

PhD Meeting SINS

Pisa 2025

*“Neuroscience Reimagined:
Young, Bright, and Excellent Minds at Work”*

Teatro Verdi, Via Palestro, 40,
56127 Pisa (PI)

10 September 2025

h09:00 – h14:00

The event is entirely free for PhD
applicants that are part of the
SINS community and already
possess a **SINS** membership

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CONGRESS VENUE

The PhD Student National Meeting in neuroscience will be held at **Teatro Verdi** located in Via Palestro, 40, Pisa.

Arriving by plane 

Nearest airport: **Galilei International Airport** (4 km from venue)

- By bus
 - Take the PISAMOVER from Piazzale d'Ascanio (outside the airport) to Central Railway Station
 - Take bus line 3+ at via Cesare Battisti and get off at **Mediceo 1** (170m from the venue)
 - Ticket price: €8,20 (available on the bus)
- By taxi
 - Taxis are available in front of Arrivals; ask for "Teatro Verdi"
 - Estimated Fare: €10-20

Arriving by train 

Nearest train station: **Pisa Centrale** (2 km from venue)

- By bus
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- By foot
 - You can easily walk to the venue from the station, about a 15-minute walk (1 km from the venue) along Pisa's main street (Corso Italia) and over the beautiful Ponte di Mezzo (200m from the venue)

DETAILED PROGRAM

09:00 Opening Remarks

Session 1: "From Development to Disease Building and Breaking the Nervous System"

Chairs: Dr Borgonovo Giulia & Dr Serra Marcello

09:10 **Dr Bonato Martino, University of Turin**

Targeting oligodendroglia sustains brain development in MCPH17 microcephaly models

09:25 **Dr Amoruso Federica, University of Milan**

Targeting Semaphorin Signaling to Unveil Novel Therapies for CHARGE Syndrome

09:40 **Dr Licastro Ester, University of Naples Federico II**

Timing Matters: How Daily Rhythms Affect Neuroprotection Therapy for Stroke

09:55 **Dr Mongiardi Brunella, Telethon Institute of Genetics and Medicine (TIGEM)**

Identification of Sex-Specific Autophagy Enhancers for Dementia

Session 2: "The Thinking Brain - Circuits Behind Cognition and Behavior"

Chairs: Dr Campanelli Federica & Dr Mancini Giulia

10:10 **Dr Spinola Alessio, University of Milan**

GluA3 Shapes Synaptic Plasticity and Neuronal Structure

10:25 **Dr Palla Giulia, Scuola Normale Superiore**

If You Drink | Drink-Uncovering Social Facilitation Process in Mice



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- 10:40 **Dr Rominto Anita Maria, University of Turin**
Chronic Social Defeat Stress Reduces Prefrontal Cortex Excitability in Mice
- 10:55 **Dr Ferretti Elena, University of Parma**
Cortical Signatures of Yawning: a Possible Mechanism for Social Gating?

Session 3: "Next-Gen Neuroscience - Innovations Transforming Brain Research"

Chairs: Dr Mingardi Jessica & Dr Raffaele Stefano

- 11:10 **Dr Rossi Eros, University of Verona**
Bioinformatic Analysis Identifies a Novel Nerve-Growth Signature of Tumor-Associated Macrophages
- 11:25 **Dr Turrini Giacomo, University of Turin**
Unraveling the Developmental Origins of Cerebellar Astrocyte Heterogeneity
- 11:40 **Dr Lucidi Benedetta, University of Verona**
Generation of Rat Cerebral Organoids as Tools for Regenerative Medicine
- 11:55 **Dr Zanelli Alessio, University of Pisa**
Building the Corticospinal Tract with Cortical and Spinal Organoids

12:10 Coffee/Lunch Break

12:30 Poster Session

13:50 Concluding Remarks

14:00 End of works



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This event was organized by:

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Abstracts selected for oral presentation

Session 1: “From Development to Disease Building and Breaking the Nervous System”

Targeting oligodendroglia sustains brain development in MCPH17 microcephaly models

Bonato, Martino⁽¹⁻²⁾; Khastkhodaei Ardakani, Maryam⁽¹⁻²⁾; Astigiano, Cecilia⁽¹⁻²⁻⁴⁾; Di Cintio, Niccolò⁽¹⁻²⁾; Di Cunto, Ferdinando⁽¹⁻²⁾; Ferrini, Francesco⁽³⁾; Buffo, Annalisa⁽¹⁻²⁾; Boda, Enrica⁽¹⁻²⁾

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Primary autosomal recessive microcephaly-17 (MCPH17) is caused by mutations in CIT, encoding for Citron Kinase (CIT-K), a serine-threonine kinase involved in DNA repair and cytoskeletal dynamics. MCPH17 patients display microcephaly, simplified gyration, seizures, cognitive and motor deficits, and - in case of CIT truncating mutations - neonatal mortality. Cit-k KO mice recapitulate the main features of MCPH17, including epilepsy and early mortality. Besides neuronal defects, both MCPH17 patients and Cit-k KO mice show a severe CNS hypomyelination and reduction of oligodendroglial cells. Hypomyelination, oligodendrocyte apoptosis and senescence were also found in conditional mutant mice where Cit-k was deleted only in oligodendroglia, in line with a cell-intrinsic role of Cit-k loss in producing oligodendrocyte alterations. Of note, hypomyelination and oligodendroglial defects contribute per se (i.e. independently of neuronal defects) to some aspects of pathology (i.e. perinatal susceptibility to epileptic seizures and cognitive impairment at later stages). Thus, we tested the hypothesis that restoring oligodendroglia may positively impact on the neuronal/circuit development and on the functional outcome of Cit-k KO MCPH17 mouse model. Pharmacological and cell-based interventions aimed at restoring oligodendroglia resulted in a reduced epileptic phenotype, better motor performances along with a partial rescue of the cortical inhibitory neurotransmission and upregulation of markers indicative of interneuron maturation. In case of oligodendroglial cell transplantation, Cit-k KO mouse lifespan was also remarkably increased. In conclusion, although in MCPH17 glial cell dysfunctions have been mostly neglected, our data show that targeting oligodendroglia – although not correcting microcephaly - is an effective option to sustain brain development and ultimately ameliorate the functional phenotype of the animal model of the disease.

