Abstract Book

30th Meeting of the HSfN

HELLENIC SOCIETY FOR NEUROSCIENCE

24-26 November Athens

NATIONAL CENTER FOR SCIENTIFIC RESEARCH "DEMOKRITOS"

2023

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The front page was designed by Yassemi Koutmani & Panagiotis Politis. Central to this design is an experimental image, originally captured and provided by Yassemi Koutmani.

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Overview of the 30th Meeting of the HSfN

The Program and Organizing Committees of the 30th Meeting of the HSfN have made every effort for a well-balanced schedule, with a blend of plenary lectures, symposia, oral presentations, and poster sessions that cover various aspects of neuroscience research and therefore cover the interests of the anticipated 400 attendees of this Meeting and the ISN-ESN Symposium. Moreover, the HSfN is committed to the principles of equity, gender equality, diversity, and fairness and seeks to create a welcoming and inclusive environment for all attendees. We believe that diversity drives innovation and that everyone deserves an equal opportunity to participate in and contribute to the field of neuroscience. We are dedicated to ensuring that all voices are heard and that every attendee has an equitable chance to engage with their peers and present their research. We also strive to mentor our many graduate students and foster the careers of early-stage investigators to the best of our abilities. Consequently, we anticipate that the 30th Meeting of the HSfN will serve this purpose as well, as it will help our young scientists increase the visibility of their research while informing them more about cutting-edge topics in neuroscience.

The Hellenic Society for Neuroscience was founded in 1985. Members of the HSfN are scientists from almost every subfield of neuroscience research, including molecular, cellular, developmental, behavioral, cognitive, and systems neurosciences. We are a vibrant community of 400 active members, including professionals from academia and industry across various disciplines, including physicians, biologists, pharmacologists, psychologists, epidemiologists, electrophysiologists, geneticists, and bioinformaticians, among others. We are proud to be a constituent member of the Federation of European Neuroscience Societies (FENS) and a founding member of the International Brain Research Organization (IBRO).

Registrations

For registration, please follow the link:

Register here

Registration Fees

Member:	**40 €**
Non-Member:	**60 €**
Ph.D Students Member:	**10 €**
Ph.D Students Non-Member:	**20 €**
Postgraduate Students:	**free**
Undergraduate Students:	**free**

For Student Registration, a Valid Student ID (with reference of current valid enrollment), needs to be provided through the online registration process.

We would like to encourage participants to register via this online platform rather than onsite during the conference, as it will save time and resources for the HSfN.

Scientific Program

Friday, 24 November 2023

9:00 - 9:30 **Registration**

9:30 - 11:30 **Symposium S1 Unravelling the Complex Landscape of Neurodegenerative Disorders: Pathways and Therapeutic Targets**

Chair: *Ioannis Charalampopoulos & Panagiota Papazafiri*

Maria Xilouri
Title: Targeting the Autophagy-Lysosome Pathway in a-Synucleinopathies

Epaminondas Doxakis
Title: Intricacies of α-synuclein: Post-Transcriptional Control and Aggregation in Parkinson's Pathogenesis

Selected oral presentations

Andreas Giannisis
Title: APOEε4 phenotypical signatures in the periphery

Olympia Apokotou
Title: Astrocyte-neuron interactions: game changers in Parkinson's disease?

Katerina Melachroinou
Title: Endogenous alpha-synuclein is essential for the transfer of pathology by exosome-enriched extracellular vesicles, following inoculation with preformed fibrils in vivo

Konstantina Chanoumidou
Title: Harnessing a human iPSC-based model to study the effects of hyperglycemia on neurodegeneration and inflammation; the involvement of p75 neurotrophin receptor

Anastasia Vamvaka Iakovou
Title: Monitoring the potential therapeutic role of cannabidiol against Stress and Alzheimer's disease brain pathologies

11:30 - 12:30 **Plenary lecture #1 Panayiota Poirazi**
Title: Dendritic contributions to biological and artificial learning and memory

Chair: *Fotini Stylianopoulou*

12:30 - 14:10 **Coffee Break and Poster Viewing - Poster session #1**

14:10 - 14:30 **Biotech in Focus – Session #1: An Expert Showcase of New Products and Technologies – Antisel**

Chair: *Myrto Denaxa*

Agnieszka Ciesielska
Science & Technology Advisor, 10X Genomics
Title: Reimagine neurobiology with single cell and spatial transcriptomics applications

14:30 - 16:30 **Symposium S2 Origins in Neurodevelopment: The Underpinnings of Nervous System Diseases**

Chair: *Maria Gaitanou & Christos Gkogkas*

George Leondaritis
Title: Neuronal lipid signaling in development and disease

Kiriaki Sidiropoulou
Title: Development of the mouse prefrontal cortex: from neurons to networks

Selected oral presentations

Platon Megagiannis
Title: Epigenetic control of reactive gliosis and neuroinflammation via ASD-associated chromatin remodeler-CHD8 in astrocytes

Leonidas J. Leontiadis
Title: Network activity alterations of the dorsal and the ventral hippocampus in a rat model of fragile X syndrome

Sofia Notopoulou
Title: Multi-omics analysis in a neural stem cell model of Parkinson's disease provides insights into the disease mechanisms

Georgia Lokka
Title: McIdas is fundamental for ependymal cell generation

Sofia Pasadaki
Title: Role of developmental regulators of axonal local translation in adult axons.

16:30 - 18:30 **Workshop – Mentoring in Neuroscience**
Chair: *Fotini Stylianopoulou, Mimika Mangoura, Spyros Efthimiopoulos, Ioannis Sotiropoulos, Alexia Polissidis, Vasiliki Tsata*

18:30 - 19:30 **Plenary lecture #2 Georgios Skretas**
Title: Bacteria to the rescue: engineering microorganisms to function as a living early-stage drug discovery platform for diseases caused by protein misfolding and aggregation

Chair: *Panagiotis Politis*

19:30 - 21:00 Welcome Reception

Saturday, 25 November 2023

9:00 - 11:00 **Symposium S3 Mechanisms of Neuronal Regeneration and Homeostasis**

Chairs: *Rebecca Matsas & Ilias Kazanis*

Lida Katsimpardi
Title: Bloody brain: systemic control of brain aging and rejuvenation

Vasiliki Tsata
Title: Exploring the Neuro Cardiac Axis-Defining local mechanisms and long-range signals that promote organ regeneration

Selected oral presentations

Athena Boutou
Title: Microglia-specific therapeutic modulation of solTNF-TNFR1 pathway promotes cortical remyelination.

Maria Anesti
Title: Investigation of the functional interaction between Neural Stem/Progenitor Cells and platelets using mouse models of thrombocytopenia and co-culture assays

Christos Karoussiotis
Title: Effects of κ-opioid receptor in stress-driven synaptic alterations due to autophagy induction

Niki Ktena
Title: Autophagy degrades myelin proteins and is essential for maintaining CNS myelin homeostasis

Konstantinos Varvaras
Title: Towards a biophysical model of a single neuron exhibiting critical dynamics

11:00 - 12:00 **Plenary lecture #3 Ewelina Knapska**
Title: Neuronal correlates of social behavior in health and disease

Chair: *Antonios Stamatakis*

12:00 - 12:30 **Coffee Break**

12:30 - 14:10 **Lunch and Poster Viewing - Poster session #2**

14:10 - 14:30 **Biotech in Focus – Session #2: An Expert Showcase of New Products and Technologies - Lab Supplies**

Chair: *Panagiotis Politis*

Susan Wu
Title: ReWarD Customers Through Technology, Quality and Talents

14:30 - 16:30 **Symposium S4 Molecular Pathways to Neuronal Health and Disease: Understanding Function at its Core**

Chair: *Iro Georgousi & Dimitra Thomaidou*

Konstantinos Palikaras
Title: The yin and yang of mitophagy in neuronal homeostasis

Dimitra Dafou
Title: RNA editing defines neurodegenerative disease manifestations

Selected oral presentations

Avgis Hadjipapas
Title: Modest frequency differences in gamma oscillations across laminar compartments in macaque V1

Emmanouela Leandrou
Title: α-Synuclein oligomers potentiate neuroinflammatory NF-κB activity and induce Cav3.2-mediated calcium signaling in astrocytes.

Irini Thanou
Title: Exploring the Brain's Response to Chemotherapy: Neurogenesis at the Forefront

Eirini Georganta
Title: Unraveling Behavioral Deficits in Neurofibromatosis Type 1: Insights from Drosophila Models

16:30 - 16:50 **Coffee Break**

16:50 - 18:30 **Symposium S5 The Dynamic Brain: Unraveling the Links between Behavior and Neuroplasticity**

Chair: *Vasilis Raos & Manolis Froudarakis*

Nikolaos Smyrnis
Title: 1/f noise in human cognition: a signature of predictive processes

Georgia Gregoriou
Title: Filtering out distractions while focusing attention: Two sides of the same coin

Selected oral presentations

Ioanna Zioga
Title: Alpha and beta oscillations shape language comprehension and production

Ermis Ryakiotakis
Title: Neonatal maternal neglect effects on rat reward-anticipatory behavior, social status stability, and reward circuit activation in adulthood

Giannis Lois
Title: Tracking politically motivated reasoning in the brain

Christos Samsouris
Title: Cerebral lateralization in writing: comparing handwriting, and typing using computer and smartphone keyboard

18:30 - 19:30 **Plenary lecture #4 Aaron D. Gitler**
Title: Expanding mechanisms and therapeutic strategies for neurodegenerative diseases

Chair: *Alexia Polissidis*

19:30 - 20:30 **General Assembly of the Hellenic Society for Neuroscience**

Sunday, 26 November 2023

9:00 - 11:00 **Symposium S6 Neurobiological Mechanisms and Intervention Strategies in Neurological Disorders**

Chair: *Leonidas Stefanis & Christina Dalla*

Panos Zanos
Title: NMDA receptor activation underlies ketamine's rapid antidepressant efficacy

Vasiliki Kyrargyri
Title: Role of microglia in neuroinflammatory and demyelinating diseases

Stella Giakoumaki
Title: Neuropsychological profiles of high schizotypal individuals: implications for cognitive remediation programs

Selected oral presentations

Angeliki Chroni
Title: Time-dependent effects of currant (Vitis vinifera) consumption on neuroinflammation and oxidative stress in the 5xFAD mouse model of Alzheimer's Disease

Charalampos Brakatselos
Title: Cannabidiol restores ketamine-induced schizophrenia-like symptomatology by multi-level action on the underlying neurobiological substrate.

Evangelia Chrysanthi Kouklari
Title: Cross-sectional Developmental Trajectories of cool and hot Executive Function in Autism Spectrum Disorder

Pavlina Pavlidi
Title: Sex-specific effects of pharmacological agents targeting the estrogen membrane receptor GPER1 on anxiety levels and monoaminergic activity of male and female rats

11:00 - 12:00 **Plenary lecture #5 Andreas Papassotiropoulos**
Title: Human genetic signatures of remembering and forgetting as a basis for drug discovery

Chair: *Efthimios Skoulakis*

12:00 - 12:30 **Coffee Break**

12:30 - 14:30 **Lunch and Poster Viewing - Poster session #3**

14:10 - 14:30 **Biotech in Focus – Session #3: An Expert Showcase of New Products and Technologies – Bioanalytica**

Chair: *Christina Kyrousi*

Aida Freire Valls
Spatial Biology Specialist, NanoString Technologies, Inc.
Title: Transforming neuroscience: from digital spatial profiling to single cell imaging

14:30 - 16:30 **ISN Symposium Mechanistic Advances and New Perspectives on Brain Metabolism (S7)**

Chair: *Mimika Mangoura & Kostas Vekrelis*

Blanca I. Aldana
Title: Metabolic interventions in glial cells: Implications for Neurodegenerative disorders

Juan P. Bolaños
Title: Astrocyte metabolism: energy or signaling?

Johannes Hirrlinger
Title: Brain metabolism: from heterogeneity to pathophysiology

16:30 - 17:30 **Young scientists awards & closing remarks**

END of the 30th MEETING of the HSFN

Abstracts from the Invited Symposium Speakers

Symposium S1 Unravelling the Complex Landscape of Neurodegenerative Disorders: Pathways and Therapeutic Targets

FRIDAY – 24 NOVEMBER 2023

Targeting the Autophagy-Lysosome Pathway in α-Synucleinopathies

Maria Fouka[1], Fedra Arvanitaki[1], Panagiota Mavroeidi[1], Anastasia M. Bougea[2], Marie-Laure Arotçarena[3], Elena-Georgia Gialinaki[1], Erwan Bezard[3], Leonidas Stefanis[1,2], Benjamin Dehay[3] and Maria Xilouri[1]

[1] Center of Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation of the Academy of Athens (BRFAA) 4, Soranou Efesiou Street, Athens, 11527, Greece
[2] 1st Department of Neurology, Eginition Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece
[3] Univ. de Bordeaux, CNRS, IMN, UMR 5293, F-33000 Bordeaux, France

A growing body of evidence, encompassing neuropathology studies in human brain, animal models of concomitant proteinopathies and studies employing sophisticated methods of probing protein-protein interaction, cumulatively suggest that the neuronal protein α-Synuclein is well positioned to exert a strong influence on the pathogenesis of the neurodegenerative comorbidities. Accumulation of α-Synuclein-positive inclusions in neurons and oligodendrocytes is the main histopathological hallmark of Parkinson's disease (PD) and multiple system atrophy (MSA), respectively.
In addition, various pieces of data indicate that components of the autophagy lysosomal pathway (ALP) are altered in the context of α-Synucleinopathies. α-Synuclein itself is degraded by the ALP, but at the same time aberrant protein species may impair ALP function. Genetic PD often involves components of the ALP, including common genetic mutations in *GBA1*, which encodes for the lysosomal enzyme *β*-glucocerebrosidase (GCase). Alterations in ALP components that correlate with a commensurate increase in α-Synuclein deposition have been widely observed in PD brains. However, corresponding data in the context of MSA are scant.
In the current talk, recent advances regarding ALP contribution to the development of PD and MSA, as well as, experimental paradigms of the therapeutic potential of ALP enhancement in rodent and non-human primate models of α-Synucleinopathies will be discussed.
There is distinct promise in the field of neurodegenerative diseases for therapeutics that aim to enhance protein degradation systems, so as to remove toxic aggregated protein species. Our data insinuate that ALP enhancement might open new therapeutic opportunities for slowing down the degenerative process in patients with α-Synucleinopathies.

This work is funded by a Michael J Fox grant (ID: 16887, 024029) and by a Multiple System Atrophy Trust grant (ID: 2019/MX60185). Partial support is also provided by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "Second Call for H.F.R.I. Research Projects to support Faculty members and Researchers" (ID: 3661).

Intricacies of α-synuclein: Post-Transcriptional Control and Aggregation in Parkinson's Pathogenesis

Epaminondas Doxakis[1]

[1]Biomedical Research Foundation of the Academy of Athens, Soranou Efesiou 4, 11527, Athens, Greece

Abnormal accumulation of alpha-synuclein (SNCA) is a pivotal characteristic of Parkinson's disease (PD). Linkage disequilibrium within the *SNCA* 5' and 3' untranslated region (UTR) blocks suggests their relevance in the more common sporadic form of PD. Nevertheless, the precise molecular mechanisms through which the 5'UTR and 3'UTR influence SNCA expression require further clarification. In this study, we present data elucidating the role of the 5'UTR in *SNCA* mRNA translation and the influence of different-sized 3'UTRs on *SNCA* mRNA subcellular localization, stability, and protein expression. Additionally, we provide insights from mass spectrometry analysis, identifying the proteins that interact with *SNCA* UTRs. Two RNA-binding proteins are selected and described for their impact on the biology of *SNCA* transcripts. We conclude with the development of an antisense oligonucleotide-based strategy for the treatment of PD.

This work was supported by the Michael J. Fox Foundation for Parkinson's Research (Grant ID16186) and the Greek General Secretariat for Research and Innovation (T2EDK-01291, TAEDR-0535850).

Symposium S2 Origins in Neurodevelopment: The Underpinnings of Nervous System Diseases

FRIDAY – 24 NOVEMBER 2023

Neuronal lipid signaling in development and disease

George Leondaritis

Department of Pharmacology, Faculty of Medicine and Institute of Biosciences, URCI, University of Ioannina, Ioannina, Greece

Dysregulation of signaling pathways that integrate genetic, environmental, and developmental signals to orchestrate brain development, function and connectivity has been highlighted as a key pathophysiological driver for neurodevelopmental disorders. We discuss the intricacies of PIP_3, a prominent signaling lipid, synthesized by PI3K lipid kinases and dephosphorylated by PTEN lipid phosphatase, that activates the Akt/GSK3β/mTOR pathway. This pathway integrates inputs from growth factor and neurotransmitter receptors, and inputs from nutrients, energy status and stress, to regulate growth and metabolism. During neuronal development, it controls almost all aspects of growth and differentiation including dendrite and axon branching, synapse formation, and neuronal connectivity/plasticity. In our previous studies, we have shown that the transmembrane axonal protein PLPPR3 locally modulates PTEN-dependent generation of axonal filopodia and branches. In our ongoing work, we expand our studies on the functions of PLPPR3 as a putative effector of lysophosphatidic acid, an extracellular bioactive lipid essential for CNS development and function. In parallel projects investigating the involvement of Akt/GSK3β/mTOR in neuropsychiatric diseases, we have focused on its recently reported hypofunction in chronic schizophrenia patients. We have begun to assess the phosphorylation of key signaling proteins in PBMCs from first episode of psychosis (FEP) patients. Our results suggest that FEP patients are characterized by two distinct signaling endophenotypes: GSK3β hypofunction is normalized after antipsychotic drug treatment, whereas mTOR hypofunction represents a stable state. Intriguingly, our ongoing studies point to a mechanistic heterogeneity of mTOR downstream signaling in schizophrenia, culminating on differential regulation of mTORC1-specific effectors controlling protein synthesis.

Development of the mouse prefrontal cortex: from neurons to networks

Kyriaki Sidiropoulou [1,2]

[1]Department of Biology, University of Crete
[1]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas

The prefrontal cortex (PFC) is characterized by prolonged maturation. The cellular mechanisms controlling the early development of prefrontal circuits are still largely unknown. In this talk, I will discuss the development of cellular and synaptic mechanisms from the neonatal period to adulthood. I will present data showing the development of excitatory and inhibitory mechanisms in a cell-type specific manner, and how these mechanisms shape the network properties at different ages. Furthermore, I will discuss the role of specific developmental adaptations (such as the change in the GABA-A reversal potential and synaptic pruning) on neuronal network activity and the maturation of behavioural functions mediated by the prefrontal cortex. Finally, I will discuss how the knowledge regarding the developmental processes in the prefrontal cortex can help us better understand the pathophysiology of an animal model of schizophrenia.

This work was supported by H2020-Marie Curie RISE action, “Network Analysis in Neocortex during Passive and Active Learning-neuronsXnets” and the European Innovation Council project “Minimally-Invasive Soft-Robot-Assisted Deep-Brain Localized Therapeutics Delivery for Neurological Disorders (SOFTREACH)”

Symposium S3 Mechanisms of Neuronal Regeneration and Homeostasis

SATURDAY – 25 NOVEMBER 2023

Bloody brain: systemic control of brain aging and rejuvenation

Lida Katsimpardi[1,2]

[1]Institut Necker Enfants Malades, INSERM UMR-S1151, Paris, France
[2]Faculté de Médecine, Université Paris-Cité, Paris, France

Research over the last couple of decades has shown that aging is not an irreversible process as once thought, but instead is subject to manipulation. This plasticity of aging extends to a different degree for each organ, but there are ways to slow down- or even reverse- aging.
Previously, we showed that infusion of young systemic factors in the aged organism through heterochronic parabiosis can rejuvenate several organs, including those with low regenerative capacity, such as the brain. We identified different blood factors with pro- or anti-aging potential, among which Growth Differentiation Factor 11 (GDF11), which has a pleiotropic role in the organism, acting as a calorie restriction-mimetic through stimulation of adiponectin secretion in the periphery. We recently demonstrated that GDF11 also has a crucial central role in attenuating the depression-like phenotype and improving memory in aged mice via activation of neuronal autophagy. Interestingly, serum levels of GDF11 were inversely associated with major depressive disorder (MDD) in patients. These findings highlight the important role of blood factors in aging and disease, as well as the correlation between levels of blood factors and the progression of disease. Our current projects focus on identifying new pro- and anti-aging blood and CSF factors in human physiological and pathological aging, deciphering the exact molecular mechanisms of brain neurovascular aging in mouse models, as well as developing novel translational tools, such as the organ-on-chip, with the objective of therapeutic applications.

Exploring the neuro cardiac axis – defining local mechanisms and long-range signals that promote organ regeneration

Vasiliki Tsata

Experimental Surgery, Clinical and Translational Research Center and Center of Basic Research, Biomedical Research Foundation, Academy of Athens, 11527, Athens, Greece

Diseases of the heart and central nervous system (CNS)/brain are the 1st and 2nd leading causes of death worldwide, resulting in disability-adjusted life for more than half of surviving individuals each year. Intriguingly, cardiovascular complications after CNS injury and/or neurological damage after myocardial infarction suggest a causal link between conditions that affect the CNS and the heart. Yet, our current inability to explain the underlying mechanisms regulating such inter-organ communication in both homeostatic and post-injury conditions, poses a major challenge to effective disease prognosis and medical treatment. In contrast to mammals, zebrafish exhibit a remarkable regeneration capacity for both the brain and heart, which enables the investigation of the injury responses and interactions between these organs in a regenerating vertebrate. Here, we use a stab lesion and cryoinjury model to mimic CNS and heart injury conditions respectively, and advanced imaging to map cellular responses in the distant organ. Utilizing behavioral readouts and RNAseq at different timepoints post-injury in both organs, we seek to acquire a mechanistic understanding of the brain-heart axis in an adult vertebrate. We propose that identifying the cues that orchestrate brain-heart interactions in zebrafish, could reveal targets to reprogram their mammalian counterparts with implications to regenerative medicine.

Funding: Hellenic Foundation for Research and Innovation (H.F.R.I), 3rd Call for H.F.R.I. Research Projects to Support Post-Doctoral Researchers (Project ID: 7903 grant to V.T.)

Symposium S4 Molecular Pathways to Neuronal Health and Disease: Understanding Function at its Core

SATURDAY – 25 NOVEMBER 2023

The yin and yang of mitophagy in neuronal physiology

Konstantinos Palikaras[1]

[1]Unit of Neurogenetics and Ageing, Department of Physiology, Medical School, National and Kapodistrian University of Athens

Ensuring mitochondrial homeostasis is essential for the high-energy demands of nerve cells. Post-mitotic neurons rely extensively on robust mechanisms to maintain mitochondrial quality control and sustain their function. Mitochondrial selective autophagy, known as mitophagy, plays a pivotal role in neuronal development and survival during stress and aging. Numerous neurological disorders are associated with dysfunctional mitochondria and deregulated mitophagy. While mitophagy often shields neurons, excessive and uncontrolled activity can lead to a reduced mitochondrial count, energy depletion, and ultimately cell death. Unveiling the molecular mechanisms of neuronal mitophagy and its intricate role in neuronal survival and demise will aid in developing novel mitophagy modulators to promote cellular and organismal physiology in health and disease.

RNA editing defines neurodegenerative disease manifestations.

Dimitra Dafou

School of Biology, Department of Genetics Development and Molecular Biology, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece

RNA editing contributes to transcriptome diversification through RNA modifications in relation to genome-encoded information (RNA–DNA differences, RDDs). The deamination of Adenosine (A) to Inosine (I) or Cytidine (C) to Uridine (U) is the most common type of mammalian RNA editing. It occurs as a nuclear co- and/or post-transcriptional event catalyzed by ADARs (Adenosine deaminases acting on RNA) and APOBECs (apolipoprotein B mRNA editing enzyme catalytic polypeptide-like genes). RNA editing may modify the structure, stability, and processing of a transcript. We provide comparative transcriptome and editome analyses between human disease and corresponding animal models of several neurodegenerative disorders focusing on 'proteinopathies'. Data suggest RNA editing to be an emerging mechanism in disease development, displaying common and disease and cell type-specific patterns. The potential use of RNA editing events as disease biomarkers and available tools for RNA editing identification, classification, ranking, and functional characterization that are being developed will enable comprehensive analyses for a better understanding of disease(s) pathogenesis and potential cures.

This work was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "4th Call for H.F.R.I. PhD Fellowships" (Project Number: 10829), by the IKYDA Greek-German collaboration Project 2018/39, and the Action RESEARCH – CREATE - INNOVATE co-funded by the European Regional Development Fund of the European Union and National Resources through the OP Competitiveness, Entrepreneurship & Innovation (EPANEK) (project code: T1EDK- 03884).

Symposium S5 The Dynamic Brain: Unraveling the Links between Behavior and Neuroplasticity

SATURDAY – 25 NOVEMBER 2023

1/f noise in human cognition: a signature of predictive Processes

Alexandros Smyrnis[1] and Nikolaos Smyrnis[1]

[1] Laboratory of Cognitive Neuroscience and Sensorimotor Control, University Mental Health, Neurosciences and Precision Medicine Research Institute "COSTAS STEFANIS", Athens, Greece.

It has been suggested that 1/f noise in human cognition arises as a signature of Self-Organized Critical-ity (SOC). SOC predicts universality of 1/f noise in the power spectrum of response time (RT) time series in cognitive tasks and persistent serial correlations (long-range dependence, LRD) in these time series. We hypothesized instead, that 1/f noise arises only when responding without having to process sensory information, thus predicting upcoming stimuli. We designed a stimulus response task with constant interstimulus intervals (ISI) and increased the ISI in different blocks from 0.5s to 5.0s. In ac-cordance with our hypothesis, we found significant modulation of the power spectrum that varied from 1/f noise for short ISIs where effective stimulus prediction resulted in average RTs ~ 0s, to white noise for long ISIs where ineffective prediction was replaced by reaction after stimulus presentation with corresponding average RTs ~ 0.25s. We then modelled time dependence in short ISIs, starting with a simulation of autoregressive (AR) moving average (MA) and autoregressive fractionally inte-grated moving average (ARFIMA) models, focusing on the properties of ARFIMA when simulating series of limited number of data points. We show that ARFIMA models with different LRD parameter (d) values, can fit experimental data equally well, by manipulating the AR, MA parameters. We thus focused on modelling the group average RT time series resulting in a range of possible d values, that correlate to the strength of temporal binding, which can be thought to reflect the varying effectiveness of predictive processing across different cognitive tasks.

Filtering out distractions while focusing attention. Two sides of the same coin

Georgia G. Gregoriou[1,2]

[1]Medical School, University of Crete, 70013, Heraklion, Greece and
[2]Institute of Applied and Computational Mathematics, Foundation for Research and Technology Hellas, 70013, Heraklion, Greece

Despite the overwhelming number of stimuli in our visual fields we are able to selectively process stimuli that are most relevant for our current goals and behavior while filtering out irrelevant distractors. This is implemented through selective visual attention. Although research in the last two decades has shed light on the neural correlates of visual attention at the level of single neurons in visual brain areas, we lack a comprehensive understanding of how selection of relevant and suppression of irrelevant stimuli is brought about. In this talk, I will present experimental results from our lab that implicate frequency specific interactions between the prefrontal cortex (PFC) and visual area V4 during selection of behaviorally relevant stimuli. Specifically, I will argue that enhanced rhythmic activity in theta frequencies (4-8 Hz) that originates in PFC, influences activity in V4 populations that encode the attended stimulus. Our results suggest that PFC theta rhythmic input facilitates processing of attended stimuli in V4 by releasing from inhibition the relevant V4 population resulting in enhanced spiking and gamma band (40-70 Hz) activity. In parallel, the representation of unattended distracting stimuli is suppressed through local mechanisms within area V4 manifested on one hand by enhanced V4 theta activity, indicative of enhanced lateral inhibition, and decreased gamma activity, and on the other hand by processing of the distractor at a non-optimal phase of the theta oscillation. The relevance of these neural signatures for successful attention is further supported by the analysis of error trials.

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: 41).

Symposium S6 Neurobiological Mechanisms and Intervention Strategies in Neurological Disorders

SUNDAY – 26 NOVEMBER 2023

NMDA receptor activation and not inhibition underlies ketamine's antidepressant actions

Panos Zanos[1]

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Ketamine is a non-competitive NMDA receptor antagonist. A single sub-anesthetic dose of ketamine is effective in producing rapid and long-lasting antidepressant effects, particularly in patients who do not respond to typical antidepressants. Since ketamine cannot be widely prescribed outside clinical settings due to its side effects, including dissociation and abuse potential, there is a need to elucidate the exact mechanism of its antidepressant action to develop the next generation of rapid-acting antidepressants. Here, we will present novel findings that demonstrate, for the first time, the crucial role of NMDA receptor activation, rather than inhibition, in mediating the therapeutic effects of ketamine and other potential rapid-acting antidepressant compounds. Our data reveal that NMDA receptor activation is imperative for ketamine's antidepressant actions, requiring an NMDAR receptor activation-dependent initiation of canonical long-term potentiation (LTP) at excitatory glutamatergic synapses, subsequently leading to a sustained enhancement of synaptic strength. These conclusions are further supported by our novel findings, showing that sub-effective doses of ketamine exert synergistic antidepressant-relevant behavioral actions when combined with sub-effective doses of NMDA receptor positive allosteric modulators. Importantly, our results highlight the necessity and sufficiency of the NMDA receptor subunit GluN2A activation to drive the aforementioned effects. Therefore, in vivo activation of GluN2A-NMDA receptor-dependent LTP could serve as a promising and innovative strategy for developing the next generation of rapid-acting antidepressant interventions.

IKKβ deletion from CNS macrophages increases neuronal excitability and accelerates the onset of EAE, while from peripheral macrophages reduces disease severity

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Multiple Sclerosis (MS) is a neuroinflammatory autoimmune disease characterized by motor deficits and cognitive decline. Many immune aspects of the disease are understood through studies in the experimental autoimmune encephalomyelitis (EAE) model, including the contribution of the NF-κB transcription factor to neuroinflammation. However, the cell-specific roles of NF-κB to EAE and its cognitive comorbidities still needs further investigation. We have previously shown that the myeloid NF-κB plays a role in the healthy brain by exerting homeostatic regulation of neuronal excitability and synaptic plasticity and here we investigated its role in EAE. We used constitutive MϕIKKβKO mice, in which depletion of IKKβ, the main activating kinase of NF-κB, was global to CNS and peripheral macrophages, and MgIKKβKO mice, in which depletion was inducible and specific to CNS macrophages by 28 days after tamoxifen administration. We subjected these mice to MOG_{35-55} induced EAE, measured pathology by immunohistochemistry, investigated molecular mechanisms by RNA sequencing analysis and studied neuronal functions by in vivo electrophysiology in awake animals. We show that IKKβ-mediated activation of NF-κB in myeloid cells has opposing roles in EAE depending on the cell type and the disease stage. In CNS macrophages it is protective while in peripheral macrophages it is disease-promoting and acts mainly during chronic disease. Although clinically protective, CNS myeloid cell IKKβ deletion dysregulates neuronal excitability and synaptic plasticity in EAE. These effects of IKKβ on brain cognitive abilities deserve special consideration when approved NF-κB inhibitors are used in MS.

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Neuropsychological profiles of high schizotypal individuals: implications for cognitive remediation programs

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Schizotypy is a latent construct including a set of personality traits indicating proneness to schizophrenia-spectrum disorders. There is currently ample evidence for a significant overlap between schizotypy and schizophrenia-spectrum disorders in genetic, neuroanatomical, neuropsychological and a range of other indices. Schizotypal traits are increased in schizophrenia-spectrum patients, in clinical and genetic high-risk groups for these disorders and in 5-10% of the general population; longitudinal studies have revealed significant conversion rates of high schizotypal individuals into disease states over time. In our first neuropsychological studies, we found (a) a differential profile of associations between the schizotypal dimensions and cognitive functions (i.e. paranoid and negative schizotypy were associated with poor cognitive functions mediated by a frontal-temporal-parietal network) and (b) that differences in executive working memory and verbal fluency between unaffected relatives of schizophrenia patients and control individuals are sensitive to the effects of negative and paranoid schizotypy. As the findings of these studies can have applications in cognitive intervention programs, the associations of the different schizotypal dimensions with the near- and far-transfer gains of executive working memory are currently examined in a translational study in man and mice. Based on our previous findings, we hypothesize that (a) paranoid and negative schizotypy will have negative effects while cognitive-perceptual schizotypy will facilitate the gains of executive working memory training in man and (b) schizotypal mice will not benefit from executive working memory training; this effect is expected to be associated with reduced dendritic spine density in the prefrontal cortex and hippocampus.

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Abstracts

ORAL Presentations (OP) - Symposium S1

FRIDAY – 24 NOVEMBER 2023

OP01-APOEε4 phenotypical signatures in the periphery

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The APOEε4 allele is the strongest genetic risk factor for neurodegenerative diseases like Alzheimer's disease (AD), with the risk varying between racial/ethnic groups[1]. Studies aiming to elucidate this link have focused on the central nervous system, with the rationale that apolipoprotein E (apoE) cannot cross the blood-brain barrier[2]. However, we recently demonstrated in APOEε3/ε3 humanized-liver mice, that plasma apoE3 levels were linked to cognitive and behavioural phenotypes[3], while in APOEε4/ε4 humanized-liver mice, a hepatic APOEε4/ε4 genotype and plasma apoE4 levels were related to brain pathological alterations (i.e. impaired synaptic integrity)□. Using liquid-chromatography/mass-spectrometry on plasma samples from cognitively healthy individuals, or patients with mild cognitive impairment or AD, we found a correlation between total plasma apoE levels or specifically the apoE4 isoform levels, with cerebrospinal fluid AD biomarkers, cognition, brain imaging, and plasma lipoproteins, as well as an overall reduction in the apoE levels in patients with Alzheimer's disease and APOEε4-carriers□-□. Comparisons of apoE levels between racial/ethnic groups revealed differences in plasma apoE levels, with APOEε4/ε4 Black/African-Americans exhibiting the highest□. The mechanisms regulating plasma apoE levels are not fully known, however, we have observed altered expression of 624 genes, and an APOEε4-dependent 73%-increase in the levels of the apoE-cleaving enzyme cathepsin-D in primary human hepatocytes obtained from liver donors that were APOEε4-carriers versus non-carriers. APOEε4-carriers also exhibited specific lipidomic signatures and elevated levels of plasma triglycerides□. Overall, our studies provide evidence of peripheral phenotypical traits in APOEε4-carriers which may be associated with their increased risk of neurodegeneration.

