironment restrictions than oligodendrogenesis and constitutes a new tool for the investigation of the effects of possible therapeutic strategies in specific properties of neurogenesis and oligodendrogenesis.

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abstracts

Topic: Development

CHARACTERIZATION OF MENA SPATIOTEMPORAL EXPRESSION AND SYNAPTIC LOCALIZATION IN THE POSTNATAL MOUSE CNS

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Neurons have adapted various mechanisms that enable semi-autonomous spatiotemporal regulation of protein homeostasis in distal parts of their neurite networks, in order to maintain their structural and functional polarity, and to effectively coordinate multivariate responses to intra- and extracellular signals. Local mRNA translation is one of those key mechanisms that has been shown to play crucial roles both in the developing and mature nervous system (NS). During neuronal development, local mRNA translation, in concert with active actin cytoskeleton remodelling, are required for axon guidance, and synapse formation, while being pivotal for synaptic plasticity in the postnatal/adult NS. Despite the fact that dysregulation of those processes is implicated in many neurodevelopmental and neurodegenerative disorders, there is relatively limited knowledge on their regulation and coordination at the molecular level. Previous work from our lab in developing neurons has already established a dual nature of the protein Mena in actin cytoskeleton remodelling and the regulation of local translation of specific mRNAs in developing axons. However, the exact mechanism of Mena function, as well as its potential role beyond development in the CNS remain elusive. Our hypothesis is that Mena could function in synapses by coordinating actin dynamics and shaping the local synaptic proteome, via regulation of local mRNA translation. In an initial attempt to address this, we investigated the spatiotemporal expression pattern of Mena in the postnatal CNS, in relation to different neuronal and synaptic markers. We observed a highly heterogeneous intracellular and extracellular distribution of Mena across distinct isolated neuronal populations with strong indications for synaptic localization in areas. By employing *in vivo*, *ex vivo* and *in vitro* approaches, we were able to detect the presence of Mena protein and some of its main interactors in synaptic compartments, underlying its potential contribution in synapse formation and function, which raises further assessment.

THE ENVIRONMENTAL IMPACT ON THE MATURATION OF MGE-DERIVED CORTICAL INTERNEURONS

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GABAergic interneurons (cIN) comprise one of the two main classes of cortical neurons that are essential for the assembly and function of cortical neural circuits. In the rodent cortex, cINs comprise at least 20 functionally distinct subtypes, which arise from three proliferating regions of the embryonic basal telencephalon-the medial and caudal ganglionic eminences (MGE, CGE) and the preoptic area (POA). Around 60% of cINs are born in the MGE and include two cardinal subtypes defined by the expression of the calcium-binding protein Parvalbumin (PV) and the neuropeptide Somatostatin (Sst). The place of origin and early genetic programs implemented at the IN-progenitor level restrict IN fates into cardinal types, but their distinct mature identity is defined later, within their cortical environment, possibly by genetic programs which might be activity-dependent.

Indeed, recent work from our and other labs provides evidence that increased levels of intra-cortical activity can affect the survival of certain inhibitory subtypes in the cortex (Denaxa et al., 2018a; Denaxa et al., 2018b). Here we examine whether the cortical environment in terms of sensory or intra-cortical input affects other aspects of the mature identity (defined by cellular and molecular criteria) of PV- and SST-expressing cINs.

For this we are using two different protocols: In the first one we manipulate sensory input *in vivo*, in the mouse somatosensory barrel cortex, via chronic unilateral whisker plucking to attenuate activity, or whisker stimulation, which leads to enhancement of neural activity. In the second one we explore whether intra-cortical activity levels contribute to the maturation of PV- and SST-expressing interneurons by employing an *in vivo* virus-based approach in combination with chemogenetics.

Knowledge of the mechanisms controlling the maturation of different IN subtypes in the infant brain should provide insight into how the abnormal function of specific INs contributes to distinct neurodevelopmental and neuropsychiatric illnesses.

INVESTIGATION OF THE ROLE OF THE CENTRIOLAR SATELLITE PROTEIN, OFD1, IN THE DEVELOPMENT OF THE HUMAN CEREBRAL CORTEX AND ITS MALFORMATIONS

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Development of the brain is a highly orchestrated process depending on the correct balance of neural progenitors' proliferation and their differentiation into neurons and glial cells. In animal models, primary cilia have been shown to be critical for the development of the brain, while mutations in genes controlling cilia formation and function lead to ciliopathies which are associated with brain malformations and dysfunction. OFD1 is a cilia-related gene encoding a centrosomal protein which was found mutated both in the ciliopathy oral-facial-digital syndrome and in a cortical malformation disease called periventricular heterotopia. Single-cell-RNA sequencing datasets from the developing mouse brain, cerebral organoids cultures of different species and the human fetal brain, were examined in respect to OFD1 expression profile, to pinpoint potential differences of its expression during brain development. Further, we provoked ectopic OFD1-overexpression or silencing in the developing mouse cerebral cortex via *in utero* electroporation, to investigate its role *in vivo*. By immunofluorescence, we examine the morphology and numbers of RGs, BPs and the newly formed migrating neurons. Our results showed that OFD1 ectopic overexpression in the developing cortex led to changes in progenitor cells' morphology highlighting the importance of primary cilia. To study primary cilia function, specifically in the human cortex, human iPSCs-derived cerebral organoids will be used. We aim to manipulate OFD1 endogenous expression acutely or permanently in cerebral organoids and compare our analysis to the *in vivo* model to identify potential species-specific mechanisms of primary cilia. Number and position of the human-specific basal radial glial cells (bRGs) will be also examined to identify the role of cilia on this population. This will allow us to pinpoint any contribution of primary cilia on mechanisms that control both the proliferation and differentiation of neural progenitor cells and to understand the mechanisms controlling normal human brain development and its malformations.

PLATELETS' REGULATORY ROLE ON POSTNATAL BRAIN NEURAL STEM CELLS OF THE SUBEPENDYMAL ZONE AND THEIR NICHE

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Postnatal brain Neural Stem Cells (pbNSCs) reside in specialized microenvironments, called stem cell niches, such as the Subependymal Zone (SEZ) of the lateral ventricles walls'. We have previously shown specific aggregation of platelets (PLTs) within the niche's vasculature after focal demyelination in the adjacent corpus callosum (CC)[1] and we have reported evidence of interaction between PLTs and pbNSCs, affecting the behaviour of the latter, based on a co-culture system of these two cell populations that allow us to assess the effects of their direct cell-to-cell interaction. Here, we extended our investigation by evaluating additional cell fate markers of pbNSCs in the typical co-culture protocol, as well as, in the presence of platelet lysate, revealing that high densities of platelets affect pbNSCs proliferation and differentiation (both neurogenic and oligodendrogenic) potential, depending on the presence/absence of mitotic factors. When co-cultures were set up using Nbeal2$^{-/-}$-derived PLTs, characterized by non-functional α-granules, both effects were abolished. Moreover, experiments of CC demyelination in thrombocytopenic (Nbeal2$^{-/-}$, Crlf3$^{-/-}$) and thrombophilic (JAK2V6$^{fl/+}$) mice, followed by histological analysis of cellular and non-cellular components of the SEZ and the CC, showed deficient activation of oligodendrocyte progenitor cells (OPCs), without changes in neurogenesis, in thrombocytopenic mice and a significantly reduced response of the SEZ vasculature in mice with altered numbers of circulating PLTs. Finally, after direct grafting of labelled PLTs in the SEZ and in the adjacent striatum, we observed activated grafted PLTs around blood vessels of the SEZ. Altogether our results indicate a functional role of PLTs, as cellular entities, in the regulation of both pbNSCs and their niche, partially dependent on α-granules and their compartments.

Acknowledgements
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THE ROLE OF Prox1 IN METABOLISM-MEDIATED REGULATION OF EMBRYONIC AND ADULT NEUROGENESIS

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Emerging evidence suggests that a metabolic switch from glycolysis to oxidative phosphorylation in neural stem/progenitor cells (NSCs) is a key regulatory event for the induction of neurogenesis. The self-renewing NSCs rely on glycolysis, while down-regulation of glycolysis and enhancement of oxidative phosphorylation (OXPHOS) is required for their differentiation into neuronal cells. How this reprograming of cellular metabolism is controlled at the transcriptional level is largely unknown. Here we identified Prox1 (Prospero-related homeobox 1) as a candidate transcription factor for such a function. Prox1 expression is induced during the switch of NSCs from glycolysis to oxidative phosphorylation in embryonic and adult neurogenesis. Moreover, our experimental observations suggest that Prox1 suppresses the expression of genes encoding for enzymes and transporters with critical roles in glycolysis of Neuro 2A cell line. In agreement, Prox1 is sufficient and necessary to promote neurogenesis in the embryonic and adult NSCs. Collectively, these data raise the intriguing hypothesis that Prox1 may orchestrate at the transcriptional level the reprogramming of metabolism in NSCs by inhibiting glycolysis to allow the initiation of neurogenesis. In this project we aim to further investigate the Prox1-dependent metabolic profile of N2A cells and embryonic NSCs. Our results will offer novel insights into how metabolism is coupled with gene regulation networks that control neurogenesis.

Mirk/Dyrk1B MINIBRAIN KINASE OVEREXPRESSION ALTERS LATERAL COLUMNAR ORGANIZATION OF MOTOR NEURONS IN THE EMBRYONIC CHICK SPINAL CORD

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Dyrk1B is a dual-specificity kinase involved in growth arrest, differentiation, and cell survival. Dyrk1B has a major role in tumorigenesis and cancer progression. Our studies have implicated Dyrk1B kinase for the first time in neurogenesis. To elucidate the role of Dyrk1B in spinal cord development we performed *gain-of-function* studies in the easily amenable embryonic chick neural tube by applying unilateral *in ovo* electroporation at E2 followed by analysis at E4 and E6. Endogenous Dyrk1B is expressed in the embryonic chick spinal cord by cycling NPCs of the ventricular zone (VZ) and by post-mitotic motor neurons of the mantle zone. Dyrk1B expression is decreased during embryonic CNS development.

At E4, forced Dyrk1B expression promotes dramatically cell cycle exit and apoptosis, while neuronal differentiation is slightly induced. Increased apoptosis was observed in the VZ and motor neuron (MN) domain, as indicated by the increased number of Caspase3$^+$ and Caspase3$^+$/Islet1$^+$ cells by 100% and 108%, respectively. Premature cell cycle exit combined with increased apoptosis resulted in the reduction of the total number of motor neuron progenitors (pMNs), as well as of motor neurons (MNs). Especially, we observed a reduction in the number of Nkx6.1$^+$ and Olig2$^+$ pMNs by 16.24%, and 26.98% respectively, and a reduction of MNR2$^+$ and Islet1$^+$ MNs by 26.88% and 18.28% respectively. This intense ventral phenotype of Dyrk1B function indicates the possible involvement of Shh signaling. In agreement, real-time qRT-PCR analysis revealed that Dyrk1B overexpression reduces Shh and Gli3 mRNA levels by 65% and 79% respectively.

Consistently, Dyrk1B forced expression alters MN columnar organization at E6, as we observed that FoxP1$^+$/Islet 1/2$^+$ cells were decreased by 26.10% in the lateral motor column (LMC) in Dyrk1B-GFP electroporated side compared with the non-electroporated side, while in GFP-electroporated embryos no differences were observed contralaterally. Possible changes in medial motor column (MMC) are being investigated.

AUTOPHAGY IN OLIGODENDROCYTE MATURATION AND MYELIN HOMEOSTASIS

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Autophagy comprises a major lysosome-dependent degradation mechanism which engulfs, removes, and recycles unwanted cytoplasmic material, including damaged organelles and toxic protein aggregates. Although a few studies implicate autophagy in CNS demyelinating pathologies, its role, particularly in oligodendrocytes and CNS myelin, remains poorly studied.

We will present data on the significance of macroautophagy in the nervous system, focusing on myelinating glia of the CNS and myelin homeostasis. To this end, we have used both *in vitro* and *in vivo* approaches. *In vitro*, pharmacological and genetic inhibition of autophagy have revealed severe defects in myelin sheet formation, delayed maturation and altered cellular distribution of major myelin protein constituents. In parallel, we are currently examining the role of autophagy in vivo, utilizing a new conditional mutant mouse line that we have generated, in which a core gene of autophagic machinery (*atg5*) is specifically ablated in the myelinating glial cells after tamoxifen administration (*plp-Cre^{ERT2}; atg5$^{fl/fl}$*). Biochemical and ultrastructural analysis of this mouse line has revealed differences in myelin protein levels as well as morphological alterations in conditional mutant animals compared to age-matched controls.

In summary, our data support the novel principle that the progression of myelination in the CNS requires the involvement of a fully functional autophagic machinery.