Targeting Semaphorin Signaling to Unveil Novel Therapies for CHARGE Syndrome

Amoruso, Federica⁽¹⁾; La Rocca, Federica⁽²⁻³⁾; Santonicola, Pamela⁽²⁾; Zampi, Giuseppina⁽²⁾; Paganoni, Alyssa Julia Jennifer⁽¹⁾; Cristofani, Riccardo⁽¹⁾; Di Schiavi, Elia⁽²⁾; Oleari, Roberto⁽¹⁾; Cariboni, Anna⁽¹⁾

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(3) *Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "L. Vanvitelli", Caserta, Italy*

CHARGE syndrome (CS) is a rare genetic disorder characterized by multi-organ malformations, including hypogonadism, developmental delays, and neurological dysfunction, for which no pharmacological treatments currently exist. CS is primarily caused by heterozygous loss-of-function mutations in Chromodomain Helicase DNA binding protein 7 (CHD7), a chromatin remodeler essential for neural crest and neuronal development. Despite CHD7 mutations accounting for most CS cases, the wide clinical variability suggests the involvement of genetic modifiers. Recent evidence implicates semaphorins (SEMA) and their receptors as potential CHD7 genetic interactors. To identify compounds that can restore SEMA gene expression and ameliorate CS phenotypes while bypassing CHD7 haploinsufficiency, we developed a tailored pharmacological screening pipeline using two disease models: a *Caenorhabditis elegans* (*C. elegans*) *chd-7* mutant strain and a CRISPR-engineered neuronal cell line. A screening of 234 chromatin-modifying compounds in *C. elegans* identified five promising candidates that selectively rescued the aberrant developmental phenotypes of mutant worms. To complement this approach, we generated a *Chd7*-depleted mouse neuronal model via CRISPR/Cas9 genome editing. This *in vitro* CS model revealed cellular and genetic signatures linked to impaired cell migration and proliferation pathways, alongside significant dysregulation of SEMA gene expression. To explore SEMA modulation *in vivo*, we also examined the spatiotemporal expression of candidate genes during embryonic head and brain development in a *Chd7*^{Gt/+} gene-trap mutant mouse line that models CS. Our ongoing work focuses on testing candidate compounds identified in *C. elegans* on *Chd7*-depleted neuronal cells to evaluate their potential to rescue aberrant phenotypes via modulation of SEMA gene activity. Preliminary results identified one compound that restored both proliferative and migratory defects of *Chd7*-KO cells, and another that selectively corrected migration abnormalities. These findings highlight the potential of targeting SEMA signaling as a therapeutic strategy for CHD7-related pathologies and lay the groundwork for the development of pharmacological treatments for CS.

Timing Matters: How Daily Rhythms Affect Neuroprotection Therapy for Stroke

Licastro, Ester⁽¹⁻²⁾; Viscardi, Viviana⁽²⁾; Cuomo, Ornella⁽²⁾; Hayakawa, Kazuhide⁽¹⁾; Lo, Eng H⁽¹⁻³⁾; Pignataro, Giuseppe⁽²⁻³⁾; Esposito, Elga⁽¹⁻³⁾

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Despite decades of promising preclinical findings, most neuroprotective strategies have failed in clinical stroke trials. Remote limb ischemic postconditioning (RLIP), a non-invasive intervention involving brief occlusion of a limb artery after cerebral ischemia, has demonstrated efficacy in reducing infarct size in animal models. However, its clinical translation remains limited. One potential reason for this failure may be the circadian mismatch between preclinical studies—typically conducted in nocturnal rodents—and clinical trials involving diurnal humans. To investigate the impact of circadian timing, male C57BL/6J mice were housed under normal or reversed light cycles for three weeks. Transient focal cerebral ischemia was induced either during the mice's inactive phase (ZT1–5/ZT3–9) or active phase (ZT13–17/ZT15–21), followed by RLIP. Infarct volumes were assessed by TTC staining, and striatal expression of PER2, a core circadian clock protein, and neuronal nitric oxide synthase (nNOS), a proposed mediator of RLIP, were measured by Western blot at 1- and 3-days post-ischemia. RLIP significantly reduced infarct volumes when applied during the rodent inactive phase but failed to confer protection during the active phase. RLIP also improved neurological scores when administered during the mice's inactive phase. This time-dependent protection was associated with decreased PER2 and increased nNOS expression, observed only during the inactive phase. These findings suggest that diurnal rhythms critically influence RLIP efficacy, potentially through PER2-nNOS signaling pathways. Given that most strokes in humans occur during the early active phase (morning), which corresponds to the rodent inactive phase, a circadian mismatch may underlie the gap between animal data and clinical outcomes. This study highlights the importance of aligning circadian timing in experimental models of cerebral ischemia. Reassessing RLIP protocols in the context of biological timing may enhance translatability and help optimize the therapeutic window for stroke treatment.

Identification of Sex-Specific Autophagy Enhancers for Dementia

Mongiardi, Brunella⁽²⁻³⁾; Loffredo, Vittorio⁽⁴⁾; Monaco, Mariagrazia⁽²⁾; Somma, Cristina⁽²⁾; Fabiani, Greta⁽¹⁾; Sanzari, Maria⁽¹⁾; Torromino, Giulia⁽¹⁻²⁾; De Risi, Maria⁽¹⁻²⁾; De Leonibus, Elvira⁽¹⁻²⁾

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Aging leads to the accumulation of misfolded proteins, such as β -amyloid and α -synuclein, which are believed to underly the onset of neurodegenerative disorders. Most of the treatments for brain disorders come from pre-clinical evidence in males, although women are more vulnerable than men to Alzheimer's disease (AD), which is the first cause of age-related dementia. However, the mechanisms underlying these sex-differences are not known. To date, two therapeutic approaches are predominantly used to prevent and/or slow neurodegeneration; they include autophagy pharmacological enhancers and exercise training. Autophagy is one of the most relevant catabolic process whereby misfolded proteins are degraded. However, most of the preliminary observations of the efficacy of these treatments have been obtained in male mouse models, although sex-differences in genes expression and response to pharmacological treatments have been reported for non-brain pathologies. In this study, using a mouse model of early aging, we report that female mice have similar cognitive deficits to males, but they occurred earlier. In both sexes this cognitive decline is associated with the accumulation of misfolded protein and impaired autophagy-lysosomal function. However, they differently respond to the precognitive effects of exercise training and autophagy agonists. Similar results were obtained using a genetic mouse model of AD. These findings provide novel and translational relevant preclinical evidence of sex-dependent efficacy of anti-dementia treatments, filling a major knowledge gap in gender medicine for AD.