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Acknowledgments

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OP02-Astrocyte-neuron interactions: game changers in Parkinson's disease?

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Parkinson's disease (PD) is characterized by progressive loss of midbrain dopaminergic neurons while the major histopathological hallmark is the presence of α-synuclein (αSyn) inclusions, termed Lewy bodies and Lewy neurites(1). Approximately 10% of PD cases are associated with mutations in specific genes, such as the p.A53T-αSyn mutation. Despite intensive research on neuronal dysfunction, the role of astrocytes in PD remains largely unexplored. Our aim is to investigate the contribution of astrocytes and their interactions with neurons in PD pathology. In this context, we generated ventral midbrain-patterned iPSC-derived astrocytes and neurons, and developed a co-culture system of p.A53T-αSyn or healthy neurons on either p.A53T-αSyn or healthy astrocytes. We observed that the p.A53T-αSyn astrocytes introduced neuropathology in co-cultured healthy neurons, including compromised neuronal viability, impaired neuritic outgrowth and neurodegeneration features. In contrast, the viability and neurite outgrowth of p.A53T-αSyn neurons were improved when co-cultured with healthy astrocytes and their neurodegenerative phenotypes, such as Lewy bodies and Lewy neurites, were alleviated. Accordingly, the synaptic connectivity of healthy neurons was compromised on p.A53T-αSyn astrocytes, whereas healthy astrocytes improved the connectivity of p.A53T-αSyn neurons, as assessed using a rabies virus-based retrograde monosynaptic tracing system. Interestingly, most aforementioned phenotypes were also observed in a non-contact mediated setup, in which healthy or p.A53T-αSyn neurons were treated with conditioned medium from healthy or p.A53T-αSyn astrocytes. Our data reveal a critical role of mutant astrocytes in PD pathology and a remarkable ability of healthy astrocytes in rescuing neurodegeneration. These effects are mediated, at least partially, in a paracrine manner.

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OP03-Endogenous alpha-synuclein is essential for the transfer of pathology by exosome-enriched extracellular vesicles, following inoculation with preformed fibrils in vivo.

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The main pathological hallmark of Parkinson's disease (PD) and related synucleinopathies is the presence of intracellular proteinaceous aggregates, enriched in the presynaptic protein alpha-Synuclein (α-Syn). α-Syn association with exosomes has been previously documented both as a physiological process of secretion and as a pathological process of disease transmission, however, critical information about the mechanisms governing this interplay is still lacking. To address this, we utilized the α-Syn preformed fibril (PFF) mouse model of PD, as a source of brain-derived exosome-enriched extracellular vesicles (ExE-EVs) and assessed their pathogenic capacity following intrastriatal injections in host wild type (WT) mouse brain. We further investigated the impact of the fibrillar α-Syn on the exosomal cargo independent of the endogenous α-Syn, by isolating ExE-EVs from PFF-injected α-Syn knockout mice. Although PFF inoculation does not alter the morphology, size distribution, and quantity of brain-derived ExE-EVs, it triggers changes in the exosomal proteome related to synaptic and mitochondrial function, as well as metabolic processes. Importantly, we showed that the presence of the endogenous α-Syn is essential for the ExE-EVs to acquire a pathogenic capacity, allowing them to mediate disease transmission by inducing phosphorylated-α-Syn pathology. Notably, misfolded α-Syn containing ExE-EVs when injected in WT mice were able to induce astrogliosis and synaptic alterations in the host brain, at very early stages of α-Syn pathology, preceding the formation of the insoluble α-Syn accumulations. Collectively, our data suggest that exosomal cargo defines their ability to spread α-Syn pathology.

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OP04-Harnessing a human iPSC-based model to study the effects of hyperglycemia on neurodegeneration and inflammation; the involvement of p75 neurotrophin receptor

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Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized by high glucose levels. Accumulating evidence indicate T2DM as a high-risk factor for neurodegenerative Alzheimer's Disease (AD), however the underlying interlink is still unclear. Increasing evidence support that neuroinflammation represents a central adversity in both pathologies. Here, we aim to study the neurological manifestations of hyperglycemia and investigate its relationship with AD pathogenesis emphasizing on the pan-neurotrophin receptor p75 (p75NTR). p75NTR belongs to the TNF-receptor superfamily and signals apoptosis in a cell- context dependent manner. We use mono- and co-cultures of human iPSC-derived neurons and astrocytes in 2D and 3D (porous collagen scaffolds) conditions to decipher the involvement of p75NTR signaling in the direct and astrocyte-mediated effects of hyperglycemia on neurodegeneration. Our results show that high glucose triggers an upregulation of p75 NTR expression accompanied by a dose-dependent neuronal cell death which is rescued upon pharmacological inhibition of the receptor. Furthermore, we demonstrate that high glucose downregulates key synaptic proteins, indicating a deregulation of synaptic plasticity. We are currently analyzing the effect of hyperglycemia on astrocyte activation and the involvement of p75 NTR signaling. Collectively, our study provides insights into the neural deficits caused by hyperglycemia investigating in parallel the therapeutic potential of p75NTR targeting.

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OP05-Monitoring the potential therapeutic role of cannabidiol against Stress and Alzheimer's disease brain pathologies

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Alzheimer´s disease (AD) is a complex neurodegenerative disease without an effective disease-modifying therapy; Given the high prevalence of AD and the predicted triplication of AD patients by 2050, there is an urgent need for innovative therapeutic approaches. In light of recent attention paid to the potential beneficial role of endocannabinoid signaling against cognitive and mood deficits (1), our work focuses on the therapeutic role of cannabidiol (CBD), an exogenously administered phytocannabinoid, against AD brain pathology and its protective significance against the AD risk factor of chronic stress (2,3). Hereby, 4-5 month-old Tau transgenic mice and their wild-type littermates were subjected to chronic unpredictable stress with simultaneous CBD treatment. Our findings show that chronic CBD treatment ameliorated stress-driven cognitive impairment and mood deficits in Tau Tg mice while ongoing molecular, neurostructural, and proteomic analysis provides further evidence for the molecular underpinnings of the beneficial impact of CBD treatment. These results support the potential therapeutic use of CBD treatment against AD-related Tau pathology and precipitating factors of the disease, contributing to our limited knowledge of how cannabinoid signaling can modulate brain pathology.

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ORAL Presentations (OP) - Symposium S2

FRIDAY – 24 NOVEMBER 2023

OP06-Epigenetic control of reactive gliosis and neuroinflammation via ASD-associated chromatin remodeler-CHD8 in astrocytes

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Glial cells respond to injury and disease through reactive changes that play central roles in orchestrating neuroinflammatory processes. Neuroinflammation has an intrinsic role in many neurological diseases such as neurodegeneration, often excreting deleterious effects in disease progression. Elucidating the molecular and cellular mechanisms that control reactive gliosis during neuroinflammation is crucial for understanding brain pathophysiology and improving disease outcomes. Here, we report that global deletion of ASD-associated Chd8 in adult mice, unexpectedly prevents reactive gliosis. Conditional Chd8 deletion in astrocytes but not microglia is sufficient to suppress reactive gliosis by impeding astrocyte proliferation and morphological elaboration. Furthermore, astrocyte Chd8 ablation alleviates Lipopolysaccharide (LPS)-induced generalized neuroinflammation, conferring protective roles in mice. Gene expression profiling revealed that Chd8 ablation in astrocytes attenuates neuroinflammation by perturbing astrocyte-microglia crosstalk while altering metabolic and lipid-associated pathways. Moreover, we designed and validated an astrocyte-specific AVV-mediated CRISPR-based Chd8 gene editing toolkit to mitigate reactive gliosis directly in vivo. These findings uncover a new role of CHD8 in the adult brain and highlight the translational potential of targeting epigenetic pathways for alleviating reactive gliosis and neuroinflammation during injury and neurological disorders. Future efforts will center on dissecting the CHD8-controled mechanisms in the diverse reactive astrocyte subtypes in the mouse brain at the transcriptional and epigenetic levels. Our findings pave the way for the future implementation of CHD8 perturbation towards a potential therapeutic avenue against the excessive, neurotoxic effects of neuroinflammation in neurodegenerative diseases such as Alzheimer's Disease and Multiple Sclerosis.

OP07-Network activity alterations of the dorsal and the ventral hippocampus in a rat model of fragile X syndrome

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One of the most common causes of inherited intellectual disability is Fragile X Syndrome (FXS). FXS is a severe neurodevelopmental disorder and also the most prominent genetic cause of autism spectrum disorder. FXS is caused by the lack of expression of Fragile X messenger ribonucleoprotein (FMRP). The absence of this protein has been proposed to be associated with alterations in neuronal network properties such as excitability and inhibition, which ultimately change the balance of excitation/inhibition within several brain areas including hippocampus. In order to assess these phenomena, we used a rat model of FXS that does not express the Fmr1 gene (Fmr1 knock-out, KO) and proceeded in studying by means of in vitro electrophysiological methods the spontaneous recurring activity of the dorsal and ventral hippocampus, and also the evoked neural activity of the same brain areas. Our studies showed that sharp wave-ripples (SWRs), an endogenous hippocampal pattern contributing to memory consolidation, and multiunit activity are decreased specifically in the dorsal but not ventral KO hippocampus. Furthermore, network excitability is generally increased on the KO hippocampus, but inhibition exerts an increased effect exclusively on the ventral KO hippocampus. In addition, increased α1GABAA receptor expression and reduced susceptibility to epileptiform activity were only associated with the ventral KO hippocampus. These results suggest that the ventral hippocampus of the adult KO rat may have developed changes that balance excitation/inhibition to protect it from a hyperexcitability course.

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OP08-Multi-omics analysis in a neural stem cell model of Parkinson's disease provides insights into the disease mechanisms

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Parkinson's disease (PD) is the second most common neurodegenerative disease and the fastest-growing neurological disorder for people over 65 years old. The molecular mechanisms underlying PD remain poorly understood, hindering the development of effective treatments. Here, we sought to investigate the dysregulated proteome, metabolome and lipidome in a novel cellular model of familial PD driven by the aggregation of the pathological α-synuclein (SNCA) A53T. Genetically modified human neural progenitor cells (hNPCs) which inducibly overexpress the mutant gene recapitulate a robust PD-like molecular phenotype accompanied by mitochondrial dysfunction and decreased differentiation potential. Quantification of deregulated protein abundance using data-independent acquisition (DIA)-mass spectrometry revealed major impairment in lipid metabolism and neuronal signaling. Additionally, metabolomics analysis highlighted significant perturbation in energy production, phospholipid metabolism and amino acid biosynthesis. Finally, the integration of proteomics, metabolomics and lipidomics data indicated mitochondrial deficiency and elevated fatty acid peroxidation, leading to oxidative stress, DNA damage and eventually programmed cell death through ferroptosis. A multi-omics-driven network highlighted the key players of these critical pathways which may be promising targets for therapeutic intervention in PD.

OP09-McIdas is fundamental for ependymal cell generation

Georgia Lokka[1], Konstantina Kaplani[1], Ioanna Papadionysiou[1], Maria Mpakogianni[1], Maria-Eleni Lalioti[1], Zoi Lygerou[2], Stavros Taraviras[1]
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Ependymal cells (ECs) line the walls of the brain ventricles. Multiciliated ECs contribute to cerebrospinal fluid flow and have been related to the development of neurological conditions such as hydrocephalus. A rare subpopulation of ECs, called E2, have two distinguishable basal bodies bearing two cilia. However, little is known about their origin and their function.
The differentiation of multiciliated ECs is strictly regulated by a molecular pathway that coordinates the generation of multiple basal bodies and cilia. Previous studies from our research group have pointed out GemC1 as the most upstream regulator for the commitment towards the ependymal lineage. Currently our research is focused on the role of McIdas in ependymal cell generation. Our data provide evidence that upon McIdas deletion, progenitor cells are committed towards the ependymal cell fate. However, multiciliated ependymal cells are absent and hydrocephalus is developed in McIdas mutants. Interestingly, increased number of bi-ciliated cells with characteristics of E2 ependymal cells are detected upon McIdas deletion, indicating that McIdas may play a role in the formation of those cells. To conclude, McIdas has been shown to be fundamental for the early steps of ependymal cell differentiation but not for the commitment towards the ependymal cell fate. Furthermore, evidence has been provided that the balance between different populations of ependymal cells may be affected by McIdas. Our data will contribute in unravelling the mechanism underlying the formation of E2 ependymal cells and use this knowledge for the development of novel therapeutic approaches for hydrocephalus

OP10-Role of developmental regulators of axonal local translation in adult axons.

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Adult axon regeneration is a complex process that recapitulates axonal development and relies on cell extrinsic and intrinsic factors for successful structural and functional repair of the nervous system (NS). Axonal protein synthesis is one such process that in coordination with cytoskeletal dynamics, appears to be of utmost importance for the initial stages of the regenerative procedure. However, our understanding of the regulatory mechanisms behind axonal local translation (LT) is still very limited. We have recently identified a dual role for Mena in axonal LT and actin cytoskeleton dynamics in developing NS. Our hypothesis is this dual Mena's function is preserved in the adult NS and might play a role in the regeneration after injury. Using the sciatic nerve as a model with intrinsic capacity for regeneration, we employed an unbiased biochemical approach to study the interactions of Mena in adult axons and uncovered a strong correlation with the ribosomal machinery and the mRNA translation. In good agreement with a potential role for Mena in LT, we demonstrate that Mena-KO sciatic nerves fail to acutely initiate injury-induced translation of hallmark proteins, which are necessary for the regenerative response. This is probably largely due to the inability of Mena-KO axons to induce the mTOR pathway, as they exhibit significantly low levels of mTOR translation and activation, while also having elevated levels of PTEN. Additionally, in vivo regeneration assays with wt and Mena-KO mice have been employed, to assess the contribution of Mena in regeneration.

ORAL Presentations (OP) - Symposium S3

SATURDAY – 25 NOVEMBER 2023

OP11-Microglia-specific therapeutic modulation of solTNF-TNFR1 pathway promotes cortical remyelination.

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Microglia have a critical role in CNS pathology controlling the disease environment, either by secreting neurotoxic mediators promoting neuroinflammation or by acquiring pro-regenerative phenotypes enhancing repair. A major pro-inflammatory cytokine secreted by microglia is TNF. In the TNF system, solTNF mainly contributes deleteriously via TNFR1 receptor signaling, while tmTNF has opposing beneficial functions through TNFR2 receptor in the CNS. Here we apply pharmacological and conditional gene editing tools, together with 3D high resolution immunofluorescent brain analysis, functional motor performance tests and brain transcriptomics in in vivo and in vitro models of demyelination and remyelination, focused on enhancing the beneficial regenerative microglia responses to promote CNS repair. The results show that selective blocking of solTNF by XPro1595, a brain-penetrating pharmacological inhibitor, promotes remyelination in the cortical grey matter by modulating microglia activation. Cell- and time-specific conditional gene editing, using CX3CR1-cre tamoxifen inducible mice, indicate that microglia-specific TNFR1 depletion, not TNFR2 depletion, was sufficient to promote remyelination in the cortex and improve Rotarod motor coordination performance, reproducing the effects of XPro1595 treatment and indicating a beneficial cell-autonomous microglia role. Focusing on glia involvement in CNS repair on a cellular level, single cell phenotypic analysis showed altered activation of microglia and astrocytes in the absence of solTNF-TNFR1 signaling. Last, brain RNA-seq transcriptomics revealed distinct gene signatures which indicate the important contribution of glial cells during CNS pathogenesis and recovery. Overall, enhancing beneficial microglia responses, by modulating major pro-inflammatory pathways, is a promising therapeutic strategy to promote CNS repair.

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OP12-Investigation of the functional interaction between Neural Stem/Progenitor Cells and platelets using mouse models of thrombocytopenia and co-culture assays

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Postnatal Neural Stem/Progenitor cells (NSPCs) reside in specialized microenvironments, called stem cell niches, such as s the Subependymal Zone (SEZ) of the lateral ventricles. Previous in vivo work revealed that focal demyelination in the corpus callosum (CC) induces specific platelet aggregation within the activated vasculature and that exposure of NSPCs to platelet lysate enhances their survival [1]. Here, we investigate direct cell-to-cell interactions, performing cocultures between platelets and NSPCs, as typical monolayer cultures, or using a polymorphic assay with different microenvironments (3D & 2D) [2]. We observed that platelets increased NSPC undifferentiated/progenitor state in the absence of growth factors and enhanced the distribution of neuroblasts and proliferating cells in the proximity of 3D structures, without changes in apoptotic markers. However, when we exposed NSPCs in a conditioned medium enriched with platelet factors, the progenitor state was not affected, reinforcing the role of cell-to-cell interactions. We also performed experiments of focal demyelination on the CC in transient chemical platelet depleted- and thrombocytopenic Nbeal2 knockout mice. Histological analysis showed increased oligodendrocyte density in both the SEZ and CC, and a reduction in the percentage of proliferating oligodendrocyte progenitor cells (OPCs) in the SEZ after platelet depletion, without changes in neurogenesis. Finally, we successfully grafted platelets into the CC in order to investigate their direct interaction with progenitors in homeostasis. In conclusion, we demonstrate a functional role of cell-to-cell interaction between platelets and NSPCs in vitro, and an altered behavior of OPCs in the niche and the CC of thrombocytopenic mice after demyelination.

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OP13-Effects of κ-opioid receptor in stress-driven synaptic alterations due to autophagy induction

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Stress has been shown to increase the endogenous neuropeptide dynorphin A levels, upregulate κ-opioid receptor (κ-OR) signaling which in turn modulates anxiety related behavioral responses in various brain regions (1). Neuronal autophagy controls the quality of cytoplasmic proteins, modulates synaptic organization and morphogenesis an effect that is enhanced upon chronic stress responses (2). Knowing that κ-OR blockade impedes the effects of stress in animal studies and that today κ-OR antagonists are in Phase III clinical trials as antidepressive drugs we should to determine the signaling constituents responsible for these neurobiological responses (3). We demonstrate herein with in vitro and in vivo studies that κ-OR agonists induce the autophagic machinery through a Gαio/ERK1,2/CREB novel signaling pathway (4). Moreover, our studies demonstrate that sub-chronic U50,488H administration in mice causes profound increases of specific autophagic markers in hippocampus with a concomitant decrease of several pre- and post-synaptic proteins, plausibly engulfed in the κ-OR-induced autophagic cargo (4). In addition, U50,488H-κ-OR activation promoted anxiogenic effects and cognitive impairment as measured by behavioral assessments such as elevated plus maze and novel object recognition. Moreover, using forced swim, a stressor known to increase the levels of the endogenous κ-OR ligand dynorphin, we are demonstrating that administration of the κ-OR selective antagonist, nor-binaltorhimine, blocks the induction of autophagy and the stress-evoked reduction of synaptic proteins in the hippocampus. These results provide novel insights that κ-OR-induced autophagy plays a key role in synaptic function that contributes to mood disorders targeted by the dynorphin/κ-OR system.

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OP14-Autophagy degrades myelin proteins and is essential for maintaining CNS myelin homeostasis

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(Macro)autophagy is a major lysosome-dependent degradation mechanism that 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 mature oligodendrocytes and CNS myelin, remains poorly studied.
Here, using both pharmacological and genetic inhibition of the autophagic machinery, we provide evidence that autophagy is an essential mechanism for oligodendrocyte maturation in vitro. Our study reveals that two core myelin proteins, namely proteolipid protein (PLP) and myelin basic protein (MBP) are incorporated into autophagosomes in oligodendrocytes, resulting in their degradation. Furthermore, by ablating atg5, a core gene of the autophagic machinery, specifically in myelinating glial cells in vivo by tamoxifen administration (plp-Cre^{ERT2}; atg5 ^{f/f}) we examined the contribution of autophagy in both adults (6 months-old) and aged animals (22 months-old). Autophagy depletion results in differences in myelin protein levels and PLP accumulation. Significant morphological defects in the myelin membrane, such as decompaction, accompanied by increased axonal degeneration are also observed. As a result, the mice exhibit behavioral deficits. In summary, our data highlight the fact that the maintenance of myelin homeostasis in both the adult and aged CNS requires the involvement of a fully functional autophagic machinery.

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OP15-Towards a biophysical model of a single neuron exhibiting critical dynamics

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Complex systems near continuous phase transitions display thermodynamic properties depending only on a small number of features, such as dimensionality and symmetry, and are insensitive to underlying microscopic properties. At such critical points, system dynamics can be described by a small set of parameters known as critical exponents. Accumulating experimental and theoretical work over the last decades suggests that the brain operates near a critical point. The "criticality hypothesis" is especially attractive in neuroscience as operation at or near a critical point has been shown to maximize information capacity and transmission and allows for communication and coordination of anatomically distant parts of the brain. In this context, single neurons have received little attention regarded as mere components of the complex system. Using ex vivo intracellular recordings from rat CA1 pyramidal cells, our group has shown that membrane potential fluctuations of single neurons show signs of critical behavior. The underlying biophysical mechanism generating critical fluctuations remains unknown as the original Hodgkin & Huxley model fails in reproducing the experimental findings. Here, we build upon non-deterministic, Markovian Hodgkin & Huxley models which are based on the stochastic nature of membrane ion channels. We use the method of critical fluctuations to analyze the produced timeseries and we show that this class of models is able to reproduce several experimental results. A biologically plausible mechanism is suggested for the emergence of self-organized criticality in single neurons. We envision a radically new class of complex systems, composed of complex units, with unexplored capabilities.

ORAL Presentations (OP) - Symposium S4

SATURDAY – 25 NOVEMBER 2023

OP16-Modest frequency differences in gamma oscillations across laminar compartments in macaque V1

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Gamma activity in monkey V1 is observed across the laminar probe[3]–[6], however, whether multiple gamma generators exist(s) is not fully elucidated. Early studies indicated that laminar compartments are uncorrelated, consistent with the notion of quasi-independent components[5]. Here we systematically address there are multiple laminar gamma generators and if their frequencies differ. Data across multiple sessions from two monkeys, which view grating stimuli of varying luminance contrasts for two attention conditions is analyzed. LFP across 16 laminar depths were converted into Current Source Density estimates [9] and then converted to time frequency representations (TFRs). These were segmented based on microsaccades, as these play an important role in organizing gamma activity [10], [11]. We identified two main gamma components: 1) a transient component (0-0.1s post microsaccade offset) and 2) a sustained component (0.1-0.35s post microsaccade offset), both of which, have generators in all supragranular(SG), granular (G) and infragranular compartments(IG). Frequency differences between the compartments are in the order of 2-5Hz but remain statistically significant after control for multiple comparisons. Significant frequency differences exist in both monkeys, although these vary somewhat between them, and are not significant for all experimental conditions. Transient and sustained components exhibit different patterns of significant laminar frequency differences. Thus, multiple gamma generators exist, which exhibit modest cross-laminar frequency differences; which, in turn, is consistent with a recent study [12], which however, utilized different stimuli/paradigm. The frequency differences reported here may have important consequences for synchronization across layers and information flow across the cortical module[7], [8].

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OP17-α-Synuclein oligomers potentiate neuroinflammatory NF-κB activity and induce Cav3.2-mediated calcium signaling in astrocytes.

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α-synuclein aggregation has been linked with sustained neuroinflammation in Parkinson's Disease (PD) aggravating neuronal degeneration [1]. Even though α-synuclein can be phagocytosed by microgla and/or astrocytes, the molecular pathways that trigger and prolong neuroinflammation in the PD brain remain unclear [2]. Aberrant astrocytic calcium signaling has been linked with the pathogenesis of several neurodegenerative diseases and could contribute to the initiation or maintenance of neuroinflammation[3]. In the present study we investigated the role of calcium signalling in α-synuclein-induced inflammation in vivo, using the human α-synuclein A53T transgenic mouse model, where the presence of α-synuclein oligomers, but not monomers, is correlated with sustained inflammatory responses as indicated by significant elevations in the levels of pro-inflammatory cytokines and the produced endogenous antibodies. 3D cell reconstruction and morphometric analysis of the GFAP+ astrocytes in the striatum revealed increased number and distinct morphological alterations in the A53T mice compared to wild type mice. Further analysis of the striatum of A53T mice revealed an activation of the p38/MAPK pathway in microglia that stimulated the NFκB pathway in astrocytes. Such activation resulted in the upregulation of astrocytic T-type Cav3.2 voltage gated Ca2+ channel (VGCC). Proteomic analysis in quiescent astrocytes overexpressing the Cav3.2 VGCC, highlighted the secretion of Insulin-like growth factor-binding protein-like 1 (IGFBPL1), a potent regulator of axonal growth through IGF1 induction [4]. Our findings, combined with the absence of neuronal death in A53T mice, suggested that the elevation of astrocytic Cav3.2 levels could act, at least in part, as a neuroprotective mechanism against α-synuclein-induced neuroinflammation.

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OP18-Exploring the Brain's Response to Chemotherapy: Neurogenesis at the Forefront

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Recent research has identified side effects of chemotherapy on brain function. These include reduced neural stem cell growth, white matter degeneration, and brain inflammation, collectively known as 'Chemobrain.' We studied the impact of intraventricular infusion of chemotherapeutic mito-toxic agent arabinoside-C (Ara-C) on adjacent neurogenic and non-neurogenic brain areas.
Characterization of the spatio-temporal distribution of neuronal and glial cells at multiple time points following the chemical lesion (4, 15 days, and 6 weeks) revealed that Ara-C infusion leads to persisting ependymal cell layer disruption and impaired neurogenesis in both neurogenic niches (Subventricular Zone and Subgranular Zone). Moreover, it triggers doublecortin+ (DCX+) neuroblasts' ectopic presence clustered within white matter tracts of striatal parenchyma, as well as in deep areas of the granule layer of the dentate gyrus (DG). Using transmission electron microscopy (TEM) to evaluate brain micro-structure we observed myelination defects and axon loss which is accompanied by impairment of the oligodendrocyte lineage. Our data also support a strong neuroinflammatory response with extensive astro- and microgliosis and the presence of CD3+ T cells in the SVZ and choroid plexus, suggesting a disruption of the blood–CSF barrier in this site.
Our studies are ongoing to follow the lineage trajectories, origin, and molecular profile of ectopic neuroblasts.

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OP19-Unraveling Behavioral Deficits in Neurofibromatosis Type 1: Insights from Drosophila Models

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Neurofibromatosis type 1 (NF1) is an autosomal dominant multi-systemic disorder, affecting □1 in 2000-3000 individuals worldwide. It results from mutations in the Nf1 tumor suppressor gene, leading to a wide array of symptoms. Nf1 encodes Neurofibromin (Nf1), a large multifunctional protein, preferentially expressed in the central and peripheral nervous system, crucial for regulating multiple signaling pathways. Although typically considered a tumor predisposition syndrome, it is also associated with skeletal and skin pigmentation abnormalities, short stature and broad cognitive/behavioral presentations, including impaired learning, attention deficit hyperactivity disorder, autism spectrum disorder, social/communicative disabilities and disturbed sleep. Progress towards amelioration of these behavioral deficits requires understanding the cellular and molecular impact of particular Nf1 mutations that govern such behaviors. For this reason, appropriate animal models emulating human phenotypes are necessary. Loss of the highly conserved Drosophila dNf1 ortholog mimics human NF1 pathology, causing reduced size, impaired learning, synaptic defects, behavioral inflexibility, and abnormal activity and sleep patterns. Furthermore, particular Nf1 point mutations are associated with specific behavioral deficits that implicate distinct molecular mechanisms than those affected upon total dNf1 loss. Our evidence thus far, suggests that different Nf1 mutations may impact differentially both established and previously unidentified functions of the protein, possibly in a cell-type-specific manner. These allele-specific effects could be a contributing factor to the variability observed in NF1-related pathologies. Elucidating the determinants of these phenotypes will contribute significantly to the development of novel, potentially personalized ameliorative strategies for these defects.

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ORAL Presentations (OP) - Symposium S5

SATURDAY – 25 NOVEMBER 2023

OP20-Alpha and beta oscillations shape language comprehension and production

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Brain oscillations are prevalent in all species and are involved in numerous perceptual operations. In particular, alpha oscillations are thought to reflect a mechanism of active inhibition, which fine-tunes sensory processing by guiding attention and suppressing distracting input, while beta oscillations are proposed to support the reactivation of content representations, by synchronizing local cell populations and long-distance networks. Can the proposed functional role of alpha and beta oscillations be generalized from low-level operations to higher-level cognitive processes? To answer this question, we present two magnetoencephalography (MEG) studies investigating brain oscillatory responses during (i) naturalistic story comprehension, and (ii) a novel rule-switching task incorporating linguistic demands. Briefly, results show that alpha and beta band dynamics seem to subserve high-level operations (inhibition, as well as reactivation and rule-switching, respectively), during language comprehension and linguistic task demands. Overall, our findings support the generalizability of the role of alpha and beta oscillations from perceptual to complex linguistic processes.

OP21-Neonatal maternal neglect effects on rat reward-anticipatory behavior, social status stability, and reward circuit activation in adulthood

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Maternal neglect, a common form of human early life adversity, has been associated with the emergence of long-lasting psychopathological symptoms including mood disorders, reward-learning deficiency, and inability to form stable social bonds. Few studies have explored the impact of maternal neglect on social interactions and reward learning during group reward anticipation tasks. Using a rodent model that closely resembles human parental neglect where anticipated care is delayed (the DER experience), we measured reward association in a group food anticipation training task that included a context-dependent and a cue-dependent learning module for 7 consecutive days. The experimental setup employed in our study integrated social interactions as a modulating factor. At the end of training, the activity of reward system areas was assessed by pCREB protein levels determination.
We found that the DER experience induced alterations in exploratory behaviors and lower overall context-reward association during context-dependent learning as well as delayed cue-reward association and increased locomotion during cue learning. These reward-context/cue association deficiencies were accompanied by increased food access competition and reward system overactivation. Collectively, we showed novel effects of the DER experience highlighting the comorbidity of social abnormalities with reward association deficiency and abnormal reward system activation. Notably, this phenotypical novelty highlights the importance of using group tasks in early life adversity studies.

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OP22-Tracking politically motivated reasoning in the brain

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The increasing politicization of scientific topics have raised crucial questions about the neurocognitive mechanisms that underlie politically motivated reasoning. Using an experimental design that identifies motivated reasoning as directional deviations from a Bayesian benchmark, we asked members of opposing opinion-based groups to what extent they endorse factual messages on a polarizing political topic. Both groups exhibited a desirability bias by over-endorsing desirable messages and under-endorsing undesirable messages and an identity bias by over-endorsing messages from ingroup sources and under-endorsing messages from outgroup sources. In both groups, brain activity in regions implicated in encoding value, error detection and adjustment, and mentalizing tracked the degree of desirability bias. In contrast, less extensive activation within the mentalizing network tracked the degree of identity bias. These findings provide direct neural evidence of the cognitive architecture of two distinct forms of politically motivated reasoning and highlight the universality of these processes across ideologically opposing groups.

OP23-Cerebral lateralization in writing: comparing handwriting, and typing using computer and smartphone keyboard

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The neural underpinnings of written language, similarly to the neural underpinnings of oral language, are left-lateralized, with distinct patterns between left- and right-handers. However, cerebral lateralization for written language has only been studied using handwriting tasks; the cerebral lateralization of typing on a computer or a smartphone keyboard, popular alternatives for writing, have not been explored. Therefore, it remains unanswered whether handwriting and keyboard typing follow similar cerebral laterality patterns. We pre-registered two functional trans-cranial Doppler (fTCD) ultrasound studies aiming to explore (i) the cerebral lateralization patterns of typing using a computer (completed study with n= 53, left-handers = 23) and a smartphone (registered report in the stage of data collection) keyboard by comparing those patterns to the ones of handwriting and (ii) handedness differences in these lateralization patterns. Our initial hypothesis was that the cerebral lateralization patterns would not differ between the methods of writing. We also hypothesized that right-handers would exhibit a greater leftward cerebral lateralization during writing (regardless of the method) compared to left-handers, consistent with the findings from studies on oral language and handwriting. In this conference we are going to present the results on the cerebral laterality of typing on a computer keyboard, the expected results from typing on a smartphone keyboard, and discuss the implications of using electronic means of writing on neuroscience.