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EFFECTS OF AUTOPHAGY IMPAIRMENT ON PREFRONTAL CORTICAL DEVELOPMENT IN MICE

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Autophagy is a cellular process that results in the removal of dysfunctional or unnecessary products via lysosome-dependent pathways. Autophagy has been associated with the mechanisms underlying pruning of excitatory synapses that normally occurs during development and it is believed that its dysregulation could lead to neurodevelopmental diseases. The prefrontal cortex is a brain area involved in higher-order cognitive function and exhibits slower maturation compared to other cortical areas. Here, we aimed to understand whether inhibiting autophagy during the synaptic pruning period of the PFC (post-natal day (P)35-p45) affects dendritic spine density in the PFC and its function. For this purpose, *thy1-Cre^{ERT2};atg5$^{f/f}$* (KO) mice were generated, which lack ATG5-a gene necessary for the elongation of the phagophore during the process of autophagy-in specific neural lineages expressing Thy1. Tamoxifen (tmx) was post-injected to mice on P31-35 or P61-65 (as a control), to induce Cre activity and lead to autophagy impairment. A milder autophagy impairment was enabled in *thy1-Cre^{ERT2};atg5$^{+/fl}$ (*heterozygotes or het*)* animals*.* After P80, all mice were tested in the following: Novel Object Recognition (NOR), Object To Place (OTP), Temporal Order Object Recognition (TOR) tasks, and Three-Chamber Sociability test. Dendritic spine morphology and density of these animals' pyramidal neurons were studied using optical microscopy following Golgi-cox staining. KO mice that had received Tmx at P31-35 (KO P30) exhibited reduced discrimination index in the NOR, OTP and TOR tasks, compared to het P30 mice as well as control groups. No differences among groups were observed in the sociability task. Golgi-cox staining results indicate a tendency towards enhanced densities of mature dendritic spines in KO and het animals in both P30 and P60 treatments. Therefore, our results suggest that deficient autophagy after P30 results in increased dendritic spine density in the PFC and impaired performance in NOR, TOR and sociability tests.

UNRAVELLING THE MECHANISM OF Satb1 FUNCTION ON CORTICAL INTERNEURON DEVELOPMENT

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GABAergic interneurons comprise 20-30% of all neurons in the cortex and are essential for cortical circuit function. They have multiple roles, from maintaining excitation/inhibition balance and synchronizing brain activity, to refining cortical processing in unique and multiple ways. Their functional diversity is enabled through their remarkable heterogeneity at the molecular, morphological and electrophysiological level. This diversity may become evident from early embryonic stages, and depends on the unique combinatorial expression of transcription factors that characterize the place of origin of each interneuron type, but it is better manifested at postnatal stages, when interneurons acquire their mature properties, a process that is controlled by genetic programs which are initiated upon the response of immature interneurons to emerging environmental cues, such as network activity.

Satb1 is an activity-regulated transcription factor that is expressed at late embryonic stages in the lineage of parvalbumin (PV) and somatostatin (SST) expressing interneurons and previous studies have suggested that it might play a role in their differentiation. Here, we investigate the mechanism of Satb1 function on cortical interneuron development. Using a number of approaches, such as state-of-the-art genetics, unbiased gene expression profiling, *in vitro* and *in vivo* viral-based morphological labelling of interneurons, we provide evidence that Satb1 acts as a molecular switch that promotes the maturation of PV- and SST-expressing interneurons. In addition, we suggest that Satb1 might be a regulatory node for a number of genes implicated in autism susceptibility.

ANTISENSE OLIGONUCLEOTIDES AS NOVEL RNA-TARGETED THERAPEUTIC APPROACH FOR Tau PATHOLOGY IN AND BEYOND ALZHEIMER'S DISEASE

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Despite that worldwide research has been increasingly focusing on Alzheimer´s disease (AD) and its two characteristic molecules, Aβ and hyperphosphorylated Tau over the last decades, it was only until recently that Tau become a key regulator protein of AD brain pathology with promising therapeutic properties. In AD brain, Aβ overproduction triggers Tau hyperphosphorylation and its accumulation leading to neuronal malfunction while Tau reduction (by deletion of Tau gene) blocked the Aβ detrimental effects on AD brain. Interestingly, recent work from our team and others suggest that Tau mediates neuronal malfunction found in various neuropathologies (e.g. epilepsy, traumatic brain injury, chronic stress) suggesting that Tau may trigger brain pathology beyond AD. Recently, antisense oligonucleotides (ASOs) emerged as novel pharmacological approach to control the expression of abnormal/pathological proteins in different neurological disorders; some received FDA/EMA approval for human use. Thus, this project designed, synthetized and tested 58 ASOs against both human and mouse Tau for future testing in animal models as well as human iPSCs. In addition, two ASOs categories were designed for 1) lowering total Tau levels (found to be pathologically elevated in different Tau-related pathologies) and 2) reducing the increased levels of 4R-Tau isoform; splicing-corrector ASOs). ASOs were tested for toxicity and their efficiency to reduce Tau mRNA and protein levels in neuronal cell lines - N2A (mouse origin for testing mouse ASOs) and SHSY5Y-P301LTau cells (human origin that express also mutant P301LTau protein for testing human ASOs) –the most efficient and less toxic ASOs were further tested in primary neurons from wild-type and P301LTau Tg mice. Using different molecular and cellular techniques, we identified 4 ASOs with great Tau reduction efficacy. This project provides the first *in vitro* and *in vivo* evidence of the beneficial use of ASOs against Tau-related neuronal malfunction in diverse brain pathologies.

RNF113A REGULATES CELL DEATH, PROLIFERATION AND DIFFERENTIATION OF NEURAL STEM CELLS DURING BRAIN DEVELOPMENT

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RNF113A (Ring Finger Protein 113A) is a newly discovered multifunctional molecular player, genetically associated with autism spectrum disorders and X-linked trichothiodystrophy (TTD) syndrome. TTD is characterized by neurodevelopmental impairments including abnormal development of central nervous system and mental retardation. How RNF113A affects mammalian brain development is not known. Here we identify Rnf113a1 as a critical regulator of cell death and neurogenesis during mouse brain development. Rnf113a1 gene exhibits widespread expression in the embryonic murine brain and spinal cord. Gain and loss-of-function experiments in embryonic cortical neural stem/progenitor cells (NSCs) and knockdown studies in the mouse cortex suggest that Rnf113a1 controls survival, proliferation and differentiation properties of progenitor cells. Importantly, Rnf113a1 deficiency triggers cell apoptosis via a combined action on essential regulators of cell survival, including p53, Nupr1 and Rad51. Collectively, these observations establish Rnf113a1 as a regulatory factor in nervous system development and provide insights for its role in neurodevelopmental defects associated with TTD and autism.

This research is co-financed by Greece and the European Union (European Social Fund—ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project “Strengthening Human Resources Research Potential via Doctorate Research” (MIS-5000432), implemented by a State Scholarships Foundation (IKY) PhD studentship to MT. This work was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the “First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant” (Project Number: 1782).

Topic: **Behavioral Neuroscience**

THE ROLE OF Ca/CALMODULIN ADENYLATE CYCLASE IN HABITUATION AND ITS RELATION TO ASSOCIATIVE LEARNING

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The *rutabaga* gene encodes for a calmodulin dependent adenylate cyclase that converts ATP to cyclic AMP (cAMP) in Drosophila melanogaster. As cAMP is a major signal transducer and is involved in the majority of neuronal responses the function of rutabaga is funtamental for many biological processes, including behavioral responses like habituation and associative learning.

Habituation is an adaptive behavioral outcome of processes engaged in reducing responsiveness to non - reinforced repeatitive or prolonged stimuli. Even though habituation is a conserved crucial for survival mechanism, the neuronal circuits and molecular mechanisms that underlie this process are not well understood in most experimental systems and humans alike. In the brain of in D. melanogaster previous publications have demonstrated that the brain circuits known as mushroom bodies (MBs) play key roles both in habituation to footshock and in olfactory aversively (footshock) reinforced associative learning. Associative learning involves the establishment of a predictive link between two stimuli as a consequence of their temporal pairing. Previous publications provide considerable evidence that associative learning depends critically on neural activity and cAMP signaling in the MBs.

In this study we investigated the importance and nessecity of rutabaga in habituation and associative learning by studying the effects of loss and partial reduction of rutabaga in various neuronal subsets of the MBs and we explored whether the two processes require signaling within the same or distinct neuronal circuits. This is particularly important as loss of the cyclase results in associative learning deficits, but also in premature, but also in lack of habituation to repeated footshocks. We complement these studies with a pharmacogenetic approach aiming to reverse habituation deficits and ask whether they also ameliorate associative learning deficits.

CONTEXTUAL FACIAL EMOTION RECOGNITION IN DIFFERENT DIMENSIONS OF SCHIZOTYPY

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Objective: High schizotypy is associated with disturbed facial emotion recognition. Although emotion recognition accuracy of healthy individuals improves when the face is presented in a congruent context, the role of schizotypy in this process has not been described. This study aimed to investigate the effects of four schizotypal dimensions (disorganized, negative, paranoid and cognitive-perceptual), on contextual facial affect recognition.
Participants and Methods: Sixty-eight community participants were administered the Schizotypal Personality Questionnaire and the Assessment of Contextualized Emotions (ACE) task. The ACE consists of 16 photos with a central person expressing one of three emotions (sadness, anger, disgust). This person is surrounded by others expressing either congruent or incongruent emotions. Participants are asked to rate the central character's emotion. We split our participants into groups (with a quartile-split) according to their scores in each schizotypal dimension and conducted extreme groups' comparisons.
Results: We found significant recognition accuracy x group interactions for the paranoid, negative and disorganized dimensions (all p values <.05): the groups with higher traits had increased accuracy in the recognition of sadness and disgust compared with the groups having lower traits; the opposite pattern was found for anger. We also found significant recognition accuracy x condition (i.e. faces presented in congruent or incongruent context) interactions (all p values <.005): while sadness was more accurately recognized in the incongruent condition, anger was better identified in the congruent.
Conclusions: Individuals with high paranoid, negative or disorganized traits demonstrated a dual profile of contextual facial emotion recognition. The findings (a) suggest that anger is a core component of emotion processing disturbances in schizotypy and (b) could have implications in early-intervention schemes for the schizophrenia-spectrum.
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A MODEL OF OLANZAPINE-INDUCED METABOLIC SYNDROME IN FEMALE SPRAGUE-DAWLEY RATS: PRELIMINARY RESULTS

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Despite their effectiveness, second-generation antipsychotics (SGAs), including olanzapine, are associated with weight gain, dyslipidemia, and glucose intolerance (1). The mechanism by which olanzapine disrupts metabolic regulation is unclear and there is a lack of a well-established rodent model in the literature (1). In this study, we investigate a novel model of olanzapine-induced metabolic syndrome (MetS) in rats based on the parameters that appear as criteria for MetS in humans: body weight, systolic pressure, body mass index (BMI), glucose levels, insulin, triglycerides and, cholesterol (2). Twenty-four adult female Sprague-Dawley were treated with either olanzapine-chocopills (OLA) or vehicle-chocopills (VEH) for 4 weeks and their MetS parameters were assessed weekly. No olanzapine effects were found on the body weight (VEH: 260,83±18,32, OLA: 255±16,76, p = ns) and BMI (VEH: 0,63±0,54, OLA: 0,61±0,04). However, a trend was observed regarding the glycose change levels with a slight increase in the OLA group in the 4th week and the overall change after treatment seems to be higher in the OLA group (median = 18) compared to the VEH (median = -1,5, d = 0,64) though not significant. Finally, a possible effect of OLA was also found on the systolic pressure. Overall, the results suggest that weight might not be a useful parameter for assessing MetS in this strain. However, more research could further examine the possible effects of olanzapine on glycose and systolic pressure. Finally, results on insulin, cholesterol and triglycerides that might add on the effects of olanzapine are still under process.

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ADAPTIVE TRAINING OF EXECUTIVE WORKING MEMORY IMPROVES COGNITIVE FLEXIBILITY

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Working memory training (WMT) induces fundamental improvements to a cognitive system critical for everyday functioning across the lifespan. The present study aimed to examine whether WMT can enhance cognitive flexibility, which is classified into executive functions, in healthy adults.

The study included one hundred and forty-three healthy participants, who were divided into a) control (no cognitive training), b) partially adapted [partial administration of the Letter Number Sequencing (LNS), an executive working memory task, for six consecutive days] and c) fully adapted (administration of the entire LNS for six consecutive days) groups. Upon completion of the training/no-training period, participants were tested with the Intra-Extra Dimensional Set Shifting task (ID/EDS), which assesses cognitive flexibility. Between-group differences were examined with non-parametric (Kruskal-Wallis) analyses, due to the absence of normal distribution. Significant differences were followed-up with Mann-Whitney test. Alpha was set at $p<0.05$.