Session 2: “The Thinking Brain – Circuits Behind Cognition and Behavior”

GluA3 Shapes Synaptic Plasticity and Neuronal Structure

Spinola, Alessio; Italia, Maria; Caruso, Sofia; Gómez-Blanco, José Ignacio; Pelucchi, Silvia Cecilia; Zianni, Elisa; Marcello, Elena; Di Luca, Monica; Gardoni, Fabrizio

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AMPA-type glutamate receptors (AMPA) are composed of GluA1-GluA4 subunits, which ultimately mediate fast synaptic transmission. Among AMPARs subunits, the physiopathological role of GluA3 remains mostly unveiled. Here, to get a grasp on its function, we downregulated GluA3 through an shRNA based approach in both an in vitro and in vivo model. Primary hippocampal rat neurons were infected with either shRNA-GRIA3 or a scrambleRNA at DIV10, and at DIV20 we performed biochemical and molecular evaluations. GluA3 downregulation selectively reduced the postsynaptic enrichment of GluN2B-containing NMDA receptors, which suggests lateralization to extrasynaptic sites. Live calcium imaging revealed a significant reduction in calcium transients, which is independent of glutamate release as indicated by glutamate imaging analyses. In addition, high frequency single spine stimulation led to aberrant calcium accumulation at dendritic spines. Moreover, dendritic arbor complexity was significantly reduced. Transcriptomic analysis via RNA-seq showed the dysregulated expression of key activity-related genes (e.g., CAMK2A, MAPK1), as well as pathways related to protein trafficking and neuronal development. Notably, the impact of GluA3 silencing was time-dependent, with earlier silencing (DIV3) producing stronger effects than DIV10, while later silencing (DIV14) was no longer detrimental. To further validate the contribution of GluA3 to brain function, we are currently moving to an in vivo model. Considering the time-dependent role of GluA3 in primary hippocampal neurons, we are downregulating its expression in the dorsal CA1 (dCA1) region of the hippocampus in both adolescent and adult mice. These animals will undergo behavioral assessments combined with fiber photometry recordings, as well as dendritic arborization analyses. Altogether, our findings reveal that GluA3-containing AMPARs are key modulators of postsynaptic calcium signaling and neuronal differentiation; they orchestrate NMDAR localization and activation of the CaMKII-ERK pathway. Additionally, in vivo investigations will provide a substantial contribution to the understanding of its role in complex brain functions.

If You Drink, | Drink-Uncovering Social Facilitation Process in Mice

Palla, Giulia⁽¹⁻²⁾; Giordano, Nadia⁽¹⁾; Fruzzetti, Lorenzo⁽²⁾; Mazziotti, Raffaele⁽²⁾; Pizzorusso, Tommaso⁽¹⁾; Bonini, Luca⁽³⁾; Caleo, Matteo⁽⁴⁾; Alia, Claudia⁽²⁾

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The observation of actions performed by conspecifics can trigger the repetition of the same behavior—a phenomenon known as social facilitation (SF). SF is thought to underlie a fundamental role in shaping social interaction and communication within species and it is observed not only in humans but also in a wide range of animals including rodents. However, the neural mechanisms underlying SF, as well as the influence of developmental and environmental regulators, remain poorly understood. In this study, we developed a novel behavioral paradigm to investigate SF in mice, based on the observation of a conspecific performing a drinking-foraging task. We found a robust facilitation of drinking behavior in head-fixed observer mice, significantly greater than in control conditions where the observed mouse was not engaged in the task. Strikingly, we found that SF in adult mice is shaped by early-life social experiences. In fact, juvenile social isolation (JSI) from postnatal days 21 to 35—a critical period for social development—markedly impaired SF compared to mice reared in enriched environments (EE). Furthermore, chemogenetic inhibition of the anterior cingulate cortex (ACC) in EE adult mice similarly disrupted SF, suggesting a key role for this region in mediating socially driven behaviors. Finally, we are currently studying the role of the ACC in shaping social behaviour during the social critical period. To further investigate the contribution of the ACC and the impact of early-life social experiences on its functional properties, we are currently characterizing electrophysiological activity of ACC during observation of the drinking task. Together, our findings support the existence of a dedicated neural system for detecting and responding to socially salient behaviors, with the ACC emerging as a central hub.

Chronic Social Defeat Stress Reduces Prefrontal Cortex Excitability in Mice

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Chronic social defeat stress (CSDS) is a widely used rodent model for studying the neurobiological mechanisms underlying depression, as it reliably induces depressive-like behaviors such as social avoidance, despair behavior and anhedonia. The medial prefrontal cortex (mPFC) is a central hub in the neural circuitry regulating mood and adaptive responses to stress, and its dysfunction has been implicated in the pathophysiology of depressive disorders. However, the cellular adaptations in mPFC neuronal activity caused by chronic stress remain not fully understood. In this study, we subjected C57BL/6j male mice to a 10-day CSDS protocol and assessed behavioral outcomes using the social interaction test, allowing classification into stress-susceptible and resilient phenotypes. Whole-cell patch-clamp recordings were performed on pyramidal neurons in acute mPFC slices to investigate intrinsic excitability. Our results demonstrate that only stress-susceptible mice exhibited robust social avoidance, while resilient and control mice maintained normal social interaction. Electrophysiological analysis revealed that, although passive membrane properties were unchanged, mPFC pyramidal neurons from susceptible mice displayed a significant reduction in action potential firing in response to depolarizing current injections. This hypoexcitability likely arises from impaired sodium channel activation and enhanced potassium-mediated hyperpolarization, disrupting the neuron's ability to sustain repetitive firing. These findings provide direct evidence that chronic stress leads to a downregulation of intrinsic excitability in mPFC pyramidal neurons specifically in mice that show depressive-like behavior. Our data support the hypothesis that reduced mPFC activity is a key cellular correlate of susceptibility to chronic stress and may contribute to the emergence of depressive symptoms. Understanding these adaptations may help identify novel targets for therapeutic intervention in stress-related mood disorders.

Cortical Signatures of Yawning: a Possible Mechanism for Social Gating?