ORAL Presentations (OP) - Symposium S6

SUNDAY – 26 NOVEMBER 2023

OP24-Time-dependent effects of currant (Vitis vinifera) consumption on neuroinflammation and oxidative stress in the 5xFAD mouse model of Alzheimer's Disease

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Alzheimer's disease (AD) is associated with brain Aβ accumulation, neuroinflammation and oxidative stress. Currants, a low glycemic-index dried fruit, and their components display pleiotropic neuroprotective effects in AD. We examined whether diet containing 5% Corinthian currant (Vitis vinifera) paste (CurD) administered in 1-month-old 5xFAD mice for 1, 3, and 6 months affects Aβ levels, neuroinflammation and oxidative stress in comparison to control diet (ConD) or sugar-matched diet containing 3.5% glucose/fructose (GFD). CurD for 3 months reduced brain Aβ42 levels in male mice compared to ConD and GFD, but after 6 months, Aβ42 levels were increased in mice both on CurD and GFD compared to ConD. CurD for 3 months also reduced TNFα and IL-1β levels in both male and female mouse cortex homogenates compared to ConD and GFD. However, after 6 months, TNFα levels were increased in mice both on CurD and GFD compared to ConD. IL-1β levels were increased in the brain of all groups after 6 months. Furthermore, the activity of antioxidant enzyme paraoxonase 1 (PON1) was increased and the levels of reactive oxygen species (ROS) were decreased in the cortex of male and female mice on CurD for 1 and 3 months compared to GFD and ConD. After 6 months though, PON1 activity was reduced and ROS levels were increased in all mice groups. These findings show that currant consumption by 5xFAD mice, at early stages of disease, reduces Aβ42 levels, neuroinflammation and oxidative stress, but longer-term intake, during later stages of disease, diminishes the protective effects

OP25-Cannabidiol restores ketamine-induced schizophrenia-like symptomatology by multi-level action on the underlying neurobiological substrate.

Charalampos Brakatselos[1], George Ntoulas[1], Michail-Zois Asprogerakas[1], Olga Tsarna[1], Anastasia Vamvaka-Iakovou[2], Gerasimos Nakas[1], Anastasios Delis[3], Joanna Silva[2], Foteini Delis[1], Joao Filipe Oliveira[2], Ioannis Sotiropoulos[2], Alexia Victoria Polissidis[3], Katerina Antoniou[1]
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The neurobiological underpinnings of repeated ketamine (KET) model of schizophrenia remain poorly understood. Cannabidiol (CBD), a non-addictive phytocannabinoid has been reported to present antipsychotic potential, but the mechanisms involved remain elusive. This study aims to investigate the KET-induced bio-phenotype, and the potential therapeutic effect of CBD in the manifestation of psychopathology from the local microcircuit to network function and behavior.
After a repeated subanesthetic KET exposure, rats received a 5-day long treatment with CBD. Subsequently, they underwent behavioral analyses exploring positive, negative, and cognitive symptomatology. HPLC-ED provided estimates of dopaminergic and glutamatergic activity, NMDA and AMPA receptors' synaptic phosphorylation state was evaluated via western blot, specific interneuron densities or synaptic receptor localization have been estimated using immunofluorescence. LFPs have been recorded from sevoflurane-anesthetized rats' mPFC (medial prefrontal cortex), simultaneously with dorsomedial striatum (DMS), and ventral hippocampus (VH).
KET-treated rats displayed a schizophrenia-related behavioral bio-phenotype, with a parallel impairment of the glutamatergic neurotransmission in the PFC and DMS, and excitation/inhibition imbalance in the VH. CBD counteracted positive, negative, and cognitive symptomatology, while also robustly modulated prefrontal glutamatergic dysregulation and hippocampal E/I imbalance.
Overall, our study provides novel insights into the neurobiological substrate of the repeated-ketamine model of schizophrenia and contributes to the elucidation of pathophysiology. Importantly, we pinpoint specific properties of CBD's action towards the development of novel therapeutic strategies focused on the endocannabinoid system and E/I imbalance restoration.

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the HFRI PhD Fellowship grant (Fellowship Number: 1203).

OP26-Cross-sectional Developmental Trajectories of cool and hot Executive Function in Autism Spectrum Disorder

Evangelia Chrysanthi Kouklari[1,2], Evdokia Tagkouli[1], Stella Tsermentseli[3], Vassiliki Ntre[1], Artemios Pehlivanidis[1], Katerina Papanikolaou[1]
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Developmental theories of Executive Function (EF) have postulated the existence of separate EF domains, known as "cool" and "hot" EF, yet the evaluation of EF development in Autism Spectrum Disorder (ASD) has primarily relied on assessments of cool EF. Limited attention has been given to the examination of hot EF development in ASD. This current research endeavor aimed to explore the cross-sectional developmental patterns of both cool and hot EF during middle childhood and adolescence within ASD. Our study involved the assessment of 82 children and adolescents aged 7-16 years, employing measures to evaluate cool EF facets (i.e., inhibition, working memory, planning, and cognitive flexibility) as well as hot EF (i.e., affective decision-making and delay discounting). Our findings unveiled linear age-related improvements in all facets of cool EF among individuals with ASD. Conversely, the developmental trajectories of hot EF exhibited non-linear age-related patterns. The scrutiny of these developmental trajectories of cool and hot EF holds the potential to delineate cognitive phenotypes that evolve across different age stages within the ASD population.

OP27-Sex-specific effects of pharmacological agents targeting the estrogen membrane receptor GPER1 on anxiety levels and monoaminergic activity of male and female rats

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Affective and anxiety disorders disproportionately affect adult women, with the G protein-coupled estrogen receptor 1 (GPER1) emerging as a potential player in these disorders through rapid neuroestrogen signaling. Notably, similarities exist between the rapid effects of ketamine and the GPER1 signaling pathway. This study aimed to elucidate the role of GPER1 in the central nervous system and its potential as a new therapeutic target for mood disorders. 180 adult male and female Wistar rats received of treatments of either vehicle, fluoxetine, ketamine, GPER1 agonist (G1), GPER1 antagonist (G15), or their combinations. Behavioral tests (Open field, Light/Dark test, Novelty suppressed feeding test, and Forced swim test) were conducted, followed by monoamine level analysis of the prefrontal cortex and hippocampus using HPLC-ED. Sex differences were observed in vehicle-treated animals, as males exhibited lower anxiety levels than females. In males, acute ketamine and coadministration of G1 and ketamine exerted anxiolytic/antidepressant effects in the NSFT. Moreover, the combination of G15 and fluoxetine revealed an anxiolytic effect exclusively in males. In contrast, female rats demonstrated anxiogenic responses when treated with both G1 and ketamine, emphasizing the sex-specific effects of GPER1 modulation. Furthermore, treatment effects on serotonin (5-HT) and dopamine turnover ratios varied between sexes in the PFC, with fluoxetine elevating 5-HT levels in males and G1 influencing dopaminergic activity in females. The study underscores the sex-specific effects of GPER1 modulation on mood and neurochemical pathways, emphasizing its potential as a mood disorder therapeutic target.

Abstracts

Poster Presentations (PP) - Poster session #1

FRIDAY – 24 NOVEMBER 2023

PP001-Investigating the Synaptic Dynamics of Adaptive Behavior in the mouse frontal cortex

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In this world of unexpected change, animals face a critical trade-off between retaining older information and facilitating new learning. Dendritic spines, where excitatory synapses are located, represent the basic structural and functional unit of learning and memory at the neuronal level; spines are formed and/or modified during learning to support behavioral demands. However, how the interplay between plasticity and stability in dendritic spines is implemented to accommodate adaptations in behavior remains unclear. We investigated this question in head-fixed mice performing a rule-switch behavioral task. We found that the secondary motor cortex (M2) of the dorsal prefrontal cortex (dPFC) is essential for changing strategy in response to external stimuli, but not for the maintenance of a strategy. Bilateral inactivation of M2 impaired the switch to the new rule by increasing the perseverative errors. Moreover, in-vivo imaging of spines in M2 pyramidal neurons across behavioral sessions after the rule switch, revealed an increase in spine dynamics (spine turnover) that was significantly higher in mice learning to adapt compared to mice maintaining a previously learned strategy. These results uncover an important role of M2 persistent synaptic changes in adaptive behavior.

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PP002-Exploring Gene-Drug Interactions for Personalized Treatment of Post-Traumatic Stress Disorder (PTSD)

Konstantina Skolariki[1], Marios Krokidis[1], Aris Vrahatis[1], Themis Exarchos[1], Panagiotis Vlamos[1]
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Post-Traumatic Stress Disorder (PTSD) is a mental disorder that can be developed after exposure to traumatic events. This study is separated into two main axes. The first axis of this work focuses on the genes and genetic variations that contribute to PTSD. Using three methodological approaches, 122 genes and 184 Single Nucleotide Polymorphisms (SNPs) were identified as PTSD-related. Consequently, the complex genetic interactions were examined through various networks and functional analysis. The ontological analysis provided insights into the biological processes, cellular components, and molecular functions involved in PTSD. The KEGG and Reactome analysis provided insights into the different pathways associated with the disorder. The protein-protein interaction network (PPI) helped visualize complex molecular relationships related to PTSD. This was the groundwork on which the focus of the second axis was based upon. One of the aims of this work is to propose a more personalized and effective treatment for PTSD, utilizing drug repurposing approaches. In this regard, by using the PTSD-related gene set identified, and utilizing two distinct computational approaches, potential drug candidates for repurposing were distinguished. The top 16 drug candidates were then further validated for oral bioavailability and drug-likeness. Classifying, thus, the compounds that exhibited drug- like properties and had favorable characteristics for oral bioavailability.

PP003-Adaptations in prefrontal cortical and hippocampal function in adolescent MAM mice

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Schizophrenia is a severe neurodevelopmental psychiatric disorder evident in adulthood. Reduced prefrontal cortical (PFC) – hippocampal (HPC) connectivity associated with poor cognitive performance in human patients and animal models, is believed to emerge earlier in development. During adolescence, we aim to understand adaptations in behaviour, neuronal activity and anatomy of PFC and HPC pyramidal neurons (PN) in the methylazoxymethanol acetate (MAM) mouse model of schizophrenia relatively to control mice.
Adolescent (~P40) male and female MAM and control mice were tested in the temporal order (TOR) and object-to-place (OTP) recognition tasks in, followed by Golgi-cox staining to study CA1 HPC PN spine morphology or electrophysiology to study synaptic properties and reversal potential of GABAA receptor of L2/3 PFC PN or power of oscillations in both L2/3 PFC PN and HPC CA1 PN in brain slices up to P45.
We found a significant reduction of sIPSCs in MAM PFC compared to controls. Increased excitation in MAM PFC is further supported by a more depolarized reversal potential of GABAA receptors. These neurophysiological adaptations could underlie impaired performance in the TOR task in MAM mice. Likewise, MAM mice displayed deficits in the OTP task accompanied by reduced dendritic spines in the HPC. However, no differences were detected in synchronization to oscillations in neither PFC nor HPC brain slices between the two groups.
Overall, MAM mice exhibit neurophysiological and anatomical adaptations in PFC and HPC in adolescence, a period of extensive circuits maturation, potentially associated with deficits in PFC-dependent recency and HPC-dependent spatial memory.

PP004-Description and quantification of apoptosis during in vitro mouse brain Neural Stem Cell differentiation

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One of the best-described neurogenic regions of the adult mammalian brain is the Subependymal Zone (SEZ), which is located at the lateral walls of the lateral ventricles. Neural Stem Cells (NSCs) found therein are the descendants of the embryonic NSCs called Radial Glia cells and remain mainly in an inactive (quiescent) state. Because of their ability to preserve their proliferation and differentiation potential, NSCs can be isolated and cultured in vitro. The absence of growth factors from the culture medium, can lead to NSCs' differentiation towards Neural Progenitor Cells, which can give rise to astrocytes, oligodendrocytes and neurons (Kriegstein and Alvarez-Buylla,2009). However, the removal of pro-mitotic factors can have a negative effect on stem cells, in general, either via apoptosis or necrosis.
In this study we followed for three days adult mouse NSCs, cultured in differentiation conditions in order to describe and quantify apoptotic cell death (Ceccatelli et al.,2004). Apoptotic nuclei and apoptotic bodies were identified based on DAPI staining, while at the same time the expression of SOX2 and NESTIN (to mark progenitor cells), GFAP (to mark astrocytes), DCX (to mark neuroblasts) and OLIG2 (to mark oligodendrocytes) was assessed immunocytochemically. The results indicated a significant increase of apoptosis after 24 and 48h in culture, with a parallel significant switch in the presence of apoptotic bodies at the expense of whole apoptotic nuclei. In addition, we observed a surprising fluctuation in the cell profile of the cultures; a finding that has to be thoroughly investigated.

PP005-Definition of the Neural Representation/Code of Actions in the Population Activity of Mirror Neurons (MirNs).

Konstantinos Chatzimichail[1,2], Eleutheria Tzamali[1], Vassilis Papadourakis[1], Vassilis Raos[1,2,3]
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The type of information encoded within the ensemble activity of MirNs and the way this information is distributed across the constituent elements of the ensemble has not been previously investigated. To address this gap, we utilized our MirNs database*, which includes spiking neural activity data recorded from the forelimb representations of dorsal and ventral premotor cortical areas, where MirNs are located. This data was collected while macaque monkeys were either observing or performing reaching-to-grasp actions. We applied pattern classifiers to analyze this dataset.
Initially, we sought to define the nature of the information, referred to as the "code", and determine when and for how long this code is represented within the population activity. We also examined the contribution of individual neurons to this population code, aiming to ascertain whether the code is distributed among many neurons or confined to a specific group of neurons.
Subsequently, we investigated the temporal generalization of decoding to determine whether the code remains consistent throughout the trial (referred to as "stationary", where each neuron maintains a persistent state of activity and selectivity) or whether it changes over time (referred to as "dynamic", indicating shifts in neural selectivity or different neurons becoming selective at different times).
Our findings reveal that grip-related information can be reliably decoded from the population activity of MirNs in both areas and under both observational and action-based conditions. During movement, observed or executed, a distributed dynamic code is employed. These results imply that the variables encoded within MirNs populations are grip dependent.

* Papadourakis V, Raos V. 2017. Evidence for the representation of movement kinematics in the discharge of F5 mirror neurons during the observation of transitive and intransitive actions. J Neurophysiol 118(6):3215-3229
* Papadourakis V, Raos V. 2019. Neurons in the macaque dorsal premotor cortex respond to execution and observation of actions. Cereb Cortex 29(10):4223-4237

This research was supported by a grant to VR co-financed by Greece and the European Union (European Social Fund-ESF) through the Operational Programme “Human Resources Development, Education and Lifelong Learning 2014-2020” in the context of project MIS 5048512.

PP006-Mirror neurons (MirNs) in the premotor cortex of the macaque monkey encode the kinematics of both observed and executed actions.

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Unfolding actions involve dynamic stimuli that continuously evolve. The question of what aspects of these stimuli are encoded by MirNs remains a debated issue. The prevailing belief suggests that MirNs encode the "goals" of observed actions, even though this idea lacks empirical proof. This belief emerged from the notion that MirNs only activate during the observation of transitive actions, presumed to have specific goals, contrasting with intransitive actions, seen as goalless. In contrast to this belief, we* demonstrated that most MirNs respond to both transitive and intransitive actions, indicating that MirNs may encode the kinematics of these movements. Our study aims to test this hypothesis through techniques like principal component analysis, regression, and decoding.
To do so, we employed neural data from MirNs in the ventral and dorsal premotor cortex of the macaque brain recorded during the execution and observation of grasping actions. We also utilized offline-recorded kinematic data from both the monkey and the experimenter during grasping actions. Employing multiple linear regression, we examined the relationship between MirNs' discharge and kinematics, modeling kinematic parameters as linear functions of neural activity and vice versa. We subsequently conducted a decoding analysis to determine whether grip-related information is present in the predicted variables.
Our results demonstrate that a relatively simple linear model accurately predicts various kinematic parameters from neural activity and vice versa. This suggests that kinematic information is indeed encoded by MirNs at the population level, supporting our hypothesis.

* Papadourakis V, Raos V. 2017. Evidence for the representation of movement kinematics in the discharge of F5 mirror neurons during the observation of transitive and intransitive actions. J Neurophysiol 118(6):3215-3229

This research was supported by a grant to VR co-financed by Greece and the European Union (European Social Fund-ESF) through the Operational Programme “Human Resources Development, Education and Lifelong Learning 2014-2020” in the context of project MIS 5048512.

PP007-Investigating the Correspondence between Neural Population Codes for Observed and Executed Actions in Premotor Areas of the Macaque Brain.

Konstantinos Chatzimichail[1,2], Christos Paschalidis[1,2], Vassilis Papadourakis[1], Vassilis Raos[1,2,3]
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Mirror neurons (MirNs) respond not only when a monkey actively performs an action but also when it passively observes a similar action by another. The expected congruence in the actions they encode, whether during observation or execution, has been challenged by our recent findings*. We demonstrated that individual MirNs inconsistently exhibit congruency, prompting us to suggest that this alignment may occur at the population level. While not a feature of every individual MirN, the mirror property might be a characteristic of the MirN ensemble.
To explore this hypothesis, we used decoding to assess neural code congruency, employed neural manifold optimization to study the similarity in neural population structure, and quantified neural population dynamics using the "tangling" metric.
Our cross-conditional decoding analysis in the ventral premotor activity, revealed an extended period spanning from the end of the movement to the middle of the holding period, with significant readout. In the dorsal premotor activity, significant readout occurred but for a shorter duration around the end of the movement. Although cross-conditional decoding accuracy was lower than within-condition decoding, its presence suggests some degree of similarity in population codes for both conditions. Neural manifold optimization identified a "shared" space where cross-conditional readout improved, supporting the idea of a common neural representation. Analysis of neural dynamics showed low tangling values in both execution and observation conditions, possibly indicating similar neural computations in both contexts. These findings offer compelling evidence that neural population codes for observed and executed actions align, endorsing our initial hypothesis.

* Papadourakis V, Raos V. 2019. Neurons in the macaque dorsal premotor cortex respond to execution and observation of actions. Cereb Cortex 29(10):4223-4237

This research was supported by a grant to VR co-financed by Greece and the European Union (European Social Fund-ESF) through the Operational Programme “Human Resources Development, Education and Lifelong Learning 2014-2020” in the context of project MIS 5048512.

PP008-Adaptations in excitation-inhibition balance in the medial prefrontal cortex during postnatal development could

contribute to working memory deficits and corresponding neuronal activation changes in adulthood in the MAM model of schizophrenia

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Schizophrenia is a severe, neurodevelopmental psychiatric disorder lacking effective treatment. We aim to understand synaptic properties of layer 2/3 (L2/3) pyramidal neurons (PN) of medial prefrontal cortex (mPFC) during postnatal development to provide insights regarding cognitive deficits in the methylazoxymethanol acetate (MAM) mouse model of schizophrenia[1].
Voltage-clamp recordings were performed in mPFC brain slices from neonatal, juvenile, adolescent and adult saline and MAM-treated C57BL/6 male mice to investigate spontaneous inhibitory and excitatory postsynaptic currents (sI/E-PSC) in L2/3 PN. Working memory was evaluated in adulthood using the forced alternation T-maze task, followed by c-Fos immunofluorescence to study neuronal activation.
We found a significant increase of sIPSC frequency in neonatal, but a significant decrease in juvenile and adolescent MAM mice compared to controls, whereas no difference was observed in adult mice. Frequency of sEPSC was significantly reduced in juvenile, but significantly increased in adult MAM mice relative to controls, while no changes were observed in neonatal and adolescent mice. Excitation/inhibition (E/I) ratio based on the frequency of sEPSC/sIPSC revealed a shift towards inhibition for controls, whereas towards excitation for MAM animals. These differential developmental alterations are likely responsible for the reduced performance in the T-maze task of adult MAM mice, compared to controls. Furthermore, c-Fos immunofluorescence revealed adaptations in neuronal activation during the working memory task, possibly stemming from increased E/I balance in MAM mice.
Overall, early-life adaptations in synaptic properties affect E/I balance in mPFC and potentially its maturation, leading to cognitive deficits and impaired mPFC activation in adult MAM mice.

1. Chalkiadaki, K. et al. (2019) ‘Development of the MAM model of schizophrenia in mice: Sex similarities and differences of hippocampal and prefrontal cortical function’, Neuropharmacology, 144, pp. 193–207.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

PP009-DendroTweaks: An interactive approach for unraveling dendritic dynamics

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Neurons rely on the interplay between dendritic morphology and ion channels to transform synaptic inputs into a sequence of spikes. While detailed biophysical models with active dendrites have proven useful for exploring this interaction, creating and validating such models is challenging due to the large number of parameters involved. Often, it is not straightforward how changing these parameters affects the resulting voltage dynamics of a neuron.
In this work, we introduce DendroTweaks, a framework that provides an intuitive and interactive approach to adjusting model parameters. Through a graphical interface equipped with widgets and interactive plots, users can easily modify any parameter of the model with real-time visual feedback. Furthermore, we encourage users to optimize the set of adjustable parameters by simplifying the model. This simplification is achieved through built-in functionality for standardizing ion channel models and reducing dendritic morphology while preserving voltage responses of the original model. As a result, the behavior of the model, and dendritic integration in particular, becomes easier to understand and design on demand.

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PP010-Human Tau transgenes functionally compensate for the loss of Drosophila Tau in memory formation, in a cell-type specific manner.

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Accumulation of highly post-translationally modified tau proteins is a hallmark of neurodegenerative disorders known as tauopathies, the most common of which is Alzheimer's disease. Human Tau isoforms are microtubule-associated proteins with varying capabilities for stabilizing microtubules. Additionally, these isoforms have non-standard functions, including the regulation of filamentous actin stability, genomic stability, and synaptic plasticity. They are predominantly expressed in neurons, yet the precise function of each Tau isoform remains poorly understood. Previously, we identified a novel role for dTau in behavioral plasticity and demonstrated that dTau null mutants exhibit enhanced memory performance. In this study, we expressed each of the six human Tau isoforms in different neuronal subsets, all within a dTau null background. Our findings reveal that only 4R isoforms functionally complement dTau loss and reverse the memory phenotype of the mutants, in a cell-type specific manner. These results may help improve the understanding of how each isoform contributes to neuronal function and dysfunction.

Funding: State Scholarship Foundation (IKY)

PP011-The inhibition of α-synuclein aggregation using marine-derived bacterial metabolites as a novel neuroprotective approach for Parkinson's Disease

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Inclusions consisting of aggregated α-synuclein (α-syn), an abundant neuronal protein physiologically found in the central nervous system, are the most common histopathological finding in Parkinson's Disease (PD). The soluble high molecular weight oligomers resulting from multimerization of monomeric α-syn are considered detrimental for the viability of neuronal cells and therefore are considered potential therapeutic targets. The vast and largely unexplored biodiversity of the marine environment presents an untapped resource for discovering novel biochemicals with unique chemical structures and potentially potent biological activities. In this work, we have screened a panel of marine-derived bacterial extracts to discover agents that can drive the removal of aberrant α-syn assemblies. Initially, the homogenates of marine-derived bacteria were selected by their ability to hinder the elongation of α-syn pre-formed fibril seeds in vitro using a specific thioflavin T fluorescence assay. The most potent extracts were subsequently administered in a well-established SH-SY5Y cell system in which the inducible expression of α-syn results in oligomer formation and cell death. Effects in the levels of oligomeric α-syn were assessed by western blotting and an oligomer-specific ELISA assay. The secondary metabolites isolated from the most potent extracts were administered to SH-SY5Y cells to assess which compounds contain anti-aggregation activity in a cellular context. Our results demonstrated that the extract BIO904 and its component BIO904-09, a 2,5-diketopiperazine, exhibited a significant dose-dependent decrease in the levels of aggregated α-syn. We are currently investigating the molecular mechanism underlying the BIO904-09-induced decrease of aggregated α-syn by assessing whether BIO904-09 affects proteasome activity.

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PP012-Pancreatic Cancer-Associated Depression (PCAD) is linked to adult neurogenesis impairment

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Pancreatic Cancer (PC) is a very aggressive type of cancer, associated with a high incidence of major depression manifesting before formal diagnosis (PCAD). Although the connection between PC and depression is known, the mechanism underpinning this correlation remain unexplored. Our study aims to elucidate how PC affects adult neurogenesis, leading to PCAD. We behaviorally tested a PC mouse model, generated by orthotopically injecting human pancreatic Panc-1 cells in immunocompromised mice. Our results demonstrated that these mice exhibit a depressive-like phenotype when compared to sham-operated controls. High-performance-Liquid-Chromatography (HPLC) analysis on brain lysates, demonstrated an unprecedented imbalance in the serotonin levels of PC mice. Furthermore, we employed a genetic mouse model (Pdx1Cre-AKrasG12D) that mimics human PC development by constitutively expressing KrasG12D specifically in the pancreas. HPLC analysis of these brain lysates confirmed the imbalance in the serotonin pathway. Moreover, immunofluorescent analysis on brain cryosections showed impaired hippocampal neurogenesis, as demonstrated by the reduced number of DCX+ and GFAP+/radial glia-like neural stem cells (NSCs), compared to controls. To assess the involvement of systemic factors on adult hippocampal NSCs behavior during progression of PC, NSCs isolated from the dentate gyrus of wild-type mice were cultured in the presence of sera collected from Pdx1Cre-AKrasG12D or control mice. Exposure of NSCs to Pdx1Cre-AKrasG12D serum reduced their proliferative capacity (BrdU assay) and survival (TUNEL assay). Our findings suggest that PC affects the brain by altering the serotonin pathway and by reducing the neurogenic capacity of hippocampal NSCs, possibly via the secretion of systemic factors, leading to PCAD.

PP013-Mical modulates Tau toxicity via cysteine oxidation in vivo

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Tau accumulation is clearly linked to pathogenesis in Alzheimer's disease and other Tauopathies. However, processes leading to Tau fibrillization and reasons for its pathogenicity remain largely elusive. Mical emerged as a novel interacting protein of human Tau expressed in Drosophila brains. Mical is characterized by the presence of a flavoprotein monooxygenase domain that generates redox potential with which it can oxidize target proteins. In the well-established Drosophila Tauopathy model, we use genetic interactions to show that Mical alters Tau interactions with microtubules and the Actin cytoskeleton and greatly affects Tau aggregation propensity and Tau-associated toxicity and dysfunction. Exploration of the mechanism was pursued using a Mical inhibitor, a mutation in Mical that selectively disrupts its monooxygenase domain, Tau transgenes mutated at cysteine residues targeted by Mical and mass spectrometry analysis to quantify cysteine oxidation. The collective evidence strongly indicates that Mical's redox activity mediates the effects on Tau via oxidation of Cys322. Importantly, we also validate results from the fly model in human Tauopathy samples by showing that MICAL1 is up-regulated in patient brains and co-localizes with Tau in Pick bodies. Our work provides mechanistic insights into the role of the Tau cysteine residues as redoxswitches regulating the process of Tau self-assembly into inclusions in vivo, its function as a cytoskeletal protein and its effect on neuronal toxicity and dysfunction.

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PP014-Mapping the signature of pain in the posterior insular and secondary somatosensory cortex in mice by cFos expression

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The insular cortex (IC) and the secondary somatosensory cortex (SII) are the only two brain regions that when stimulated produce pain sensation in humans. Both areas are activated with painful stimuli and have been suggested to play a role in stimulus intensity coding, recognition of the noxious nature of the stimulus and even in pain memory. Except of pain, they are also implicated in several other functions, raising the question if pain processing occurs in specific subregions. In order to map the pattern of activation throughout the mouse posterior IC and SII during pain, we used immunofluorescence to detect cFos expression, a marker of neuronal activity. Mice were divided in two groups, a control (sham operated) and a neuropathic group (spared nerve injury model). Each group was subdivided into two subgroups, one that received pain stimulation and one without stimulation. Expression of cFos was quantified spatially along the rostrocaudal axis (120 μm intervals) and across the cortical layers. This study could provide insight in the roles of the pIC and the SII in normal and pathologic pain.

PP015-Multiple posterior insula – descending pain modulatory system projections might regulate pain perception

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Several lines of evidence support a major role of the insula in pain. The insular cortex is activated by noxious cutaneous stimuli, conversely stimulation of the insular cortex produces pain sensation, while insular cortex damage causes pain deficits. Part of the insular function in pain might be mediated by connections of the insula to the descending pain modulatory system (DPMS). Here we use the Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-based chemogenetic activator hM3Dq expressed in mouse posterior insular cortex pyramidal neurons to investigate the role of the insula in pain. The DREADD agonist DA21 was used to activate the insula during a battery of behavioral tests, including a Diffuse Noxious Inhibitory Control (DNIC) paradigm as an assay of the DPMS function. Our experiments show that insula activation prevents DNIC, thus indicating that insular cortex mediated pain exacerbation involves the DPMS. Anterograde axonal tracing from the posterior insula showed projections to all major brainstem areas involved in the DPMS, the periaqueductal grey, the serotonergic raphe magnus and the adrenergic locus coeruleus and A5 nucleus. These results show taht the insula might engage multiple descending pathways to modulate pain.

PP016-Investigating plasticity in the mouse posterior insular and secondary somatosensory cortex in neuropathic pain

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Pain is an unpleasant sensory and emotional experience associated with or resembling that associated with actual or potential tissue damage. Pain that persists becomes a pathological condition known as chronic pain. Neuropathic pain is a chronic pain type that is usually caused by a lesion or disease of the somatosensory nervous system. Neural plasticity in the brain is involved in the development and maintenance of neuropathic pain. A number of reports have suggested that plastic changes in different brain areas that are responsible for central sensitization and the behavioral alterations in neuropathic pain. The posterior insula (pIC) and the secondary somatosensory cortex (S2) are consistently found activated during pain; We therefore investigate their state of plasticity. We used the spared nerve injury (SNI) neuropathic pain model in the mouse, to test chronic pain mediated modifications in pIC and S2 plasticity. Extracellular electrophysiological recording in acutely isolated coronal brain slices were performed. We found that the NMDA dependent synchronous activity in the pIC and S2 was altered in SNI mice, indicating aberrant plastic changes. In addition, we test long term potentiation induced by theta burst stimulation and a low frequency stimulation protocol to induce long term depression. Further investigations might provide promising information about cellular and molecular underpinnings of pathological pain and could form the basis of improved treatment options.

PP017-The nitric oxide (NO) donor molsidomine attenuates memory impairments induced by the D1/D2 dopaminergic receptor agonist apomorphine in the rat

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Several lines of evidence suggest that scarcity of the gaseous molecule nitric oxide (NO) is associated with the pathogenesis of schizophrenia. Therefore, compounds, such as NO donors, that can normalize NO levels might be of utility for the treatment of this pathology. It has been previously shown that the NO donor molsidomine attenuated schizophrenia-like behavioral deficits caused by glutamate hypofunction in rats. The aim of the current study was to investigate the efficacy of molsidomine and that of the joint administration of this NO donor with sub-effective doses of the non-typical antipsychotics clozapine and risperidone to counteract memory deficits associated with dysregulation of the brain dopaminergic system in rats. Molsidomine (2 and 4 mg/kg) attenuated spatial recognition and emotional memory deficits induced by the mixed dopamine (DA) D1/D2 receptor agonist apomorphine (0.5 mg/kg). Further, the joint administration of sub-effective doses of molsidomine (1 mg/kg) with those of clozapine (0.1 mg/kg) or risperidone (0.03 mg/kg) counteracted non-spatial recognition memory impairments caused by apomorphine. The present findings pro-pose that molsidomine is sensitive to DA dysregulation since it attenuates memory deficits induced by apomorphine. Further, the current findings reinforce the potential of molsidomine as a com-plementary molecule for the treatment of schizophrenia.

PP018-Cholinergic Modulation of Synaptic Transmission in the Dorsal and the Ventral Hippocampus.

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Cholinergic transmission plays a major role in neural information processing supporting several brain functions, including attention, learning - memory and sleep-wake behavior, by activating muscarinic and nicotinic receptors. Cholinergic neurotransmission is fundamentally involved in modulating flow of information in brain neural circuits including the hippocampus which displays a remarkable functional segregation along its longitudinal axis. Aim of this study was to investigate whether and how muscarinic and nicotinic neuromodulation contributes to this segregation. We found that the agonist of muscarinic acetylcholine receptors (mAChRs) carbamoylcholine chloride, suppresses excitatory transmission in the dorsal and ventral CA1 hippocampal synapses similarly. This suppression is controlled by M4 mAChRs (M4Rs). However, activation of type a7 nicotinic acetylcholine receptors (α7nAChRs) by their highly selective agonist PNU 282987 induced a gradually developing increase in field excitatory postsynaptic potential only in the dorsal hippocampus. This long-term potentiation of synaptic transmission was not reversed upon application of nonselective nicotinic receptor antagonist mecamylamine, but the induction of potentiation was prevented by prior blockade of α7nAChRs by their specific antagonist MG624. We also examined the expression of M4Rs and α7nAChRs and we found that they are similarly expressed in the two segments of the hippocampus. We conclude that muscarinic transmission powerfully controls excitatory synaptic transmission along the hippocampus acting through M4Rs. In contrast, α7nAChRs enhance long-term plasticity exclusively in the dorsal hippocampus suggesting that participate in modulating functions that depend on the normal function of this segment of the hippocampus.

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PP019-Examining the Visual Perception of Historical Maps via Eye Movement Analysis

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Among the different types of cartographic products, historical maps play an important role in several domains, including education and research. Hence, the examination of map users' reaction during the observation of historical maps could reveal critical findings about how such products are perceived and interpreted. In cartographic research, several experimental methods are utilized in order to capture and analyze map users' visual behavior and strategies, mainly adopted from the fields of psychology and neuroscience. In the present study, an experimental work in progress is presented. The aim of the study is to examine the visual perception of map users during the observation of historical cartographic stimuli. Specifically, an eye tracking experiment is designed and conducted to record the visual response of 32 participants during the observation of 150 experimental stimuli, under free-viewing conditions. Each experimental stimulus is presented randomly for three seconds, while participants' eye movements are recorded at 1000Hz. Participants' visual behavior is described using typical eye movement metrics based on both fixation and saccade events, as well as techniques that indicate the aggregated visual patterns (statistical grayscale heatmaps). Gaze data analysis highlights the salient locations on the observed stimuli. Although existing saliency models can predict salient locations on natural images, they are not suitable for modeling visual saliency in artificial images (such as maps). Therefore, both raw and analyzed gaze data could be used as an objective ground truth towards modeling the visual perception of historical maps.