There were significant differences between the groups in the number of total errors (Kruskal-Wallis $x^2 = 8.007$, $p = 0.018$), number of completed stages (Kruskal-Wallis $x^2 = 7.014$, $p = 0.030$), latency (Kruskal-Wallis $x^2 = 8.358$, $p = 0.007$) and total trials (Kruskal-Wallis $x^2 = 9.860$, $p = 0.007$). Specifically, the fully adapted group presented with superior performance compared to the control group as they made fewer errors (Mann-Whitney U = 868.0, p = 0.007) and completed more stages (Mann-Whitney U = 1080.0, p = 0.009). Also, the control group had increased response latency and needed more trials to complete the stages of the task compared to both the partially adapted (Mann-Whitney U = 812.0, p = 0.009 & Mann-Whitney U = 860.5, p = 0.023 & Mann-Whitney U = 830.5, p = 0.003) and the fully adapted groups (Mann-Whitney U = 932.0, p = 0.025).

These findings further support the value of WMT in cognitive enhancement approaches as cognitive skill learning.

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SEXUAL DIMORPHIC EFFECTS OF RESTRAINT STRESS ON PREFRONTAL CORTICAL FUNCTION ARE MEDIATED BY GLUCOCORTICOID RECEPTOR ACTIVATION

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Stress, a major regulator and precipitating factor of cognitive and emotional disorders, differentially manifests between males and females.

Our aim was the investigation of the mechanisms underlying the sexual dimorphic effects of acute restraint stress on males and females on the function of the prefrontal cortex (PFC).

For this study, adult male and female mice were subjected to restraint stress (RS) or left in their home-cage (NR), and then tested in the light-dark test followed by the temporal order object recognition (TOR) task immediately or 24hrs after restraint stress (RS24). In a different cohort of animals, evoked field excitatory postsynaptic potentials (fEPSPs) were recorded in layer II of acute PFC slices, immediately or 24hrs after restraint stress. In some cases, PFC slices of NRs were incubated with corticosterone. In a third cohort of mice, mifepristone (corticosterone receptor antagonist) was administered 1hr before the restraint stress.

Our results shown that female RS mice exhibited increased anxiety-like levels, while male RS mice only showed deficits in the TOR task, while only the reduced performance in the TOR task in male RS24 mice persisted. Long-term synaptic potentiation (LTP) was significantly reduced in RS and RS24 males, but not females, compared to their respective NR group. PFC slices incubated with corticosterone showed significantly reduced LTP only in males. The mifepristone prevented the effects of restraint stress on the TOR task in males, but not anxiety in females.

In conclusion, restraint stress has differential effects on recency memory and anxiety, in regards to sex, which are partly mediated by the effects of corticosterone signaling on synaptic plasticity.

DIAMETRICALLY DIFFERENT ASSOCIATIONS OF AUTISTIC AND SCHIZOTYPAL TRAITS WITH HIGHER COGNITIVE FUNCTIONING

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Previous studies suggest that autism and schizophrenia co-occur at a trait level. However, the theoretical models proposed and findings on their association are inconclusive. The aim of this study was to examine the association of autistic and schizotypal traits as well as their joint effect with higher cognitive functioning, such as response inhibition, planning/complex problem solving, working memory, and abstract reasoning. One hundred and ninety-five healthy community individuals were assessed with the Schizotypal Personality Questionnaire (SPQ), Autism Spectrum Quotient (AQ), Stockings of Cambridge task (SoC), Stroop Colour-Word Test, N-Back task and Raven's progressive matrices (RPM). Hierarchical regression analyses were performed, controlling for age. Dependent variables were the metrics of the neuropsychological tasks; predictors were AQ and SPQ total scores and their standardized interaction term. We found that AQ total score was a significant positive predictor ($b=0.186$, $p<0.05$) of the RPM total score, whereas SPQ total score negatively predicted the same measure ($b=-0.309$, $p< 0.001$). AQ total score was also positively associated ($b=0.165$, $p<0.05$) with higher total correct responses in the N-Back task, while SPQ total score was associated with lower performance in this measure ($b=-0.255$, $p<0.001$). Stroop interference score was predicted by both SPQ ($b=-0.165$, $p<0.05$) and AQ ($b=0.184$, $p<0.05$) scores in an opposing manner. SPQ total score was associated with poorer performance in SoC (fewer 5-moves problems solved $b=-0.209$, $p<0.05$ and more moves $b=0.180$, $p<0.05$). All the interactions of the AQ and the SPQ total scores were not significant predictors of executive functioning. These findings (a) highlight that autistic and schizotypal traits have diametrically opposing effects on executive functioning and (b) support the diametric theoretical model suggesting balanced cognition when these conditions co-occur.

Topic: **Neurophysiology and neuroimaging**

HIGH CONTENT SCREENING AND PROTEOMIC ANALYSIS IDENTIFY A KINASE INHIBITOR THAT RESCUES PATHOLOGICAL PHENOTYPES IN A PATIENT-DERIVED MODEL OF PARKINSON'S DISEASE

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Combining high throughput screening approaches with induced pluripotent stem cell (iPSC)-based disease modeling represents a promising unbiased strategy to identify therapies for neurodegenerative disorders. We have previously established a model of iPSC-derived neurons from patients with familial PD harboring the p.A53T αSyn mutation (G209A in the SNCA gene) that displays disease-relevant phenotypes at basal conditions. These included protein aggregation, compromised neuritic outgrowth, and contorted or fragmented axons with swollen varicosities containing αSyn and Tau (1). In this study we successfully adapted the p.A53T-iPSC-based cellular system in 384-well plate format and launched a screening campaign on a small kinase inhibitor library using high-content imaging. We thus identified the multi-kinase inhibitor BX795 that at a single dose effectively restores disease-associated neurodegenerative phenotypes. Proteomics profiling mapped the molecular pathways underlying the protective effects of BX795, comprising a cohort of 118 protein-mediators of the core biological processes of RNA metabolism, protein synthesis, modification and clearance, and stress response, all linked to the mTORC1 signaling hub. In agreement, expression of human p.A53T-αSyn in neuronal cells affected key components of the mTORC1 pathway resulting in aberrant protein synthesis that was restored in the presence of BX795 with concurrent facilitation of autophagy. Taken together, we have identified a promising small molecule with neuroprotective actions as candidate therapeutic for PD and other protein conformational disorders.

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EX VIVO PERTURBATIONS ASSOCIATED WITH GLUTAMATERGIC SIGNALLING IN PATIENTS WITH MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system (CNS), characterized by white matter demyelination and intense perivascular infiltration by macrophages and auto-reactive T cells that migrate into the CNS and initiate myelin destruction, following their activation in the peripheral blood (PB). It is known that T cell activation, which is highly dependent on the activity of the voltage-gated potassium channel Kv1.3, is regulated by glutamate via metabotropic receptors. Combining these data with the glutamatergic hypothesis of pathogenesis concerning neurodegeneration in MS, we present an *ex vivo* study of the glutamatergic transmission in freshly-isolated T lymphocytes of MS patients. For that reason, we went on studying the biophysical properties of Kv1.3 channels in both patients and healthy individuals using patch clamp technique in whole-cell configuration with specific voltage protocols. Furthermore, in the T lymphocytes of the same MS patients and healthy controls we used semi-quantitative PCR in order to assess the group II metabotropic glutamate receptor mRNA expression (mGluR 2 and 3).
Interestingly, we found that Glu failed to exert its action on Kv1.3 currents in T lymphocytes of MS patients. The underlying mechanism of this phenomenon is probably related to group II metabotropic glutamate receptors (mGluR 2 and 3), a hypothesis which is consistent with the reduced mRNA expression of these receptors in T cells of MS patients compared to healthy individuals. In this study we show that MS patients present aberrations in glutamate transmission due to a decrease in the expression levels of group II metabotropic glutamate receptors. As a result, despite the higher concentrations of glutamate in MS patients' serum, there is an increase in both "tonically active" Kv1.3 channels and Kv1.3 channels that are available for activation, thus promoting T lymphocyte responsiveness and therefore the inflammatory processes observed in MS.

DISRUPTED NEUROGENESIS AND INCREASED NEUROINFLAMMATION FOLLOWING BRAIN CHEMICAL LESION

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Long-term adverse side effects of chemotherapy, also known as "Chemo-Brain", have been only recently anticipated and several mechanisms are proposed to be involved in changes regarding brain structure and function following systematic use of chemotherapeutic agents. These include reduction of Neural Stem Cell (NSC) proliferation rates in adult brain neurogenic zones, white matter degeneration and inflammation. This phenomenon is particularly pronounced in cancers, such as glioblastoma, which are inherently resistant to chemotherapy requiring high doses of chemotherapy to eliminate them, resulting in its high concentration in the cerebrospinal fluid.

Our preliminary data indicate that multiple stereotaxic intraventricular injections of the chemotherapeutic mito-toxic agent Cytosine Arabinoside (Ara-C) lead to impaired neurogenesis in both neurogenic niches and increased inflammation. Moreover, it triggers doublecortin+ (DCX+) neuroblasts' ectopic presence in the adjacent non-neurogenic striatal parenchyma, the majority of which cluster inside myelinated white matter tracts. In order to acquire a different perspective on the effect of Ara-C in brain connectivity and RMS integrity, we proceeded to sagittal sectioning of the brain tissues, and we observed RMS disorganization and migration of DCX+ neuroblasts from RMS towards striatum. The next step of our study was to evaluate the neurogenic potential of the hippocampus and further characterize the neuroinflammatory response. To this end, we also examined the potential infiltration of peripheral cells into the brain, caused by blood-cerebrospinal fluid (B-CSF) barrier disruption. Our study is ongoing to fully characterize the morphological and cellular alterations imposed by Ara-C to forebrain structure and connectivity.

CEREBRAL LATERALITY FOR WRITING: CURRENT UNDERSTANDING AND FUTURE DIRECTIONS

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The cerebral lateralization of language (also known as laterality or hemispheric dominance / asymmetry) refers to the fact that language is predominantly processed by the left hemisphere of the brain in the majority of individuals. The neural underpinnings of *written* language in particular are of great interest, as writing is a skill that demands the contribution of several cognitive and motor functions. Moreover, disorders of writing are implicated in special learning disabilities, such as dyslexia and dysgraphia. However, the literature focuses on *oral* language; research on cerebral lateralization during writing is extremely limited. What is more, only four studies to date have studied left-handers, who constitute approximately 10% of the population making it important to account for this variation. Furthermore, cerebral laterality for writing has not been studied in atypical populations (e.g., dyslexia and dysgraphia). The limited evidence available to date on cerebral laterality for writing points to the direction of a clear leftward lateralization in right-handers, but the picture is not clear in the case of left-handers. Work in progress from our laboratory addresses cerebral laterality for writing in left-handers, using tasks that disentangle the linguistic and motor components of writing. Other questions that are being addressed include cerebral lateralization during typing (in PC keyboards and mobile phones), the relationship of writing quality with cerebral lateralization, the possible cortical re-organization after non-dominant hand training, and cerebral laterality in dyslexia and dysgraphia. For neurophysiological purposes we employ functional transcranial Doppler ultrasound (fTCD), a reliable measurement of continuous blood flow in the middle cerebral arteries with excellent temporal resolution, which lends itself to the study of writing as its signal is not disrupted by movement artifacts. Overall, this interdisciplinary work contributes to the broader question of individual differences in brain organization and function, both in typical and atypical populations.

ROLE OF DEVELOPMENTAL REGULATORS OF AXONAL LOCAL TRANSLATION IN ADULT AXONS

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Local mRNA translation (LT) is vital for axon development. Disruption of the process is implicated in multiple neurodevelopmental disorders, while numerous studies show that axonal mRNA translation is crucial in adulthood, particularly during plastic responses like axon regeneration. Adult PNS axons maintain high levels of LT and can regenerate after injury, while CNS axons that lose their intrinsic ability for LT as they mature, are unable to regenerate successfully. Therefore, developing CNS and adult PNS axons retain crucial growth programs that are absent from, or different in adult CNS axons. However, the regulatory mechanisms behind axonal LT remain elusive. We have previously identified a ribonucleoprotein complex (Mena-RNP) that regulates axonal LT in the developing brain. It requires the cytoskeleton-associated protein Mena that interacts with known regulators of translation (i.e. HnrnpK, PCBP1) and controls translation of the RNP-bound mRNAs. Mena deficiency results in severely reduced levels of the respective proteins in axons due to perturbed LT. Here, we explore the presence and conservation of the Mena-RNP in the adult nervous system, in an attempt to elucidate the role of Mena and Mena-dependent translation in adult axon regeneration. We observe that the components of the Mena-RNP are different not only between developing and adult axons, but also between axons of the CNS and PNS. Interestingly, novel interactors of Mena were also identified, like Vimentin and β-Catenin that have been long related to post-injury mechanisms, implying a potential role of a developmental program (Mena-RNP complex and LT) in processes like axon regeneration.

EFFECTS OF WORKING MEMORY TRAINING ON THE SYNAPTIC PROPERTIES OF THE PREFRONTAL CORTEX AND HIPPOCAMPUS: A STUDY IN FEMALE ADULT MICE

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Working memory (WM) is a cognitive function that refers to the ability of short-term storage and manipulation of information necessary for the accomplishment of a task. Two brain regions involved in WM are the prefrontal cortex (PFC) and the hippocampus (HPC). Several studies have suggested that training in WM (WMT) can improve performance in several cognitive tasks. However, our understanding of the changes that WMT induces in neurons is very limited. Previous work from our lab has shown that WMT enhances synaptic and structural plasticity in the PFC and HPC in male mice. In this study, we investigate the effect of WMT on synaptic properties in PFC and HPC in adult female mice.