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Macaques provide an invaluable model for investigating neuronal systems underlying perception, motor control, cognition, and social behaviours, surpassing the resolution possible in human studies. Advances in wireless recording systems have enabled neuroethological research in freely moving primates, yielding ecologically valid results and opening avenues to explore species-specific and social behaviours previously inaccessible using traditional methods. Primate behaviours are influenced or facilitated by observing conspecifics, as seen in phenomena like contagious yawning. Although controlled by subcortical structures, yawning in primates varies with endogenous and social factors, playing a role in communicating the physical and mental state of individuals and/or synchronizing their behaviour, likely requiring cortical intervention to adapt flexibly to context. Based on the motor representation of face and mouth actions in the ventral Premotor Cortex (PMv) and the presence of neurons with sensorimotor and social functional properties in this region, we hypothesized that PMv contributes to regulating spontaneous and contagious yawning for communicative and social functions. To address this issue, we used wireless neural data loggers to record neuronal activity from the PMv of two freely moving *Macaca mulatta*, tested separately, while monitoring their behaviour with a multi-camera system. We recorded PMv neurons during a variety of behaviours, including yawning, threatening, sucking, biting, and bringing food to the mouth. We found PMv neurons modulated during yawning, either exclusively or concurrently with at least one other mouth-related action. Through Intracortical microstimulation, we found that sites hosting yawn-responsive neurons exert direct control over mouth movement. Despite this association, kinematic analysis showed that this control operates beyond basic motor parameters, as no significant correlation was observed between the peak firing rate and mouth or head kinematics during yawning. These findings suggest that PMv likely plays a role in shaping yawning display, paving the way to identifying possible neural mechanisms for contagious yawning.

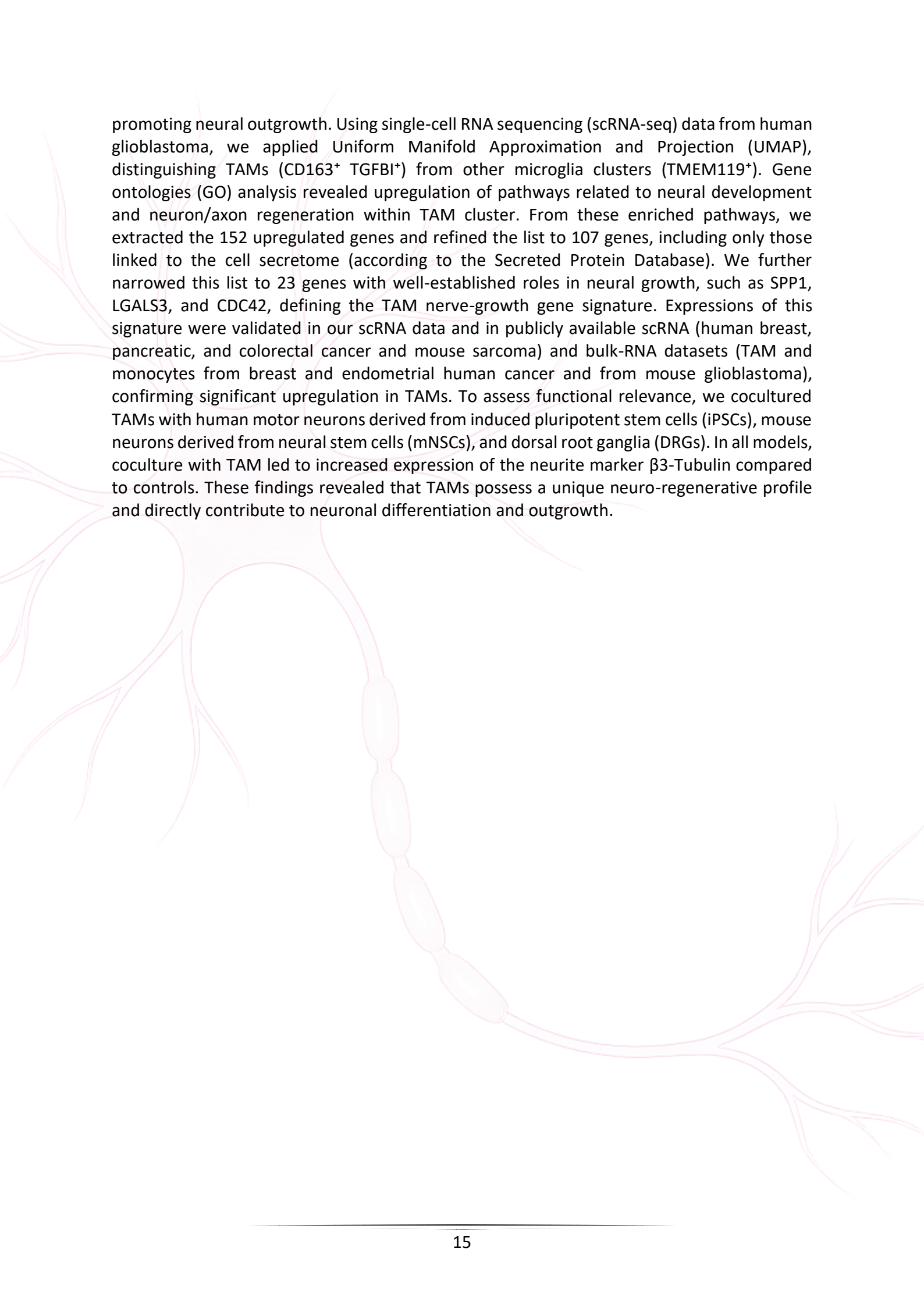
Session 3: “Next-Gen Neuroscience – Innovations Transforming Brain Research”

Bioinformatic Analysis Identifies a Novel Nerve-Growth Signature of Tumor-Associated Macrophages

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Beyond their primary role in neurotransmission, nerves have been shown to support tumor growth and metastasis. Similarly, many cancers have been shown to promote nerve growth and infiltration, creating a self-sustaining cycle that fosters mutual progression. Tumor-associated macrophages (TAMs) are key players in the tumor microenvironment, contributing to angiogenesis, extracellular matrix remodeling, and immune suppression. However, their role in nerve growth remains poorly understood. In this study, we identified a novel nerve-growth gene signature specific to TAMs and demonstrated their direct involvement in



promoting neural outgrowth. Using single-cell RNA sequencing (scRNA-seq) data from human glioblastoma, we applied Uniform Manifold Approximation and Projection (UMAP), distinguishing TAMs (CD163⁺ TGFBI⁺) from other microglia clusters (TMEM119⁺). Gene ontologies (GO) analysis revealed upregulation of pathways related to neural development and neuron/axon regeneration within TAM cluster. From these enriched pathways, we extracted the 152 upregulated genes and refined the list to 107 genes, including only those linked to the cell secretome (according to the Secreted Protein Database). We further narrowed this list to 23 genes with well-established roles in neural growth, such as SPP1, LGALS3, and CDC42, defining the TAM nerve-growth gene signature. Expressions of this signature were validated in our scRNA data and in publicly available scRNA (human breast, pancreatic, and colorectal cancer and mouse sarcoma) and bulk-RNA datasets (TAM and monocytes from breast and endometrial human cancer and from mouse glioblastoma), confirming significant upregulation in TAMs. To assess functional relevance, we cocultured TAMs with human motor neurons derived from induced pluripotent stem cells (iPSCs), mouse neurons derived from neural stem cells (mNSCs), and dorsal root ganglia (DRGs). In all models, coculture with TAM led to increased expression of the neurite marker β 3-Tubulin compared to controls. These findings revealed that TAMs possess a unique neuro-regenerative profile and directly contribute to neuronal differentiation and outgrowth.