PP020-Neural Stem Cells and Platelets: allies or rivals? an investigation of cell viability, apoptosis, mitosis and neurogenesis in co-cultures

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Pools of postnatal brain Neural Stem Cells (NSCs) are clustered in specialized microenvironments called stem cell niches. One such niche is located at the Subependymal Zone (SEZ, or Ventricular - Subventricular Zone, V-SVZ) at the lateral walls of the lateral ventricles. Platelets (PLTs) are oval-shaped fragments that circulate through the Vasculatory System, playing an important role in clot formation. The SEZ is characterized by very specialized vasculature that participates in the regulation of NSC activity (Kazanis et al., 2010). We have previously shown the specific aggregation of PLTs within the vasculature of the niche in response to a focal demyelinating lesion in the adjacent corpus callosum and the pro-survival effects that PLT-derived factors exert on NSCs in vitro (Kazanis et al., 2015). In this project we investigated the effects of the direct co-culture of NSCs with different densities of PLTs, by examining basic cellular functions of NSCs, such as viability, apoptosis, mitosis, maintenance of stemness and induction of neurogenesis. We found that the highest PLTs density led to decreased viability of NSCs, while the percentage of apoptotic cells was not affected. This implies that PLTs affect NSCs when maintained in direct contact, depending on their density. High densities of PLTs are toxic to NSCs, indicating the emergence of necrotic cells death. Additionally, high density of PLTs led to increased mitosis, which may be enhanced by the secretion of platelet-derived factors. Finally, the percentage of Doublecortin+ cells, as well the maturation of neuroblasts (Sox2+ cells) was not affected by the PLTs.

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PP021-Septotemporal variation of information processing in the hippocampus of Fmr1 KO rat

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Fragile X messenger ribonucleoprotein (FMRP) is a protein involved in many neuronal processes in the nervous system including the modulation of synaptic transmission. Loss of FMRP produces the fragile X syndrome (FXS), a neurodevelopmental disorder affecting synaptic and neuronal function and producing cognitive impairments. However, the effects of FXS on short-term processing of synaptic inputs and neuronal outputs in the hippocampus have not yet been sufficiently clarified. Furthermore, it is not known whether dorsal and ventral hippocampus are affected similarly or not in FXS. We used a Fmr1 knock-out (KO) rat model of FXS and recordings of evoked field potentials from the CA1 field of transverse slices from both the dorsal and the ventral hippocampus of adult rats. Following application of a frequency stimulation protocol consisting of a ten-pulse train and recordings of fEPSP, we found that the dorsal but not ventral KO hippocampus shows altered short-term synaptic plasticity. Furthermore, applying the frequency stimulation protocol and recordings of PS, both segments of the KO hippocampus display altered short-term neuronal dynamics. These data suggest that short-term processing of synaptic inputs is affected in the dorsal, not ventral FXS hippocampus, while short-term processing of neuronal output is affected in both segments of the FXS hippocampus in a similar way. These FXS-associated changes may have significant impact on the functions of the dorsal and ventral hippocampus in individuals with FXS.

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PP022-Neuronal dysfunction and toxicity are differentially linked to the aggregation properties of Tau

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Tau is a microtubule-associated protein, known for its involvement in neurodegenerative diseases. Tau aggregates are a hallmark in a range of diseases called Tauopathies, with Alzheimer's disease being the most common one. Mutations in the gene MAPT lead to familial Tauopathies with early age of onset. It is thought that these mutations exacerbate Tau's ability to aggregate, leading consequently, sooner to neurodegeneration. In Drosophila we have shown that toxicity induced by wild-type and mutated Tau is linked to the aggregant properties of the protein. Inversely, we have shown that a non-aggregant form of Tau, while non-toxic can lead to pronounced neuronal dysfunction. Moreover, toxic FTDP-17 mutations induce mild neuronal dysfunction. The results separate two core aspects of Tau-induced phenomena, those of toxicity and of neuronal dysfunction and link them to its aggregant nature. Pairing Tau's distinct properties with specific phenotypes is essential for understanding the pathophysiology of the protein.

PP023-The role of placental CRH in human brain development

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Corticotropin-Releasing Hormone (CRH) was first identified as a neurohormone secreted by the hypothalamus in response to stressful stimuli. However, large amounts of CRH are also secreted by the placenta of anthropoid primates during pregnancy. Although the role of hypothalamic CRH has been extensively studied, there is a remarkable lack of evidence regarding the role of placental CRH, while the biological significance of its unique expression pattern in anthropoid primates remains elusive.
In order to investigate the effects of placental CRH on human brain development and to overcome the limitations raised in experimenting with human tissue, we have generated human 3D-neural spheroids and human cerebral cortical organoids from human embryonic stem cells (hESCs).
Exposure of neural spheroids or/and cortical organoids to CRH results in significant differences in their size and cellular composition. In addition, immunohistological analyses using cortical layer-specific antibodies, revealed differences in the cytoarchitecture of the organoids exposed to CRH as compared to control. Pharmacological disruption of the CRH signaling using the specific CRH receptor 1 antagonist, NBI, reverses the effects of CRH. In addition, RNA sequencing analysis of the CRH and NBI-exposed organoids revealed altered expression of genes related to neurodevelopmental processes such as HOXB9 and FOXG1, depicting CRH as an essential modulator of human brain development.
The key role of CRH in stress physiology and the human-specific pattern of placental CRH expression, suggest that this in vitro approach provides a unique tool for our understanding of the mechanisms underlying the role of stress hormones in human brain physiology.

PP024-Early life maternal neglect alters microglia: Dopaminergic system regulation of microglia response to adversity

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Early life experiences can affect the development of the brain, thus affecting behavior of the adult organism. Even a mild form of neonatal neglect has been shown to be capable of leading to a dysfunctional prefrontal cortex.
We employed a mildly stressful neonatal experience to simulate maternal neglect in rats, the DER model (Denial of Expected Reward through contact with the mother). DER experience leads to low prefrontal cortex (PFC) dopamine (DA) while increased levels of microglia in the medial orbitofrontal cortex (MO). Since microglia express dopamine receptors, we investigated the link between reduced dopamine and increased microglia both by attempting to reverse the effects of "maternal neglect" by administering dopamine agonists in the PFC of DER pups and by pharmacologically mimicking "maternal neglect" by administering dopamine antagonists in the PFC of control pups. During Postnatal days (PND) 10-13, we administered in the PFC of DER pups either a D1 agonist (SKF 38393) or a D2/3 agonist (Quinpirole) and in the PFC of control pups either a D1/5 antagonist (SCH 23390) or a D2/3 antagonist (Raclopride). In all pups, we determined on PND13 the number and arborization extend of microglial cells (Iba-1 immunopositive cells) in the MO.
Based on our results, the effects of "maternal neglect" on microglia can be reversed by activation of D2/3 dopamine receptors in the PFC, but not by activation of D1 dopamine receptors. Conversely, administration of D2/3 receptor antagonists in the prefrontal cortex of control animals mimics the effects of “maternal neglect” on microglial cells.

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PP025-Early life experiences have long-term effects on the oxytocinergic system in the male rat brain

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Early life experiences are associated with alterations in behavioral responses later in life. These behavioral changes have been linked to altered brain neurochemistry. Among other systems, it is well known that early life experiences exert a profound influence on the oxytocin system (OXT/OXTR), a complex network intricately interwoven with social and emotional behavior. Here, we employed a paradigm of neonatal experience in which during a neonatal T- maze training one group of pups is allowed contact with their mother (Receipt of Expected Reward, RER-positive experience), while the other group is denied contact with the mother (Denial of Expected reward, DER-adverse experience). RER, DER as well as control animals underwent the 2-choice social interaction (SI) test during early adulthood, followed by immunohistochemical and epigenetic analyses in the medial amygdala (MeA), hippocampus (HPC) and medial orbital prefrontal cortex (MO). In the SI test, DER rats exhibited reduced social interaction compared to RER and control animals. Immunohistochemical analysis showed a decrease in oxytocin receptor-expressing cells in the MeA of DER animals, an increase in the HPC of RER rats, and no significant difference in the MO between groups. Moreover, epigenetic analysis of the proximal promoter of the oxytocin receptor gene revealed increased methylation in the amygdala of DER animals. In summary, our findings suggest that different early life experiences can distinctly influence adult social behaviors, possibly through changes in the oxytocinergic system.

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PP026-Pharmacological inhibition of ATXN1 protein aggregation

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Spinocerebellar ataxia type 1 (SCA1) is a neurodegenerative disorder resulting from trinucleotide (CAG) repeat expansions within the coding region of the ATXN1 gene. These expansions result in longer polyglutamine (polyQ) chains in the produced ataxin-1 (ATXN1) protein. A hallmark of polyQ-expanded ATXN1 is its propensity to misfold into insoluble inclusions within the nucleus. The formation of polyQ inclusions correlates with selective neurodegeneration, primarily observed in the Purkinje cells of the cerebellum. Despite the fact that the polyQ tract is the main driver of aggregation, experimental evidence indicates that the AXH domain of ATXN1 is crucial for its dimerization and oligomerization.
Here, we employed a computational and experimental pipeline for the identification of chemical compounds which would bind to the AXH domain of ATXN1 and might suppress polyQ protein aggregation. First, we performed an in silico/virtual screening against the AXH domain and identified 42 chemical compounds. These molecules were tested whether they inhibit the dimerization of both AXH domain and full-length ATXN1 in LuTHy assays. Hit compounds were further evaluated for their ability to reduce ATXN1 protein aggregation in a relevant cell model. These experiments indicated a novel compound which effectively suppresses the aggregation of the polyQ-expanded ATXN1 and might be neuroprotective for SCA1.

PP027-Generation and characterization of SCA1 iNSCs from human PBMCs

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Spinocerebellar ataxia type 1 (SCA1) is a neurodegenerative disease which belongs to the group of polyglutamine (polyQ) disorders. It is characterized by progressive degeneration that mainly affects the Purkinje cells of the cerebellum. SCA1 is caused by CAG repeat expansions in the ATXN1 gene and is associated with an abnormally large polyglutamine tract in the encoded ataxin-1 (ATXN1) protein. The mutant protein forms toxic oligomers which slowly aggregate into larger insoluble inclusions within the nucleus. These aggregates correlate with disease progression and the age of the patient. Despite recent advances in SCA1 modeling, a reliable cell model is still missing.
Here, we present the generation of a patient-derived neuronal cell model, partially retaining the aging signature of the donor. To this end, peripheral blood mononuclear cells (PBMCs) from a SCA1 patient were directly trans-differentiated into induced neural stem cells (iNSCs). Reprogramming was implemented using non-integrating Sox-2 and c-Myc Sendai viruses. The generated iNSCs exhibit neural stem cell properties, including the expression of neural markers, self-renewal and differentiation ability into neurons and astrocytes. The pathogenic phenotype of iNSC-derived SCA1 neurons will be assessed using various cell-based assays. We expect that SCA1-iNSCs will be suitable for the in vitro modeling of SCA1, offering a platform for the development of novel therapeutic interventions.

PP028-Analyzing Object- and Grip-Related Information within Population Activity Recorded Across Various Behavioral Tasks in the Anterior Intraparietal Area (AIP) of the Macaque Brain.

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AIP comprises neurons that are engaged in distal hand movements, some of which exhibit robust visual properties. To elucidate the integration of signals related to object characteristics, hand configurations, and motor plans within AIP we* recorded the activity of 140 neurons while the monkeys performed two manipulation and three fixation tasks. In one manipulation task, the monkeys performed grasping actions while observing an online image of their hands and the objects (ML), whereas in the other, no visual input of the object or hand was provided during grasping (MD). The three fixation tasks involved the monkey watching a replay of its own grasping action (FHM), observing an experimenter's grasping action (FHE), and fixating solely on the object (FOB) without engaging in any grasping activity.
To ensure we did not overlook critical aspects of neuronal responses, which might occur with traditional epoch-based analysis, we employed unsupervised phenograph clustering. This method allowed us to categorize the temporal activity profiles of the neural population into three distinct clusters. To overcome potential masking effects regarding the involvement of a brain region in specific tasks that could result from trial averaging, we employed cross-temporal and cross-conditional decoding techniques. These analyses were applied to both the entire neural population and its subpopulations. Through this approach, we revealed the existence of both stable and dynamic neural codes for objects, hand shapes, and motor plans within AIP. These findings provide valuable insights into the encoding and representation of information within the neural ensemble of AIP.

* Maeda K, Ishida H, Nakajima K, Inase M, Murata A. 2015. Functional properties of parietal hand manipulation-related neurons and mirror neurons responding to vision of own hand action. J Cogn Neurosci 27(3):560-72.

PP029-Neurosteroids adjust the translocation of Tau protein to mitochondria

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The main feature of many neurodegenerative diseases is the aggregation of proteins, with a prominent example being the microtubule-associated protein tau. In contrast, neurosteroids are thought to have neuroprotective effects regulating the brain function, also, by rapid non-genomic actions. The present study aims at exploring the effect of allopregnanolone, (allo) and dehydroepiandrosterone, (DHEA) on the levels of total and phosphorylated at the Ser404 residue (pS404) tau, using C57BL/6 mice brain slices. Following tissue fractionation, we found that in the fraction enriched in mitochondria, the levels of both pS404 and total tau were increased while, in the cytosolic fraction total levels appeared to decrease significantly. Accordingly, we investigated the effect of high concentration of calcium ions ([Ca2+]) and, interestingly, the resulting pattern was similar to that of neurosteroids. Assessment of allo or DHEA effect on high [Ca2+] perfused-tissue resulted in a decrease of tau levels in the mitochondrial fraction, showing that neurosteroids exert a regulatory role upon stress. Finally, we investigated the effect of all the aforementioned conditions on two (PP2A, CDK5) of the enzymes that modify the phosphorylation pattern of tau. We found that their levels remain unaffected, supporting that translocation rather than phosphorylation is involved in the regulation of the pS404/total tau ratios in different subcellular compartments.

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PP030-Validation of KLK-6 as a new therapeutic target for Parkinson's Disease

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Alpha-synuclein (AS) is a presynaptic neuronal protein genetically and biochemically implicated in a group of neurodegenerative diseases, collectively termed synucleinopathies. Aberrant forms of AS have been linked to the development of Parkinson's disease (PD) and have been shown to facilitate the transmission of PD pathology to neighboring healthy cells. Consequently, effective clearance of extracellular AS is crucial for slowing down or halting PD. Kallikrein-6 (KLK-6) is a serine protease, abundantly expressed throughout the central nervous system and secreted into the extracellular space. KLK-6 has been implicated in the proteolytic clearance of extracellular AS, although its role in the formation of AS fragments and their effect on toxicity remain unclear. In this project, the role of KLK-6 will be validated as a potential therapeutic target for PD. To this end, recombinant human AS fibrils (PFFs) were produced and injected in the dorsal striatum of adult wild type (wt) mice. Preliminary data indicate that PFFs induce the expected robust AS pathology and that KLK-6-mediated proteolysis of recombinant AS leads to the formation of PFFs with reduced pathogenic capacity in vivo. Moreover, AAV-mediated KLK-6 overexpression along the nigrostriatal axis was optimized via stereotactic injection of different titers of KLK-over expressing AAVs in the ventral midbrain and assessment of the respective toxicity and transduction efficiency. Finally, the effects of KLK-6 on the development of PD-like pathology will be evaluated biochemically and biophysically, following striatal inoculation of adult wt mice with PFFs and synchronous injection of KLK-expressing AAVs in the ipsilateral midbrains.

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PP031-Extracellular vesicles as vehicles for immunotherapy in neurodegenerative disorders

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Synucleinopathies, like Parkinson's Disease (PD), Dementia with Lewy Bodies (DLB) and Multiple System Atrophy (MSA) are characterised by the deposition of a misfolding-prone protein called alpha-synuclein (α-syn). Under these pathologic conditions, misfolded α-syn spreads to healthy neighboring cells, inducing the aggregation of the endogenous protein and thus impairing cellular homeostasis. Immunotherapies aiming to neutralise toxic species of α-syn have been studied for their disease modifying effects. In our study, we developed a passive immunization therapeutic scheme, combining conformation specific antibodies and nanobodies, targeting oligomeric and fibrilar α-syn forms, and exosomes, a subtype of cup-shaped nanosized extracellular vesicles. Exosomes contain a wide range of micro-molecules and are secreted by all cell types, including brain cells, thus serving as mediators of inter-cellular communication. In the current study, we have developed a protocol for the generation of brain-derived exosome-antibody/nanobody complexes, as a means to favour the delivery of the conformation-specific agents to aberrant α-syn-bearing target cells.

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PP032-In vivo microglial Bin1 deletion following LPS stimulation regulates neuroinflammation in the mouse hippocampus and Adult Hippocampal Neurogenesis

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The hippocampal formation and its cortical inputs - crucial for memory formation - are affected early in the development of Late Onset Alzheimer's Disease (LOAD). Adult Hippocampal Neurogenesis (AHN), taking place in the Dentate Gyrus (DG), declines drastically during early stages of LOAD, and correlates to the patients' cognitive status. Numerous Single Nucleotide Polymorphisms (SNPs) linked to LOAD by Genome-Wide Association Studies (GWAS) express isoforms in microglia - the brain's innate immune cells that actively remodel AHN. Notably, SNPs near the Bridging Integrator 1 gene, encoding the BAR protein family member Bin1, have been significantly associated with an elevated risk for LOAD development, surpassed only by Apolipoprotein E. Bin1's microglial isoform is related to the endolysosomal network - a system which is necessary for microglial modulation of AHN. To this end, we generated a double transgenic mouse model (Cx3CR1Cre-ERT2//Bin1fl/fl) which allows the conditional knockout (cKO) of Bin1 in microglial cells. To investigate Bin1 deletion in the murine hippocampus in homeostasis and inflammation, we treated both control and microglial Bin1 cKO mice with lipopolysaccharide (LPS). In hippocampi lacking microglial Bin1, exposure to LPS caused a pronounced upregulation of pro-inflammatory genes and enhanced astrogliosis. Concurrently, microglial Bin1 deletion led to elevated proliferative capacity of DG microglia upon LPS stimulation, and an inherent increase of microglial phagocytic capacity under homeostatic conditions. Finally, the absence of microglial Bin1 in the mouse hippocampus was responsible for alterations in cell populations within the neuronal lineage, most notably a significant rise in the number of neuroblasts.

PP033-Glial alterations in the midbrain of the human neonate after Perinatal Hypoxia- Ischemia

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Perinatal Hypoxia-Ischemia (PHI), playing a crucial role in brain development, is considered a risk factor for the onset of childhood disabilities and/or neuropsychiatric deficits later in life. Postmortem human neonate studies showed a dramatic reduction of tyrosine hydroxylase expression in the substantia Nigra (SN) after prolonged PHI, as well as a significant reduction in cell size indicating delayed development or early degeneration. However, increased expression only of the cytoplasmic Apoptosis-Inducing Factor was revealed, exclusively in the subgroup of PHI neonates with concommitant infection. Since microglia play a key role in neural damage after acute or chronic PHI we applied immunohistochemistry for calcium-binding adapter molecule 1 (Iba1) and transmembrane protein Cd68, considered as markers for microglial activation and phagocytosis, respectively. The quantification of cell number and grey density was performed on digital microscopy and whole-slide imaging data. Activated microglia in neonates with severe/abrupt PHI reveal higher Iba1 positive cell number and grey density. Subjects with very severe/prolonged or chronic PHI with infection comorbidity showed activated Cd68 positive phagocytosing microglia indicating early signs of SN neurodegenerative process in this group. Females expressed more Iba1 positive cell number and grey density than males, suggesting a gender difference in microglia maturation and immune reactivity after PHI insult (Panayotacopoulou, Papageorgiou, Pagida et al., 2022). Since astrocytes influence the phagocytic activity of microglia (Jung and Chung, 2018), we also applied Glial Fibrillary Acidic Protein (GFAP) immunohistochemistry. Detailed quantitative morphometric analysis is in process to reveal possible factors affecting GFAP expression in the perinatal period.
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PP034-Ca2+ signaling dysregulation in human iPSC-derived astrocytes and neurons from Parkinson's disease patients

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Astrocytes play crucial roles in the brain, such as maintaining homeostasis and regulating connectivity of neural circuits, while their dysregulations may contribute in neurodegenerative conditions(1). Parkinson's disease (PD) models suggest that disturbances in neuronal and astrocytic Ca2+ signaling may lead to disruptive neuronal networks providing insights into PD pathology(2,3). Here, we differentiated human induced pluripotent stem cells toward midbrain neurons (iNeurons)(4) and astrocytes (iAstrocytes) to investigate whether the PD-causal autosomal dominant mutation p.A53T in alpha-synuclein (αSyn), may affect the intrinsic Ca2+ activity of astrocytes and co-cultured neurons. Within this scope, after Fluo4-AM labeling, we acquired time-lapse images of live iAstrocytes in monocultures or iNeurons co-cultured with iAstrocytes. Interestingly, the p.A53T-αSyn iAstrocytes displayed spontaneous Ca2+ activity with decreased amplitude and peak latency and increased frequency as compared with healthy iAstrocytes. Proteomic analysis of healthy and p.A53T-αSyn iAstrocytes highlighted several differentially expressed proteins critically involved in important Ca2+-mediated pathways. Furthermore, in a mixed neuron-astrocyte setup, the p.A53T-αSyn iNeurons presented with increased peak amplitude and firing frequency compared with healthy iNeurons, and notably, in the presence of p.A53T-αSyn astrocytes, both types of iNeurons displayed augmented firing frequency. Altogether, our data support that pA53T-αSyn iAstrocytes develop malfunctions in Ca2+ homeostasis that alter their spontaneous Ca2+ activity. Moreover, they contribute to abnormal neuronal Ca2+ firing, in relevance with PD pathology. Next, along with our proteomic data, we will investigate the ER, mitochondria, and plasma membrane of the p.A53T-αSyn iAstrocytes to elucidate the observed Ca2+ disturbances and explore neuron-astrocyte cross-talk to ultimately uncover novel therapeutic targets.

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PP035-In vivo study of the effect of microglial BIN1 deletion in homeostatic and neuroinflammatory conditions

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Genome-Wide Association Studies have identified several Single Nucleotide Polymorphisms (SNPs) strongly associated to increased risk of developing LOAD, many of which are related to microglial activation. SNPs in the locus harboring Bridging Integrator 1 (Bin1) gene show the strongest association with AD, after Apolipoprotein E. BIN1 is an adaptor protein implicated in cell membrane modelling dynamics. Although, its role in neurons has been studied both in vitro and in vivo, the role of BIN1 in microglial activation state and its contribution in LOAD pathology remains to be clarified. To this end we developed a conditional double transgenic Cx3CR1 Cre-ERT2//Bin1 fl/fl mouse, in which BIN1 is knocked-out in microglial cells. Furthermore, we have challenged Bin1 cKO mice with LPS, to investigate the effect of microglia-specific BIN1 deletion under homeostatic and inflammatory conditions.We performed snRNA-Seq in somatosensory cortex in our model to reveal novel targets related to microglial Bin1. Our analysis indicates that a number of signaling pathways regulated by microglia are differently impacted by LPS treatment in Bin1 cKO and control animals. Bin1 deletion resulted in the enrichment of microglial subpopulations exhibiting enhanced proliferative capacity and IFN-type I - mediated inflammatory response after LPS treatment, findings that were confirmed by subsequent real time RT-PCR and immunohistochemical analysis. Moreover, Bin1 deletion in resting microglia was sufficient to elicit transcriptional changes in astrocytes related to the expression levels of other LOAD risk factors.

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PP036-Dissecting the Role of the Autophagy-Lysosome Pathway in Multiple System Atrophy Pathogenesis

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Multiple system atrophy (MSA) is a fatal neurodegenerative disorder characterized by the presence of glial-cytoplasmic inclusions (GCIs), consisting primarily of the neuronal protein α-Synuclein (αSyn) and the oligodendroglial-specific phosphoprotein TPPP/p25α within oligodendrocytes. This abnormal accumulation may result from a failure of the intracellular proteolytic systems, such as the autophagy-lysosome pathway (ALP) thus leading to oligodendroglial degeneration. Up to date the role of autophagy in the context of MSA remains unexplored.
Herein, we are biochemically analyzing the ALP pattern in the context of human MSA, by utilizing multiple brain regions harboring different degrees of GCI pathology, in an attempt to correlate alterations in the levels/activity of key lysosomal markers with αSyn-related pathology. To assess whether ALP alterations may also be present in the periphery of MSA patients, a similar analysis is ongoing using peripheral blood mononuclear cells (PBMCs) derived from MSA patients and age- and sex-matched controls.
Our findings so far demonstrate that alterations in lysosomal indices are present in human MSA brains, coinciding with the detection of aberrant αSyn conformations/species in detergent-insoluble fractions. The LC3-II/-I ratio, widely used to measure macroautophagic flux, was significantly increased in MSA-derived PBMCs, probably suggesting either excessive induction of macroautophagy or impaired formation of autophagosomes in MSA PBMCs. To further elucidate the underlying mechanisms, we are utilizing human MSA or control iPSC-derived oligodendrocytes.
Collectively, this line of research seeks to uncover a possible role of the ALP in regulating αSyn accumulation in the context of MSA and to provide potential therapeutic targets and disease biomarkers.

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PP037-Oligodendroglial-derived Extracellular Vesicles: The Missing Link between Neuronal and Oligodendroglial Interplay

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Multiple system atrophy (MSA) is a rare neurodegenerative disorder, characterized by the formation of proteinaceous inclusions within oligodendrocytes, encapsulating an array of aggregated proteins, notably the neuronal protein α-Synuclein (SNCA, αSyn) and the oligodendroglial phosphoprotein TPPP/p25α. Under baseline conditions, mature oligodendrocytes express low to undetectable αSyn levels, leading to the hypothesis of aberrant intracellular aggregation in MSA-oligodendrocytes. Proposed mechanisms include excessive SNCA gene expression or uptake of extracellular αSyn secreted by neighboring neurons. Emerging evidence suggests a pivotal role of exosomes in αSyn secretion, implicating them in the transmission of αSyn-related pathology.

This study explores the potential impact of exosomes derived from oligodendroglial cells inoculated with human MSA brain-amplified fibrils on the propagation of MSA-related pathology and on neuronal and oligodendroglial function. Towards this direction, exosomes isolated from control or human αSyn- or TPPP/p25α-overexpressing rat oligodendroglial cell lines treated with patient-derived fibrils for 48h (or PBS as control) were applied to murine primary neuronal and oligodendroglial cultures. The protein cargo, size and distribution of these extracellular vesicles were correlated with the manifestation of αSyn-related pathological features within the primary cultures.

Our results indicate that MSA fibril-treated oligodendroglial exosomes operate as carriers of pathological αSyn and TPPP/p25α proteins and are readily taken up by neurons and oligodendrocytes. Their delivery to rodent cortical or oligodendroglial cells triggers the formation of pathology-related αSyn assemblies within recipient cells, without altering cell survival.

These findings underscore the importance of oligodendroglial-derived exosomes on neuronal-oligodendroglial communication and spread of MSA-like pathology, providing potential landscapes for therapeutic interventions.

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PP038-Oligodendroglial-derived Exosomes in the Intersection Between Neurons and Oligodendrocytes

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Accumulation of the neuronal protein alpha-synuclein (αSyn) within proteinaceous aggregates is a hallmark of alpha-Synucleinopathies, such as multiple system atrophy (MSA) and Parkinson's disease (PD). Intriguingly, in MSA the protein aggregates predominantly within oligodendrocytes, forming the glial cytoplasmic inclusions (GCIs). The prevailing hypothesis for this ectopic assemblage is that the protein enters oligodendrocytes from the extracellular space, following its release by neighboring neurons, mediated at least partly by exosomes.

Our aim is to elucidate the contribution of oligodendroglial-derived exosomes in the development and spread of αSyn-related pathology in murine alpha-Synucleinopathy models. To this end, we are utilizing rat oligodendroglial cell lines - stably overexpressing human αSyn or the tubulin-promoting protein TPPP/p25α and control cells expressing undetectable αSyn or p25α levels. These cells are inoculated with human recombinant αSyn preformed fibrils (PFFs) or brain-derived fibrils amplified from PD patients. At selective time points following fibril addition, comprehensive biochemical and immunocytochemical analyses were performed, to assess the intracellular and extracellular αSyn and p25α protein levels and conformations.

Our findings indicate that both αSyn and TPPP/p25α are released via oligodendroglial exosomes and this release is augmented upon hαSyn or TPPP/p25α overexpression. Importantly, exosomes isolated from fibril-treated cells contain aberrant (high molecular weight-aggregated, Ser129-phosphorylated) αSyn species, suggestive of their contribution to cell-to-cell spreading of toxic molecules between brain cells. Ongoing experiments assess the impact of these nanovesicles on primary neuronal and oligodendroglial cultures.

This line of research may potentially offer novel insights into factors that mediate cell-to-cell communication, which may represent potential therapeutic targets.

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PP039-Modeling CNTNAP2 loss of function using human induced Pluripotent Stem Cell-derived cerebral organoids

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Autism spectrum disorder (ASD) is one of the most common neurodevelopmental disorders (NDDs), with an increasing prevalence (1 in 36 children in the US). ASD diagnostic hallmarks include altered social interactions/communication, repetitive behavior and language impairments, accompanied by severe comorbidities such as epilepsy, sleep and feeding disorders. ASD is a multifactorial disorder, caused by genetic, epigenetic and environmental factors. Contactin associated protein 2 is encoded by the CNTNAP2 gene and has a strong association to ASD and other NDDs such as schizophrenia, epilepsy and intellectual disability. CNTNAP2 mutations were first reported in Cortical Dysplasia-Focal Epilepsy (CDFE) syndrome, a syndromic form of ASD. Since then a plethora of mutations in CNTNAP2 were shown. A convergent pathway perturbed in ASD and other NDDs is the mammalian/mechanistic target of rapamycin (mTOR) signaling pathway. Increased mTOR activity was reported in ASD/NDD patients and rodent models of ASD, including Cntnap2 KO mice. We hypothesized that CNTNAP2 ablation alters early brain development, through dysregulation of mTOR signaling pathway. We generated human cerebral organoids from CNTNAP2 knockout (KO) iPSCs. We found that KO cerebral organoids exhibit pronounced morphological and cellular deficits after 30 days (D30) in culture. Proteomics analysis in D30 and D60 revealed differential expression of several ASD risk genes in CNTNAP2 KO organoids. Furthermore, CNTNAP2 KO cerebral organoids recapitulate dysregulated mTOR signaling. Taken together this work provides us with an in vitro tool of human origin to develop new therapies based on deep mechanistic understanding.

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PP040-Unveiling the Role of PLPPR3 in neuronal LPA Signaling and Morphological Dynamics

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Phospholipid-phosphatase-related proteins (PLPPRs) are a five-member family of neuron-enriched, developmentally regulated membrane proteins that control glutamatergic synapses, filopodia and branch formation, as well as growth cone navigation. Despite sharing homology and topology with Lipid Phosphate Phosphatases, they lack Lysophosphatidic Acid (LPA) phosphatase activity. Previous studies have suggested the involvement of PLPPR4 in neuronal LPA signaling via an LPA transporter or scavenger function, but the role of other PLPPRs, including PLPPR3, a close PLPPR4 relative, in LPA signaling, remains elusive.
The aim of present work is to study the molecular interactions of PLPPR3 and LPA and how this interaction could affect LPA-induced morphological effects in neurons.
We investigated the physical interactions between 18:1 LPA and PLPPR3 utilizing Microscale Thermophoresis and we assessed the extent of LPA uptake in different cell lines overexpressing PLPPRs through Flow Cytometry. Finally, we evaluated the effect of LPA treatment on neuronal morphology and axonal growth in primary hippocampal WT and PLPPR3 KO neurons in different developmental stages and substrates.
Our results suggest multiple physical interactions between 18:1 LPA and PLPPR3. Furthermore, while PLPPR4 increases LPA uptake of cells, PLPPR3 exhibits biphasic effects dependent on concentration, time, and cell type. Importantly, WT and PLPPR3 KO neuronal response to LPA, is substrate and developmental stage-dependent. Collectively, our results suggest that PLPPR3 functions by enabling or fine-tuning LPA-responses in neurons potentially mediated via direct interactions with LPA.

PP041-Performance in detecting approaching objects in a simulation of thalamic artificial vision

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Simulating artificial vision and psychophysical tasks performed by normal-sighted humans are useful for predicting the experience of seeing through visual prostheses. While previous work has explored several daily activities (reading[1], watching television[2], face recognition[3], object recognition, manipulation[4,5]), those tasks might not reflect the demands of navigating in the three-dimensional world, such as detecting impending collision with an approaching object[6]. To approximate such a real-world experience, we simulated the visual angle a car on a collision course subtends, and asked subjects to press a button when they detected the approaching motion of a car-sized disk. We varied the disk's speed (15, 30, 45, 60 km/h), its starting distance (10, 20, 30, 40, 50, 60 m) and the prosthetic device's resolution (125, 250, 500, 1000 phosphenes, Natural view). Object distance at the time of response exhibited a saturating limit that depended on the phosphene resolution and not on the object's initial distance or speed, once a given reaction time was considered. Thus, approaching motion was perceived only after the disk had crossed a distance threshold: as the phosphene resolution increased, the distance of the object at the time of response increased as well, with the behavior for higher phosphene resolution resembling that for the Natural view. Our findings suggest that -depending on the device's resolution- only within a proximity range would the recipients of a visual prosthesis be able to safely respond to an approaching object, reflecting a limitation for daily life in settings of more embodied nature that importantly, require action.