To this end, 50 female adult mice were split into 3 groups: a) naïve which remained in their home cage, b) non-adaptive which learned to alternate the arms in the T-maze but without any delays and c) adaptive which were trained in the delayed alternation task for 9 days. The delayed alternation task was used for WMT. Following the behavioral experiments, evoked field excitatory post-synaptic potential recordings were performed in PFC and HPC brain slices. Our results show that there was no difference in the long-term potentiation (LTP) induced in the PFC following tetanic stimulation among the three groups. In the HPC, the adaptive group exhibited enhanced long-term synaptic potentiation following theta-burst stimulation compared to the other two groups. Similarly, dendritic spine density was not different among the three groups in the PFC but were increased in the adaptive group in the HPC. These results are different from our results in males, indicating the possibility for sex differences in the effect of WMT in synaptic plasticity in the PFC and HPC.

Topic: **Neurological and psychiatric disorders**

DETECTION OF EARLY PROTEOSTATIC FAILURE AND DISRUPTION OF NEUROTRANSMISSION IN A MOUSE MODEL OF SYNUCLEINOPATHY

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Parkinson's disease is the second most common neurodegenerative disorder and its main neuropathological hallmark are inclusions called Lewy bodies containing mostly aggregates of α-synuclein (αSyn). Parkinson's disease is divided into sporadic and familial and the *SNCA* gene that encodes α-Synuclein is mostly associated with the sporadic form of the disease. However, point mutations and multiplications of this gene are connected with the familial form of the disease. The main mutation that has gained the most interest is the p.A53T-αSyn mutation that has Greek-Italian ancestry. It is inherited in an autosomal dominant way and through aggregation of αSyn, it leads to degeneration of the dopaminergic neurons in the substantia nigra pars compacta and eventually to known motor symptoms of the disease. Nevertheless, the mechanisms leading to neuronal death remain inconclusive. The non-motor symptoms of PD and major cellular processes that are impaired in the disease are yet to be described in this particular model. Most importantly, it is crucial to examine the onset of those dysfunctions. This work aims to investigate how p.A53T αSyn affects the entity of the dopaminergic system and others -less described in PD- such as the glutamatergic system. Furthermore, this study focuses on identifying dysfunctions in proteasomal activity, autophagy and mitochondrial biogenesis along with determining the onset of Lewy body formation and how this process correlates to the deterioration of multiple cellular mechanisms. In this study distinct brain regions of p.A53T transgenic mice are characterized histopathologically and molecularly at different diseases stages. Overall, we expect to gain a better understanding of the spatiotemporal events that lead to widespread neurodegeneration attributed to the expression of pathological αSyn species.

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UNRAVELING THE MECHANISMS OF TUBULIN POLYMERIZATION PROMOTING PROTEIN TPPP/P25A DEGRADATION IN OLIGODENDROCYTES

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Our aim is to identify the proteolytic pathways responsible for the clearance of TPPP/P25A in oligodendrocytes, which may represent potential therapeutic targets for Multiple System Atrophy (MSA).

MSA is a neurological disorder of unknown etiology and rapid progression. The main pathological hallmark of the disease is the presence of Glial Cytoplasmic Inclusions (GCIs) within oligodendrocytes, mainly composed of alpha-synuclein (SNCA) and the oligodendroglial-specific phosphoprotein TPPP/P25A. TPPP/P25A is critical for the aggregation of oligodendroglial SNCA in MSA; therefore manipulation of its expression levels will provide a rational approach to combat the accumulation of SNCA within oligodendrocytes.

For the purposes of the current study we utilized a rat immortalized oligodendroglial cell line stably overexpressing human TPPP/P25A (OLN-p25α) and murine primary oligodendrocytes expressing endogenous TPPP/P25A and treated them with either PBS (control) or human SNCA Pre-Formed Fibrils (PFFs) as pathological seeds. To assess the role of the autophagy-lysosome pathway (ALP) in TPPP/P25A proteolysis, we used pharmacological inhibitors (NH_4Cl, 3-MA) and enhancers [Atypical retinoid 7 (AR7), rapamycin] of the autophagic pathways or we performed siRNA-based gene silencing of autophagy-related genes (*Lamp2a*, *Atg5*) and assessed the levels of TPPP/P25A by western immunoblotting and immunocytochemistry.

The pharmacological and molecular inhibition of ALP resulted in a significant accumulation of TPPP/P25A in both cellular models, upon their treatment either with PBS or PFFs. Moreover, our data show that TPPP/P25A bears a KFERQ-like motif and is effectively cleared via Chaperone-mediated autophagy (CMA) in an *in vitro* system of isolated rat brain lysosomes. Interestingly, pharmacological enhancement of CMA or macroautophagy resulted in a decrease of TPPP/P25A levels both under physiological and under pathological conditions of PFF-induced seeding of SNCA.
The ALP mediates the clearance of TPPP/P25A in oligodendrocytes; therefore manipulation of autophagic pathways may represent a successful approach for the removal of pathologically accumulated proteins in the context of MSA.

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INTRANASALLY ADMINISTERED NOVEL CARBON NANOFORMS ENCAPSULATED IN POLYMERIC CARRIERS EFFICIENTLY DELIVER GALANTAMINE TO THE TRANSGENIC ALZHEIMER RAT BRAIN

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Oral administration of the Alzheimer's disease (AD) treating agent galantamine (Gal) produces severe gastrointestinal effects and low Gal accumulations in the brain. Intranasal (IN) administration of Gal-loaded nanoparticles for the direct nose-to-brain delivery of Gal could serve as an excellent alternative. Although IN delivered nanoparticles are able to enter the brain, their distribution in the various brain regions over time and their ability to efficiently deliver Gal to AD-affecting neurons are still unknown.

In the present study, Hierarchical Porous Carbon (HPC) loaded with Gal and rhodamine-B and nanoencapsulated in poly-(lactic-co-glycolic acid) (PLGA) 65/35 were prepared by solid-oil-water modified double emulsification method. They were administered IN to 24 Fischer-344 rats, sacrificed at 1h, 2h, 4h, 24h, 48h or 72h post-delivery. Treated brains were either immunohistochemically stained and examined under the confocal microscope or processed for HPLC Gal quantification. *In vitro* evaluation of PLGA65/35-HPC-Gal nanoparticles on primary neuronal cell cultures was also performed. Experimentation was approved by competent regulatory authorities (66629(261)/02.04.2019).

Histological examination revealed that prepared PLGA65/35-HPC-Gal nanoparticles, with an average size of 241.598±0.31nm, entered the brain and were dispersed all along it's rostro-caudal axis as early as 1h after their IN delivery. Nanoparticles were detected mainly along the olfactory pathway (olfactory bulb, piriform cortex, entorhinal cortex), neocortical areas (orbitofrontal, motor and somatosensory cortex), hippocampus, amygdala and cerebellum. Neuronal (NeuN), microglial (Iba-1) and astroglial (GFAP) marker immunostaining and *in vitro* experimentation revealed an intra-cellular localization of PLGA65/35-HPC-Gal nanoparticles and their distribution pattern over the 72h period. HPLC successfully quantified Gal in all treated brains and showed that Gal was not detectable in the circulating blood at any examined time point.

Conclusively, PLGA65/35-HPC-Gal nanoparticles, after a single IN administration, are capable to efficiently reach and deliver Gal to AD-affected brain regions and neurons. Their therapeutic potential is under evaluation in AD-transgenic rats.

* equal contribution

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THE ROLE OF CANNABIDIOL ON A SCHIZOPHRENIA-LIKE BIO-PHENOTYPE INDUCED BY REPEATED KETAMINE

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Ketamine (KET) has been associated with the induction of schizophrenia-like profile, while accumulating evidence from clinical and experimental studies support the antipsychotic potential of cannabidiol (CBD). This study aims to investigate specific behavioral, neurochemical, and neurobiological aspects of the schizophrenia-like bio-phenotype induced by repeated subanesthetic KET, and to explore the potential mitigating role of CBD.

Male adult Sprague-Dawley rats have been treated with 30 mg/kg/day KET or saline (SAL) for 10 days. Subsequently, rats were treated with 10 mg/kg/day of CBD, or vehicle (VEH). Two days after the last injection, rats underwent a battery of behavioral tests consisting of spontaneous and habituated open-field activity, object recognition task, social interaction, and pre-pulse inhibition. Moreover, the dopaminergic activity in specific brain regions involved in the neurobiological substrate of schizophrenia was estimated with HPLC. In parallel, protein expression levels of neurobiological indices associated with neuroplasticity processes and their downstream signaling were evaluated with western blot.

KET-treated rats displayed defected motor habituation, impaired recognition memory, social dysfunction, and sensorimotor gating deficits. Subsequent CBD treatment ameliorated the behavioral alterations. Neurochemical analyses have shown region-specific dopaminergic alterations while western blot analyses demonstrated an affected expression pattern of downstream signaling related to glutamatergic function and neuroplasticity following KET treatment. These neurobiological alterations were modulated in KET-CBD treated rats.

Repeated KET administration induced a schizophrenia-related bio-phenotype in terms of behavior and the subsequent neurochemical and neurobiological analyses. CBD ameliorated or reversed the behavioral aspects of this schizophrenia-like model while affected KET-induced neurochemical alterations and modulated the neurobiological underpinnings of the bio-phenotype in a region-specific manner. The abovementioned findings characterize further the schizophrenia-like bio-phenotype induced by repeated KET, provide insights regarding schizophrenia pathophysiology, and enrich our understanding of the antipsychotic potential of CBD.

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INVESTIGATION OF PREFRONTAL CORTICAL NEUROPHYSIOLOGICAL MECHANISMS DURING NEONATAL, JUVENILE AND ADOLESCENT PERIODS OF DEVELOPMENT IN A MOUSE MODEL OF SCHIZOPHRENIA

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Schizophrenia is a common, severe and multifactorial neuropsychiatric disorder, for which current medication mainly focuses on treating the positive symptoms of the disease. In our study we aim to identify early-life neurophysiological changes conceivably evident in the methylazoxymethanol acetate (MAM) mouse model of schizophrenia compared to control mice (saline-treated) [1]. Our experiments included neonatal (P8-P11), juvenile (P15-P21) and adolescent (P40-P45), female and male C57BL/6J mice. MAM or control mice were decapitated and prefrontal cortical (PFC) brain slices were acquired for extracellular local field recordings, followed by analysis for neuronal oscillations present in the recordings. Adolescent MAM and control mice performed the temporal object recognition (TOR) task, and afterwards the mice were used for electrophysiological recordings. Our results indicate a significant reduction regarding the baseline neuronal oscillations of delta, theta, alpha and beta rhythms in neonatal MAM mice, but not in juvenile or adolescent MAM mice, compared to controls. In control adolescent mice, ketamine application in PFC brain slices tended to increase the beta and gamma frequencies; however, in MAM adolescent mice ketamine tended to reduce the contribution of these frequencies. Finally, adolescent MAM mice exhibit a significantly reduced discrimination index compared to control mice in TOR task. In conclusion, early-life alterations of neuronal oscillations could affect prefrontal cortical development and lead to cognitive deficits (TOR deficits) observed in adolescence.

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BNN27 MICRONEUROTROPHIN EFFECTS AFTER OPTIC NERVE CRUSH

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Optic Nerve Injury (ONI) is a leading cause of irreversible blindness worldwide. Currently, there is no available pharmacological or surgical intervention capable of preventing ONI consequences including retinal ganglion cell (RGC) death and eventual loss of vision [1]. The objective of this study is to evaluate the effects of BNN27 microneurotrophin (MNT), a small-molecule analog of endogenous NGF [2], in an established mouse model of optic nerve crush (ONC) that resembles clinical ONI. BNN27 was delivered via eye drops (administered daily) or via porous collagen scaffold grafts (PCS) placed once around the optic nerve at the injury site. BNN27 effects were evaluated 2 and 10 weeks post injury (wpi), focusing on behavior, neuronal survival, axonal elongation and inflammation.

Experimental results show that ONC led to significant RGC death, astrogliosis, axonal degradation in the optic nerve and vision impairment, as expected already at 2 wpi. BNN27 administration resulted in significant improvement on RGC survival. Although the method of BNN27 delivery did not affect RGC survival, delivery via grafts resulted in more consistent effects. While RGC rescue was maintained at 10 wpi, BNN27 delivery did not enhance axonal regeneration at 10 wpi and consequently did not improve optomotor response, in agreement with previously published effects of BNN27 on neurite extension [3].

While several studies have probed MNT effects in various animal models of neurodegeneration [4], this study presents the first evidence on MNT neuroprotective effects on ONI. It demonstrates that BNN27 administration can prevent ONC-induced RGC apoptosis. This study also presents the first administration of MNT via biomaterial grafts, which provides a more targeted way of delivery at ONC sites that does not induce further damage. The observed neuroprotective effects of BNN27 can be utilized to develop novel treatments for ONI, complemented by other compounds that can enhance the elongation of surviving axons towards their targets.