Unraveling the Developmental Origins of Cerebellar Astrocyte Heterogeneity

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Understanding astrocyte heterogeneity is crucial for gaining insights into the development and function of the central nervous system. While the main types of cerebellar astrocytes can be identified based on their morphology and location, it remains unclear whether this classification reflects the full extent of cerebellar astrocyte heterogeneity. Furthermore, our overall knowledge on their molecular profile, development, and functions is very limited. We recently provided the first evidence that an ontogenetic program, tightly regulated in space and time, generates the main types of cerebellar astrocytes from embryonic and postnatal progenitors with distinct fate potencies. However, the molecular profiles of these progenitor pools and the mechanisms governing the ontogenesis of different astrocyte types remain to be elucidated. Through single-cell RNA sequencing and spatial transcriptomics in both postnatal and adult mouse cerebella, we unveiled the transcriptome of established astrocyte types and identified novel subtypes. Moreover, by developing a multimodal computational approach, we were able to reconstruct their maturation trajectories from postnatal progenitors. This analysis highlighted previously unrecognized progenitor sources for some astrocyte types and uncovered the gene expression cascades along distinct trajectories. Intriguingly, we shed new light on the ontogenesis and physiology of cerebellar nuclei astrocytes (CNA), which had previously received limited attention. Our transcriptomics data, complemented by clonal analyses, suggest that CNA arise from a distinct embryonic lineage compared to other astrocytes, and share a common origin with oligodendrocytes. Hence, the developing cerebellum appears to include two astrocyte lineages, clearly segregated at birth. Yet, they eventually converge into mature astrocyte profiles that despite spatial segregation show in some cases a high degree of transcriptional overlap, particularly in genes associated with homeostatic functions. This suggests that environmental cues play a crucial role in shaping cerebellar astrocyte heterogeneity, driving astrocytes of different embryonic origins toward shared homeostatic roles.

Generation of Rat Cerebral Organoids as Tools for Regenerative Medicine

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Department of Diagnostic and Public Health, Section of Pharmacology, University of Verona, Italy

Cerebral organoids are emerging as state-of-the-art tools for the study of neurodevelopment, disease modelling, and recently, they have drawn attention to their potential use in the regeneration of damaged brain, overcoming all the drawbacks of neural stem cells (NSCs) transplantation e.g., low survival rate and poor differentiation. Indeed, cerebral organoids are 3D in vitro structures that can recapitulate the in vivo brain complexity both functionally and structurally. Based on the protocol already published in our lab (Ciarpella et.al 2021,2023), we succeeded in generating rat cerebral organoids starting from NSCs isolated from the subgranular zone (SGZ), which progressively organize and differentiate, reaching maturation in 32 days in vitro (DIV). Immunofluorescence characterization of cerebral organoids revealed a progressive neuronal maturation and differentiation (DCX+, TUBb3+ cells), and a different cellular composition (GFAP+ cells), mirroring the in vivo neurodevelopment. Moreover, at later stages of culture, we observed mature neurons (MAP2+ cells) forming synapses and functional neuronal networks, evaluated by high-density multi-electrode array recordings. As proof of concept of their potential use as regenerative tools, we set up organoids' transplantation grafting immature organoids (14 DIV), obtained from hippocampal NSCs transduced with LV-GFP, into the brain of healthy adult rodents. Organoid-derived cells were successfully detected 30 days after transplantation in different brain regions (i.e. cortex, hippocampus) expressing markers of neuronal differentiation, thus suggesting their integration and maturation within the host brain. Overall, these data highlight the possible application of organoids for regenerative medicine purposes, opening the possibility of generating rat CA3 region-specific organoids to restore neural circuitry following transplantation into epileptic animals.

Building the Corticospinal Tract with Cortical and Spinal Organoids

Zanelli, Alessio; Siddig, Khallafalla; Moiz Jawed, Syed; Dell'Amico, Claudia; Onorati, Marco

University of Pisa, Italy

Our ability to move voluntarily is finely controlled by the corticospinal tract. This key system is composed of long-range connections between the cerebral cortex and the spinal cord neurons. Then, the motor signal is delivered to the muscles via synapses established with spinal motor neurons. A wide variety of affections, either developmental, degenerative, or traumatic, can severely impair this system. We aimed to generate in vitro 3D human models mimicking the corticospinal tract. First, we derived and validated brain and spinal cord organoids generated from healthy human iPSCs. Brain organoids showed a progressive switch from neural stem cell to neuronal marker expression. At later developmental stages, organoids were also enriched in neurons with different cortical layer identities, including cortical motor neurons. Spinal cord organoids were generated following an accelerated protocol oriented toward motoneuronal patterning. After only 28 days of differentiation, we achieved a high expression of mature motor neuron markers. Subsequently, we obtained corticospinal assembloids by keeping the two organoids in contact. Within this system, we observed neurons projecting from the brain to the spinal cord portion. Moreover, we placed the two organoids in a device composed of two chambers connected by a channel. Through this innovative approach, we obtained corticospinal connectoids with a clear axonal bundle spanning between the two chambers. In addition, we are currently applying these platforms to explore amyotrophic lateral sclerosis and spinal cord injuries to identify new targets and develop new therapeutic strategies.