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PP042-A miR-124-mediated transcriptional and post-transcriptional mechanism controlling the cell fate switch of astrocytes to induced-neurons

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Direct astrocytic reprogramming holds therapeutic promise for the amelioration of neuronal loss during neurodegeneration or brain trauma. For this, many neurogenic factors, namely transcription factors, miRNAs or chemical compounds have been employed for the direct conversion of astrocytes to induced-neurons (iNs) in various in vitro and in vivo approaches. The miRNA miR-124 has been employed in several reprogramming strategies, however its independent mechanism of action remains largely unexplored. Here we show that miR-124 drives the reprogramming switch of mouse cortical astrocytes towards an immature neuronal fate by repressing many astrocytic regulatory genes and upregulating gene signatures characteristic of cortical intermediate progenitors and cortical layer neurons. Identification of miR-124 direct targets in our analysis using publicly available Ago HITS-CLIP data from mouse cortex, revealed the RNA-binding protein Zfp36l1 – implicated in ARE-mediated mRNA decay – as a prominent target. Further experiments highlighted the importance of miR-124:Zfp36l1 interaction during the neurogenic conversion. To this end miR-124 action recapitulates endogenous neurogenesis pathways, being further enhanced upon addition of the neurogenic compound ISX9, which greatly improves iNs' maturation. Additionally, gene regulatory network analysis revealed the major TFs that dictate the reprogramming process. Importantly, AAV-mediated overexpression of miR-124 in a mouse model of cortical trauma pointed towards the competence of miR-124 in directly converting reactive astrocytes to immature iNs, while ISX9 supplementation conferred a survival advantage to iNs, without reinforcing their maturation. However, certain downstream effectors of miR-124 and ISX9 identified here could amplify their combined in vivo reprogramming action and enhance iNs' maturation.

PP043-Mitochondrial function in Neurofibromatosis type 1 models

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Neurofibromatosis type 1 (Nf1) is a prevalent autosomal dominant disorder that affects individuals across various population groups, occurring at a rate of 1 in 3500 people. Nf1 results from mutations in the Neurofibromin gene. The disease's manifestations are predominantly localized in the skin, eyes, bones and nervous system. Cognitive abnormalities and specific impairments in learning and memory are associated with this condition. Nf1 models exhibit increased activation of the Ras pathway, as Neurofibromin typically maintains Ras within normal levels. Furthermore, alterations in the levels of different metabolites are evident in type 1 Neurofibromatosis models. This research employs the fruit fly Drosophila melanogaster as the experimental model. This study examines point mutations C1045Y (E4) and R1809C, as well as the null mutation E2 of Neurofibromin (dNf1). In Nf1 flies, reduced body size, impaired circadian rhythms, learning and memory deficits and decreased lifespan are observable. The primary objective of this specific study is to assess mitochondrial function in Neurofibromatosis type 1 flies, given that the absence of Neurofibromin has been demonstrated to impact metabolism. This study involves the evaluation of mitochondrial respiration in the aforementioned Nf1 models. Additionally, levels of mitochondrial proteins were determined using proteomic analysis, Western blot analysis and confocal microscopy.

Poster Presentations (PP) - Poster session #2

Saturday – 25 NOVEMBER 2023

PP044-Computational modeling and conformational accuracy of wild type and mutated protein variants in Alzheimer's Disease

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Predicting the three-dimensional structure of proteins directly from their amino acid sequence remains a challenge in biomedical research. Recent advances in AI-power algorithms provide a transformative effect in solving this problem. AlphaFold, a deep learning tool that produced groundbreaking results in CASP14, has since been used for highly accurate structural predictions even when there are very little to no known homologous structures. In this study, a set of proteins and mutated protein variants that are ranked pathogenic for Alzheimer's disease was performed and their structural simulations were evaluated using the ColabFold implementation of AlphaFold which utilizes MMseqs2 algorithm to quickly figure multiple sequence alignments. Our findings suggest that potent conformations could be accurately modeled at a molecular level in their wild-type form while compared regions were correctly folded and the estimated metrics were almost exclusively in the "high accuracy prediction" range. Our study also demonstrated that only a few residues with sufficiently strong destabilization effects can exert detectable structural (backbone) deviation from the wild-type model.

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Acknowledgements

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PP045-Comparative analysis of telencephalic Tle4/Grg4 expression between domestic cat and mouse embryos reflects on a common repression pattern, with subtle differences

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Tle4 is a member of the Groucho family (known as TLE in human or Grg in mouse); Groucho (Gro), the family prototype, encodes a co-repressor that was first identified in Drosophila melanogaster in 1968. Gro co-repressors are involved in several signaling pathways, including BMP, Wnt and Notch and mediate repression either directly, through interaction with transcription factors, or indirectly, through histone acetylation or chromatin modifications. Previous studies of Grg4 expression in the embryonic murine telencephalon, have revealed a dynamic spatiotemporal pattern; functional analysis suggested a role in cell migration mechanisms and in the temporal regulation of neuronal specification. In this work, we have analyzed the spatiotemporal pattern of the Tle4 expression in the embryonic feline telencephalon at E26/27 and E24/25 using in situ hybridization and a battery of subpalial (Lhx6, Lhx7/8, Dlx2, Nkx2-1, Ascl1, Er81) or pallial (Pax6, Emx1, Lhx2, Tbr1, Tbr2) markers. Moreover, the expression pattern of the feline Tle4 was compared to that of Grg4 in E13.5 mouse embryos. Tle4 exhibited a complex expression pattern, overall conserved to that of the mouse, under the transcriptional control of Nkx2-1 and further involved in the regulation of migration of distinct progenitor populations, from the diagonal domain to the basal magnocellular complex and the globus pallidus primordia. Despite the similarities reflecting highly conserved patterning and regional specification mechanisms, careful comparison between the expression patterns of the feline and the murine homologs, revealed subtle differences, possibly associated with the evolutionary emergence of the more elaborate gyrencephalic feline brain.

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PP046-Expression of Neurod1 and Neurod6 in the embryonic pallium of the domestic cat reveals conserved spatiotemporal neurogenic gradients.

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Basic helix-loop-helix (bHLH) transcription factors Neurod1 and Neurod6 belong to the Neurod family of bHLH neuronal differentiation factors, that bear similarity with the atonal group of Drosophila melanogaster and play pivotal roles in the regulation of differentiation, migration and maturation of the glutamatergic neurons of the embryonic pallium. Neurod1 and Neurod6 present distinct, yet highly overlapping expression patterns in the developing telencephalon of the mouse. Neurod1 is expressed in the subventricular (SVZ) and the adjacent intermediate (IZ) zone of the pallium, regulating the differentiation of intermediate progenitors to postmitotic neurons, downstream of Tbr2 and upstream of Tbr1. Neurod6 is expressed by postmitotic pyramidal neurons, all along their migration to the cortical plate (CP), with a role in mitochondrial biogenesis, axonal growth and navigation. In this work, the expression patterns of Neurod1 and Neurod6 were studied in various stages of the developing feline pallium. To this end, in situ hybridization was performed using riboprobes for the aforementioned genes, as well as for well-established markers Tbr2, for the SVZ-residing, cycling progenitors, and Tbr1 for the postmitotic cells of the CP. Our results indicate an overall-conserved expression pattern for both Neurod1 and Neurod6 reflecting on common mechanisms underlying neurogenesis. Neurod1 was detected mainly in the SVZ and the CP, while Neurod6-expressing cells were found in the mantle and the SVZ, but most prominently in the CP. Finally, we observed spatial and temporal expression gradients, confirming the gradual progression of neurogenesis across the distinct sectors of the feline pallium, as defined for the mouse.

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PP047-Increased ability of induction LTP through β-adrenergic receptors in the dorsal compared with ventral hippocampus

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Noradrenaline (NA) strongly modulates hippocampal activity acting mainly through β adrenergic receptors (β-ARs). Here we aimed to investigate whether noradrenergic activation can differently induce LTP of synaptic transmission and neuronal activity in the dorsal and the ventral hippocampus without high-frequency stimulation. Using slices from dorsal and ventral hippocampus and recordings of evoked field excitatory potentials (fEPSP) and population spikes (PS), we found that application of either 10 μM NA or β-adrenergic agonist isoproterenol (ISO, 10 μM) produced LTP in synaptic effectiveness (fEPSP) or neuronal excitability (PS/fEPSP) in both segments of hippocampus. The effects of both NA and ISO on LTP of PS/fEPSP were greater in the dorsal than the ventral hippocampus and greater under ISO than NA. Although the magnitude of LTP of synaptic transmission induced by NA was greater in the ventral hippocampus, this was higher in the dorsal when induced by ISO. ISO-induced LTP in either fEPSP or PS/fEPSP could not be reversed following blockade of β-ARs by propranolol or after specific blockade of β1 or β2 adrenergic receptors by CGP 20712 or ICI 118,551, respectively. However, blockade of β1or β2 adrenergic receptors by CGP 20712 or ICI 118,551 prior to applying NA showed that both adrenergic receptors contribute to induction of LTP in both segments. These results indicate that relatively mild β-AR activation leads to a similar LTP of neuronal excitability in both hippocampal segments. In contrast, intense activation of β-ARs leads to an increased long-lasting enhancement of neuronal excitability more in dorsal than in ventral hippocampus.

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PP048-The role of a5 subunit of nicotinic receptors and the effect of environmental enrichment on the emission of ultrasonic vocalizations (USVs) in normal (WT) and transgenic (ACNA5) mice

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Ultrasonic vocalizations (USVs) are a crucial form of communication among mice, reflecting their emotional states and motivations [1]. They serve as a valuable tool for studying the influence of environmental conditions and genetic deficits on animal behavior and physiology. This, in turn, aids in gaining a better understanding of the foundations of neurodevelopmental and psychiatric disorders. In our current project, we examined the combined effects of environmental enrichment and α5-nAChR deletion on mouse vocalizations.

Prior research has demonstrated that enriching animal habitats improves their cognitive abilities, reduces anxiety levels, and alters their social interactions [2]. Additionally, nicotinic receptors (nAChRs) play a role in various cognitive functions, and their dysregulation is linked to addiction and schizophrenia pathophysiology [3,4].

We utilized a 2X2 experimental design, housing adult male WT mice and ACNA5 mice (mice lacking the α5 subunit of nAChR) in either standard or enriched environments. Pairs of genetically identical but unfamiliar mice were tested in a novel arena while their USVs were continuously recorded and later analyzed.

Our results showed differences in USV duration and various amplitude and frequency parameters between WT and ACNA5 mice, demonstrating that α5-nAChR deletion influences the way mice perceive communication. Moreover, housing type also affected USVs. Enrichment appeared to counteract the negative impact of the lack of the α5 subunit on USV duration.

These findings underscore the influence of α5-nAChR absence on vocalization emissions and the beneficial effects of enriched environmental conditions on both WT and ACNA5 mice.

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PP049-Facial emotion recognitionin Thatcherized and non-Thatcherized faces across four schizotypal dimensions

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Aims:To examine Thatcherization and inversion effects in facial emotion recognition (FER) across groups with high schizotypal personality traits, as assessed with the four-factor model of schizotypy. Methods:238 participants were allocated into a control or one of four schizotypal groups according to their factor scores in the Schizotypal Personality Questionnaire and were administered two FER tasks (Thacherized and non-Thatcherized faces) including faces presented in upright or inverted position. Between-group comparisons were examined with repeated measures ANCOVA. Significant group main effects were followed up with Bonferroni post-hoc analyses, and significant valence x group interactions with ANCOVAs and Bonferroni post-hoc tests. Results:Disorganized schizotypes scored lower compared with controls in all valences in the upright presentation of the non-Thatcherized faces, in surprise recognition of inverted faces in both tasks, and anger recognition in the upright presentation of Thatcherized faces compared with cognitive-perceptual schizotypes (all p values <0.05). Negative schizotypes scored lower compared with all other groups in sadness recognition in the inverted presentation of the non-Thatcherized faces and in both presentations of the Thatcherized faces (all p values <0.05). Conclusions:Disorganized schizotypes had a generalized impairment in FER in the upright presentation of the non-Thatcherized faces, which becomes specific to surprise after inversion and/or Thatcherization effects. A potential explanation of the finding is that it reflects trait-specific processes. Negative schizotypes had poor configural processing of sadness, affected by inversion and Thatcherization. The finding follows previous studies revealing patterns of social stimuli avoidance when they are associated with undesirable emotions in negative schizotypes.

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PP050-Circuits and molecules that govern habituation to repeated footshocks in Drosophila.

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Habituation is a conserved adaptive process essential for incoming information assessment, which drives behavioral response decrement to recurrent inconsequential stimuli and does not involve sensory adaptation, or fatigue. We have established a number of habituation protocols to persistent odor stimulation and recurrent mild electric footshocks for Drosophila. Herein we report on the role of distinct mushroom body neurons to the latency to habituate and habituation to recurrent footshocks. To understand the molecular mechanisms that govern the transitions among the distinct habituation phases, we have performed genetic screens for mutants presenting shortened or prolonged habituation latency and we report on molecular pathways engaged within mushroom body neurons and are essential for progression through the proposed phases of habituation to footshocks.

PP051-A neuronal circuit approach to Dishabituation Mechanisms in Drosophila melanogaster

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The Nervous System orchestrates body functions, processing sensory input, and enabling adaptive responses. Neuronal circuits universally recognize novel stimuli, but unpredicted features in a stimulus amplify its importance, shaping attentive reactions. Habituation, the reduction of responses to repetitive stimuli, and its counterpart, dishabituation, the reversal of habituated responses upon exposure to novel stimuli, are fundamental processes that organisms employ to navigate their environment efficiently. As the communication within neuronal circuits is an exciting emerging field and the mechanisms governing the exquisitely orchestrated mechanisms driving Habituation/Dishabituation remain partly understood, we used Drosophila melanogaster to explore them. We use the Drosophila because it is a prime model allowing sophisticated behavioral studies through the manipulation of neuronal subsets driving behaviors, to explore the circuitry and conditions that two different stimuli, another odor and footshocks, bypass or reset osmotaxis after olfactory habituation. As the molecular mechanisms of habituation appear to be disrupted in individuals with Schizophrenia (SD), understanding the neural circuits and mechanisms driving these phenomena is expected to contribute to our understanding of essential behavioral plasticity and its potential implications in psychiatric disorders.

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PP052-Sex Differences in Oxycodone's Neurochemical and Behavioral effects in rats.

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Oxycodone is considered to be the most commonly abused prescription opioid over the last decade, with women being disproportionally affected by opioid use disorder (OUD) in comparison to men. To further investigate sex-differences in oxycodone's effects, we used in vivo microdialysis coupled with LC-MS/MS analysis to measure oxycodone induced dopamine levels in brain regions associated with reward and Intracranial Self Stimulation to delineate the reinforcing effects of the drug in female and male rats. In the nAcc shell of female rats more dopamine was present compared to males, after oxycodone was administered. Moreover, preliminary data indicate that acute administration of oxycodone decreases the ICSS threshold in some female rats while in other female rats it causes severe sedation. To the contrary in all male rats tested there was no significant alteration in ICSS threshold. Overall, our findings indicate distinct sex differences in the neurochemical and behavioral properties of oxycodone in rats.

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PP053-Effects of the novel synthetic cannabinoids AM1710 and AM6527 on brain stimulation reward and the reward-facilitating effects of cocaine

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Activation or blockade of the endocannabinoid system has been shown to exert complex effects on reward mechanisms and modulate the rewarding effects of non-cannabinoid psychotropic drugs. Here, we evaluated the effects of two novel synthetic cannabinoids, the CB2 receptor agonist AM1710 and the neutral CB1 receptor antagonist AM6527, on brain stimulation reward and on the reward-facilitating effects of cocaine using the intracranial self-stimulation (ICSS) paradigm.
Male Sprague-Dawley rats were trained to self-stimulate using a rate-frequency paradigm. In the first experiment, rats were injected with AM1710 (0, 0.32, 1.0 and 3.2mg/kg, i.p.) or AM6527 (0, 0.1, 0.3 and 1.0mg/kg, i.p.) and their effects on ICSS were determined. In the second experiment, we examined whether an acute injection of AM1710 (1.0mg/kg, i.p.) and AM6527 (1.0mg/kg, i.p.) could counteract the reward-facilitating effects of cocaine (5 mg/kg, i.p.).
The highest doses of AM1710 (3.2mg/kg) and AM6527 (0.3 and 1.0mg/kg) significantly increased ICSS threshold. All other doses of the tested cannabinoid drugs did not affect ICSS thresholds or the asymptotic rate of responding. Cocaine significantly reduced ICSS threshold, without altering maximal rates of responding. The CB2 receptor agonist AM1710 (1.0mg/kg), but not the neutral CB1 receptor antagonist AM6527 (1.0mg/kg), partially reversed this action of cocaine.
The present data indicate that both AM1710 and AM6527 do not exhibit reinforcing properties in the ICSS paradigm. Importantly, CB2 receptor agonists, such as AM1710, may play a modulatory role in cocaine's reinforcing actions and, thus, may represent potential therapeutic targets for the treatment of cocaine addiction.

PP054-Combining a pharmacophore and an artificial intelligence approach to predict ORco ligands antagonizing mosquito odorant receptor function

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Insect odor receptors are heterotetrameric ligand-gated cation channels composed of a highly conserved receptor subunit, ORco, and one of many variable subunits, ORx, in as yet undefined molar ratios. When expressed alone ex vivo, ORco forms homotetrameric channels gated by ORco-specific agonists. Based on our previous studies, we developed a ligand-based pharmacophore describing the 3D arrangement of orthosteric antagonist features necessary for blocking ORco's biological response. The pharmacophore model was required to match all orthosteric antagonists we identified, while keeping the number of false positives at a minimum. Four features met these requirements best, two hydrophobic centroids, one hydrogen bond acceptor and one hydrophobic atom. On a collection of 49 volatile organic compounds (VOCs) that was computationally and functionally screened, the pharmacophore's sensitivity was 86%, while its specificity was 57%.
To enhance the pharmacophore's performance, we established a set of 2D descriptors for all pharmacophore hits and generated a support vector machine (SVM) capable of discriminating between true (ex vivo confirmed) orthosteric antagonists from false positive hits. The best SVM model, trained on a collection of 104 functionally-characterized compounds, included a topological descriptor encoding the branching of molecules and one reflecting the extend of hydrophobic or hydrophilic effects on the surface area of molecules.
The validity of the two-step in silico protocol was tested, with encouraging results, on a third collection of natural compounds using as validation tools ex vivo activity and in vivo repellence assays. A parallel approach is currently in progress for the prediction of ORco allosteric antagonists.

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PP055-Revealing the neurogenic properties of p75 neurotrophin receptor in mouse and human Neural Stem Cells, pharmacologically targeting Alzheimer's Disease.

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The pan-neurotrophin p75 receptor (p75NTR) is a member of the TNF death receptor superfamily, expressed in adult neural stem cells (aNSCs). Its remarkable upregulation during neurodegeneration and its controversial signaling, ranging from survival to cell death, have attracted a special interest on this receptor. Several studies have implicated p75NTR in Alzheimer's Disease (AD), by demonstrating its ability to serve as a mediator of Amyloid-β in neuronal degeneration and in diminished adult hippocampal neurogenesis, although the exact mechanisms remain poorly understood, especially in human specimens. Our study focuses on revealing the p75NTR functions, by examining adult neurogenesis levels on p75NTR knock-out mice crossed to 5xFAD mouse model in order to identify the aNSCs proliferation and survival. p75KO mice exhibit decreased NSCs proliferation as indicated by the number of Brdu+/Sox2+ cells and attenuated neuronal differentiation in the hippocampal Dentate Gyrus (DG) suggesting key neurogenic properties of p75NTR. In addition, we generated NSCs from human iPSCs, derived from healthy individuals, examining changes in expression level and activity of p75NTR signaling using co-immunoprecipitation and blotting analyses. Our results show p75NTR signaling-dependent regulation of survival in the presence of Amyloid-β peptides. In summary, our research suggests receptor's necessity for intact neurogenesis and demonstrates for the first time the expression of p75NTR in human NSCs and its involvement in AD pathology. Deciphering the specific actions of p75NTR on aNSCs' properties, we aim to reinforce endogenous ability of neurogenesis and thus strengthening repairing capacity against AD-induced neuronal loss.
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 Act "Enhancing Human Resources Research Potential by undertaking a Doctoral Research" Sub-action 2: IKY Scholarship Programme for PhD candidates in the Greek Universities». Financed also by SoftReach (EIC Pathfinder-EISMEA) and HFRI.

PP056-Cell-Based Assay for Alpha-4 Nicotinic Receptor Antibodies in Autoimmune Encephalitis Syndromes

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Aims: Autoimmune encephalitis syndromes (AES) comprise a group of disorders where the host immune system attacks self-antigens expressed in the central nervous system (CNS). Antibodies to membrane receptors, like NMDAR1, have been identified in AES, but many AES patients have yet unidentified autoantibodies. Neuronal nicotinic acetylcholine receptors (nAChRs) are abundant in the CNS playing critical roles in brain function, thus making them candidate autoantigens in AES. This study aimed the improvement of a cell-based assay (CBA) that detects the potentially pathogenic antibodies to the α4β2-nAChR subtype, based on our α3β2-nAChR CBA2, and its use for the identification of such antibodies in “orphan” AES cases.
Methods: The study involved the screening of sera from 1752 patients from Greece, Turkey and Italy, who requested testing for AES, and from 1203 “controls” with other neuropsychiatric diseases from the same countries or from Germany. A live CBA with α4β2-nAChR–transfected cells was developed to detect antibodies against the cell-exposed α4β2-nAChR and positive samples were confirmed quantitatively with flow cytometry.
Results: Three patients were found positive by CBA and FACS. No serum bound to control cells, and no control serum was positive by the transfected cells. The clinical characteristics of the 3 nAChR-antibody-positive patients fall into the AES spectrum. Specifically, one patient had Rasmussen encephalitis while another one had meningoencephalomyelitis.
Conclusions: Using live CBA we detected serum antibodies against the α4-nAChR in patients with AES. Future studies should be focused on larger AES cohorts to characterize the clinical phenotype and the role of those antibodies.

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PP057-Unraveling the role of TGF-β superfamily signaling in the pathogenesis of α-Synucleinopathies

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Extensive evidence highlights the regulatory role of the Transforming Growth Factor–β (TGF-β) superfamily in the central nervous system pathophysiology. Ligands of this superfamily, including TGF-βs, Activins, and Bone Morphogenetic Proteins (BMPs) modulate key events during brain development and brain tissue injury repair. The transcriptional effects of TGF-β signaling are mainly mediated by members of the Smad protein family, which, upon binding to TGF-β receptors, accumulate to the nucleus to regulate gene expression. Although, dysregulation of the TGF-β pathway has been linked to various diseases, including cancer, fibrosis, and autoimmune disorders, little is known about its connection to a-Synucleinopathies, such as Parkinson's disease and multiple system atrophy (MSA).
Herein, in order to clarify the role of TGF-β/Activin and BMP signaling pathways in the context of alpha-Synuclein (aSyn)-related pathology, we utilized rat oligodendroglial cell lines treated with recombinant human pre-formed aSyn fibrils (PFFs) or PD patient brain-derived fibrils as pathological seeds. Our results revealed differential phosphorylation patterns of the effector Smad proteins, and thus differential activation of the TGF-β pathway. In addition, intrastriatal injections of PFFs or PD fibrils in transgenic mice carrying fluorescent reporters of TGF-β/Activin (TRE-RFP) and BMP (BRE-eGFP) signaling, allowed the visualization of the response of both branches of the TGF-β pathway under pathological conditions and provided new insights about its potential role in a-Synucleinopathies.
Overall, we anticipate that our study will contribute to a deeper understanding of the role of the TGF-β superfamily signaling in the context of a-Synucleinopathies and facilitate the exploration of innovative therapeutic approaches.

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PP058-Chronic stress disrupts dentate gyrus plasticity in Tau pathology affecting complex neuron-microglia interplay

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Chronic stress and inflammation are increasingly recognized to be involved in the precipitation of Alzheimer's disease (AD). One of the primary brain areas affected in AD is the hippocampus with the dentate gyrus (DG), the input subarea of the hippocampus, being the only one that combines newly-born neurons, pre-existing neurons, and microglia. However, the exact role of chronic stress and glucocorticoid receptor (GR) signaling in stress-driven DG damage of AD brain remains unclarified. Our study aims to unravel the interplay of chronic stress and the aforementioned DG cell types and how this interplay precipitates DG dysfunction in Tau pathology, using P301L-Tau transgenic mice with specific deletion of GR in forebrain neurons or microglia. We found that chronic stress suppresses neurogenesis causing a decrease of newly-born neurons and this effect isn't altered by microglial GR deletion, whereas stress-induced spine loss in pre-existing neurons is blocked in microglial GR-depleted mice. In addition, stress-driven microglial hyper-ramification is blocked by microglial GR deletion in the DG hippocampal area, whereas these microglia plasticity changes also depend on neuronal GR signaling. Together, these findings provide novel insights into the impact of chronic stress on the complex neuron-microglia interplay that damages DG plasticity in Tau-related AD pathology.

PP059-Mirk/Dyrk1B kinase is involved in neuroinflammation in SOD1G93A mice

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Dyrk1B regulates via Sonic Hedgehog (Shh) pathway the generation and survival of motor neurons (MNs) and V2A interneurons, as well as lateral-medial motor columns (LMCm) that innervate ventrally the muscles of limbs. Dyrk1B suppresses Shh/Gli pathway, while its specific inhibitor, AZ191 increases conversely Shh/Gli signaling, resulting in increased populations of ventral progenitors and MNs. Amyotrophic lateral sclerosis (ALS) is characterized by selective loss of LMC MNs. Notably, MNs and V2A are firstly affected in the spinal cord of SOD1G93A mice. Shh has been reported to be down-regulated in ALS, while its cytoprotective role in ALS has been previously demonstrated. Here, we investigate the role of Dyrk1B in MN disease by using SOD1G93A mice. We developed a primary cell culture protocol for E12.5 spinal MNs, that yields 90% Tuj1+/Islet1/2+ MNs, including all MNs subtypes. Addition of 1μM AZ191, resulted in Tuj1+/Islet1/2+ MNs with increased axonal length and number of branching, suggesting the neurotrophic effect of AZ191. Moreover, E12.5 SOD1G93A Tuj1+/Islet1/2+ MNs exhibit shorter and fragmented axons indicating an early axonopathy. Dyrk1B expression was investigated in P30 and P140 SOD1G93A spinal cord. In wt spinal cord, Dyrk1B is mainly expressed by neurons. Only few astrocytes and microglia express in their nucleus the Dyrk1B kinase. Similarly, at P30 SOD1G93A spinal cord only few GFAP+ and Iba1+cells express Dyrk1B. In contrast, at P140 SOD1G93A spinal cord, where neuroinflammation is intense, GFAP+/Dyrk1B+ and Iba1+/Dyrk1B+ cells are increased by 3.84-fold and 8.45-fold respectively, suggesting a potential involvement of Dyrk1B kinase in neuroinflammation in SOD1G93A spinal cord.

PP060-Dissecting the cell-specific contribution in the precipitating role of chronic stress on Tau pathology

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Accumulating clinical and experimental evidence supports the detrimental impact of lifetime stress and microglia-driven inflammation in the precipitation and progression of Alzheimer's disease (AD) brain pathology. Experimental studies have shown that exposure to chronic stress and high levels of stress hormone glucocorticoid (GC) trigger the accumulation of pathological forms of Tau leading to downstream neuronal atrophy and memory deficits. Despite that chronic stress impacts both neuronal and microglial populations and triggers inflammatory responses in the brain, the exact stress-driven signaling on neurons and microglia and the individual cell-specific contribution in the precipitation of Tau pathology under stressful conditions remains unexplored. Hereby, we use a novel approach of attenuating the stress signaling in either brain neurons or microglia via conditional deletion of the GC receptors (GR) in the brain of P301LTau Tg mice. Our findings demonstrate that conditional deletion of GR in neurons blocked the precipitating role of chronic stress on Tau neuropathology, synaptic loss, and related memory impairment in P301LTau mice while similar deletion of GR in microglia reduced, but not completely abolished, the detrimental effect of stress on Tau pathology. The above data support the mediating role of GC and their neuronal signaling in the stress-driven Tau neuropathology while identifying the essential contribution of microglia, through their GR signaling, in the orchestration of stress-driven mechanism(s) that precipitate(s) Tau pathology in the AD brain. As modern life exposes individuals to high stress, understanding the links between chronic stress and Tau pathology is crucial for advancing AD treatment.

PP061-Differential sleep-like deficits of Neurofibromatosis 1 mutations in Drosophila melanogaster.

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Neurofibromatosis 1 (NF1) is a hereditary autosomal dominant disorder affecting 1 in 3500 individuals. It presents with skin abnormalities, Lisch nodules, and neurofibromas. Patients often exhibit macrocephaly, short stature, learning disabilities, and attention deficits. Children and adults with NF1 are also at increased risk for sleep disturbances. The human NF1 gene (17q11.2) encodes neurofibromin, a 2818-amino-acid protein expressed in various nervous system cells throughout life. Nf1 best-understood and evolutionarily conserved role is as a GTPase Activating Protein (GAP) for Ras, which reduces Ras biological activity. The GRD domain comprises about 10% of the protein (229 amino acids), while the functions of other protein regions remain largely unidentified. Mutations outside the GAP domain are known to precipitate pathological effects in patients. To understand the disturbed mechanisms underlying sleep defects we use the genetic power of Drosophila (ortholog of the human, dNf1), to investigate locomotor activity and a sleep-like state in mutants and patient mimicking point mutations. Using the Trikinetics automatic monitoring system, we report circadian and several sleep deficits, which also present mutation specificity. We are investigating the neuronal circuitry that drives these behaviors and is affected by dNf1 mutations and the affected molecular pathways therein. Our data thus far reveal complex regulation of activity and sleep by dNf1, probably mediated by distinct neurons in the fly central nervous system. This aligns with the observed diversity of patient phenotypes, especially when considering that various mutations can lead to distinct tissue-specific consequences, contingent upon the specific Nf1 domain that is affected.
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PP062-Properties and predictive potential of the pre-ictal oscillatory dynamics in an ex vivo model of seizure-like activity in the different hippocampal subregions

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Seizure-like activity (SLA) results from synchronous neuronal activity in the mouse hippocampal subregions, namely: CA1, CA3 and DG. While many studies have proved the anticonvulsant properties of the administration of anti-epileptic drugs, such as diazepam (DZP), a GABA-A receptor agonist, and carbamazepine (CBZ), a sodium channel blocker, their role in modulating the oscillatory patterns within these three regions remains unclear. Furthermore, the role of oscillatory dynamics in the pre-ictal period in predicting the emergence of a seizure events require further investigation. In our study, we used a high [K+] artificial cerebrospinal fluid (aCSF) solution to induce SLA in mouse hippocampal slices, followed by bath application of CBZ or DZP. Using spontaneous field potential recordings, we detected differential DZP and CBZ-induced changes not only in the number of spontaneous events, but also in the oscillatory patterns across the CA1, CA3 and DG regions. Imaging of neuronal activity in the ex vivo model of SLA following DZP and CBZ perfusion also revealed a subregion-dependent modulation of neuronal activity, which resembled the pattern of modulation of the oscillatory dynamics. Moreover, LFP analysis in the pre-ictal period revealed significant changes that the oscillatory profiles in this period significantly differed from the oscillatory dynamics at the start of the ictal event and in the absence of SLA. Furthermore, a classification algorithm revealed that using the oscillatory dynamics, the emergence of an ictal event can be predicted with high accuracy. Therefore, the oscillatory dynamics could serve a potential electrophysiological biomarker for predicting seizure activity.

PP063-The role of microglia in a familial in vivo model of Parkinson's disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder (ND) with multiple clinical manifestations. A common denominator of most NDs is synaptic dysfunction caused by changes in synaptic structure and function, which can be either the cause or the effect of the disease. Recent data suggest a neurodevelopmental origin for synaptic dysfunction and challenge the decades-old postulate that synaptic dysfunction is among the ultimate sequelae of NDs. These findings suggest that synaptic dysregulation is a result of inappropriate glial interactions, not only in adulthood, but as early as critical embryonic and postnatal developmental stages, where the importance of microglia is well established.
In this study, we use a mouse model (M83) that overexpresses in neurons the human pA53T-alpha synuclein, which is linked to familial PD. We perform extensive immunohistochemical characterization and morphometric analysis of microglia in multiple brain regions of M83 mice at early developmental stages (E14-17 & P7-10), in young adults (P30), older adults (4-5 months) and symptomatic animals (>1-year-old). In parallel, we study how the neuronal expression of pA53T-αSyn alters the microglial subpopulations and signaling pathways associated with PD, even before the onset of symptoms, by flow cytometry of M83 and wild-type microglial cells of animals at the respective developmental stages.
The present study uses the pA53T-αSyn familial PD model to identify interactions between microglia and neurons during the presymptomatic stages of Parkinson's disease. Using this non-neurocentric approach, we aim to elucidate the role of microglia in disease progression, disease-associated cellular subpopulations and potential therapeutic targets.

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PP064-Priming target color affects activity in visual and prefrontal cortex differently

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Considerable evidence suggests that the brain processes predictable stimuli differently than unpredictable ones. We investigated how target color repetition in a color-cued covert attention task affects activity in prefrontal cortex (PFC) and visual area V4 of macaque monkeys. We recorded neuronal activity in PFC and V4, and compared responses between trials where target color was repeated within a block of 20-25 trials or changed randomly across trials.
Behaviorally, animals responded faster and more accurately in blocked trials, showing a decrease in reaction times within a block. At the neuronal level, when attention was directed to the receptive field (RF), firing rates in V4 and PFC and theta (4-8Hz) as well as gamma (80-120Hz) band activity in PFC were enhanced in random relative to blocked trials, while beta band (15-29Hz) activity in PFC was decreased. When attention was directed outside the RF, distractors were more effectively suppressed in blocked trials as indicated by a decrease in firing rates. This was also evident as an enhancement in low frequency (15-29Hz) activity, indicating a suppression mechanism. During the cue period, V4 exhibited enhanced low-frequency activity (4-14Hz) in blocked trials but no difference in firing rates, whereas in PFC, an increase in firing rates and gamma band activity (30-80 Hz) was evident in random trials. Interestingly, PFC firing rates carried information about the blocked/random task mode even before cue presentation.
Overall, our results suggest that priming of attentional selection based on a color cue is implemented by different mechanisms in V4 and in PFC.