Acknowledgments

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NR5A2 AS A POTENTIAL DRUG TARGET IN NERVOUS SYSTEM MALIGNANCIES

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Abstract

Nervous system malignancies are characterized by rapid progression and poor survival rate. Glioblastoma multiforme is the most aggressive nervous system malignacy and despite recent advances in the provided therapy the average survival time remains low, between 12 to 15 months. These clinical observations underscore the need for novel therapeutic insights and pharmacological targets. Towards this direction, here we identify the orphan nuclear receptor NR5A2/LRH1 as a negative regulator of cancer cell proliferation and promising pharmacological target for nervous system-related tumors. In particular, by meta-analysing clinical data from TCGA and Oncomine databases, we find that high expression levels of NR5A2 are associated with favourable prognosis in patients with glioblastoma or tumors. Here, we experimentally show that NR5A2 is sufficient to strongly suppress proliferation of both human and mouse glioblastoma (U87-MG, GL261) and neuroblastoma cells (SH-SY5Y, Neuro2A) without affecting apoptosis. The anti-proliferative effect of NR5A2 is mediated by the transcriptional induction of negative regulators of cell cycle, CDKN1B ($p27^{kip1}$), CDKN1A ($p21^{cip1}$) and Prox1. In contrast, silencing of NR5A2 induces proliferation and suppresses the previous mentioned genes. Interestingly, two well-established pharmacological agonists of NR5A2, DLPC and DUPC, are able to mimic the anti-proliferative action of NR5A2 in human glioblastoma cells. Most importantly, NR5A2 overexpression or treatment with DLPC inhibits neuroblastoma and glioblastoma tumor growth in vivo respectively, in xenograft mouse models. These data indicate a tumor suppressor role of NR5A2 in nervous system and render this nuclear receptor a potential pharmacological target for the treatment of nervous tissue related tumors.

INVESTIGATION OF THE POTENTIAL NEUROPROTECTIVE ROLE OF BNN-20 IN PARKINSON'S DISEASE PATIENT-DERIVED NEURONS

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Parkinson's disease (PD) remains an incurable neurodegenerative disorder with variable clinical characteristics, age of onset and course of progression. PD is characterized by motor dysfunction related to the progressive loss of midbrain dopamine neurons while non-motor symptoms are also present. BNN-20 is a synthetic, BDNF-mimicking, microneurotrophin that has been shown to exhibit a pleiotropic neuroprotective effect on dopaminergic neurons of the substantia nigra pars compacta (SNpc) in the "weaver" mouse model of PD. Here, we assessed its potential effects in a unique human setting for the identification and interpretation of PD phenotypes. In particular, we used an induced pluripotent stem cell (iPSC)-based model of PD from patients bearing the p.A53T-α-synuclein mutation that simulates disease-relevant phenotypes, including protein aggregation, compromised neuritic growth, axonal pathology and reduced synaptic connectivity. BNN-20 treatment seems to increase slightly the percentage of TUJ1+ neurons in the mutant cultures and ameliorate axonal pathology. At the same time, we did not observe significant differences in neurite outgrowth between untreated and BNN-20 treated p.A53T TH+ neurons. Finally, exposure to BNN-20 could not restore the mRNA levels of genes associated with axon guidance and synaptic function that were dysregulated in p.A53T cells. Calcium imaging and induced stress experiments are in progress. Further investigation is needed to identify BNN-20 as a potential neuroprotective agent.

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ASSESSMENT OF CHRONIC STRESS EFFECTS ON ADULT NEUROGENESIS IN A HUMAN α-SYNUCLEIN OVEREXPRESSION MODEL

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Parkinson disease (PD) is a progressive neurodegenerative disorder characterized pathologically by the accumulation of α-synuclein in Lewy bodies. Although PD is a movement disorder, it frequently manifests with non-motor symptoms, including anxiety, depression, and cognitive impairment- symptoms that correlate with deficits in adult hippocampal neurogenesis. In PD animal models, manipulation of α-synuclein levels demonstrates its crucial role in the regulation of neurogenesis. Furthermore, stress is considered a risk factor in neurodegenerative diseases and it is detrimental to adult neurogenesis. However, the complex interplay between chronic stress and an enhanced α-synuclein burden in Lewy bodies on adult hippocampal neurogenesis, remains yet unexplored. Herein, we assessed the effects of chronic unpredictable stress in male BAC-transgenic rats overexpressing human α-synuclein (BAC) on adult hippocampal neurogenesis and local stress-related markers. We further extended our analysis of stress markers to postmortem hippocampal samples from PD patients. Our data indicate a dysregulation of the mRNA expression of glucocorticoid receptors (GR, MR) and corticotropin-releasing hormone (CRH) in the hippocampus, demonstrating a baseline deficit in stress responsivity in both BAC rats and PD patients. Hippocampal GR and MR levels in PD patients demonstrated significant correlations with a-synuclein levels. Furthermore, BAC animals demonstrated impaired adult neurogenesis, however, chronic stress did not further worsen the stress system imbalance observed or the neurogenesis deficit. Our study demonstrates that aberrant α-synuclein expression/accumulation drives stress system dysregulation, leading to impaired adult neurogenesis. In conclusion, enhanced α-synuclein burden leads to stress homeostasis deficits and over time, as the disease progresses, could potentially enhance stress susceptibility.

CONSEQUENCES OF CHRONIC CORTICOSTERONE ADMINISTRATION IN A PARKINSONIAN MODEL OF BAC-TRANSGENIC RATS OVEREXPRESSING HUMAN α-SYNUCLEIN

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Increasing evidence indicate that chronic stress and HPA axis activation play a role in the pathogenesis of Parkinson's disease (PD). The effects of chronic corticosterone in a prodromal PD rat model were assessed by examining non-motor and motor behaviors, brain-region specific alpha-synuclein and monamine profiles and dopaminergic system integrity. 9-month old BAC-transgenic Sprague Dawley male rats overexpressing human alpha-synuclein (AS-BAC) and their wild type (WT) littermates were administered corticosterone (50 μg/ml in drinking water, 14 days). Subsequently, subjects underwent a battery of behavioral tests followed either by brain dissection (striatum, hippocampus, hypothalamus and amygdala) for neurochemical and biochemical analysis using HPLC and Western immunoblotting, respectively, or by perfusion for TH+ stereological cell counts in the substantia nigra and VTA. Corticosterone administration reduced adrenal gland weights, increased thymus gland weights, and decreased olfactory discrimination and open arm time in the elevated plus maze in WT rats. These alterations were present in AS-BAC rats at baseline and not further affected by corticosterone. In the open field, previously reported novelty-induced hyperactivity in AS-BAC rats (Polissidis et al., 2021) was reversed and postural instability was exacerbated following corticosterone. The integrity of the dopaminergic system was impaired by corticosterone administration which led to a loss of TH+ neurons in the Substantia nigra in both WT and AS-BAC rats. Noradrenaline levels were increased in the hypothalamus of corticosterone-administered WT and AS-BAC rats and reduced in the hippocampus of AS-BAC rats, irrespective of corticosterone treatment. Finally, corticosterone reversed enhanced dopamine turnover in the hypothalamus of AS-BAC rats. Quantification of AS protein levels in the aforementioned brain areas is currently underway. Our results demonstrate that chronic corticosterone recapitulates key non-motor behavioural features of PD and exacerbates motor symptoms in AS-BAC rats, indicating that chronic stress may contribute to and/or exacerbate PD pathology associated with an enhanced alpha-synuclein burden.

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GENERATION OF A PARKINSON'S DISEASE CELL MODEL OVEREXPRESSING YFP-SNCA A53T

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Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting millions of people worldwide. Despite the enormous scientific efforts, the pathological mechanism remains unclear, whereas no treatment has been developed to reverse disease progression effectively. Therefore, reliable cellular models are needed for the detailed study of affected biological mechanisms.

Here, we inducibly overexpressed YFP-tagged SNCA A53T in neural precursor cells (NPCs) using the Sleeping Beauty transposon. Additionally, NPCs were differentiated into neuronal cells. The recombinant protein was detected in all cell types forming visible cytoplasmic vesicles. We also found that the metabolic activity of NPCs was significantly reduced in parallel with the activation of caspases. However, no such activation was detected in neuronal cells, indicating that different mechanisms may function in different developmental stages. Additionally, the pathological protein was secreted from genetically-engineered cells and endocytosed by healthy cells. YFP-SNCA A53T-expressing cells show an altered phenotype and might be relevant cell models for the study of familial PD.

EXAMINING THE EFFECT OF PRENATAL ALCOHOL EXPOSURE ON EXPERIMENTAL SEIZURE PARAMETERS IN GROWING AND ADULT RATS

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Prenatal alcohol exposure (PAE) has been linked to developmental neurological disorders, including an increased risk of seizures, a correlation not widely studied, particularly in mild PAE models. We investigated the effects of mild PAE in seizure susceptibility & severity of the offspring, using a model that has shown neuronal excitability changes *in vitro* in our lab.

Methods: Young adult nulliparous female Sprague-Dawley rats, received an ethanol solution (first 10% v/v, then 15% v/v in water) as their drinking water for 2 weeks before breeding and throughout gestation; after parturition, the ethanol concentration was gradually decreased and was replaced by clean water the 15th day. Experimental seizures were induced in the offspring at the 20th (Young, Y) or the 60th postnatal day (PND, Adult, A), and same age healthy rats as controls (Normal, N), by an i.p. injection of $GABA_A$ antagonist pentylenetetrazol (PTZ) in gradually increasing concentrations (50-70-80 mg/kg) until generalized tonic-clonic seizures with balance loss were observed (animals were monitored for a 4hour period). A total of n=161 male (M) and female (F) rats were used; 71 of these were Y (PAE, 28M & 17F; N, 13M & 13F) and 86 A (PAE, 21M & 20F; N, 28M & 21F). In order to describe accurately seizure behavior ("stages", characteristics), we devised a new classification scale, based on previously published ones*. Data were then statistically analyzed by Fisher's exact test (or Chi-square) and with student's t-test (unpaired samples, Prism program).

Preliminary data analysis indicated seizure differences between N and PAE animals, depending on age (Y, A) and sex (M, F), indicating that mild but continuous PAE could change seizure potential in growing animals. Furthermore, these differences diminish in adulthood, suggesting the presence of compensatory mechanisms that may, at least partially, reverse the detrimental effects of PAE.

**(Racine 1972, Pinel & Rovner 1978, Lüttjohann et al. 2009, Velíšková & Velíšek 2017, Jan Van Erum et al. 2019)*

EARLY OLFACTORY BULB PATHOLOGY IN ALPHA-SYNUCLEIN TRANSGENIC RATS

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Parkinson's Disease (PD) is characterized by the aberrant deposition of α-synuclein (AS). We have shown that induction of the lysosomal degradation process of Chaperone Mediated Autophagy (CMA) via upregulation of its selective transmembrane receptor LAMP2A is associated with enhanced AS clearance and amelioration of its pathological effects on the rodent nigrostriatal axis (Xilouri et al., 2003). However, effects of CMA manipulation in other brain areas affected in synucleinopathies, such as the olfactory system, have not been studied. Furthermore, it is unknown whether the strategy of CMA enhancement may be beneficial in synucleinopathy models in which pathology is already established, foreshadowing potential clinical use. The aim of the current overall study is to identify the time point at which abnormal AS accumulation commences in the olfactory bulb of WT human AS overexpressing BAC transgenic rats, and to investigate whether the AAV-mediated overexpression of LAMP2A will counteract and/or reverse the aberrant AS deposition and its resultant behavioral effects both in early and later stages. We demonstrate here via fractionated Western blotting the accumulation of total, human and phosphorylated AS in both Triton- and SDS-soluble fractions of the olfactory bulb of 4, 8 and 12 week-old hAS homozygous BAC rats, relative to WT littermates. The amount of AS remained constant over 4 to 8 weeks, while a slight increase was observed at 12 weeks. An olfactory discrimination test revealed potential impairment of olfaction only at 12 weeks. Our data suggest that early aberrant AS accumulation appears in the olfactory bulb at the age of 4 weeks, whereas at the age of 12 weeks there is slight enhancement of pathology and initial signs of olfactory dysfunction. These findings set the stage for the assessment of the effects of the manipulation of the CMA pathway on the olfactory system of this synucleinopathy model.