Abstracts selected for posters

POSTER ID: 1.L.01 – Psilocybin Improves Cognition Via BDNF-TrkB in Fragile X Syndrome

Ascone, Fabrizio⁽¹⁻²⁾; Buzzelli, Valeria⁽¹⁾; Rava, Alessandro⁽¹⁾; di Trapano, Melania⁽¹⁻²⁾; Trezza, Viviana⁽¹⁾

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(2) *Center for Neuroscience, University of Camerino, Camerino, Italy*

Fragile X Syndrome (FXS) is the most common inherited intellectual disability and a leading monogenic cause of autism spectrum disorder (ASD). Serotonergic neurotransmission, crucial for neuronal development, has been implicated in the pathophysiology of ASD and related conditions, including FXS. Given serotonin's role in synaptic remodeling, early-life serotonergic insufficiency may exacerbate neurodevelopmental disorders, leading to behavioral and emotional disturbances. As a synaptic disorder, FXS involves the loss of Fragile X messenger ribonucleoprotein (FMRP), essential for dendritic plasticity. Without FMRP, dendrites develop abnormally, leading to an excess of immature dendritic spines, a hallmark of FXS. This study explores the therapeutic potential of psilocybin, a serotonergic compound, in addressing cognitive deficits in a rat model of FXS. Our previous research demonstrated that repeated psilocybin microdoses mitigated short-term memory deficits in a rat model of FXS (Buzzelli et al., 2023). To elucidate the underlying mechanisms, we pretreated FXS rats with 0.25 mg/kg and 1 mg/kg volinanserin (5HT_{2A} receptor antagonist) or 0.3 mg/kg WAY-100635 (5HT_{1A} receptor antagonist) prior to each psilocybin dose. The positive cognitive effects of psilocybin persisted despite these pretreatments, suggesting that its benefits may not be mediated by 5HT_{2A} or 5HT_{1A} receptors. Next, we investigated the role of the brain-derived neurotrophic factor (BDNF) and its receptor TrkB in the positive effects of psilocybin in FXS rats. Pretreatment with ANA-12, a TrkB receptor antagonist, blocked the positive effects of psilocybin. Furthermore, protein expression analysis revealed increased levels of BDNF and TrkB in brain regions associated with learning and memory following psilocybin treatment. These findings indicate that the therapeutic effects of psilocybin in FXS are mediated, at least in part, by the BDNF-TrkB signaling pathway. Our results support the potential of psilocybin as a novel therapeutic strategy for neurodevelopmental disorders such as FXS and ASD.

POSTER ID: 1.L.02 – Cortical-Hippocampal Circuits Mediate Prosocial Behaviors

Cerchiaro, Ilaria⁽¹⁾; Carrea, Alexandre⁽¹⁾; Gusmini, Michela⁽¹⁾; Zianni, Elisa⁽¹⁾; Zippo, Antonio⁽²⁾; DiLuca, Monica⁽¹⁾; Scheggia, Diego⁽¹⁾

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Social behaviors and decision-making processes encompass all interactions within the social domain that are influenced by or directed toward others. Among these, social learning, such as the ability to acquire behaviors by observing conspecifics, represents a fundamental mechanism for the transmission of adaptive behaviors. Like humans, mice learn from their

conspecifics to associate stimuli and cues that predict threats or rewards for self and others, representing a fundamental aspect of inter-individual relationship across mammals. The prefrontal cortex is known for its involvement in regulation of higher-order cognitive functions, including social cognition. Furthermore, using a social decision-making paradigm, we previously revealed that the dorsal CA1 region (dCA1) of the hippocampus is a critical area for learning of prosocial behaviors through observation. However, the current understanding of the brain circuits and molecular mechanisms of if and how prosocial behaviors are acquired and its integration between cortical regions and the hippocampus, remains unclear. To investigate the neural circuits involved in the observation of prosocial behaviors we first used retroviral tracing in the dCA1 using Fos2A-iCreER mice that allow permanent labeling of activated neurons. We revealed activation of dCA1 direct inputs from cortical regions, primarily the prelimbic (PL) and retrosplenial (RSC) cortices. Furthermore, silencing PL→dCA1 projections, while did not affect social learning, resulted in a shift toward selfish choices, reinforcing the importance of prefrontal-hippocampal communication in social decision-making. Therefore, we implemented the analysis of the PL→dCA1 and RSC→dCA1 neural activities using fiber photometry recordings during observation of prosocial behaviors. Finally, to build upon this circuit identification we will explore synaptic plasticity changes within these regions that could enable the encoding of socially-transmitted information. In conclusion, this study advances our understanding of the neural basis of social decision-making that may contribute to improve insights into disorders characterized by social and cognitive impairments.

POSTER ID: 1.L.03 – Brain Circuits and Mechanisms for Social Learning: from Emotion to cognition

Carrea, Alexandre; Cerchiaro, Ilaria; Gusmini, Michela; La Greca, Filippo; Imperato, Gabriele; Di Luca, Monica; Marcello, Elena; Mitro, Nico; Scheggia, Diego

Università degli studi di Milano, Italy

The brain's high energy demands reflect the complexity of its networks and the metabolic cost of sustaining synaptic plasticity and cognition. While neuronal activity has been extensively studied, the specific metabolic pathways underlying learning and memory remain less well understood, especially in the context of brain disorders. In this project, we aim to identify key metabolic substrates and pathways engaged during fear learning, using both self-experienced and observational learning paradigms in mice. Observational fear learning — wherein subjects acquire fear response by witnessing a conspecific being conditioned, provides a framework to investigate how socially transmitted information impacts brain metabolism compared to direct experience. We further examine how contextual cues (social and non-social) regulate such processes. To characterize the coupled metabolic profiles, we used liquid chromatography and tandem mass spectrometry (LC-MS/MS) to perform targeted metabolomic analysis on brain tissue collected after the fear conditioning. This allows for high-resolution mapping of metabolite distribution in brain regions implicated in fear memory, the amygdala, hippocampus, and prefrontal cortex. We extended our analysis to aged mice and a mouse model of Alzheimer's disease (APP/PS1 transgenic mice) to assess how age and pathological states alter metabolic engagement during learning. We hypothesize that specific disruptions in these pathways may contribute to the cognitive deficit characteristic of neurodegenerative conditions. This study integrates behavioral neuroscience

with high-throughput metabolomics to offer new insights into how learning uses energy. Our findings could help redefine the role of metabolism in learning and pave the way for metabolically targeted interventions for cognitive dysfunctions in both aging and disease.