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PP065-Mechanisms of early synaptic dysfunction in p.A53T-αSyn pathology

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Alpha-synuclein (αSyn) is a highly expressed and conserved presynaptic protein, which is considered a critical factor in the development of Parkinson's disease (PD). Synapse dysfunction is presumed to be an early yet progressive pathological feature in PD, with the triggering mechanisms remaining undefined. This study focuses on the p.A53T-αSyn mutation, which has been found to affect synapse formation and function, even from the neural precursor cell stage [1] and aims to investigate the early events leading to synaptic dysfunction. Herein, a transgenic mouse model that expresses the human p.A53T-αSyn in brain neurons under the control of the PrP promoter (Prnp-SNCA*A53T) [2], as well as a human-derived neurons bearing the p.A53T mutation [3] are used. Proteomics implementation in synaptosomes derived from 6-month-old mouse brains reveals dysregulation of presynaptic proteins mainly related to synaptic vesicle trafficking. In agreement, investigation of p.A53T synapse ultrastructure at 4months shows diminished number of synaptic vesicles and impairment of PSD formation, which worsens with age. Moreover, immunocytochemical analysis of mouse and human p.A53T-αSyn neurons reveals aberrant connectivity, alterations in the numbers of excitatory and inhibitory synaptic contacts, and a largely compromised network. The early appearance of these defects is further supported by the partial inability of p.A53T neurons to form artificial synapses. However, the administration of dual-allosteric NMDAR antagonists, Memantine and Nitrosynapsin, potentially reverses the observed synaptic dysfunction. Altogether, a body of spatiotemporal evidence demonstrates that early synaptic dysfunction is a key feature of p.A53T-αSyn pathology, which can be reversed by the use of appropriate neuromodulatory compounds.

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PP066-Ventrolateral prefrontal theta influences trigger theta oscillations phase shift and gamma amplitude modulations within visual area V4 in attention.

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The prefrontal cortex is considered to play a central role in selective attention by biasing activity in visual areas in favor of attended stimuli. To examine the role of ventrolateral prefrontal cortex (vlPFC) in spatial attention, we performed simultaneous extracellular recordings with multiple electrodes from vlPFC and visual area V4 in two macaques engaged in a covert spatial attention task.

Attention modulated spiking activity in both areas, with attention effects emerging significantly earlier in vlPFC compared to V4. Within V4, LFP power was significantly enhanced by spatial attention in gamma frequencies (>30Hz) and reduced in low frequencies (4-30Hz). Within vlPFC, theta power (4-8Hz) was significantly enhanced with attention. Across areas, effects in theta coherence emerged significantly earlier compared to gamma coherence. Granger causality analysis indicated a vlPFC origin for theta interactions and a V4 origin for gamma interactions. Within V4, gamma oscillatory activity was locked to the phase of the theta oscillations and this locking was stronger with attention outside the receptive field, i.e. during the filtering of the distractor. Moreover, the theta phase at which enhanced gamma activity was found, changed with the location of attention. This effect was mediated at least in part by a shift of theta rhythmic activity in V4 with attention, possibly reflecting lateral inhibition mechanisms and enhanced competitive interactions. Thus, PFC induced modulations in V4 theta power that affect the amplitude and timing of gamma oscillations, may facilitate the processing of the target and the filtering of the distractors.

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PP067-Myelin Regeneration: A Promising Approach for Battling Alzheimer's disease

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Recent studies 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 toxic stimuli. In the present study, we compared neuronal myelination in 2, 6, and 12 months old 5xFAD (animal model of AD) mice to their wild type littermates, revealing a significant decrease of myelin in the hippocampus of 5xFAD mice. Further immunohistochemical analysis showed decreased number of PDGFRa+ oligodendrocyte progenitor cells (OPCs) supporting the hypothesis of defective oligodendrogenesis. Endogenous BDNF, acting selectively through TrkB receptor, enhances myelination in the CNS and tends to increase the density of oligodendrocyte progenitor cells (OPCs), both in vitro and in vivo (Xiao et al., 2012; Wong et al., 2014). However, natural neurotrophins are characterized by short half-life, inability to access the blood-brain barrier and a low pharmacokinetic profile. Therefore, we attempted to target OPCs and study the oligodendrogenic properties of two novel small-sized, non-steroid, synthetic neurotrophin analogs, the microneurotrophins TC508 and TC509, which selectively activate the TrkB receptor. Our results indicate that both analogs highly promote primary OPCs differentiation to oligodendrocytes and accelerated adult neural stem cells differentiation to PDGFRa+ OPCs under both physiological and AD related conditions (presence of Amyloid-β). In summary, our study shows that myelination process constitutes an appealing therapeutic target against neuronal loss in AD and suggests two novel BDNF-mimetics as promising lead therapeutic agents in the field of myelin regeneration and restoration.

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PP068-RNF113A as a potential drug target for brain tumors

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Glioblastoma multiforme (GBM) is a highly aggressive brain cancer with a poor prognosis and limited treatment options. The standard of care for GBM tumors has remained unchanged for decades, including surgery, followed by radiation and chemotherapy with alkylating agents. GBM tumors soon become resistant to chemotherapy, making long-term remission challenging(1–4). To this end, we propose here that RNF113A is a drug target that can sensitize GBM cells to alkylating agents. In particular, recent studies suggest that RNF113A is sensing alkylating DNA damage and initiating the DNA repair pathway(5,6). Here we show that knockdown of RNF113A induces apoptosis in human glioblastoma cell lines. Moreover, treatment of GBM cells with alkylating agents, such as temozolomide, and siRNA against RNF113A significantly enhances DNA damage. Although RNF113A exerts DNA repair action via its E3 ligase activity, this factor is currently undruggable. Fortunately, it can be indirectly targeted by inhibiting SMYD3 methyltransferase, which methylates RNF113A and activates its ability to sense alkylating DNA damage. Meta-analysis of TCGA data, reveals a significant positive correlation between RNF113A and SMYD3 expression in GBM tumors, supporting their cooperative roles. Gene Set Enrichment Analysis of the co-expressed genes further indicates involvement in pathways like DNA repair and apoptosis. Most importantly, our experiments indicate that GBM cells treated with SMYD3 inhibitors, when paired with alkylating agents, display enhanced DNA damage and apoptosis. Our results demonstrate a beneficial role for SMYD3 and RNF113A inhibition suggesting a promising therapeutic approach to enhance the efficacy of alkylating treatment in GBM.

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PP069-Mirk/Dyrk1B kinase controls ventral spinal cord development via Shh pathway

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Cross-talk between Mirk/Dyrk1B kinase and Sonic hedgehog (Shh)/Gli pathway affects physiology and pathology. Here, we reveal a novel role for Dyrk1B in regulating ventral progenitor and neuron subtypes in the embryonic chick spinal cord (SC) via the Shh pathway. Using in ovo gain-and-loss-of-function approaches at E2, we report that Dyrk1B affects the proliferation and differentiation of neuronal progenitors at E4 and impacts apoptosis specifically in the motor neuron (MN) domain. Especially, Dyrk1B overexpression decreases the numbers of ventral progenitors, MNs, and V2a interneurons, while the pharmacological inhibition of endogenous Dyrk1B kinase activity by AZ191 administration increases the numbers of ventral progenitors and MNs. Mechanistically, Dyrk1B overexpression suppresses Shh and Gli3 mRNA levels, while conversely, Shh and Gli3 transcription is increased in the presence of Dyrk1B inhibitor AZ191 or Smoothened agonist SAG. Most importantly, in rescue phenotype experiments, SAG restores the Dyrk1B-mediated dysregulation of ventral progenitors. Further at E6, Dyrk1B affects selectively the medial lateral motor column (LMCm), consistent with the expression of Shh in this region. Collectively, these observations reveal a novel regulatory function of Dyrk1B kinase in suppressing the Shh/Gli pathway and thus affecting ventral subtypes in the developing spinal cord. These data render Dyrk1B a possible therapeutic target for motor neuron diseases.

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PP070-The nuclear receptor NR5A2 as a potential regulator of HIF-1α

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Glioblastoma multiforme (GBM) is one of the most fatal primary brain tumors with extremely low patient survival rates. A key feature of glioblastoma is the altered tumor metabolism. In order to support rapid proliferation, cancer cells favor glycolysis even in the presence of oxygen (Warburg effect) to provide the necessary macromolecules for the synthesis of nucleotides, fatty acids and amino acids, rather than fueling the citric acid cycle and oxidative phosphorylation. Hypoxia-inducible factor-1α (HIF-1α) is a main regulator of this metabolic adaptation and its high expression indicates poor prognosis in glioblastoma. Therefore, understanding the GBM adaptive HIF-1α signaling pathway might be critical for improving therapeutic strategies against malignant tumors. In this study, we show that NR5A2 (Nuclear Receptor Subfamily 5 group A Member 2) is sufficient and necessary to repress HIF-1α protein expression in GBM cells independently of prolyl hydroxylase (PHD) inhibition-mediated stabilization. Consistently, NR5A2 inhibits the glycolytic pathway as well as associated genes, which are HIF-1α direct targets. Moreover, NR5A2 exhibits strong antiproliferative, antigliogenic, and anti-tumorigenic activity in these cells. Most importantly, DLPC, a well-established agonist of NR5A2, can recapitulate these effects by repressing HIF-1α expression and tumor-associated properties in GBM cells. These data suggest that NR5A2 action in HIF-1α holds a protective role against GBM.

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PP071-Reversal of memory and autism-related phenotypes in Tsc2+/− mice via inhibition of Nlgn1.

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Loss of function mutations in the TSC1 and TSC2 genes is the cause of the rare monogenic disorder Tuberous sclerosis complex (TSC), which is frequently co-diagnosed with autism. The mammalian/mechanistic target of rapamycin complex 1 (mTORC1) pathway, which regulates cap-dependent mRNA translation, is overstimulated in TSC and leads to aberrant exaggerated protein synthesis. We previously showed that downstream of mTORC1, the eukaryotic translation initiation factor 4E (eIF4E) preferentially stimulates the synthesis of Neuroligin 1 (Nlgn1) in mice, engendering synaptic hyperexcitability and behaviours reminiscent of autism. Herein, we identified enhanced mRNA translation and protein expression of Nlgn1 in the hippocampus of a mouse model of TSC (Tsc2+/-). Genetic inhibition of Nlgn1 using lentiviruses expressing short-hairpin RNAs or pharmacological inhibition of eIF4E-dependent translation, restored Nlgn1 expression in the hippocampus, rescued impaired hippocampal metabotropic Glutamate Receptor Long Term Depression, context discrimination and social behaviour deficits in Tsc2+/- mice. Remarkably, this rescue was achieved without restoring mTORC1 hyperactivation or elevated general protein synthesis in Tsc2+/- mice. Thus, we propose that inhibition of Nlgn1 expression is a viable therapeutic strategy for syndromic forms of ASD such as TSC, and, plausibly idiopathic ASD and other neurodevelopmental disorders, where mTORC1 is hyperactivated.

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PP072-Dissecting the molecular interplay between Tau & RNA-binding proteins in stress-driven brain pathology

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Long lifestyle stress and high levels of glucocorticoid (GC) have been recognized as well-known brain sculptors that can disrupt cognitive function, mood regulation and even contribute to mental and neurodegenerative disorders (Vyas et al., 2016). Our research builds upon previous findings indicating that chronic stress leads to increased Tau hyperphosphorylation and accumulation, suggesting a potential involvement of RNA granules in Tau pathology (Silva et al., 2019). In addition, previous studies of our team reveal that animals lacking Tau (Tau-KO) were protected against stress-driven brain pathology (Lopes et al., 2016). Despite the regulatory role for Tau in stress-driven neuronal damage and the emerging interrelationship between RNA granules and Tau homeostasis, it remains unclear if and how chronic stress affects the regulating mechanisms of RNA-binding Proteins (RBPs) and the formation of Stress Granules (SGs). To dissect this interplay in stress response, we used 5–8-month-old mice of different Tau genetic lines (overexpressing hP301L-Tau or Tau-KO) and their wild-type littermates and exposed them to a chronic unpredictable stress protocol (CUS; 6 weeks of randomly assigned daily stressors) followed by a detailed molecular and histological analysis of different RBPs. Our findings show that chronic stress affects differently RBPs and their intracellular trafficking towards the formation of RNA granules and SGs, while this stress-induced impact can be modulated by the presence or absence of Tau and its pathological status. These results highlight the importance of Tau-RBPs interplay on the stressed brain helping to the identification of novel molecular targets against stress-driven brain pathology.

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PP073-Elucidating the intrinsic regenerative capacity of axons through a comparative study

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Axon regeneration in the mature central nervous (CNS) system is extremely limited. Consequently, traumatic CNS injury leads to irrecoverable neuronal damage, resulting in permanent neurological deficits. On the contrary, mature peripheral nervous system (PNS) and developing CNS axons, can readily regrow and reconnect to their targets, allowing sufficient regain of function after nerve damage. Over the course of years, the differential regeneration dynamics of these axonal populations, have mainly been attributed to extrinsic factors. Recent studies support that intrinsic processes such as axonal mRNA translation are vital for regeneration. In fact, an increasing number of studies suggest that the high levels of local translation maintained in mature PNS and developing CNS axons, are associated to an enhanced capacity for growth and regeneration. Despite the amount of evidence, the specific intrinsic mechanisms and regulatory factors responsible for the mature CNS axons diminished regenerative potential, are yet to be elucidated. This project's main aim is to shed light on the differences and similarities of the developing and adult CNS and PNS axons' molecular repertoires. A series of high-throughput assays will allow the elaborate comparison of the axonal transcriptomes and proteomes and define how these are altered as an initial response to axotomy, independently of microenvironmental factors. Our focus on RNA-binding proteins and their potentially different roles across our axonal populations could contribute to providing an explanation regarding the low intrinsic ability of adult CNS axons to regenerate, as well as in identifying specific molecules related to the regenerative capacity of axons.

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PP074-Deciphering the role of OFD1, a primary's cilium protein, in cortical development and malformations using mouse models

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The cerebral cortex development is a process that involves the coordinated sequence of neural progenitor proliferation, neuronal differentiation and migration. Possible dysregulation of such processes results in malformations of cortical development (MCDs) such as periventricular heterotopias and polymicrogyria which are characterized by morphological and functional cerebral complications. MCDs can be caused by gene mutations, many of which with unknown functions. Recent evidence suggests the involvement of some MCDs' causative genes with the organization and function of the primary cilium (PC). Aiming to shed light on this direction we selected OFD1, a centriole and centriolar satellite protein coding gene, reported mutated in a patient with extensive polymicrogyria and heterotopia. By comparing single-cell RNA sequencing datasets, we observed that species-specific differences in its expression are suggestive of its plausible important role in human corticogenesis and malformations. Next, we sought to investigate OFD1's role in cortical development by manipulating its expression in vivo. Our data suggest that ectopic OFD1 manipulation leads to apical and basal progenitors' numbers and distribution changes in the developing cortex. To ascertain if PC plays role in the prementioned differences, we studied PC's length using appropriate cilia markers and we observed that PC's length seems disrupted upon OFD1 manipulation. Knowing that MCDs cannot be fully recapitulated in animal models, our current and future strategy is to unmask human-specific mechanisms of cortical development using human brain organoid models.

PP075-The role of long non-coding RNAs in mammalian brain development

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With the advent of new sequencing technologies, a growing list of formerly unknown regulatory RNA species has emerged. Among them, long non-coding RNAs (lncRNAs) have been found to control stem cell pluripotency, carcinogenesis, and the development and function of several tissues and organs. Although thousands of lncRNAs are expressed in the adult mammalian brain in a highly patterned and specific manner, they remain poorly characterized, and their roles in brain development have not yet been studied. Therefore, we performed RNA-Seq analysis in the developing nervous system of the mouse embryo. Based on this analysis, we identified many lncRNAs highly expressed in neural cells. We focused our efforts on lncRNAs, which are transcribed from genomic loci in close proximity to protein coding-genes, encoding for transcription factors (TFs) with critical roles in brain development. We hypothesized that these lncRNAs are implicated in the regulation of neighbouring TF genes. Thus, we characterized the changes in the expression profile of the most interesting of the identified lncRNA-TF pairs during the development of the mouse brain (telencephalon). In this study, we further investigated the functional role of three lncRNAs, TCONS_00034309, LockD and AK142161, in the differentiation of neural stem cells by in vitro and in vivo overexpression and knock-down studies. Our data suggest critical roles for these lncRNAs in neuronal differentiation and astrogliogenesis during brain development. Collectively, our study provides insights into the involvement of lncRNAs in brain organogenesis and shows how lncRNAs and protein-coding genes form regulatory networks with important functions in neural cells.

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PP076-Multifaceted Post-Translational Control of KCC2 in Differentiating Neurons by Tgf-β2

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The neuron-specific K+/Cl- cotransporter 2 (KCC2; Slc12a5) establishes the intracellular chloride levels essential for fast synaptic inhibition and regulates dendritic spine formation, thus, being an integral component of neuronal development and function. Dysregulation of KCC2 is associated with neurological pathologies, including epilepsy, highlighting the necessity to elucidate the mechanisms underlying its molecular regulation. Previous research proposed Transforming Growth Factor beta 2 (TGF-β2) as a key regulator of KCC2 membrane trafficking and activity in hippocampal neuronal cultures through a CREB/Rab11b mechanism.
In the present study, we aimed to expand our knowledge on TGF-β2-dependent regulation of KCC2 by uncovering further post-translational mechanisms that putatively modulate KCC2 functions. Using tissue, slices and primary neuronal cultures from Tgf-β2 knockout mice at embryonic day 17.5, we conducted quantitative PCR, immunoblotting, co-immunoprecipitation, biotinylation of surface proteins and immunofluorescence analyses. The results showed that while total KCC2 protein remained unchanged, phosphorylated KCC2 at threonine 1007 was increased in the brainstem of Tgf-β2 mutants, accompanied by a reduction in membrane KCC2. Moreover, Tgf-β2 deficiency disrupted the interaction of KCC2 with the scaffolding protein Ankyrin-G in vivo, and impaired their co-localization in the plasma membrane of cultured hippocampal neurons. Notably, exogenous TGF-β2 was able to rescue the observed changes in phosphorylation, surface abundance and co-localization of KCC2 with Ankyrin-G. In summary, our results demonstrate multiple aspects of TGF-β2-mediated post-translational regulation of KCC2, elevating TGF-β2 in the hierarchy of factors governing KCC2 functional regulation with implications for neuronal chloride homeostasis and understanding of neurological disorders.

PP077-Age-related hearing loss (presbycusis) in Drosophila.

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Age-related hearing loss (ARHL), a form of progressive sensorineural hearing loss also known as presbyacusis, is a complex disorder resulting from the cumulative effects of aging on the auditory system. Several signaling pathways are associated to ARHL, but no treatment is yet available. Model organisms such as Drosophila melanogaster with its short lifespan and powerful genetic tools may aid our understanding of the molecular changes underlying the senescence of the auditory system. In this study, we employed electrophysiological extracellular recordings in tethered wild-type Oregon-R flies to unravel the hearing range, the hearing threshold and maximum responses from the auditory sensory neurons during early (5-10 days old) and two late life stages (flies>80 days, feet; and 80>days, at pre-death stage) The electrophysiological analysis revealed that the amplitude of sound-evoked responses declined in aged feet individuals of both sexes. Furthermore, decline is evident in pe-death stage animals. They either lose their hearing ability or respond weakly to high intensity sound only. Age and physiological stage-dependent threshold shifting is evident in both sexes as well. These findings suggest that late in life, some sensory neurons functionally collapse while others are more resilient to aging and still respond but at higher sound intensity (threshold shifting). Interestingly, this decline was more pronounced in females than males. Thus, males maintain their auditory function and courtship behavior late in life whereas females lose their hearing ability, after surpassing their reproductive age, indicating that sex plays a crucial role in the resiliency of the auditory system during aging.

PP078-Calcium homeostasis regulates neuronal mitophagy upon stress

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Mitochondria are essential for energy production and have vital roles in calcium signaling and storage, metabolite synthesis and apoptosis, among others, in eukaryotic cells. Neuronal cells are particularly dependent on proper mitochondrial function. Thus, maintenance of neuronal homeostasis necessitates a tight regulation of mitochondrial biogenesis, as well as, the elimination of damaged or superfluous mitochondria. Mitochondrial impairment has been implicated in several age-related neurodegenerative diseases. Mitophagy is a selective type of autophagy mediating elimination of damaged mitochondria, and the major degradation pathway, by which cells regulate mitochondrial number in response to metabolic state. However, little is known about the effects of mitophagy deficiency in neuronal physiology. To address this question, we developed two composite, in vivo imaging approaches to monitor mitophagy in neurons. Neuronal mitophagy is induced in response to challenged conditions. Mitochondrial dysfunction leads to transportation of axonal mitochondria towards the neuronal cell body, in calcium- and an AMPK-dependent manner. Autophagy deficiency increases mitochondrial number in neurons of age-matched nematodes and abolishes mitochondrial axonal transport upon stress. Additionally, impairment of mitophagy results in enhanced cell death in C. elegans models of neurodegeneration. Our results indicate that mitophagy contributes to preserve mitochondrial homeostasis and neuronal health.

PP079-Neuron-Microglia Interactions in Cellular Models of Parkinson's Disease

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Parkinson's Disease (PD) affects roughly 10 million individuals worldwide and is characterized by progressive degeneration of substantia nigra neurons, resulting in a spectrum of cognitive deficits and motor impairments. PD histopathological signatures manifest in the form of Lewy body inclusions, predominantly composed of α-synuclein aggregates. Familial PD cases often exhibit mutations in the α-synuclein gene, such as the p.A53T dominant mutation, linked to α-synuclein aggregation and early-onset pathology. Despite PD being considered a neuronal disorder, other cell types of the brain seem to be involved in the neurodegeneration process, including the brain immune cells, microglia. To investigate neurons and microglia, shedding light on the mechanisms underlying PD pathogenesis, neuroinflammation, and α-synuclein aggregation in the pursuit of effective PD treatments, we employ the human neuroblastoma SH-SY5Y cell line expressing either the human p.A53T α-synuclein and dsRed or dsRED alone. Concurrently, we utilize the murine microglia-derived BV2 cell line. Co-culturing these cell lines, we systematically analyze them at specific time intervals: 24 hours, 3 days, and 7 days.
Employing immunocytochemistry protocols and confocal microscopy, we examine markers for morphological changes (IBA1), phagocytic activity (pHrodo), and activation (CD68), as well as, α-synuclein localization within co-cultured BV2 cells. Preliminary findings illuminate elevated lysosomal content and clustering, along with distinct α-synuclein localization within BV2 cells in SH-SY5Y p.A53T co-cultures. Notably, reduced lysosomal clearance is observed in both co-cultures compared to individual BV2 cultures. Our future endeavors encompass assessing the proliferative rate of BV2 cells in co-cultures and cultivating primary neurons and microglia.

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PP080-Base Excision Repair promotes age-related neurodegeneration in a nematode Parkinson's

disease model.

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Aging, genomic instability, and mitochondrial damage are recognized risk factors for neurodegenerative pathologies, including Parkinson's disease (PD). A pivotal mechanism in countering genomic instability is the Base Excision Repair (BER) pathway, responsible for repairing single-base, non-helix-distorting DNA damage in both nuclear and mitochondrial DNA. BER encompasses multiple steps, commencing with the detection and removal of damaged nucleotides. While mammals exhibit significant functional overlap among BER components, Caenorhabditis elegans offers a simplified model for understanding the distinct roles of each constituent.
In our study using a C. elegans PD model, we discovered that the absence of the initial damage-recognition step, led by the NTH-1 DNA glycosylase, did not result in a decline in healthspan. Instead, NTH-1 deficiency promoted neuroprotection. Dopaminergic neurons in NTH-1-deficient animals, expressing α-synuclein, exhibited improved morphological integrity and enhanced neuronal functionality. This unexpected neuroprotection is linked to an increase in mitochondrial transcription rates and the induction of mitohormesis. Our findings underscore the potential of C. elegans as an ideal system to study the complex interplay among BER, mitochondrial transcription, and neuroprotection, thereby identifying potential therapeutic targets against age-related neurodegenerative pathologies.

PP081-Exploring the Fmr1 KO Rat: Unraveling Behavioral Changes and Glutamatergic Dysfunction

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Fragile X Syndrome (FXS) stands as a leading cause of inherited intellectual disabilities and represents a prominent monogenic factor contributing to autism. Although mouse models involving Fmr1 gene knockout (KO) are common in neurobiological research, their limited relevance to human symptomatology and pathophysiology necessitates broader exploration. Leveraging Fmr1 KO rats, we conducted an extensive study examining behavioral patterns and neurotransmission function within key brain regions linked to FXS pathophysiology. Furthermore, we examined evoked and spontaneous field potential recordings from hippocampal slices and RNA sequencing (RNA-seq) of the hippocampus. Our findings revealed hyperactivity, cognitive deficits and glutamatergic/GABAergic alterations in Fmr1 KO rats within the prefrontal cortex and hippocampus. Furthermore, disruptions in excitability and local inhibitory processes were observed in the hippocampus, accompanied by distinctive transcriptional shifts, signifying dysregulated hippocampal network activity in KO rats. This comprehensive analysis not only enhances our understanding of the biobehavioral profile of Fmr1 KO rats but also provides valuable translational insights, enriching our comprehension of FXS syndrome's symptomatology and underlying pathophysiology.

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PP082-Crosstalk between Neural Stem Cells and Platelets: Insight from co-cultures under differentiation conditions

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In the postnatal brain of mammals, there are neurogenic niches where undifferentiated, self-renewing cells, called Neural Stem Cells (NSCs), are located. The lateral walls of the lateral ventricles host one of these niches, the Supebendymal Zone (SEZ). The SEZ microenvironment is supported by specialized vascularization, which delivers nutrients and signaling molecules across the blood-brain barrier. Platelets (PLTs), which are small, disk-shaped cell fragments, besides forming the haemostatic plug, are also involved in tissue regeneration through the release of stored bioactive molecules. It has been demonstrated that PLTs aggregate in the SEZ after a focal demyelinating lesion in the supraventricular corpus callosum of mice (Kazanis et al., 2015). In this study, we isolated NCSs and PLTs from mice and cocultured them for three days under NSC differentiation conditions investigating three different PLT densities. We assessed cell viability and the occurrence of apoptotic cell death, using DAPI staining. In addition, we determined the differentiation potential of NSCs through the immunocytochemical labeling of neuroblasts (that express doublecortin (DCX)) , astrocytes (that express GFAP) and cells of the oligodendrocyte lineage (that express OLIG2). We also examined the NSC progenitor identity using the expression of SOX2 transcription factor. Our results add more information into the ongoing investigation of the presence of a functional interaction between PLTs and NSCs and the effects of different platelet densities.

The work was funded by a grant from the Hellenic Foundation for Research and Innovation (HFRI-FM17-3395) to IK.

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PP083-The role of NO and CHD4 in regulating brain development and chromatin accessibility

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CHD4 is the ATPase subunit for the nucleosome remodelling and development complex (NuRD) and is significant for epigenetic regulation during early cortical development. Loss-of-function causes CHD4-related neurodevelopmental disorder, while variants have been implicated in disorders (childhood idiopathic epilepsy, and psychiatric disorders such as schizophrenia). CHD4 has been shown to undergo S-nitrosylation, a post-translational modification carried out by nNOS. However, the sites of S-nitrosylation are still to be discovered and the effect of nitrosylation on CHD4 expression and function during cortical development is still unclear.
We created mutants of CHD4 on putative nitrosylation sites. Hek 293 cells, overexpressing nNOS, were transfected with the mutants and cells were harvested and underwent Biotin switch experiment to detect levels of nitrosylation.
nNOS-knockout mice were used to examined the expression of CHD4 via immunohistochemistry during cortical neurogenesis, at E12.5, E15.5. and E18.5.
Through in utero-electroporation of GFP-tagged plasmid vectors into E13.5 embryos, we investigated overall changes in cortical migration patterns of developing neurons.
CHD4 mutant C1011 1012S was found to abolish nitrosylation.
In nNOS knockout mice embryos, CHD4 levels are reduced throughout the developmental period, at E12.5, E15.5, and E18.5. Radial migration of neurons is also significantly decreased.
Our results show the sites of nitrosylation of CHD4 and provide evidence that S-nitrosylation is important for CHD4 expression, and radial migration during corticogenesis. We are now at the process of completing experiments with in utero- electroporation of mutants of CHD4 plasmids into E13.5 embryos of CHD4 knock out mice and check for changes in cortical migration.

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Zhong et al. The role of auxiliary domains in modulating CHD4 activity suggests mechanistic commonality between enzyme families., Nat Commun 13, 7524

PP084-GemC1 and McIdas overexpression induce direct cellular reprogramming towards the ependymal lineage

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During mammalian brain development, neural progenitors undergo a transformation, shifting from an undetermined state to a more determined one. This transition is orchestrated by lineage-specific molecular pathways. Direct reprogramming, on the other hand, enables the cell fate conversion from one lineage into another without transitioning through an intermediary pluripotent state. We have previously provided evidence that two proteins of the Geminin superfamily, GemC1 and McIdas, govern the ependymal cell fate molecular program. Ependymal cells are ciliated-epithelial cells that develop along the surface of the ventricles of the brain, and they play a critical role in the circulation of the cerebrospinal fluid and in the homeostasis of the brain. Our study aims to determine the reprogramming potential of GemC1 and McIdas towards the ependymal lineage.
Experiments on embryonic stem cells (ESCs) showed that, through the expression of GemC1 or McIdas, mouse ESCs can be programmed into the ependymal lineage. In addition, preliminary results show that combined expression of GemC1 and inhibition of the polycomb repressive complex show the potential enhancement of reprogramming efficiency towards ependyma. Ectopic expression of these proteins in cortical astrocytes indicate the successful direct reprogramming towards ependyma. The reprogrammed cells displayed unique morphological and functional features of mature ependymal cells.
Overall, we believe that further understanding of the role of GemC1 and McIdas in the molecular pathway that promotes ependymal cell reprogramming could provide new evidence for the creation of new therapeutic approaches for the directed production of ependymal cells against neurodegenerative diseases.

The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the “2nd Call for H.F.R.I. Research Projects to support Faculty Members & Researchers” (Project Number: 81998 - Elucidating Neural stem cells fate commitment decisions into the ependymal lineage (EINSTEIN)).

PP085-Mnk1/2 kinases regulate memory and autism-related behaviours via Syngap1

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Mnk1/2, the MAPK (mitogen-activated protein kinase) interacting protein kinases 1 and 2 modulate several functions in cells, mainly via phosphorylation of their best characterised substrate, eukaryotic translation initiation factor 4E (eIF4E) on Ser209. We suggest that Mnk1/2 posses additional downstream effectors in the brain because deleting of Mnk1/2 (Mnk double knockout) impairs synaptic plasticity and memory in mice yet ablation of phospho-eIF4E (Ser209) does not affect these processes. Translational profiling showed that there is only a small overlap between Mnk1/2 - and phospho-eIF4E(Ser209)-regulated translatome. Synaptic Ras GTPase activating protein 1 (Syngap1), encoded by a syndromic Autism Spectrum Disorder (ASD) gene, was identified as a downstream target of Mnk1 since Syngap1 immunoprecipitated with Mnk1 and showed reduced phosphorylation (S788) in double knockout for Mnk (Mnk DKO mice). Knockdown of Syngap1 reversed memory deficits in Mnk double knockout mice and pharmacological inhibition of Mnks rescued autism-related phenotypes in Syngap1+/− mice. Thus, Syngap1 regulates mTORC1 signalling and protein synthesis and that the Mnk-Syngap1 axis is crucial for ASD linked behaviours such as social interaction, learning, and memory.

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PP086-Hearing in Drosophila: the physiological responses of auditory sensory neurons to different types of sound stimuli.

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Insects including Drosophila melanogaster (fruit fly) use their antennas for hearing. Sound causes rotations of the last antennal segment that are detected via mechanosensory neurons. The transduction of mechanical movement to electrical activity occurs at the dendrites of sensory neurons by a mechanism that is molecularly similar to that of the vertebrate ear. In this study, we examine and compare the response characteristics of auditory sensory neurons to different stimulation regimes in young female and male animals. We have digitally created stimuli that mimic the love song of males during the courtship ritual with females. In addition, a long stimulation sequence that spans the range of 1-1000 Hz and steps of 10 Hz was delivered to the flies at five different decibel levels (60–100 dB). The electrical response of auditory sensory neurons was recorded with tungsten extracellular electrodes; the signal was amplified, digitized, and further processed using the Clampex software. The amplitude and response delay of the compound action potentials were estimated separately for male and female flies. Fast Fourier Transformation performed at the electrophysiological recordings allows us to define the frequency range of the response to different Hz and dB levels. Finally, audiograms were constructed, and hearing thresholds were determined for the different decibel levels for both sexes. Comparing the physiologic response of young drosophilae to auditory stimuli paves the way to investigate age and/or trauma-related pathologies in drosophila.