DEVELOPMENT OF A HUMAN 3D iPSC-BASED MODEL OF p.A53T-SYNUCLEINOPATHY TO MONITOR DISEASE INITIATION AND PROGRESSION

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Parkinson's disease (PD) and related synucleinopathies are a group of incurable neurodegenerative disorders associated with alpha-synuclein (αSyn) pathology, with the best-characterized mutation, G209A, in the αSyn gene SNCA resulting in the pathological p.A53T-αSyn protein. Although current model systems, including cell culture and animal models enriched significantly our understanding of PD pathology, they do not adequately recapitulate human disease-associated features. Our group has established a 2D induced pluripotent stem cell (iPSC)-based model of PD from patients bearing the p.A53T-αSyn mutation that simulates disease-relevant phenotypes, including protein aggregates, compromised neuritic growth, axonal pathology and reduced synaptic connectivity. Here, we describe the generation of forebrain and midbrain-like iPSC-based 3D cultures, which exhibit more complex cell-cell interactions and spatial organization closely resembling the in vivo situation. Forebrain organoids at 30 days in vitro (DIV) consisted of radially allocated neural progenitor structures and cortical neuron generation. In particular, immunocytochemical characterization confirmed the existence of PAX6+ and SOX2+ neural progenitors, HOPX+ outer radial glia, DCX+ early neuroblasts, and CTIP2+ deep layer early cortical neurons. On the other hand, midbrain-like organoids (MLOs) maintained for up to 60 DIV exhibited spatially organized groups of FOXA2+ midbrain floor plate progenitors, LMX1A+ early and NURR1+ late midbrain progenitors as well as TH+ dopaminergic neurons. Detailed characterization for neuronal and astroglial differentiation in p.A53T organoids vis a vis control is in progress to explore αSyn-relevant pathology and assess therapeutic interventions.

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OLIGODENDROGLIAL-DERIVED EXOSOMES AS MEDIATORS OF α-SYNUCLEIN PATHOLOGY IN MSA-LIKE MODELS

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The current study focuses in elucidating the contribution of oligodendroglial-derived exosomes in the development and spread of α-Synuclein (αSyn) pathology in Multiple System Atrophy (MSA)-like experimental models.

MSA is a neurodegenerative disease, defined by the formation of inclusion bodies within oligodendrocytes mainly consisting of the neuronal protein αSyn and the oligodendroglial phosphoprotein TPPP/p25α. Under physiological conditions, αSyn cannot be detected in mature oligodendrocytes, thus supporting the prevailing proposed pathogenic pathway of αSyn entering oligodendrocytes following its release by neighboring neurons. This release takes place partly via exosomes, highlighting their possible involvement in αSyn-related pathology transmission.

A comprehensive biochemical analysis of exosomes isolated from control OLN-93 rat oligodendroglial cell lines or cells stably overexpressing human αSyn or TPPP/p25α was performed. To recapitulate the pathological conditions *in vitro*, cells were incubated either with human αSyn Pre-Formed Fibrils (PFFs) or MSA-amplified brain-derived fibrils. Both fibril- and PBS-treated (control) cells were incubated with exosome depleted medium and exosome-related and intracellular αSyn levels were assessed by immunoblot and confocal microscopy.

Our results indicate that both αSyn and TPPP/p25α can be released via oligodendroglial exosomes. The αSyn cargo was elevated upon treatment with all aSyn fibrils however, we detected high molecular weight αSyn species in the exosomal fraction only in the presence of MSA-amplified aSyn fibrils. Moreover, the overexpression of hαSyn or TPPP/p25α accelerated the release of αSyn-containing exosomes, following PFF treatment.

Based on our findings we surmise that oligodendroglial exosomes could serve as key participants in the spread of αSyn and/or TPPP/p25α pathology in MSA. Their potential pathogenic propensity will be further investigated following their inoculation in neural and oligodendroglial cultures or in the mouse brain. If our hypothesis is proven successful, this innovative approach will pave the way for the targeting of oligodendroglial exosomes for the treatment of MSA and, potentially, as disease biomarkers.

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Topic: **Neural cell function and signaling**

"CART PEPTIDE, A NOVEL NEUROMODULATOR OF MOTOR NEURON OUTPUT"

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Mammalian locomotion depends on the organization of spinal interneuron circuits, incorporating sensory, descending, and local signals, ultimately controlling motor neuron (MN) activity and muscle contraction. To decipher the interactions between all interneurons and MNs, we focused on each subtype separately, before attempting to comprehend complex circuits. Following up to the discovery of Pitx2$^+$ V0c interneurons, as the source of cholinergic C bouton synapses on MNs, a screen was performed for genes preferentially expressed in these neurons. We show that the Cocaine and Amphetamine Regulated Transcript (*Cartpt* gene), which is overexpressed in Pitx2$^+$ neurons, coding for the CART peptide, is not only present in 97% of Pitx2$^+$ V0c somata but also abundant in the V0c-derived C bouton synapses on MNs along with acetylcholine. Previous data showed that genetic elimination of acetylcholine in C boutons led to impaired muscle activation in a task-dependent manner, suggesting that C boutons aid in the increase of MN firing in demanding tasks. We sought to explore the role of CART in neurotransmission and its relationship with acetylcholine. Electrophysiological investigation of the effects of CART on MNs' excitability revealed that CART not only reduces the recruitment current of Fast MNs but also increases their firing rate near rheobase. Behavioral analysis of CART deficient mice revealed that mice lacking the CART peptide exhibit impaired motor performance in a gender-dependent manner. Anatomical assessment of CART, acetylcholine and CART-acetylcholine deficient mice revealed that the presence of each neurotransmitter remained unaltered in the absence of the other and that C bouton synapses retain their gross anatomy in the absence of both neurotransmitters. Our data reveal a novel MN neuromodulator, CART, co-existing with acetylcholine in C bouton synapses, suggesting a modulatory role alongside acetylcholine contributing to the same function of C boutons via a different mechanism.

NOVEL MOLECULAR COMPONENTS OF PROTEIN SYNTHESIS INDEPENDENT MEMORY

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As time flies, memories remain. In *Drosophila*, there are two forms of consolidated memories acquired by aversive olfactory conditioning: Protein Synthesis Independent Memory (PSI-M) lasting 24-48 hrs and Protein Synthesis Dependent Long Term Memory (PSD-LTM) perduring for weeks. There are multiple intriguing features that differentiate PSI-M from PSD-LTM, most importantly its independence of *de novo* protein synthesis. Such mechanistic differences between the two memories, along with the evidence of their reliance on distinct molecular components, motivated a systematic search of molecular pathways engaged in PSI-M. Interestingly, even though some PSI-M properties have been described in additional invertebrates other than *Drosophila*, this evolutionary conservation suggest that analogous processes are likely operant in vertebrates, which remain tenuous for the moment.

Based on the knowledge that the DRK protein is a necessary component for PSI-M, we searched for protein interactors of DRK within *Drosophila* adult Mushroom Bodies (MBs), neurons essential for associative olfactory memories in the fly and validated their role in PSI-M. Our results indicate that there are at least two distinct molecular pathways engaged during PSI-M and support a more nuanced outline of its molecular components. We posit that further investigation of the pathways supporting PSI-M will not only provide insight into *Drosophila* memory mechanisms, but also provide potential mechanistic links to akin memory types observed in other species, thus aiding in understanding the nature and utility of PSI-M.

IN SITU PROXIMITY LABELING REVEALS DDIT4 INTERACTING PROTEOME

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DNA damage-inducible transcript (DDIT4) is a ubiquitous protein that, under physiological conditions, is transiently increased in response to a variety of stress. However, in compromised neurons in human post-mortem brains of patients with neurodegenerative disorders such as Parkinson's and Huntington's disease, it is persistently upregulated leading to cell death. DDIT4 is best recognized for repressing mTORC1, a key protein complex indispensable for cell function and survival, that is activated by nutrients and hormones. Accordingly, DDIT4 regulates metabolism, oxidative stress, and apoptosis. Despite these well-characterized biological functions, we know little about its interacting partners and specific molecular function. Here, fusing an enhanced ascorbate peroxidase 2 (APEX2) biotin-labeling enzyme to DDIT4 combined with mass spectrometry, we *in situ* defined the proteins in the immediate vicinity of DDIT4 in both control and acute stress conditions. Remarkably, the two proteomes had a quantitative but not a functional difference. DDIT4 had twice as many interaction partners during acute stress, and while the IDs of the two protein lists showed a small overlap, the molecular function and protein categories were essentially identical. Moonlighting keratins and ribosomal proteins dominated the proteomes in both unstressed and stressed conditions, with many of their members having established non-canonical and indispensable roles during stress. Keratins regulate mTORC1 signaling via recruitment of 14-3-3 proteins, whereas ribosomal proteins control cell cycle progression, DNA repair, and death by sequestering critical proteins. Overall, our findings highlight two potentially distinct mechanisms of DDIT4 molecular function and open the door to better understand neurodegenerative pathways.

IN SITU PEROXIDASE LABELING FOLLOWED BY MASS-SPECTROMETRY REVEALS TIA-1 INTERACTOME

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TIA1 is a broadly expressed DNA/RNA binding protein that regulates multiple aspects of RNA metabolism. It is best known for its role in stress granule assembly during the cellular stress response. Mutations in TIA1 cause amyotrophic lateral sclerosis with or without frontotemporal dementia, and it is thought to facilitate tau-mediated neurodegeneration. Three RNA recognition motifs mediate TIA1 functions along with a prion-like domain that supports multivalent protein-protein interactions that are yet poorly characterized. Here, fusing an enhanced ascorbate peroxidase 2 (APEX2) biotin-labeling enzyme to TIA1 combined with mass spectrometry, we defined the proteins in the immediate vicinity of TIA1, in situ. Eighty-three and 203 protein partners, mostly associated with ribonucleoprotein complexes, were identified in control and acute stress conditions, respectively. Remarkably, the two proteomes were less than ten percent similar. In control conditions, the TIA1 interactome was enriched for biological processes that are associated with cytosolic ontologies related to mRNA metabolism, including translation initiation, nucleocytoplasmic transport, and RNA catabolism, and was represented by RNA binding proteins, ribosomal subunits and eicosanoid regulators. In stress, TIA1-labeled partners displayed a broader subcellular distribution that included the chromosomes and mitochondria. The enriched biological processes included splicing, translation, and protein synthesis regulation while the molecular function of the proteins was enriched for RNA binding activity, ribosomal subunits, DNA double-strand break repair and amide metabolism. Altogether, these data highlight the TIA1 spatial environment with its different partners in diverse cellular states and pave the way to mechanistically dissect TIA1 role in these processes giving insights into the etiopathology of neurodegenerative diseases.

AGE-RELATED DIFFERENTIAL TAU EXPRESSION AND PHOSPHORYLATION

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Tau is a neuronal microtubule-associated protein, located mainly in axons and it is necessary for normal brain function, as it ensures the stability of microtubules and contributes to axonal transport. It is involved in the pathology of several neurodegenerative diseases through aberrant post-translational modifications, changes in its subcellular distribution and/or aggregation. Its abnormal phosphorylation and hyperphosphorylation play a critical role in Alzheimer's disease (AD). AD occurs mainly in the elderly (>65 years old), in 98-99% of the cases, making age a key risk factor. Since there is a great overlap between brain pathological features observed in normal aging and AD pathology, there is a need to clarify their differences. In the present study, we examined expression levels of tau and its forms phosphorylated at the residues Thr205, Ser262, Ser356, Ser396, and Ser404 in the brains of mice at different ages, ranging from 1-month-old to 36 months. The experiments were performed in total brain extracts as well as in subcellular fractions including Pellets, containing mainly the nuclei and cytoskeletal elements, postsynaptic density fractions (PSDs), and non-PSDs, obtained through differential extraction and centrifugation. The samples were analyzed by the semi-quantitative western blot technique. In aged mice, total tau expression levels were relatively reduced, whereas the opposite was observed in Pellets. Tau phosphorylation in total extracts, in non-PSDs, and especially in PSDs was generally increased at the examined residues in aged mice, while in Pellets it was decreased. Those age-related changes could shed light on the mechanism of development of age-related tau pathology.

PHARMACOLOGICAL CHARACTERIZATION OF A SMALL CRF_1R PEPTIDE ANTAGONIST

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The corticotropin releasing factor (CRF) and its type 1 receptor (CRF_1R) play a key role in anxiety and depression, which are serious neuropsychiatric diseases affecting a large percentage of the population. In an effort to develop novel CRF_1R antagonists we designed, a small peptide analogue of CRF (D-LMI), based on the crystal structure of the N-extracellular region (N-region) of CRF_1R/CRF complex. The D-LMI is a CRF antagonist, because it inhibits 1) the CRF stimulated cAMP accumulation in HEK293 cells expressing the CRF_1R, 2) the CRF-stimulated proliferation rate of RAW 264.7 cells and 3) the CRF-stimulated production of CXCL1 from adipocytes. The D-LMI was unable to block the constitutive activity of the CRF (1–16)/R1ΔN chimera, which lacks the N-region of CRF_1R. Similarly, the antagonist astressin, which binds to the N-region of CRF_1R was unable to block the constitutive activity of the CRF(1–16)/R1ΔN chimera, in contrast to the non-peptide antagonist antalarmin which binds to the transmembrane domains of the receptor. These results suggest that the D-LMI exerts its antagonist actions by binding to the extracellular N-region of CRF_1R. Determination of the degradation rate of D-LMI after its incubation with human plasma at 37 °C for different time points suggested that the D-LMI is a proteolytically stable peptide. Based on the above results the D-LMI is a promising molecule that urges for the synthesis of a new series of CRF_1R antagonists targeting the extracellular N-region of CRF_1R.