POSTER ID: 1.L.04 – Endocrine Disruptor Mixture Impacts Maternal Behavior and Offspring Neurobehavioral Development

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Endocrine Disruptors (EDs) are widespread environmental chemicals capable of interfering with the hormonal system and causing adverse health effects in exposed populations and their progeny. We investigated the effects of maternal exposure to a real-life ED mixture in a mouse model. We determined the ED mixtures based upon the Life MILCH project (Mother and Infant Dyads: Lowering the Impact of Endocrine Disrupting Chemicals in Milk for a Healthy Life), which investigated ED presence in breastmilk and their impact on infant neurobehavioral development. Analyses conducted on urine and breastmilk samples from mother-infant dyads showed the presence of bisphenols, phthalates and parabens in over 50% of the samples, with negative correlations to neurodevelopmental outcomes. Based on these results, we aimed to reproduce maternal exposure to a real-life ED mixture to investigate the molecular pathways through which ED mixture affect different brain areas involved in neurodevelopment. Pregnant CD1 females were orally treated with corn oil (control) or an ED mixture at three concentrations (1X, 10X, 100X) from gestational day 12 to postnatal day 12. Offspring were evaluated at birth and during early postnatal development for litter size, sex ratio, body weight and anogenital distance. Maternal behavior was observed during the first postnatal week and early neurodevelopmental and cognitive indicators were assessed. Preliminary results showed that both pups-directed behaviors and grasping reflexes were significantly increased in the 10X group compared to controls, following an inverse “U-shaped” response pattern; additionally, fall angle in the climbing test increased in a dose-dependent manner with the highest values observed in the 10X and 100X groups. Ongoing analyses are focusing on brain morphology, neuroinflammation and the neural systems most affected by perinatal ED exposure, particularly the hypothalamus and hippocampus. These findings suggest that perinatal ED exposure may alter maternal behavior and early neurodevelopment potentially leading to long-term consequences on offspring brain function.

POSTER ID: 1.L.05 – Early Life Adversity Alters Pain, Behavior, and Alcohol Use

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Central Institute of mental health, Germany

Early life adversity (ELA) is a critical risk factor for the development of chronic pain and neuropsychiatric disorders, including anxiety, social dysfunction, and substance use disorders. This project aims to investigate the long-term effects of ELA on pain sensitivity, emotional behavior, and alcohol use propensity in a mouse model. Male and female mice are subjected to either social isolation or group housing beginning at postnatal day 21 to model ELA. At six

weeks of age, all mice undergo sciatic nerve injury (SNI) to induce neuropathic pain. Pain sensitivity will be assessed at multiple time points—prior to injury, and at 1, 4, and 12 weeks post-SNI—to characterize the trajectory of pain development and maintenance. Six weeks post-surgery, mice will undergo a battery of behavioral tests to evaluate anxiety-like behavior (Open Field Test, Light-Dark Box, Novelty Suppressed Feeding), social interaction (Direct Social Interaction Test, Three-Chamber Test), and alcohol consumption patterns using a two-bottle choice paradigm with escalating alcohol concentrations (4% to 20%). This design allows for the assessment of how early adverse experiences influence vulnerability to chronic pain and comorbid behavioral phenotypes, including anxiety, social deficits, and alcohol use disorder (AUD)-like behaviors. The study is expected to provide insight into the sex-specific and long-term behavioral consequences of ELA in the context of chronic pain. These findings may inform future strategies for identifying individuals at heightened risk for developing pain-related psychopathologies and guide the development of targeted interventions.

POSTER ID: 1.L.06 – Irisin-BDNF Neurometabolic Pathway Disruption in the Activity-Based Anorexia Model

Parolaro, Susanna⁽¹⁾; Mottarlini, Francesca⁽¹⁾; Da Dalt, Lorenzo⁽¹⁾; Rizzi, Beatrice⁽¹⁻²⁾; Tadini, Sofia⁽¹⁾; Bonacina, Fabrizia⁽¹⁾; Vairano, Francesca⁽³⁾; D'Agostino, Armando⁽³⁻⁴⁾; Bertelli, Sara⁽³⁾; Norata, Giuseppe Danilo⁽¹⁾; Fumagalli, Fabio⁽¹⁾; Caffino, Lucia⁽¹⁾

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Patients affected by Anorexia Nervosa (AN) experience a strong sense of reward from self-imposed starvation and excessive exercise in their pursuit of weight loss. Despite its high relapse and mortality rates, the neurobiological mechanisms underlying AN pathophysiology remain unclear making treatments challenging and symptomatic. In physiological conditions, physical exercise exerts protective functions on cognitive processes mainly by enhancing BDNF expression in the hippocampus via the peripheral activation of PGC-1 α /FNDC5/Irisin pathway from skeletal muscles. Since in AN, physical exercise turns to be pathological, gaining compulsive features, our hypothesis is that AN might be driven, at least partially, by a dysfunctional muscle-to-brain crosstalk, which may drive weight loss seeking thus feeding AN maladaptive behaviour. To test this hypothesis, we took advantage of the activity-based anorexia (ABA) rat model: female rats were exposed to the combination of food restriction and wheel access during adolescence from postnatal day (P) 38 to P42. Animals were sacrificed at P40, during phenotype induction, at P42, at the acute phase, and at P49, after a 7-day recovery period. We demonstrated that ABA rats exhibit compulsive, hyperactive behaviours. At molecular level, PGC-1 α and FNDC5 protein levels in the soleus muscle and the myokine Irisin in the plasma were rapidly triggered in ABA rats. These effects were paralleled by an increase of immediate early genes expression in the hippocampus at P40 and P42, suggestive of a hippocampal hyperactivation. However, ABA rats show reduced expression of Irisin receptor subunits and of BDNF-TrkB signalling at P42, an effect that persisted till P49, suggesting a long-lasting reduced trophic support in the hippocampus of ABA rats. Altogether these data point to the hyperactivity-driven PGC-1 α -FNDC5/Irisin-BDNF pathway

dysfunctions as critically involved in AN disease pathophysiology in ABA rats and it may represent a signal of altered processing of food reward and of long-term vulnerability to relapse.