PP087-Studying spontaneous oscillations in brain slices of the mouse motor cortex via optogenetics and complex network theory

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The advent of genetically encoded voltage indicators (GEVIs) has long been praised as a potential pinnacle in the field of neuroscience, yet the impact on our understanding of circuit physiology remains elusive. Such ambiguity arises not only due to limitations regarding indicator performance and imaging technology, but also due to the lack of a general agreement on a specific method of analyzing the emerging imaging data. Here, by expressing ArcLight, a potent GEVI for in vitro and in vivo imaging, we imaged the mouse primary motor cortex pan-neuronally and visualized both the neuronal excitation and inhibition during bath application of bicuculline. Using widefield epifluorescence microscopy, we were able to optically resolve spontaneous oscillations in brain slices of the mouse motor cortex ex vivo. Next, we resorted to complex network theory techniques in order to document the functional dependencies among recording sites, with our aim being to study whether these oscillations can be predicted based on the imaged network activity. Our results suggest that alternative interdisciplinary intuitions about the functional interpretation of the data might be more appropriate, at least until technical challenges related to GEVIs are finally resolved.

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PP088-The transcriptomic landscape across 202 micro-dissected regions of the adult human brain

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The Human Protein Atlas (HPA; www.proteinatlas.org) is a public online database that provides an integrated overview of protein expression and distribution in all major human tissue types, including brain. There, a comprehensive overview of gene and protein expression in the main anatomical structures of the mouse, pig and human brain is provided, by combining in-house generated and publicly available transcriptomic data. In the current version, released in June 2023, we have added in-house generated RNA sequencing data for 967 samples from 202 micro-dissected regions and areas of the human brain. These include 10 samples from different basal ganglia, 16 thalamic and 9 hypothalamic nuclei, 9 samples from the hippocampal complex, 5 from the amygdala, over 70 from brainstem and 5 cerebellar cortical and nuclear samples. In addition, from the cerebral cortex, more than 70 areas, gyri and subregions have also been analyzed. We have classified all protein coding genes based on regional distribution and co-expression, thus providing lists of genes associated to brain regions, cell types and functions. Based on our analysis, the piriform cortex appears to have a unique transcriptomic signature, quite different from the rest of the cerebral cortex while the claustrum, although it anatomically belongs to the cortical subplate, its transcriptional profile shares more common characteristics with the subregions of the cortical plate. In addition, based on these data, the basal ganglia are further divided into 3 sub-clusters, with notable differences in the gene expression patterns between the globus pallidus (internal and external) and the striatal parts.
Funding was provided by the Knut and Alice Wallenberg Foundation

Poster Presentations (PP) - Poster session #3

SUNDAY – 26 NOVEMBER 2023

PP089-Investigating how written language is lateralized in children at risk for dyslexia and the effects of a phonological intervention on lateralization

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Cerebral lateralization of language, i.e., the differential activation of brain hemispheres during language tasks, has been widely studied using oral word generation, reading, and comprehension tasks. There is ample evidence that a pattern of left-lateralization for these language processes is established even before school for the majority of individuals. However, an atypical pattern of language lateralization, more rightward or symmetrical, can also be observed in both neurotypical individuals and in cases of specific learning disorders, such as dyslexia. Dyslexia is characterized by difficulties in spelling, reading, and writing, potentially caused by deficient phonological awareness. The causal role of phonological awareness in dyslexia is supported by an improved reading performance together with a left-ward laterality shift in children with dyslexia following interventions with a phonological component. These effects are evident even before the diagnosis of dyslexia, when interventions are more beneficial. Although writing skills are persistently affected in dyslexia, no studies have assessed the lateralization of written language in children with or at risk for dyslexia. In our studies, we assess lateralization using functional transcranial Doppler ultrasonography and we compare the lateralization for linguistic component of the generation of written words between nine children at risk for dyslexia and their typically developing peers, with inconclusive preliminary results. We also assess the effect of a phonological intervention on brain lateralization in five children at risk for dyslexia. Preliminary findings indicate that the intervention affects lateralization. Our findings could indicate an early biomarker for dyslexia and an effective intervention prior to diagnosis.

PP090-Distinct astrocyte activation profiles associated with demyelination in the cuprizone model of multiple sclerosis

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Astrocytes are not only important regulators of CNS homeostasis but also significant mediators and potential therapeutic targets in neurological diseases. In multiple sclerosis, astrocytes are involved in the development of active lesions, further to previous perceptions that they contribute to pathology only at progressive stages. Here, we applied the cuprizone(CPZ) model of demyelination to study astrocyte involvement in the initiation and progression of CNS pathology, focusing on early cell responses that might be crucial for disease advancement. We performed morphological analyses of 3D-reconstructed astrocytes isolated from the cortex of C57BL/6 mice and used specific cell morphometrics, to assess astrocyte reactivity at hallmark disease timepoints. Furthermore, based on preliminary results in our lab showing that treatment of mice with a selective soluble TNF inhibitor(XPro1595) ameliorates CPZ pathology and promotes remyelination, we extended our analysis to XPro1595-treated mice during early CPZ. XPro1595-treated astrocytes appeared larger, with longer, thicker processes and increased soma size. Quantification of GFAP signal immunoreactivity in the cortex also displayed significant increase in the XPro1595-treated compared to the control group. To investigate the contribution of astrocytes to the beneficial effects of XPro1595, we generated mice with astrocyte specific deletion of TNF receptor 1, which selectively mediates the effects of soluble TNF, and are analyzing them with the cuprizone demyelination model in ongoing experiments. We present our results concerning the contribution of astrocytes to the beneficial effects of solTNF inhibition in CPZ demyelination, that could provide new insights in the pivotal astrocyte involvement in the progression of neurological diseases.

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PP091-Antisense oligonucleotide (ASOs) therapeutic approach against Tau-driven neuronal pathology in Alzheimer's disease brain pathology

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Although Alzheimer's disease (AD) is the leading cause of dementia with a heavy societal cost worldwide, the underlying brain pathology remains poorly understood and inefficiently treated, highlighting the urgent need for identification of innovative therapeutic strategies. As accumulating evidence from preclinical and clinical studies highlights the mediating role of Tau and its aberrant accumulation in AD brain pathology, our work focuses on the use of Tau-targeting Antisense Oligonucleotides (ASOs). ASOs, a currently rising novel therapeutic tool against various neurological diseases, are small synthetic strings of nucleotides that regulate the RNA levels of the protein of interest, while they have been proven to be safe for both animal and human use. Hereby, we designed and monitored the efficiency of 22 novel ASOs against total Tau or selectively 4R-Tau in cell lines and primary neurons expressing human Tau, as well as in a pilot in vivo study with Tau transgenic mice. We have identified a set of novel and highly efficient ASOs for reducing Tau or modulating 4R/3R isoform ratio as assessed by different types of molecular and cellular, neurostructural and behavioral analysis. Altogether, these data provide in vitro and in vivo evidence of the beneficial use of ASOs against Tau-related neuronal malfunction in AD, supporting ASOs as an innovative RNA-based therapeutic approach in neurodegenerative pathologies of the brain.

PP092-Is Default Mode Network Connectivity related to having experienced Intimate Partner Violence?

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Background: Intimate Partner Violence (IPV) against women is one of the most serious and pervasive sociological issues. Specifically, one out of three women worldwide has suffered physical and/or sexual violence from a male intimate partner [1]. Research has shown that women victims of this type of violence suffer from severe psychological disorders and structural and functional brain alterations [2]. The human brain is organized into distinct networks that underlie behaviour. One of these networks is the Default Mode Network (DMN), which is active at rest, and is related to reduced connectivity in populations exposed to trauma [3]. In this study, we examine DMN connectivity in women who have suffered IPV compared to those who have not.
Methods: A sample of 39 non-IPV victims (mean age=41, SD= ±14.5) and 39 survivors of IPV (mean age=42.5, SD= ±11.6) was collected. Participants underwent a rs-fMRI scan of 6 minutes. Data was preprocessed following procedures implemented in the CONN toolbox.
Results: The present results show differences within the DMN between the two groups. Specifically, women survivors of IPV presented decreased activation of the left precuneus, the left angular gyrus and the right interior temporal region, compared to non-IPV victims. These regions has been associated, with mind-wandering, thinking of oneself, remembering the past, and envisioning the future.
Conclusions: Findings reveal differences in DMN connectivity between the two groups. More concretely, women survivors of IPV showed decreased connectivity in regions involving the posterior DMN. Future analyses may also examine how these differences relate to psychopathological conditions.

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PP093-Intrinsic pathology of induced pluripotent stem cell-derived astrocytes from Parkinson's disease patients

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The accumulation of aggregated alpha-Synuclein (αSyn) is typical in Parkinson's disease (PD)-patients' brains and the prion-like spreading hypothesis is gaining ground. However, the astrocytic contribution in PD pathology is understudied, although PD-related mechanisms including neuroinflammation and aggregate resolution pathways may involve non-cell autonomous components[1]. 5-10% of PD cases are linked with mutations in specific genes, such as the αSyn gene SNCA. Of these, p.A53T-αSyn (G209A in SNCA) is the earliest identified and best-characterized mutation causing severe, early-onset familial PD[2]. To investigate the impact of the p.A53T mutation on astrocytes and their contribution to PD, we used our previously established induced pluripotent stem cell model (iPSC) from PD patients harboring the mutation[3] and generated ventral midbrain astrocytes that display typical lineage markers and functional characteristics. p.A53T astrocytes presented pathological traits, including αSyn upregulation, accumulation of protein aggregates, also positive for toxic phosphorylated αSyn, disturbed autophagic flux, and inefficient degradation of phagocytosed cargo. Indeed, proteome profiling revealed autophagy, endocytosis, and protein catabolic processes among the most affected pathways. Given that the compromised astrocytic ability to uptake and clear neuronal waste has been proposed as culprit for pathological accumulation of αSyn aggregates in neurons[4], we treated control and mutant astrocytes with conditioned medium from p.A53T iPSC-derived neurons. Our results indicate that healthy astrocytes efficiently uptake toxic αSyn species released in neuronal conditioned medium, whilst p.A53T astrocytes do not. Overall, we show that the p.A53T-αSyn mutation causes intrinsic malfunctions in astrocytes, related to proteostasis and clearance mechanisms that may contribute to neuronal pathology.

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PP094-Investigating the neurogenic effects of BNN237 in a mouse model of stress

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Adult neurogenesis, the generation of new neurons across the lifespan, represents a dynamic field of neuroscience research. Chronic stress, a risk factor for neuropsychiatric disorders, is associated with impaired neurogenesis. Conversely, neurotrophins are linked with enhanced neurogenesis, while they are suggested to be involved in the relationship between stress and neurogenesis. The aim of the present study was to investigate the neurogenic effects of BNN237, a novel synthetic micro-neurotrophin, in a mouse model of stress. Using immunofluorescence analysis, the densities of newborn, proliferating cells and immature neurons were evaluated in the two main neurogenic brain areas, the subventricular zone (SVZ) and the dentate gyrus (DG) of mice, receiving placebo or BNN237 under control or 6-weeks-long chronic unpredictable stress conditions. Our results demonstrate that BNN237 administration can increase the number of newborn proliferating cells in SVZ. In the olfactory bulbs, the area where SVZ-derived stem cells migrate, we observed a diminishing effect of stress in the number of neuroblasts, but also a significant interaction between BNN237 treatment and stress, resulting in enhanced DCX+ neuroblast density in non-stress conditions. Interestingly, BNN237 did not affect the neurogenic capacity of the DG, while stress tended to reduce proliferation in this area. Our findings confirm the selective pro-neurogenic effects of BNN237 in SVZ, while differences between the two neurogenic niches (SVZ and DG) may explain the restriction of these effects only in SVZ. Future studies should emphasize the interplay between neurotrophins and stress hormones, as a possible mediator of the stress-related suppression of adult neurogenesis.

PP095-Aberrant neuronal activity and delayed habituation of the giant fiber escape response circuit in Drosophila Nf1 mutants

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Neurofibromatosis type 1 (NF1) is a genetic disorder caused by reduction or absence of neurofibromin due to mutations in the NF1 gene. To directly assess the consequences of NF1 deficiency on evoked neuronal activity, we utilized null mutations, point mutations, and RNA interference to manipulate NF1 expression in Drosophila melanogaster. Our study focused on assessing the functional alterations in the giant fiber system, a well-established model to study synaptic transmission and habituation, using electrophysiology. Our findings reveal that the absence or reduction of NF1 leads to significant changes in the characteristics and habituation properties of the giant fiber system, indicating synaptic dysfunction and impaired neurotransmission. More specifically, NF1 deficiency was associated with a significant delay in the habituation rate of the system, suggesting compromised neuronal plasticity and adaptive responses. In an attempt to rescue this deficit, reminiscent of autism spectrum disorder manifestations, we tried four different drugs: Crizotinib (Alk inhibitor), Ritalin (methylphenidate), Aricept (donepezil), and Gabapentin (GABA analogue). Strikingly, Ritalin and Aricept normalized the habituation rate, while, on the contrary, Crizotinib and Gabapentin exacerbated the aberrant responses. Overall, our study provides valuable insights into the functional consequences of NF1 deficiency on the giant fiber system of Drosophila melanogaster. It highlights the critical role of NF1 in maintaining synaptic integrity and proper neurotransmission, offering potential targets for therapeutic interventions in NF1-related disorders.

PP096-Exploring the mitochondrial underpinnings of early handling in high anxiety mice

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Early life experiences shape future behavior. Early handling (EH), the brief and repeated separation of pups from their dam during early life, is an intervention implemented to study how postnatal experiences affect behavioral outcomes in adulthood. To date, the impact of EH in a high anxiety background is poorly understood. Here, we used high (HAB) and normal (NAB) anxiety-related behavior mice to investigate the effects of EH on maternal behavior and on the behavior and molecular correlates of male and female adult offspring.
During the EH protocol, pups are removed from their dam for 15 min/day for postnatal days 1-14, during which non-handled (NH) pups are exposed to animal facility rearing. Maternal behavior was observed for postnatal days 2-7 and behavioral changes in EH compared to NH mice were investigated by elaborate behavioral tests. EH-induced alterations in mitochondrial function and dynamics were assessed at the protein level by western blots and other biochemical assays, and at the mRNA level by real-time qPCRs.
EH does not impact maternal behavior in either HAB or NAB mice. Interestingly, EH exerts an anxiolytic effect in both HAB male and female mice compared to their NH counterparts. This is accompanied by changes in the protein and mRNA levels of key metabolic and mitochondrial players in male mice. Currently, we are investigating these EH-induced anxiolytic effects in HAB female mice.
Unraveling the mechanisms of EH will elucidate pertinent anxiety pathways and expedite the discovery of novel therapeutic targets for anxiety disorders.
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PP097-Hilar mossy cells in human patients and a mouse model of Frontotemporal Dementia.

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Frontotemporal Dementia is a neurodegenerative disorder that involves the progressive degeneration of the frontal and temporal lobes, with the patients displaying behavioural or language symptoms. FTD and Parkinsonism linked to chromosome 17 is a subtype of FTD that falls into the category of tauopathies. Mutations in the MAPT gene are associated with this disorder. Hilar Mossy Cells (MCs), a glutamatergic subtype of neurons of the dentate gyrus, are implicated in several pathological conditions like Temporal Lobe Epilepsy, Schizophrenia and Alzheimer's Disease. In this project, we studied the changes in MCs in a transgenic mouse model of FTDP-17 (TauVLW) and in human patients of FTD. We found that, compared to control mice, TauVLW mice show decreased volume of both the dorsal and ventral DG, and decreased number of MCs in the ventral DG. We also showed that the expression of Calretinin, which is considered a gold-standard marker for MCs, follows a latero-medial gradient in the dorsal DG. In patients with FTD, the density of CR+ MCs showed no changes compared to neurologically healthy control subjects; however, we found a decrease in the densities of CR+ immature dentate granule cells and CR+ interneurons. By performing qualitative determinations, we propose the existence of distinct subpopulations of MCs, expressing different molecular markers in the human DG. Together, these data suggest that MCs are vulnerable to neurodegeneration mechanisms present in mouse models and patients with FTD. Further investigation is needed to shed light on the underlying mechanisms behind MC loss and/or dysfunction.

PP098-The role of Contactin2 in hippocampal interneuron myelination and synaptic plasticity

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Interneurons have recently been spotlighted due to their myelination pattern. Indeed, interneuron myelination reveals differences both in composition but also in the distribution of myelin along the axon and, as a consequence, in function. Contactin-2 is a cell adhesion molecule of the immunoglobulin superfamily with a dynamic spatial and temporal expression, implicated in axonal guidance and neuronal migration during development and postnatally, in the proper organization of the juxtaparanodal areas of myelinated axons. Its function has been implicated in neurodegenerative pathologies such as Multiple Sclerosis and Alzheimer's disease.

We have previously shown that a significant population of GABAergic interneurons in the hippocampus are expressing Contactin-2. Our data show a decrease in the K+ channel clustering at the nodes of Ranvier in some interneuronal populations in vivo in the Cntn-2 deficient mice. Moreover, functional experiments show that there are significant alterations in the intrinsic properties of Sst+ interneurons in the stratum oriens of the CA1 hippocampal area of the mutant mice.
Cntn2-deficient mice show functional impairments related to the hippocampus and their defects can either be attributed to myelin deficits or to alterations in synaptic regulation in the hippocampus or both. We, for the first time, are investigating the role of Contactin-2 in synapses and importantly, we observed that Cntn2-deficient mice are significantly impaired in their long-term potentiation (LTP) profile. We are currently focusing our attention on the role of Contactin-2 in synaptic plasticity and the possible correlation of its role in hippocampal interneuron physiology as well as myelination.

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References: Micheva et al.,2016, Stedehouder et al.,2017, Bonetto et al., 2019, Kalafatakis et al.,2021, Pinatel et al., 2023.

PP099-Splice-Switching Oligonucleotide (SSO)-mediated NF1 Exon 51 Skipping in Primary Neurons Reveals Novel Properties of Neurofibromin Isoforms

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The mechanism of alternative splicing-AS expands the diversity of the transcriptome and therefore the proteome, which in turn further expands by post-translational modifications of the produced protein isoforms. AS has been a major research focus for therapeutic purposes: for several monogenic diseases, splicing modulation with SSOs - short, antisense oligonucleotides of modified nucleic acids designed to block binding of spliceosomal proteins and pre-mRNAs and thus alter exon splicing - has led to approved drugs. When exon skipping is physiological, such research serves the double purpose of elucidating the function of the produced isoforms. With this rationale we have been investigating the expression of NF1 transcripts and the function of neurofibromin isoforms; when mutated, they cause Neurofibromatosis-1, a disease characterized by learning difficulties and often glioblastoma. Major alternate exons in the NF1 mRNA are exon31, regulating the established neurofibromin's potency as a RasGAP, and exon51, which bears a nuclear localization signal-NLS and allows the protein to enter the nucleus. Recently, we have documented that NLS and ΔNLS neurofibromins function also as Microtubule Associated Proteins-MAPs for both cytosolic and spindle microtubules, albeit with different characteristics. Importantly, depletion of NLS neurofibromins with shRNA in astrocytes revealed that they are indispensable for proper spindle assembly, chromosome segregation and faithful genome transmission. We now present data for effective SSO-mediated exon51 skipping in primary neurons, which strongly suggest that NLS and ΔNLS neurofibromins have different potencies as RasGAPs and that are important MAPs also in neurons, regulating cytoskeletal dynamics in axon growth cones, critical for synaptogenesis.

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PP100-The effects of Aticaprant, a κ-opioid receptor antagonist, in stress-induced deficits in mood and cognition.

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Kappa opioid receptors (κ-ORs) are extensively investigated for their emerging role in anxiety and depression, as κ-OR blockade impedes the effects of stress in animal studies (1). Aticaprant, a selective κ-OR antagonist is currently in phase III clinical trials and is used for treatment of depression (2). In the present study, mice were subjected to unpredictable chronic stress, followed by administration of Aticaprant and behavioral assessments were performed, including elevated plus maze, open field, novel object recognition, Y-maze and forced swim test. Our results demonstrate that Aticaprant produced an anxiolytic and antidepressant effect, reversed stress-induced impairments in long-term memory, but was ineffective on short-term memory deficits. Recent studies from our laboratory have demonstrated that κ-OR induces autophagy (3), a homeostatic mechanism that degrades dysfunctional proteins to modulate the morphology of neuronal cells and alter synaptic plasticity (4). We have shown that activation of κ-OR mediates the autophagic machinery via a Gαi/o-ERK1,2-CREB pathway resulting in the decrease of hippocampal synaptic proteins, under acute stress conditions (3). In this respect, herein, we demonstrate that the levels of the hippocampal synaptic proteins spinophilin, PSD95 and SNAP25 in stressed animals were restored in Aticaprant-treated animals. Moreover, Aticaprant altered the levels of the autophagic markers in chronic stressed animals compared to naïve ones, with a concomitant alteration of the ERK1/2 signaling pathway. Our data provide evidence for the mechanism via which Aticaprant exerts its therapeutic effects as a putative novel drug to alleviate stress-related disorders.

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PP101-Neuronal type-specific requirements for the Rutabaga adenylyl cyclase in Drosophila footshock habituation and associative learning

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Understanding the molecular mechanisms underpinning learning and memory in the context of the brain's complex wiring remains a fundamental challenge in neuroscience. Drosophila melanogaster provides an invaluable model for investigating these intricate processes. In this study, we investigate the role of Rutabaga adenylyl cyclase (Rut), an essential molecular component, in Drosophila Mushroom Body neurons (MBns). Our findings reveal that Rut plays a pivotal role in both habituation and associative learning in MBns. Two distinct Rut mutants, rut2080 and Mi{MIC}rutMI10033, exhibited compromised associative learning, with premature habituation to footshocks and a failure to habituate after exposure to repeated stimuli. This study also sheds light on the MBn-type-specific contribution of Rut to these processes. Notably, Rut's downstream effector, Protein Kinase A (PKA), was found to be essential for habituation latency but not habituation in certain MBn types. Also, our study on antipsychotic drugs shows that drugs such as risperidone, haloperidol, and clozapine can rescue habituation deficits in Rut mutants. However, learning deficits were only rescued by risperidone and haloperidol, highlighting the importance of inhibiting the Dop2R receptor. Interestingly, the dosage of Rut appeared to influence habituation and learning differently, emphasizing the need for precise molecular regulation. This study enhances our knowledge of how the brain processes habituation and learning, with potential relevance to conditions like schizophrenia. Finally, our findings shed light on the complex role of Rut in neural processes and offer potential therapeutic strategies for these conditions.

PP102-The effect of the antidiabetic sodium-glucose co-transporter inhibitors on hippocampal pyramidal cell synapses and their potential in Alzheimer's disease treatment.

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Alzheimer's disease and Type 2 Diabetes Mellitus represent two chronic diseases affecting a significant percentage of the population worldwide. They share common pathophysiological features, such as oxidative stress, inflammation, and signaling pathways. Given the lack of available treatments for Alzheimer's disease, the research community has increasingly focused on exploring the therapeutic potential of certain antidiabetic drugs for Alzheimer's prevention and treatment. In particular, the class of antidiabetic drugs related to inhibitors of the sodium-glucose cotransporter 2 (SGLT-2i) is in the foreground, as literature reports indicate their presence in the central nervous system and their possible neuroprotective properties. Several SGLT-2i have demonstrated anti-inflammatory and antioxidant properties within the brains of rodent animal models. They have also shown improvements in cognitive function and a positive impact on certain Alzheimer's disease biomarkers. This study aims to investigate the impact of empagliflozin on primary hippocampal pyramidal cells and their synapses. The investigation includes primary hippocampal neurons derived from both wild-type and transgenic (TgF344-AD) rats as well as oxidative stress-induced neurons under the influence of hydrogen peroxide, replicating an in vitro model of Alzheimer's disease. Morphological analysis of the dendrites as well as dendritic spine field analysis of primary hippocampal cells by immunofluorescence showed comparable results to the cell viability experiments. Primary neuron cells exhibited physiological dendritic and spine growth after treatment with empagliflozin. The combination of empagliflozin and hydrogen peroxide resulted in the reverse of oxidative stress neurodegeneration. Further experiments are necessary to evaluate the mechanism of action of empagliflozin, in Alzheimer's disease.

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PP103-PyMouse: A low cost, automated, high-throughput behavioral training system for mice

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Training mice in behavioral tasks is often hindered by laborious processes and the need for substantial resources while ensuring reproducibility remains a persistent challenge. Commercially available automated behavioral systems tend to be expensive and impose restrictions on experimental designs. To address these limitations, we present PyMouse, an innovative open-source behavioral control framework developed using Python.
PyMouse serves as a state control system, overseeing all aspects of a behavioral experiment, including the creation and presentation of stimuli, efficient hardware management, and effective data acquisition and storage. By enabling the integration of diverse experimental designs with a variety of readily available, cost-effective hardware components, PyMouse offers high flexibility and adaptability.
Our research demonstrates the successful implementation of PyMouse in training mice in various discrimination tasks, incorporating a variety of stimuli such as visual, olfactory, auditory, and their combinations. Notably, PyMouse automates the storage of all behavioral parameters, ensuring adherence to the FAIR principles without requiring manual intervention from researchers and facilitating convenient data sharing.
By minimizing the need for extensive animal-experimenter interaction and offering a cost-effective solution, PyMouse streamlines the training of numerous mice in complex tasks with minimal researcher effort. Moreover, the system's capability to facilitate stress-free learning of intricate behavioral tasks within the animals' home cages allows for the seamless transfer of acquired skills to head-immobilized setups during neural activity recordings.

PP104-Unravelling the role of GemC1 in the development of the murine hippocampus

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The murine hippocampus, situated within the inner part of the mammalian temporal lobe, constitutes a complex brain structure primarily linked to memory formation and spatial navigation. It is composed of two primary regions: the Cornu Ammonis (CA) and the Dentate gyrus (DG). Within the latter, a thin layer of cells known as the subgranular zone (SGZ) functions as a neurogenic niche. The proper development of the hippocampus holds paramount significance, since numerous neurogenetic diseases have been linked to structural irregularities within the hippocampal structure.
Our research is dedicated to investigating the GemC1 factor, a member of the Geminin superfamily, and its potential involvement in the formation and subsequent functioning of the developing hippocampus in GemC1 knockout mouse models generated within our laboratory. GemC1 plays a pivotal role in regulating the activity of p73, in the lateral ventricles. More specifically, p73 is involved in the maturation process of radial glial cells into multiciliated ones, thus influencing multiciliogenesis in that region. Simultaneously, it has been determined that p73 is crucial for the proper development of the hippocampus, since depletion of p73 has been observed to result in significant hippocampal dysgenesis in mouse models, primarily impacting the Cajal-Retzius neurons. Our current data indicates that the absence of GemC1 results in the downregulation of p73 expression during hippocampal development. These effects manifest during both embryonic and early postnatal stages, bearing similarities to the p73 knockout hippocampus model and suggesting GemC1 to be an important factor in the normal development of the murine hippocampus.

PP105-The neuroprotective role of NR5A2 under oxidative stress

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Neurodegenerative diseases are characterized by the loss of structural and functional properties of groups of neurons and their subsequent death. Oxidative stress is implicated in the most prevalent neurodegenerative disorders leading to neuronal dysregulation and death. The development of neuroprotective therapies that promote neuronal survival by reversing the damage of oxidative stress could provide novel therapeutic insights for nervous system-related diseases. Towards this direction, we focused on the orphan nuclear receptor NR5A2, which is known for inducing neurogenesis during development and maintaining neuronal properties in the adult brain. In this study, we investigated the potential neuroprotective effect of this receptor on neuronal cells that undergo oxidative stress. We demonstrate that the adenoviral overexpression of NR5A2 in ex vivo cultured murine cortical neurons promotes their survival under oxidative stress conditions, whereas knockdown of NR5A2 has the opposite effect on neuronal survival. Most importantly, dilauroyl phosphatidylcholine (DLPC), a phospholipidic agonist of NR5A2, recapitulated the effect of the overexpression of NR5A2 in decreasing neuronal apoptosis under oxidative stress. RNA-seq analysis of DLPC treated neurons, unravels a panel of significant genes and pathways that are upregulated and downregulated and are linked with neuroprotective processes. We validated which of these genes are altered the most by DLPC and could reveal the pathway(s) by which NR5A2 inhibits neuronal apoptosis in oxidative stress conditions. These findings suggest that NR5A2 promotes neuronal survival under oxidative stress conditions and its agonist DLPC could be used as a neuroprotective treatment in neurodegenerative disorders.

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PP106-Longitudinal whole brain NGS RNA sequencing reveals characteristic transcriptomic signatures at hallmark timepoints of the cuprizone model of demyelination.

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RNA sequencing has revolutionized the identification and characterization of pathogenic mechanisms in many diseases. NGS RNA-seq technologies are widely used in neuroscience research for understanding gene expression signatures involved in brain pathology. Here, we focus on Multiple Sclerosis (MS), an inflammatory demyelinating disease of the CNS, using NGS whole brain RNA-seq in a pre-clinical animal model for MS induced by dietary cuprizone.
Specifically, to document the transcriptomic profiles of mouse brains at key pathological timepoints of CPZ demyelination and remyelination, we performed NGS sequencing of whole brain RNA from wild type C57BL/6 mice, isolated at hallmark disease stages, specifically naïve (CPZ0), peak of demyelination after 5 weeks of cuprizone (CPZ5), and remyelination after 6 weeks of cuprizone and 1 week of normal diet (CPZ6+1).
Longitudinal differential gene expression analysis between the different timepoints was performed, using computational tools and bioinformatic visualization techniques, such as Protein-Protein interaction networks, Gene Ontology networks, gene expression heatmaps, volcano and balloon plots.
Brain RNA-seq revealed distinct gene clusters and transcriptomic profile that were correlated with each disease state. The genes that were predominantly affected were associated with major disease related processes including inflammation, glial responses, phagocytosis, and myelination. The pairwise gene expression comparison of the disease model stages stated the vastly differentiated brain transcriptomic during health, demyelination and remyelination.
Overall, this project highlights the power of the NGS RNA-seq approach for identifying CNS transcriptomes in both health and disease states and for characterizing specific genes and transcriptional processes involved in the pathogenesis of brain diseases.

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PP107-Unravelling the molecular and functional maturation of the SST-expressing cortical interneurons during the first postnatal month

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Brain function is inextricably linked with the activity of cortical γ-aminobutyric acid-producing (GABAergic) interneurons (INs), which participate in the formation of inhibitory circuits and control the activity of excitatory glutamatergic pyramidal neurons (PNs). The main cortical INs (cINs), which contribute to around 60% of the total cIN population, include two cardinal IN types, defined by the expression of the calcium binding protein Parvalbumin (PV) and the neuropeptide Somatostatin (SST). The timeframe and mechanisms that underlie the maturation process of PV- and SST-expressing cINs, and their subtypes, remain mostly elusive. In the current thesis, we performed bulk RNA-seq, at critical developmental stages, to identify how the transcriptomic landscape of SST+ cINs evolves during the first postnatal month. Our findings indicate that SST+ cINs undergo substantial molecular changes throughout the first month after birth, until they acquire their final mature characteristics and physiological properties. The above results were also in agreement with significant changes in the electrophysiological properties, both intrinsic and synaptic, of SST+ cINs, that were determined at the same developmental period. In summary, our results show that the maturation of SST-expressing cINs, during the critical period of the first month after birth, is a dynamic and plastic process. In addition, our work provides significant information on the mechanistic understanding of this process, which can be used to produce SST+ cINS, apt for stem cell therapies, and for elucidating the pathogenesis of interneuropathies.

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PP108-Investigating the role of the ciliary associated gene-AHI1 in cortical development using mouse models and human brain organoids

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The intricate development of the cerebral cortex is essential for brain function, and malfunctions during its development can lead to brain disorders, such as the malformations of cortical development (MCDs) in humans. Studies have shown that disruption of primary cilia (PC), cellular sensory antennae, have been implicated in MCDs, highlighting their role in cortical development. It has been proposed that PC regulate the cell cycle of neural progenitors (NPCs) and affect neuronal migration in mice, however, their role in human brain development is vague. Our study focuses on AHI1, a ciliary-associated gene, mutations of which have been identified in the MCD polymicrogyria. We aim to understand AHI1's role in cortical development and consequently to the mechanisms contributing to MCDs. Comparing single-cell RNA sequencing datasets, we showed that AHI1 expression in humans is higher in NPCs than neurons, while the opposite pattern is observed in all other animals tested. To dissect the role of AHI1 in vivo, we induced ectopic overexpression or silencing in the developing mouse cortex, which altered the number and position of NPCs and neurons. Examination of PC's morphology demonstrated that their length, orientation, and numbers are disrupted upon AHI1 manipulation. To explore the human-specific mechanisms of cortical development, we manipulated AHI1 in human brain organoids. Our preliminary data suggest changes in the numbers of NPCs and neurons differing from the mouse model. Our ongoing research in brain organoids will provide insights into the role of PC in human cortical development and in the fundamental mechanisms underlying MCDs.

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PP109-Effect of acrylamide intoxication on behavioral parameters and the cholinergic system of brain regions

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Acrylamide neurotoxicity poses a significant public health concern. This chemical compound, which is commonly formed during the high-temperature cooking of certain foods, has been linked to altered behavior and neurotransmission in mice. Studies have demonstrated that exposure to acrylamide can disrupt the balance of neurotransmitters, leading to behavioral changes such as impaired motor coordination and cognitive function. Additionally, acrylamide intoxication has been found to impact the cholinergic system in various ways, including interference with acetylcholine release. The aims of the present study were to investigate the effects of acrylamide on behavioral parameters (anxiety-like behavior, mobility), cholinergic markers (AChE activity, ACh levels), and BDNF levels in different brain regions of male mice. The mice were divided into two groups: the Acrylamide group and the control group. Acrylamide was administered intraperitoneally (20 mg/kg body weight in saline) for five days. Twenty-four hours after the final administration, behavioral analysis was conducted. Behavior was assessed using the open-field test, followed by analysis with video-tracking software (Any-maze 6.3). Acetylcholinesterase activity in the brain was determined in both salt-soluble and detergent-soluble fractions using Ellman's colorimetric method. Acetylcholine levels were determined using Hestrin's method. Brain-Derived Neurotrophic Factor (BDNF) levels were assessed through ELISA, as it is a protein responsible for neurogenesis, as well as the growth, maintenance, and survival of neurons in the nervous system. Behavioral studies revealed an increase in anxiety-like behavior and a decrease in mobility after intoxication. Furthermore, acrylamide exposure appears to reduce acetylcholinesterase activity, acetylcholine and BDNF levels.