κ-OPIOID RECEPTOR ACTIVATION LEADS TO HIPPOCAMPAL SYNAPTIC ALTERATIONS BY AN AUTOPHAGIC MECHANISM

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Autophagy is a lysosomal degradation pathway that eliminates misfolded proteins and dysfunctional organelles to safeguard cellular homeostasis. In neurons, autophagy controls the quality of cytoplasmic proteins through degradation of important synaptic proteins and modulates synaptic organization and morphogenesis (1). Emerging studies have shown that G protein coupled receptors are direct sensors regulating the autophagic machinery (2) and that opioid receptors regulate neurogenesis and neurotransmission with as yet unclarified mechanisms (3,4). In this regard, we demonstrate that κ-opioid receptor (κ-OR) agonists induce autophagy in neuronal cells *in vitro* in a PTX-sensitive G protein manner, ERK1,2 and CREB, thus proposing a novel signaling pathway involved in this process. *In vivo* studies in mice administered with the κ-OR selective agonist U50,488H display profound increases of specific autophagic markers with a concomitant decrease of proteins enriched in dendritic spines such as spinophilin, PSD-95 and SNAP25, which interact with LC3, suggesting that these proteins are plausibly engulfed in the κ-OR-induced autophagic cargo. This U50,488H-induced autophagy is region specific since it is detected only in hippocampal synaptosomes. Finally, knowing that the dynorphin/κ-OR system plays an important role in anxiety-like behaviors we demonstrate that mice administered with the κ-OR selective antagonist norBNI upon stress stimuli blocked κ-OR-induced autophagy, while leaving the levels of hippocampal synaptosomal proteins unaltered. These results demonstrate for the first time that κ-OR-induced autophagy plays a key role in synaptic function in hippocampus and may contribute to the mood disorders targeted by the dynorphin-κ-OR system under acute stress conditions.

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NEUROFIBROMIN ISOFORMS REGULATE ACUTE CANNABINOID RECEPTOR 1(CB1) PROXIMAL SIGNALLING IN NEUROBLASTOMA AND GLIOMA CELLS

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Characterized by variable clinical presentation and high predisposition to tumors, Neurofibromatosis 1 (NF-1) is a common monogenic disease, caused by mutations in the *NF1* gene. While >3000 different mutations are identified, genotype-phenotype associations remain weak. Moreover, a multitude of developmental stage- and cell type-specific alternative splicing events in *NF1*, which produce different neurofibromin isoforms, further make predictions of the functional outcome of mutations very difficult. Most notable is the insertion of exon51, which contains a NLS allowing the protein to enter the nucleus via the Ran pathway (Koliou et al, 2016). In examining the roles of NLS- or ΔNLS-neurofibromins, using transcript-specific shRNA-depletions, we have begun to examine their individual input on the established roles of neurofibromin as a RasGAP and as a MAP. We have recently documented that NLS-neurofibromins are required as MAPs for proper spindle assembly and faithful genome transmission (Peta et al, 2020). Currently, we focus on the effects of NLS-neurofibromins loss as a RasGAP, using transcript-specific shRNAs in the IMR32 neuroblastoma cells. In naïve cells, we have postulated that CB1-dependent PKCε activation leads to an acute, transient recruitment of the RasGEF SOS1 to lipid rafts for Ras activation, and onto neurofibromin recruitment to deactivate Ras. As activated Ras molecules flow out of the rafts, some are acutely deactivated by neurofibromin and flow back. This "treadmilling" mobility has documented that neurofibromin filters out the plethora of activated H-Ras molecules, controlling its downstream signalling output (Karouzaki et al, 2019). We now find that loss of NLS neurofibromins accelerates this CB1-induced treadmilling, reflected in the altered activation profiles of its downstream effectors H-Ras, Src, Raf, and ERK. As activation of CB1 is coupled to proliferation rate decreases in IMR32 in the long term, our data suggest that NLS neurofibromins impose an important regulation on the Ras pathway outcome.

THE ROLE OF THE GLOBAL ORGANIZER FACTOR Satb1 IN SYNAPTIC HOMEOSTASIS

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The balance between excitation and inhibition levels has a key role in the formation and function of cortical neuronal circuits. Among the mechanisms that are important and necessary for the maintenance of this fragile balance, homeostatic plasticity is included. In addition to transcription and translation, activity-dependent epigenetic modifications serve as molecular mechanisms for the regulation of gene expression during brain homeostasis. Satb1 is a regulator of chromatin architecture with well-defined roles in diverse cellular processes such as immune cell differentiation, cancer growth and metastasis, which are only matched by the dearth of information regarding its function in the nervous system. However, emerging evidence suggests that Satb1 has fundamental roles in brain function and homeostasis. Thus, during development, including critical postnatal stages of cortical circuit maturation, Satb1 expression is regulated by neuronal activity. Furthermore, Satb1 controls the timing and expression levels of several immediate early genes, known to be implicated in synaptic plasticity. Our aim is to investigate how Satb1 function regulates the homeostasis of synaptic efficacy. To examine this we set up an in vitro system of primary cortical neuronal cultures. Applying picrotoxin (PTX) to manipulate the levels of neuronal activity, in control and Satb1-deficient cortical neurons, we are trying to unravel the intricacies of Satb1 regulation in response to changes in network activity by using molecular and -omics approaches.

IPSC-DERIVED MIDBRAIN-PATTERNED ASTROCYTES FROM PARKINSON'S DISEASE PATIENTS

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Parkinson's disease (PD) and related synucleinopathies are a group of incurable neurodegenerative disorders associated with alpha-synuclein (αSyn) pathology, with the best-characterized mutation, G209A, in the αSyn gene *SNCA* resulting in the pathological p.A53T-αSyn protein. Although the disease mechanisms remain largely unresolved, the technology of induced pluripotent stem cells (iPSC) provides a unique human setting for the identification and interpretation of PD phenotypes. Most published work, including ours, has focused on neuron-intrinsic dysfunction in PD while the role of other cell types has only started being examined. Astrocytes are the most abundant cells in the human brain with a critical role in maintaining neuronal health, yet their neurotoxic potential is now being appreciated and could contribute to disease pathology through neuroinflammatory mechanisms, a consistent PD feature. Here we report the generation of midbrain-patterned astrocytes from p.A53T-αSyn patients using iPSC technology and their initial characterization by immunocytochemistry and qRT-PCR. Gold standard astrocytic markers were examined in p.A53T astrocytes vis-à-vis human primary astrocytes. Comparison of an isogenic pair of p.A53T-αSyn iPSC-derived astrocytes and its corresponding gene-corrected control in terms of neuroinflammatory reactivity is ongoing, before evaluating their effect on neuronal network connectivity in astrocyte-neuron co-cultures. We anticipate that our model should answer fundamental questions related to PD pathogenesis and could serve as a novel drug-testing platform.

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ROLE OF DEVELOPMENTAL REGULATORS OF AXONAL LOCAL TRANSLATION IN ADULT AXONS

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Local mRNA translation (LT) is vital for axon development. Disruption of the process is implicated in multiple neurodevelopmental disorders, while numerous studies show that axonal mRNA translation is crucial in adulthood, particularly during plastic responses like axon regeneration. Adult PNS axons maintain high levels of LT and can regenerate after injury, while CNS axons that lose their intrinsic ability for LT as they mature, are unable to regenerate successfully. Therefore, developing CNS and adult PNS axons retain crucial growth programs that are absent from, or different in adult CNS axons. However, the regulatory mechanisms behind axonal LT remain elusive. We have previously identified a ribonucleoprotein complex (Mena-RNP) that regulates axonal LT in the developing brain. It requires the cytoskeleton-associated protein Mena that interacts with known regulators of translation (i.e. HnrnpK, PCBP1) and controls translation of the RNP-bound mRNAs. Mena deficiency results in severely reduced levels of the respective proteins in axons due to perturbed LT. Here, we explore the presence and conservation of the Mena-RNP in the adult nervous system, in an attempt to elucidate the role of Mena and Mena-dependent translation in adult axon regeneration. We observe that the components of the Mena-RNP are different not only between developing and adult axons, but also between axons of the CNS and PNS. Interestingly, novel interactors of Mena were also identified, like Vimentin and β-Catenin that have been long related to post-injury mechanisms, implying a potential role of a developmental program (Mena-RNP complex and LT) in processes like axon regeneration.

NEUROPROTECTIVE ROLE OF Nr5a2 NUCLEAR RECEPTOR

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Neurodegenerative diseases, brain injury, ischemia and inflammation are only some of the CNS-impairing diseases which severely affect millions of people worldwide, leading to death, disability and a plethora of comorbidities. Throughout the human life, neural tissue is exposed to a variety of injurious and toxic stimuli. The postmitotic status of neurons, as well as their limited regenerative capacity restricts their ability to overcome cellular injury and death. Common pathophysiological mechanisms such as oxidative stress, hypoxia and ischemia, neuroinflammation, excitotoxicity, toxicity due to protein aggregation and ER stress underline the majority of neurological and neurodegenerative diseases, culminating in neuronal death. Hence, the discovery of neuroprotective factors against multiple injuries has been at the forefront of biomedical research. In the present study we focus on the potential neuroprotective role of Nr5a2, an orphan nuclear receptor, with known pharmacological agonists and transcriptional activity. Nr5a2, also known as Lrh1, has been implicated in the embryogenesis of various visceral organs and in the regulation of metabolic processes, although its role in the CNS has not been extensively described. Here, we present evidence that Nr5a2 possesses a neuroprotective capacity in vitro, as shown on both SHSY-5Y cells and on mouse embryonic primary neuronal cultures exposed to various insults. Nr5a2 overexpression increases cell survival in MTT assay following H2O2 expression, while it decreases activated caspase 3 expression at the protein level. Incubation of primary neurons with DLPC (1,2-dilauroyl-sn-glycero-3-phosphocholine), an Nr5a2 agonist, leads also to decreased expression of the activated caspase 3 protein. Nr5a2 is a druggable receptor that could become a promising neuroprotective target in various neurological disorders.

EFFECT OF NEUROPROTECTIVE AGENTS IN SYNAPTIC SIGNALING

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In the Central Nervous System, any imbalance between stimulatory and inhibitory signaling might cause a variety of dysfunctions leading to excitotoxicity, a milestone in neurodegeneration. In this context, the present study aimed at evaluating the activation of ERKs and CaMKII, as an indicator of effective depolarization and synaptic activity, in the presence of disruptive glucose levels and after administration of protective agents. Dehydroepiandrosterone and allopregnanolone, known to promote cell survival, were used, as well as promising neuroprotective agents like oleocanthal, vitamin D and creatine. Acute brain slices of C57BL/6 mice were perfused with artificial cerebrospinal fluid containing either diverse glucose concentrations or neuroprotective agents, combined with 45mM KCl for depolarization. The levels and activation of proteins of interest were examined by Western blot analysis of total extracts. Interestingly, our results showed that basal levels of pCaMKII were upregulated with creatine and basal levels of pERKs increased after perfusion with allopregnanolone and dehydroepiandrosterone. Following the induction of depolarization, levels of pERKs and pCaMKII were elevated, except for hyperglycemic conditions. As the same result was recorded in the presence of mannitol, it indicates that the increase in osmolality of the perfusion solution may account for this effect. On the contrary, in hypoglycemic conditions depolarization is not accompanied by a statistically significant difference in the activated protein levels. The screening of neuroprotective agents revealed that when both depolarization and dehydroepiandrosterone were applied, a moderate upregulation of pERKs was observed, compared to depolarization alone. This outcome corroborates the hypothesis that dehydroepiandrosterone alleviates the excessive activation of the kinases, resembling a shield towards excitotoxicity. The aforementioned results support the beneficial role of neuroprotective agents and implicate a cutoff point where activation of survival pathways becomes neurotoxic.

OLIGODENDROCYTE PROGENITOR CELLS AS A POTENTIAL THERAPEUTIC TARGET IN ALZHEIMER'S DISEASE

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Recent studies in mouse models and human patients highlight myelin breakdown and loss of myelin sheath as an early stage event in Alzheimer's Disease (AD). However, it is still unknown whether myelin loss is attributed to increased oligodendrocyte vulnerability, reduced repairing capacity or to toxic stimuli. In the present study, we compared neuronal myelination in 12m old wild type (wt) and 5xFAD mouse model of AD, revealing a significant decrease of myelin in the hippocampal regions of dentate gyrus, CA1 and CA3 of 5xFAD mice, compared to wild type littermates. Further immunohistochemical analysis showed a decrease in the number of PDGFRa+ oligodendrocyte progenitor cells (OPCs) supporting the hypothesis of defective oligodendrogenesis under neurodegenerative conditions. We then attempted to target OPCs and study *in vitro* the oligodendrogenetic properties of two small-sized, synthetic neurotrophin analogs, the microneurotrophins BNN27 and BNN237, previously characterized for their neurogenic and neuroprotective effects through the selective activation of TrkA and p75NTR neurotrophin receptors. Our results indicate that BNN27 and BNN237 highly promote oligodendrogenesis as their administration increased OPCs proliferation in primary mouse OPC cultures and accelerated adult neural stem cells (NSCs) differentiation to both PDGFRa+ OPCs and O4+ mature oligodendrocytes. In summary, our study shows that OPCs constitute an appealing therapeutic target against myelin loss in AD and suggests BNN27 and BNN237 as promising lead therapeutic agents in the field of myelin regeneration and restoration.