PP110-The effects of chronic stress on the microbiome of an in vivo experimental alpha-synuclein – associated Parkinson's disease.

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Parkinson's disease (PD) is associated with non-motor symptoms, including gastrointestinal dysfunction, often preceding the onset of motor symptoms, even by decades. With increasing evidence supporting a role of the HPA axis activation in the pathogenesis of PD, we explore the effects of two different models of chronic stress in a prodromal model of PD-rats overexpressing the human alpha-synuclein gene in a BAC construct (BAC). Since the HPA axis and the metabolites produced by gut microbiota enable an indirect bidirectional communication between the brain and the gut, to identify the effect of psychological (chronic unpredictable stress - CRUST) or pharmacological stress (chronic corticosterone - CORT), on BAC vs. wildtype animals, a 2x2 experimental design was applied. For the quantification of microbial metabolites, specifically, short chain fatty acids (SCFAs), freshly collected feces were analyzed by LC-MS/MS. To examine the diverse microbial populations residing in the gut, 16S rRNA sequencing data were analyzed to identify changes in the microbiome. While our finding of reduced fecal SCFAs -particularly of acetic acid in the BAC animals- agrees with findings in PD patient feces, stress additionally appears to significantly interact with genotype to alter the microbiota populations of Subdoligranulum, Tyzzeralla, Turicibacter, Peptococcus, Faecalibacterium and Dubosiela, depending on the stress paradigm. Overall, while the exact signaling pathways have yet to be uncovered and the nature of microbiome metabolism and population changes observed is multifaceted, the gut-brain axis represents an increasingly important source of biomarkers that aid our understanding of the neurodegenerative interplay between stress and alpha-synuclein burden.

Funding Sources: HFRI, GSRI and IKY

PP111-Fine Motor Deficits in the 5xFAD Mouse Model of Alzheimer's Disease

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Background: Alzheimer's disease (AD) is characterized by memory, cognition, and motor function deficits. AD transgenic mouse models show similar motor difficulties based on behavioral tests. The main objective of this project was to investigate the fine motor skills of the 5xFAD Alzheimer's mouse model.
Methods: 3- and 9-month-old 5xFAD mice were compared to their wild-type (WT) littermates with three motor behavioral assays (n=52 mice). Rotarod was used to assess motor coordination, and balance beam was conducted at three beam lengths (15, 10, and 6 mm-width) to evaluate the fine motor coordination and balance. Single-pellet reaching test was performed to assess the fine skilled limb movement.
Results: 9M 5xFAD mice had a lower rotation speed compared to 9M WT mice in the rotarod test. Balance beam results showed that 9M 5XFAD mice traverse each beam at greater time, and slower speed. A performance scoring revealed that 9M 5xFAD mice were the most affected group with a significant impairment at 6 mm with increased presence of dragging and foot slips. Single-pellet reaching test showed an impairment in fine limb movement mostly in 9M 5xFAD mice, since 3M, 9M WT, and 3M 5xFAD mice achieved significantly higher success rates and speeds to reach successful the food-pellet compared to 9M 5xFAD mice.
Conclusions: This study provided the first proof of fine motor deterioration in 9M 5xFAD mice. Older transgenic mice presented the most impaired performance in all the behavioral assays, suggesting that advanced AD-related pathology may have a role to play in fine movements.

Funding source: School of the Cyprus Institute of Neurology and Genetics

PP112-Chronic corticosterone treatment exacerbates pS129 alpha-synuclein pathology in the hypothalamus of a prodromal rat model of alpha-synuclein-associated Parkinson's disease.

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Parkinson's disease (PD) is the fastest growing neurodegenerative disorder affecting millions of people worldwide. PD is linked with non-motor symptoms, that often precede the manifestation of motor symptoms that typically occur in advanced stages of the disease, when there is already a significant depletion of dopaminergic neurons. Studies suggest that chronic stress may lead to an earlier onset or exacerbation of motor deficits in PD patients. We hypothesize that chronic corticosterone administration exacerbates alpha-synuclein burden in brain regions associated with the stress response, the hypothalamus and the hippocampus, and aggravate neurodegeneration along the nigrostriatal axis. Histological analysis in an animal model of PD overexpressing the human wildtype alpha-synuclein gene in a BAC construct, revealed that already enhanced phosphorylated alpha-synuclein (pS129) expression in the hippocampus of transgenic BAC rats was not further increased. Transgenic animals treated with chronic corticosterone had significantly higher levels of pS129 (threefold) in the hypothalamus, the initiator of the stress response and HPA axis activation. Additionally, immunofluorescence staining confirms the robust presence of pS129 alpha-synuclein pathology in dopaminergic neurons of the hypothalamus. Moreover, treatment with corticosterone fueled degeneration of tyrosine hydroxylase positive dopaminergic neurons in the substantia nigra and the corresponding afferents of the dorsolateral subdivision of the striatum. Further biochemical characterization of total and pS129 alpha-synuclein in the soluble and insoluble fractions of hippocampal and striatal tissue revealed significant changes associated with the overexpressed alpha-synuclein phenotype. These results contribute to a better understanding of the interplay between chronic stress and the alpha-synuclein pathology in PD.

Funding sources: HFRI, GSRI

PP113-The neuronal locus of habituation in the giant fiber system of Drosophila

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Habituation is a form of non-associative learning expressed as a response decrement after repeated stimulation. In Drosophila, the Giant Fiber System (GFS) is a sensory-motor circuit that transmits light and wind inputs through interneurons to the giant fiber neuron and sequentially to motor neurons and corresponding jump and flight muscles. The activation of the GFS circuit evokes the escape behavior of the animal and is amenable to habituation, but the neuronal locus of habituation is not known. In our study we employed the GAL4/UAS system to overexpress or downregulate particular genes and performed electrophysiological recordings to investigate possible contribution to habituation of two groups of neurons ((lobular columnar, type 4 – LC4, and lobula plate/lobula columnar, type 2 - LPLC2) that are presynaptic to the giant fiber neuron. Overexpression of tetanus toxin (cleaves synaptobrevine and blocks synaptic transmission) sequentially to the groups of presynaptic to GF interneurons revealed that silencing the LC4/giant fiber synapses, but not the LPLC2/giant fiber synapses, resulted in failure of giant fiber action potential generation, suggesting that LC4 transmission is crucial for GF supra-threshold activation. Furthermore, we used RNAi to downregulate Shaker K+ channels in LC4 and LPLC2 neurons and we examined the habituation dynamics of the GFS by delivering 1K stimuli at 5 and 10 Hz. The Shaker down-regulation caused delayed habituation but more pronounced for LC4 neurons, one more indication that the LC4 neurons are more important for habituation. These data allow us to hypothesize that the LC4 neurons are the locus of habituation.

PP114-Satb1 deletion in cortical interneurons leads to an aberrant cortical inhibitory network and autism-like behaviors

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GABAergic interneurons comprise 15-25% of all neurons in the cortex. 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, although begins from early embryonic stages and depends on the unique combinatorial expression of transcription factors that characterize the place of origin of each interneuron type, it is better manifested at postnatal stages, when interneurons acquire their mature properties via inducing additional genetic programs, as a response to emerging environmental cues, such as network activity. We have previously shown that Satb1 is an activity-regulated transcription factor that is expressed at late embryonic stages, in the lineages of MGE-derived, parvalbumin (PV) and somatostatin (SST)-expressing interneurons, and it is implicated in their differentiation[1]. Here we investigate the cell-autonomous mechanism of Satb1 function in cortical interneurons. We provide evidence that in the absence of Satb1 function, MGE-derived cortical interneurons show an aberrant transcriptional profile, with genes implicated in synapse organization, ion channel transmission, and neuropeptide regulation being down-regulated. These data are further supported, by defects in the morphology, as well as synapse number, of Satb1 mutant interneurons, both in vivo and in vitro. This disrupted inhibitory network, results in the manifestation of autism-like behaviors, in Satb1cKO mice.
We conclude that SATB1 orhestrates a late-onset developmental molecular program in cortical interneurons, that when disturbed contributes to the onset of autism.

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PP115-Assessment of the mechanisms of dying-back axonopathy following Chaperone-Mediated Autophagy malfunction in the rat nigrostriatal axis

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Chaperone-mediated autophagy (CMA) is a major pathway for α-synuclein degradation. In the rat substantia nigra (SN), inhibition of CMA via Adeno-Associated Virus (AAV)-mediated expression of shRNA targeting the rate-limiting step of the pathway, the LAMP2A receptor, leads to progressive nigrostriatal neurodegeneration and accumulation of alpha-synuclein, thus mimicking major aspects of PD.
The primary objective of this study was to explore the early impacts of CMA impairment on dopaminergic axonal-synaptic elements, at a time point preceding overt neurodegeneration. In order to inhibit CMA, we stereotaxically injected viruses expressing shRNAs targeting LAMP2A or scrambled control in the rat SN. At 2 and 3 weeks post-injection, we assessed the nigrostriatal projections and their isolated synaptosomes with immunohistochemistry and immunoelectron microscopy. We carried out the molecular cloning of a plasmid designed to express the monoamine transporter VMAT2, tagged with the fluorescent molecule mCherry.
CMA impairment triggered an early "dying-back" effect of dopaminergic neurons, accompanied by abnormal accumulation of autophagosomes at degenerating synaptic terminals. We successfully constructed a vector encoding a chimeric VMAT2-mCherry protein under the control of the neuronal promoter Synapsin1 and verified its expression in neuronal cells. This vector can be used to transduce nigral dopaminergic neurons in this model, and, through Fluorescence-Activated Synaptosome Sorting (FASS), to enable the selective isolation and study of nigrostriatal synaptic elements.
These results have significant implications for understanding the interplay between protein degradation pathways and the mechanisms of dying-back axonopathy thought to occur in PD, shedding light on synaptic alterations that eventually lead to dopaminergic neurodegeneration.

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PP116-Human Tau aggregates are permissive to Protein Synthesis Dependent Memory in Drosophila Tauopathy models

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Tauopathies including Alzheimer's disease, are characterized by progressive cognitive decline, neurodegeneration and intraneuronal aggregates comprised largely of the axonal protein Tau. It has been unclear whether cognitive deficits are consequent of aggregate accumulation which compromise neuronal health and eventually lead to neurodegeneration. We use the Drosophila Tauopathy model to reveal a specific defect in Protein Synthesis Dependent Memory (PSD□M), but not in its Protein Synthesis Independent variant. We demonstrate that these neuroplasticity defects are reversible upon suppression of new transgenic human Tau expression, but surprisingly correlate with an increase in Tau aggregates. Inhibition of aggregate formation results in re□emergence of deficient memory in animals with suppressed hTau0N4R expression. Significantly, in hTau0N3R□expressing animals with normal memory and elevated aggregates after inhibition of aggregate formation results in PSD□M deficits. Deficient PSD□M upon human Tau expression in the Drosophila CNS is not consequent of toxicity and neuronal loss because it is reversible. Furthermore, PSD□M deficits do not result from aggregate accumulation, which appears permissive, if not protective of processes underlying this memory variant.

PP117-Neurophysiological alternations in Working Memory Training

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Working Memory (WM) is the ability to store and adaptively handle information in a timescale of seconds, in order to plan and execute complex cognitive tasks. Except for the prefrontal cortex, hippocampus also has a pivotal role -especially in spatial WM. While the neural correlates of WM have been extensively researched, the effects of training, especially in animal models, have not been studied. Previous work from our lab showed that WM training enhanced synaptic plasticity in the prefrontal cortex and synaptic transmission on the hippocampus in male mice and hippocampal synaptic plasticity. Our aim was to determine the effects of WM training, using the T-maze Delayed Alternation Task on the spontaneous activity of hippocampal brain regions as well as long-term potentiation (LTP) enhancement in female mice. Mice were seperated in two training groups: the adaptive group performed the delayed alternation task, while the non-adaptive the alternation procedure. A third group remained in their homecage (control). Following training, the mouse brain was harvested for electrophysiological recordings. Spontaneous local field potentials (LFPs) were taken to measure spontaneous activity properties in the CA1,CA3 and DG subfield of the hippocampus immediately after training, while evoked field excitatory postsynaptic potentials (fEPSPs) were acquired to measure LTP 2 weeks after training. Our results showed that oscillatory properties were elevated immediately after training in CA1 and CA3 regions of the hippocampus. LTP in CA1 hippocampal region was enhanced in the adaptive and non-adaptive groups compared to controls 2 weeks after training.

PP118-Effect of cannabidiol on anxiety-like behavior from adolescent to aged male mice

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Age-related changes have been linked to behavioral manifestations and alterations in the cholinergic system. This study aimed to investigate anxiety-like behavior and mobility in three age groups of male mice as they age, after Cannabidiol (CBD) treatment. Mice were divided into the following age groups: a) adolescent (1-month-old), b) adult (3-4 months old), and c) aged (13-14 months old). Each age group was divided in 2 groups: CBD group (CBD 10mg/kg, 10%DMSO, 2% tween-80) and Control group (saline, 10%DMSO, 2% tween-80). CBD was administered intraperitoneally for 10 days. Behavioral analysis was performed 24 hours after the final administration, evaluating anxiety-like behavior and mobility using the open-field test in a 10min task, followed by analysis with video-tracking software (Any-maze 6.3). In particular, total distance travelled, time in the peripheral zone, number of entries to the center zone were some of the behavioral markers that were analysed. The results indicated that the adolescent and aged groups displayed significantly higher levels of anxiety compared to the adult group. Concurrently, mobility was observed to decrease in the adolescent and aged groups in comparison to the adult group. Furthermore, the outcomes revealed an anxiolytic-like effect and increased mobility after CBD treatment across all age groups.

PP119-Deep learning synthesis of optimal stimuli across mouse lateral visual hierarchy

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Invariant object recognition is the ability of animals to rapidly recognize objects irrespective of variations in their appearance. This remarkable ability is mediated by a set of hierarchically organized interconnected visual areas, the ventral visual stream. To identify the computational role of neurons along the ventral stream, deep neural networks have been routinely used, due to their hierarchical organization. While deep learning approaches have explored optimal stimuli in mouse visual areas, they have mostly been restricted to functional imaging data from the primary visual cortex. Here, we seek to expand these approaches by using large-scale electrophysiological data from multiple visual cortical areas to generate a digital twin of the mouse visual cortex. The digital twin model allowed us to synthesize optimal visual stimuli that maximally drive the neurons (most exciting inputs - MEIs). Specifically, by using the Neuropixels probes we simultaneously recorded the activity of hundreds of neurons in vivo in mouse visual areas in response to natural images. We then trained a convolutional neural network to predict the responses of each neuron recorded across the different areas and generated a set of MEIs that would optimally excite the recorded neurons. Subsequently, we showed the optimized stimuli back to the mice and recorded the activity of the same neurons. This enabled us to verify in vivo the results of the in silico model predictions. Such closed loop approaches will ultimately allow us to dissect the role of hierarchical processing in complex cortical computations such as invariant object recognition.

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PP120-Spontaneous and inducible CD8 T cell lesions in the brain and spinal cord of HLA-DR15-positive MS PBMC humanized mice

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Autoimmune diseases of the central nervous system such as multiple sclerosis (MS) are only partially represented in current experimental models and the development of humanized immune mice is crucial for better understanding of immunopathogenesis and testing of novel therapeutics. We describe a humanized mouse model with several key MS features. Severely immunodeficient B2m-NOG mice were transplanted with peripheral blood mononuclear cells (PBMC) from MS and healthy (HI) donors and showed rapid engraftment by human T and B lymphocytes. DR13-positive MS PBMC mice developed low levels of graft versus host disease (GVHD) and no CNS inflammation. Both DR15 MS and DR15 HI mice developed spontaneous and EAE-inducible infiltration of CNS barriers and parenchyma by CD8+ and CD4+ T cells. DR15 MS mice uniquely developed spontaneous T cell lesions in brainstem and spinal cord grey matter, and large EAE-inducible lesions in the brain corpus callosum, with relatively low GVHD levels compared to DR15 HI mice. Main limitations to be further addressed in this model are the poor monocyte engraftment and consequently lack of CNS demyelination, and absence of lymph node organization and IgG responses. These results show that PBMC humanized mice represent promising experimental tools for MS immunopathology and for testing experimental immunotherapeutics in a patient-specific approach.

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PP121-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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Parkinson's Disease (PD) is a prevalent debilitating neurodegenerative disorder, for which no disease-modifying therapy is available. One key feature of PD is the accumulation in specific brain regions of alpha-synuclein (aSyn) aggregates, which are likely to be the disease-causing entities. A widely used animal model for PD involves injecting recombinant aSyn preformed fibrils (PFFs) into the striatum, replicating key PD characteristics.
To investigate the potential of reducing aSyn levels as a treatment strategy, we employed novel brain-penetrating viral vectors (PHP.eB AAVs). These vectors, expressing a microRNA or a shRNA targeting mouse Snca, encoding for aSyn, were intravenously administered in mice, while respective scrambled control sequences were used as controls. After a week, human aSyn PFFs were injected into the dorsal striatum to assess the impact of aSyn downregulation on PFF-induced pathology, at 3 months post-injection. Our study included behavioral and histochemical analyses to evaluate any improvements in phenotype associated with aSyn downregulation following PFF injection.
Our findings demonstrate the successful widespread transduction of PHP.eB AAVs throughout the CNS, including the substantia nigra, effectively reducing endogenous mouse aSyn protein levels. Injection of aSyn PFFs led to the formation of phosphorylated aSyn cytoplasmic inclusions, resembling human Lewy body pathology, in brain regions connected to the injection site, while behavioral deficits were also evident. AAV-mediated downregulation of endogenous aSyn reduced the accumulation of phosphorylated aSyn and alleviated motor impairments.
This non-invasive delivery strategy holds promise for treating neurodegenerative diseases with widespread pathology, such as a-Synucleinopathies.

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PP122-Assessing pTau181 and pTau217 in the blood as biomarkers for Alzheimer's disease using cutting-edge SIMOA technology

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Introduction: The emergence of ultra-sensitive assays pioneered by the single molecule array (SIMOA-Quanterix®), has enabled the precise measurement of neurological biomarkers in blood (overcoming the need for cerebrospinal-fluid, CSF). Tzartos NeuroDiagnostics is already utilizing this technology to measure NFL, GFAP, and phosphorylated-Tau (pTau) levels in blood, as preliminary prognostic and diagnostic biomarkers of multiple sclerosis and Alzheimer's disease (AD). In the context of early detection of AD, the plasma pTau proteins have been shown as very promising biomarkers. Plasma pTau181 distinguishes AD from other neurodegenerative disorders (NDD) and healthy controls, and correlates with CSF pTau181 and Amyloid- and Tau-PET imaging scores[1-4]. Also, plasma pTau217 discriminates AD from other NDD with higher accuracy and was recently proposed to be incorporated into a diagnostic workflow to detect AD in memory clinic settings[5].
Goal: The independent validation of the diagnostic accuracy of SIMOA pTau181 and pTau217 plasma assays for AD patients.
Methods: Established AD CSF biomarkers (Aβ42/40, pTau, and tTau) were measured with Lumipulse immunoassays. 26 participants were classified as ADCSF if their Aβ42/40 ratio was <0.063 (N=12 for ADCSF and N=14 for non-ADCSF). Plasma pTau181 and pTau217 were measured using the SIMOA HD-X instrument in "Tzartos NeuroDiagnostics". To evaluate the efficacy of pT181 and pT217 we computed areas-under-the-curve (AUCs), Pearson correlation coefficient (r), sensitivity and specificity.
Results: Each plasma pTau181 and pTau217 was significantly higher in the ADCSF group compared to the non-ADCSF group and significantly discriminated abnormal CSF Aβ42/40 ratio. Combination of both plasma biomarkers further increased sensitivity of the approach.

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PP123-Assessment of the effects of alpha-Synuclein modulation on the dopaminergic system integrity of a mouse model of Chaperone-Mediated Autophagy inhibition

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alpha-Synuclein (AS) accumulation plays a central role in Parkinson's disease (PD). Dysregulation of the mechanisms involved in AS degradation, such as the lysosomal pathway chaperone-mediated autophagy (CMA) may underlie disease pathogenesis. We have previously shown that CMA inhibition in the rat substantia nigra (SN), led to AS accumulation and degeneration of the nigrostriatal pathway. Herein, we aim to assess the impact of AS knockdown or overexpression on the dopaminergic system integrity, in the context of CMA malfunction induced by downregulation of the CMA's rate limiting step, the LAMP2A receptor.
To this end, we down-regulated the LAMP2A receptor in the nigra of both wild-type (WT) and AS knockout mice and assessed the effects on the mouse dopaminergic system eight weeks post-injection, with immunohistochemistry and biochemical analyses. In addition, we simultaneously down-regulated the LAMP2A receptor and overexpressed the human WT AS and three weeks post-injection we examined the effects on the nigrostriatal axis, AS levels/pathological conformations.
Extensive neurodegeneration was noted along the nigrostriatal axis following CMA inhibition in both WT and AS knockout mice. AS overexpression led to increased levels of phosphorylated alpha-synuclein, considered a pathological form, which interestingly remained unchanged after CMA pathway suppression. At this early time point, no evident neurodegeneration was observed.
This study successfully replicated CMA pathway impairment as a model for AS accumulation in mice, and highlights the significance of AS-independent mechanisms in dopaminergic SN neurodegeneration subsequent to CMA inhibition. These findings contribute valuable insights into the complex relationship between CMA, AS, and neurodegenerative diseases.

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PP124-Exploring genomic variation in loci associated with Tourette Syndrome

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Tourette Syndrome (TS) is a neuropsychiatric multifactorial disease characterized by chronic vocal and motor tics. Despite the intense efforts during the past years, the origin of its clinical phenotype remains unclear. Scientists have tried to approach the etiopathological basis of TS by focusing on genetic and environmental factors, that highlight pathways likely associated with its clinical picture. In 2019 it was speculated, for the first time, that a non-functional interaction between two transmembrane proteins, namely SLITRK1 and PTPRD, of the synaptic cleft is associated with TS. An equally interesting field of study seems to be the endocannabinoid system; the inhibition of tics mediated by the use of cannabinoids, among others, seems very promising. The endocannabinoid system is an interesting target of study for TS, as it is involved in the regulation of neurotransmitters in the basal ganglia and, by extension, in the regulation of movement, possibly affecting tic-like behaviors differently in the two sexes.
Here we present our genetic association study focusing on loci that encode the interaction domains of SLITRK1 and PTPRD from cases previously diagnosed with either TS or chronic tics. In parallel, we explore the association of genetic variation of the CNR2 gene, which encodes for the CB2 receptor, with sex bias in boys and girls of Caucasian origin diagnosed with TS. Despite the careful selection and phenotyping of the study sample, the small number of cases contributed negatively to the discovery of any novel association with variation in the sequences of interest.

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PP125-The Role of Small Extracellular Vesicles in α-Synuclein Transmission

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A major pathological feature of PD is the accumulation and transmission of misfolded assemblies of alpha-synuclein (α-Syn). Small extracellular vesicles (sEVs) have emerged as key players in intercellular transfer of α-Syn in PD and related synucleinopathies. The current study aims to examine the intracellular trafficking pathway of sEVs in glial cells linked with the sEV-associated α-Syn transmission. Glial primary cultures were incubated with DiI-stained mouse brain-derived sEVs, in the absence or presence of recombinant fibrillar human α-Syn (pre-formed fibrils, PFFs). The internalization and trafficking pathways in cells treated with pharmacological reagents that block the endocytic pathways, were analyzed by immunofluorescense, using Imaris imaging analysis software. PFFs were internalized by both microglia and astrocytes at early time points of PFF incubation; however, in microglia, PFF uptake was faster. In the presence of sEVs, a delay in the PFF uptake was observed in both glial cell types. Treatment with dynasore, that inhibits dynamin-dependent endocytosis, affected PFF uptake only in the absence of sEVs. sEV-associated PFFs seemed to utilize macropinocytosis and/or phagocytosis as the main pathway of endocytosis. Fibrillar α-Syn species when associated with sEVs enter the endosomal pathway and are targeted to the lysosome for clearance. In the absence of sEVs, only a portion of fibrillar α-Syn is sorted to the endolysosomal pathway. Our data indicate that brain-derived sEVs serve as scavengers and mediate a rather cell-to-glia transfer of α-Syn which is targeted to the endolysosomal pathway, suggesting a beneficial role in glia-mediated clearance of toxic protein aggregates, present in neurodegenerative diseases.
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PP126-Overexpressing LAMP2A receptor as a means to counteract olfactory deficits in a rat synucleinopathy model.

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Accumulation of alpha-synuclein (AS) is thought to underlie Parkinson's Disease (PD) pathogenesis. Enhancement of the lysosomal degradation pathway of Chaperone-Mediated Autophagy (CMA) has been proposed as a therapeutic strategy to counteract AS pathology. Whether this strategy can be effective beyond the nigrostriatal axis, and can curtail ongoing AS deposition and reverse relevant behavioral deficits is unknown.
We assessed abnormal AS accumulation in the olfactory system of human WT AS-overexpressing BAC transgenic (ASBAC) rats, and to investigate whether overexpression of LAMP2A, the rate-limiting step in CMA, could counteract and/or reverse the aberrant AS deposition and resultant behavioral effects.
We performed Western immunoblotting and immunohistochemistry with various AS antibodies on material derived from olfactory regions such as the olfactory bulb (OB), the anterior olfactory nucleus (AON) and the piriform cortex (Piri) of WT and ASBAC rats. Olfaction was assesed using a habituation-cross-habituation task. We injected AAV-HA-Lamp2a or AAV-GFP in the AON in 3 or 5 month-old BAC Tg rats and assessed AS pathology and behavioral effects 2 months later in each case.
In ASBAC rats there was accumulation of total, human and phosphorylated AS in the OB, AON and Piri at 4, 8 and 12 weeks of age, and an olfactory dysfunction at 12 weeks. AAV-HA-Lamp2a injection led to a modest (around 20-30%) amelioration of AS accumulation and olfactory behavior, compared to AAV-GFP-injected rats. Despite the limited transduction of the olfactory system, these results demonstrate the potential of enhancing CMA as a therapeutic strategy in ongoing synucleinopathy beyond the nigrostriatal system.

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2.We acknowledge support of this work by the project "Innovational Multi-Vision, Multi-photon Laser Microscopy System for Advanced 3D Diagnostic Applications and Preclinical Studies" (MIS 5060306) funded by the Regional Operational Programme "Attica" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund.

PP127-Evaluating Peakedness Measures for Neuronal Encoding of Visual Regularity

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Regularity appears to be a ubiquitous visual attribute in patterns and textures. Assessments of partial regularity are a common part of everyday life, and observers consistently agree on their judgments [1], [2]. Ouhnana et al. [3] and Yamada et al. [4] have demonstrated that regularity is an adaptable perceptual dimension, suggesting a specialized representation in the human neural system. Ongoing research [5] is focused on modeling the neuronal encoding of regularity in the visual system. The basis of such models involves a filter-rectify-filter process in low-level vision that yields a distribution of neuronal energy responses across scale. Regular patterns, marked by distinct repetitions, exhibit a pronounced peak in this energy spectrum, aligning with the spatial scale of repetition. In contrast, less regular patterns present a less pronounced peak. By employing point patterns with varied presentation conditions (dot size, spacing, and number), we can manipulate the energy spectrum and conduct discrimination experiments on regularity [6]. This enables an exploration of the relationship between discrimination performance and the peakedness of the distribution. However, the concept of peakedness is not clearly defined. Here, I evaluate the predictive accuracy of a list of peakedness measures, with the ultimate goal of identifying a readout mechanism that enables a higher-level neuronal set to interpret the distribution of responses.

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PP128-Generation of vascularized human pluripotent stem cells derived retinal organoids as a platform to study retinogenesis and disease modeling

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Retinal diseases, such as diabetic retinopathy and age-related macular degeneration are the major causes of blindness nowadays. It has been shown that the dysfunction in the relationship between the neuroretina and the vascular system (neurovascular unit-NVU) plays a crucial role in the pathophysiology of these diseases. In vitro retinal model development has gained momentum due to the inadequacy of animal models in replicating the structure and function of the human retina. Human embryonic and induced pluripotent stem cell (iPSC)-derived retinal organoids(ROs) have demonstrated diverse applications, such as investigating human retinogenesis, modeling diseases and drug discovery. Multiple protocols have been established to generate ROs aligning with fundamental principles of forebrain and eye development, in which the consistent laminar organization and the presence of all neural cell types within the retinal structures is significant. However, they lack vascularization and thus their maturation is impaired. Our work is focused on the generation of human PSCs-derived ROs consisting of both neuronal and vascular cells. We have already generated and extensively characterized endothelial (ECs) and mural(MCs) cells derived from hPSCs/hiPSCs. Furthermore, hPSCs/hiPSCs-derived ROs have been generated and characterized using a sequential step strategy, mimicking the spatio-temporal development of the retina in vivo. Our plan is to vascularize these ROs in order to develop the rNVU in the best anatomical layout. Furthermore, our in vitro rNVU will serve as a model to elucidate the pathophysiology of Retinitis Pigmentosa (RP) (an inherited disease causing blindness) using patient-derived iPSCs with a PRPF31mutation, known to be responsible for RP development.

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PP129-Investigating the transition dynamics of endogenous cortical activity to epileptiform discharges with a phase space reconstruction methodology

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The state of a dynamical system can be depicted as a point in a multidimensional space, the Phase Space, each axis of which corresponds to a variable involved in the system's behavior. As the system evolves through time, a corresponding trajectory is created in the Phase Space which describes its behavior. One of the most powerful tools used in nonlinear dynamical systems study is the Phase Space Reconstruction (PSR) method. Takens' Embedding Theorem [1] and the work of other researchers [2] provide the mathematical foundation supporting PSR. In practice a dynamical system may be determined by several variables whose measurement can be rendered impossible. The power of the PSR method lies in the fact that using a one-dimensional time series coming from the system, a Reconstructed Phase Space can be created that, although different from the original one, preserves its dynamics.
We have used PSR to gain insights on the transition of the spontaneous UP/Down states network activity of ex vivo cortical slices, considered the default cortex activity [3,4], into a final hypersynchronous epileptiform state, through various sequences of affected neuronal behavior patterns. This is a complex phenomenon [5], not following strict stereotypical paths of evolution, and our analyses of the networks reconstructed trajectories (generated from the Local Field Potentials signals) are being performed with the use of various suitable metrics, as Lyapunov exponents, fractal dimension metrics, several entropies and informational complexity measures, etc. Our results exhibit the potential to elucidate several aspects of the mechanisms underlying this dynamical transition.

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PP130-KLK6 is as a new regulator of amyloidogenesis in Alzheimer's disease

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The gradual accumulation of extracellular amyloid β (Aβ)-containing plaques is the pathological hallmark of Alzheimer's disease (AD). KLK6 is trypsin-like serine protease highly expressed in the nervous system. The failure of γ-secretase inhibitors in treating AD has turned the interest in the identification of new mechanisms accounting for turnover of Aβ aggregates. In this direction, the identification of new enzymes that process the APP or Aβ peptides is of major importance. Here, we demonstrate that KLK6 readily cleaves soluble APP, Aβ1-42 peptides, and Aβ1-42 fibrils. The cleavage positions were mapped with mass spectrometry. The KLK6-cleaved Aβ1-42 peptides could aggregate to higher extend than the non-cleaved Aβ1-42 peptides. To provide in vivo evidence for the role of KLK6 in AD, the 5xFAD mice were crossed with the Klk6-/- mice to generate the 5xFADKlk6+/-. The 5xFADKlk6+/- had lower amyloid deposits in the hippocampus and piriform cortex at 10 months compared to 5xFAD. Also, the 5xFADKlk6+/- showed lower levels of phospho-Tau accumulation and higher ARC expression in the brain relative to 5xFAD. Further, the 5xFADKlk6+/- show better performance in elevated plus maze and clasping at 6 and 10 months. To this end, it should be noted that the heterozygous mice 5xFAD Klk6+/- better resemble the chemical inhibition that occurs during therapeutic approach with small molecules compared to the knockouts. In conclusion, KLK6 appears to exert an amyloidogenic action and provides a new target for pharmacological intervention of AD.

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PP131-Peptidergic modulation of motor neuron output via CART signaling at C bouton synapses

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Mammalian spinal circuits contain populations of interneurons that regulate the rhythm and pattern of movements such as locomotion, whilst the intensity of movements is largely determined by the properties of motor neurons that convey network output to muscles. Here we describe complementary roles in the control of movement vigour for acetylcholine and a novel signalling peptide, the cocaine amphetamine related transcript (CART), that we have identified at prominent C bouton synapses on motor neurons. Whole-cell patch clamp electrophysiology revealed distinct, yet parallel mechanisms for CART and acetylcholine in the amplification of motor output. We found that CART facilitates recruitment and firing rates at the lower end of the input range whereas muscarine increased maximal firing rates at the upper end of the input range in fast but not slow motor neurons. We also found that mice with broad genetic deletion of CART or selective elimination of acetylcholine from C bouton source cells exhibit deficits in behavioural tasks that require higher levels of motor output. Intriguingly, impaired motor function following deletion of CART or acetylcholine is not due to altered pre-or post-synaptic C bouton structure given that we find no changes in gross C bouton morphology or post-synaptic M2 receptor organization when CART or acetylcholine are removed independently or in tandem. Together these data provide novel insight into roles for spinal interneurons in the control of movement gain, through the selective modulation of motor neuron subtypes that support movements that require a high degree of muscle force.