Topic: **System Neuroscience**

NON-INVASIVE GENE DELIVERY OF AAV.PHP.eB-GFP CAPSIDS TO THE CNS AS A NOVEL THERAPEUTIC APPROACH FOR NEURODEGENERATIVE DISEASES

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Accumulation of α-synuclein (α-syn) aggregates in specific brain regions is a hallmark of Parkinson's disease (PD) and related synucleinopathies. The neuron-to-neuron propagation of α-syn has been suggested to be the underlying mechanism by which aggregates spread throughout the brain. The preformed fibril (PFF) α-syn striatal injection model has been widely utilized as an animal PD model, as it recapitulates many clinically relevant PD hallmarks, including α-syn aggregation, dopaminergic cell loss and behavioral deficits. Decreasing the levels of endogenous α-syn represents a promising approach for ameliorating PFF-induced α-syn pathology. The generation of novel brain-penetrant viral vectors (PHP.eB AAVs) opened up new horizons for the development of effective pan-CNS treatment for brain diseases. Towards this direction, we performed intravenous injections of PHP.eB AAVs engineered capsids, targeting the mouse α-syn (PHP.eB-AAVs SNCA-shRNA) and a scrambled control sequence (PHP.eB-AAVs SCR- shRNA) in 8-week old C57/Bl6 male mice. Two weeks post-injection, human α-syn PFFs were unilaterally injected in the dorsal striatum in order to assess the effects of α-syn down-regulation on the seeding of PFF-induced pathology, at 2 and 5 months post-injection. Herein, we show that α-Syn PFFs injection triggers the formation of cytoplasmic inclusions of phosphorylated α-Syn reminiscent of Lewy body pathology in brain regions directly interconnected with the injection site, with evident behavioral deficits already present by 2 months post-PFF intrastriatal inoculation. Additionally, we demonstrate that intravenous administration of PHP.eB AAVs successfully enables widespread transduction throughout the CNS, including the substantia nigra, achieving efficient downregulation of endogenous rodent α-syn. Our preliminary data suggest that AAV-mediated downregulation of endogenous α-syn reduces the accumulation of phosphorylated α-syn and mitigates motor impairment manifestations. Overall, we established a noninvasive delivery strategy, with high transduction efficacy to the CNS, which has prospects for the treatment of neurodegenerative diseases with widespread pathology such as α-synucleinopathies.

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REDUCED DISORDER IN JOINT ENTROPY MEASURES OF ANTERIOR-POSTERIOR AND MEDIAL- LATERAL BODY SWAY

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Information theoretical analyses of center of pressure (COP) fluctuations are likely to do justice to the inherent complexity of postural sway fluctuations during quiet upright stance. Does the Shannon entropy of COP trajectories change under different sensory feedback conditions in a similar way as do linear measures of postural sway? Is there a different pattern of medial-lateral (ML), anterior-posterior (AP) and joint (ML and AP) sway entropies? Static balance was assessed in 32 healthy adults during quiet stance on a force platform under different visual and proprioceptive feedback conditions: Standing on firm support with eyes open (condition 1) or closed (condition 2) and standing on foam with eyes closed (condition 3). Postural sway was analyzed by means of linear (sway area, and root-mean-square (RMS) of ML and AP sway) and nonlinear, information theoretic metrics (entropy of ML and AP sway, joint ML and AP entropy). Sway area, ML RMS and AP RMS deteriorated significantly from condition 1 through condition 3. However, ML entropy, AP entropy and joint ML and AP entropy remained stable. The values of ML and AP entropies were practically at their theoretical maximum in all conditions. On the other hand, joint ML and AP entropies were clearly submaximal. Decreasing the reliability of visual and proprioceptive input does not alter the Shannon entropy of body sway, although it does increase the magnitude of conventional linear sway metrics. Importantly, individual ML and AP sway entropies tend towards absolute randomness, whereas the joint, ML and AP, sway entropy exhibits a higher degree of regularity, suggesting its role as the actual controlled variable.

Topic: Techniques

THE USE OF SHEEP IN NEUROSCIENCE RESEARCH

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Basic neuroscience research is dominated by two animal models, the mouse and the rat. This is not only due to their convenient husbandry, but also because of available genome editing techniques to accurately simulate human neurological disorders.[1,2,3] Nevertheless the relevance of these models for the studying of certain disorders is questioned, due to developmental, histological and anatomical differences to humans.[3]

Sheep though poses as a suitable model for the study of human neurological disorders not only because of them being intelligent and easily manageable but mostly because of their similarities in the anatomy and complexity of the nervous system to that of non-human primates.[4] Sheep are already used to study Huntington's and Parkinson's disease. Even more importantly, there are naturally occurring neurological disorders observed both in humans and sheep, like neuronal ceroid lipofuscinosis and prion disease. [3]

Along with the use of sheep as animal model for neurological disorders, it has been used for other purposes, including basic and applied fetal and reproductive research. Other fields such as circadian rhythms and the interaction between olfactory cues and behavior have been also considered.

Induced models of spinal cord injury, research including blood brain barrier and vasculature of the brain have also been mentioned in the literature. Sheep plays important role as an experimental model in neuroendocrinology, including parturition and lactation. More specifically, injections into the sheep PVN have been performed for tracing of functional pathways relevant to milk-ejection reflex.

Conclusively, the tendency of the research not only of neurological disorders but into other aspects of neuroscience research, will shift from rodents to more suitable animal models and the sheep is displayed as a very promising model for future studies.

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MILKING": AN INNOVATIVE APPROACH TO INVESTIGATE THE PROPERTIES OF POSTNATAL BRAIN NEURAL STEM CELLS AND TO OBTAIN OLIGODENDROCYTE PROGENITOR CELLS FROM LIVE EXPERIMENTAL RATS

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Postnatal brain neural stem and progenitor cells (NSPCs) cluster in anatomically defined stem cell niches, one of which is the subependymal zone (SEZ) at the lateral walls of the lateral ventricles and are characterized by a wide differentiation potential, self-renewal and quiescence. Oligodendrocyte progenitor cells (OPCs) give rise to myelinating oligodendrocytes and also exhibit self-renewing potential. Here, we refine our method of "milking" the rat ventricular system in order to collect OPCs from the corpus callosum and to investigate the basic properties of SEZ-resident NSPCs. Milking consists of an intracerebroventricular injection of a release cocktail containing neuraminidase, β1-integrin blocking antibody and Fibroblast Growth Factor-2 in order to induce the controlled flow of NSPCs and OPCs in the cerebrospinal fluid. At a second "collection" step, liquid biopsies of CSF are performed from the cisterna magna of anesthetized experimental animals without the need of an incision. Liquid biopsies after milking caudal ventricular areas resulted in the isolation of cells expressing at high percentages typical OPC markers, such as Olig2 and PDGFRα. Furthermore, cells isolated after milking of the SEZ, that we have previously shown to exhibit characteristics of quiescent neural stem cells, were cultured in three different growth media. The typical NSPC medium, containing FGF2 and EGF and two media known to favor the expansion of neural stem cells without enhancing their progress towards differentiation. Our results showed significant differences in the morphology of grown cells and in their colony-formation characteristics that we investigate further using a range of NSPC markers in order to directly assess the profile of endogenous SEZ.

ISOLATION OF BRAIN NEURAL STEM CELLS BY MILKING THE SUBEPENDYMAL ZONE: INVESTIGATION OF THE HUMAN NICHE AND OF THE EFFECTS OF MILKING TO THE EPENDYMA

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Postnatally, Neural Stem Cells (NSCs) are located in specialized areas of the brain, such as the Subependymal Zone (SEZ) of the lateral walls of the lateral ventricles. These stem cell niches host neurogenesis throughout the rodents' life, but in humans the neurogenic activity of the SEZ is maintained until the first 18 months after birth. Nevertheless, this early presence of NSCs could allow their use to treat juvenile neurodegenerative diseases, such as leukodystrophies, if they could be isolated. For this purpose, the method of "Milking of the SEZ" (McClenahan et al., 2021) has been developed experimentally in rats. It involves the controlled compromise of the ependymal layer in order to allow the flow of NSCs from the SEZ into the cerebrospinal fluid whereof they can be collected via liquid biopsies. Here, we describe the structure of the human infant SEZ -using immunohistochemical analysis on paraffin sections- in order to assess the potential to transfer 'milking" to the clinic. In addition, we deepen our investigation of the effects of the "release cocktail" to the target area by setting up cultures of adult rat brain-derived ependymal cells.

McClenahan F, Dimitriou C, Koutsakis C, Dimitrakopoulos D, Arampatzis A, Kakouri P, Kourla M, Oikonomou S, Andreopoulou E, Patsoni M, Meri DK, Rasool RT, Franklin RJM, Kazanis I. **(2021)** Isolation of neural stem and oligodendrocyte progenitor cells from the brain of live rats. Stem Cell Reports, *e-pub: 23 Sept 2021.* Doi: 10.1016/j.stemcr.2021.08.015

ENGLISH LESSONS WITH THE USE OF SONGS FOR PEOPLE WITH MILD COGNITIVE IMPAIRMENT

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Background

People suffering from Mild Cognitive Impairment (MCI) need to improve their cognitive abilities in order to avoid the deterioration of their condition. Learning a foreign language is a good way for cognitive enhancement. However, since these people have memory deficits and lack of attention abilities, conventional teaching methods cannot be used. The use of songs in foreign language lessons is proved to be a very good technique for more effective acquisition of the language while also improving well-being and relaxation.

Method

The Erasmus+ funded "E.L.So.M.C.I" program is a 2 year pan-European initiative that aims to develop an educational program on teaching English to people with MCI and using English songs as a main tool for the teaching process. The methodology of this educational program is based on innovative teaching approaches such as "Communicative Language Teaching" and "Natural Approach and the method of "Neuro-linguistic Programming (NLP)". These methods place great emphasis on verbal communication, creation of a positive environment in class, reduction of stress and encouragement of learners to learn step by step in a natural and pleasant way.

Result

In this moment, the project has entered its first operational phase, that has the objective of producing:

- A Workshops' Methodological Guide which is a document provides trainers with guidelines for teaching languages that focuses on communication in English (for the learners) and preparation of the lesson plan for each lesson. The Methodological Guide includes two kinds of materials, one for the participants-students and one for the professionals (trainers).
- Several Workshops for English learning, addressed to people with MCI (MMSE between 28 and 24).
- Co-Created Methodological Guide and Training for trainers via MOOC.

Conclusion

This project and methodology can improve cognitive functions, prevent from Alzheimer's disease, support the teaching process through the use of songs, reduce stress and increase positive emotions, improve participants' socialization, as well as, develop the sense of belonging to a group.

ACUTE STRESS ALTERS CEREBELLUM METABOLOME

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[#]*equal contribution*

Psychological stress and stress-related disorders are leading causes of disability worldwide. Although several brain regions have been implicated in emotional processing, the involvement of cerebellum in stress responses still needs to be elucidated. Metabolomics, the comprehensive measurement of low molecular weight molecules, has recently emerged as an important tool for understanding the underlying mechanisms of neuropsychiatric and stress-related disorders. Here, we exposed mice to a forced swim test (FST) acute stressor and we investigated the effects on the mouse cerebellum metabolome by Nuclear Magnetic Resonance (NMR)-based metabolomics. We found a clear differentiation between stressed and unstressed mouse cerebella. Our results also showed altered levels of 19 out of the 47 annotated metabolites, implicated mainly in neurotransmission and N-acetylaspartic acid (NAA) turnover, as well as in energy and purine/pyrimidine metabolism. Pathway enrichment analysis revealed aspartate metabolism as the most affected pathway, followed by the urea cycle and purine metabolism. The metabolite signals were correlated with the FST behavioral parameters, and four of the examined metabolites exhibited significant associations with swimming and floating. Our work indicates that acute stress alters metabolic signatures in the mouse cerebellum and underlines the implication of this brain region in stress responses.

Keywords: Forced swim test (FST), Cerebellum, Metabolomics, NMR, Acute stress, Mouse

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*equal contribution

videos and documentary

Genesis, 4:45 min.

Geogenesis, 4:40 min.

RRR, Replace, Reduce, Refine, 5:15 min.

MIND THE LIMIT, FRAMES OF MIND, 4 min.

Documentary, 8.30 min.

Creation 2nd VERSION, 6 min.

The series of five video projections and the Short Documentary video are made by Stavros Panagiotakis

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