POSTER ID: 1.L.07 – Behavioral Profiling of G93A Mice in ALS Progression

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by motor neuron death, resulting in paralysis. While patients are stratified by disease stage during clinical trials, preclinical validation often relies on animal recruitment based solely on age, disregarding individual disease progression. This mismatch might contribute to the frequent failures in translating preclinical data to clinical success. Additionally, both ALS onset and progression are influenced by the patient's gender, a factor often reflected in mouse models. This suggests the importance of selecting animals based on the severity of symptoms to personalize treatment strategies. G93A mice are genetically modified rodents that express a mutant form of the human superoxide dismutase 1 (SOD 1) gene, specifically the G93A mutation. This mutation is associated with familial ALS, a progressive neurodegenerative disease that affects motor neurons. G93A mice exhibit many of the pathological and clinical features of ALS, including motor neuron degeneration, muscle weakness, and paralysis. As such, they serve as a valuable model for studying the mechanisms underlying ALS and for testing potential therapeutic interventions. Our study aimed to characterize the motor and behavioral progression of G93A mice using a comprehensive battery of tests (Open field, Rotarod, Page, Beam Balance and Grip test) throughout their lifespan. The recorded videos were analysed using two tracking software programs (ToxTrac and Optimouse) to finely characterise animal behavior. Male and female mice were assessed from 40 to 120 days. Multivariate analysis of motor and behavioral variables revealed early sex- and disease-specific characteristics. Notably, stress-related behaviors, measured by the number and duration of freezing events, distinguished G93A mice from controls. Anxiety-related symptoms seem to be precocious in male mice compared to their female counterparts. These pre-onset signals might be applicable to patient screening and the development of new therapeutic strategies.

POSTER ID: 1.L.08 – Space Worms: Adaptation to Microgravity and Low Magnetic Field Strength

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As manned spaceflight and deep-space missions have increased over the years, so has the desire to establish permanent presence on the moon and beyond. This requires the interaction of numerous organisms, from bacteria to soil-dwelling invertebrates and plants for food production and ecological services such as soil renewal. However, these processes are tuned to Earth's gravity and magnetic field (H). Although much is known the effects of microgravity on muscles, bones and fat, these same effects on organisms' interactions and behaviors such as predation still remain elusive. Here, we establish a new system to study the effects of μ -gravity on predatory behavior using *P. pacificus* as a model predator and *C. elegans* as a model prey. Using machine vision, we conduct phenotypic profiling and quantitative analysis of predatory behavior to study the effects of multigenerational exposure to μ -gravity and low H on both the physiology and behavior of prey and predator, as well as the efficiency of their interaction. We aim to reveal if adaptation confers a unilateral advantage to one species, shifting the balance between predator and prey. Moreover, we will map the neurogenetics driving behavioral adaptations by RNA sequencing of both species across multiple generations. Our phenotypic and genetic maps will allow us to correlate phenotypic adaptations with the underlying genetic drivers. Our multigenerational approach will yield insights into the long-term effects of spaceflight adaptation on predatory interactions. This knowledge will lay the cornerstone to create space-ready organisms by tuning their genetics.

POSTER ID: 1.L.09 – Extrapolation of Visual Features Occurs Early and Induces Crowding

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In everyday vision, objects often pass behind occluders, disrupting the visual input that reaches our retinas. Yet, visual system can tolerate these interruptions through well-known processes as object persistence and motion extrapolation. However, it remains unclear whether detailed visual features are also maintained and extrapolated during occlusion, and at what stage of visual processing this occurs. To investigate this, we devised a dynamic version of the crowding paradigm—a process associated with mid-level stages of visual processing—where flankers could disappear behind an occluder. In our experiments, moving colored flankers travelled across the screen before disappearing temporarily behind occluders. Half the way through the occlusion we briefly presented (83 ms) of a target color patch. Although the flankers were not visible at the time of target presentation, participants' color judgments were consistently biased toward the flanker hue. This effect occurred even when the flankers had been occluded for up to 1000 ms. Crucially, the flanker effect emerged despite their last seen positions being well outside Bouma's critical crowding zone, suggesting that color information can be extrapolated and maintained during occlusion. Our

manipulations on target's position to test the spatial specificity of the effect and showed the strongest color bias occurred when the target corresponded to the trajectory of the hidden flankers, while surrounding positions showed reduced influence. Furthermore, no strong bias was observed in a control condition where flankers stopped moving before reaching the occluders and never passed behind them. These findings indicate that the visual system preserves and utilizes color information from objects even when they are temporarily out of sight. This highlights the visual system's capacity to integrate information across space-time, and the emergence of this effect within a crowding framework suggests that extrapolation mechanisms act relatively early in the visual processing stream.

POSTER ID: 1.L.10 – Impact of EBV Infection on Cortical Neurodevelopment

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The Epstein-Barr Virus (EBV) is a DNA virus belonging to the Herpesvirus family. EBV infections are extremely common, and linked to various diseases. Recent studies have highlighted the role of herpesviruses in the central nervous system (CNS) and some reports suggest a possible link between EBV infection and Rasmussen's encephalitis. This is a progressive neurological disease that primarily affects children between the ages of 6 and 10 and it suggests a potential involvement of early-stage EBV infection. Due to EBV's inability to infect rodent cells lacking the necessary receptors, iPSC-derived cortical cultures provide a unique opportunity to study the interactions between EBV and human brain cells across neurodevelopmental stages. To this end, 2D cell cultures were infected at a neurodevelopmental stage in which mature neurons and neural progenitors coexist. Real-time PCR analysis confirmed that EBV successfully infected cortical cultures, detecting latent and lytic transcripts, including BZLF1 LMP1 and GP220. Strikingly, infected cultures displayed higher levels of the neural progenitor marker PAX6, suggesting an alteration in neuronal differentiation. Focusing on an earlier neurodevelopmental stage, we observed an increase in LAMP1 and CLEAVED CASPASE 3, associated with an abnormal structuring of neural rosettes after EBV infection. Typically, neural rosettes recapitulate neural tube closure with a well-defined organization, however, EBV infection profoundly disrupted the morphology of neural rosettes in 2D cultures leading to chaotic formations. To further investigate this phenomenon, we employed a 3D micropatterned system (RosetteArray technology) that forces cell aggregation into 3D neural rosettes. Across three different control cell lines, we observed that EBV infection impaired neural rosette formation, leading to a reduced number of rosettes with significantly smaller areas supporting the idea that EBV infection impacts neurodevelopment. To further explore its effects on neuronal networks, we will analyze morphological, molecular, and functional changes using multielectrode array recordings and calcium imaging.

POSTER ID: 1.L.11 – Modelling Reelin Role on Corticogenesis

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The development of the cerebral cortex in mammals is a process of proliferation and differentiation, ultimately leading to the formation of layered structure essential for proper brain function. A key factor in this process is the secreted glycoprotein Reelin. It has been demonstrated that Reelin interacts with receptors on the membrane of neuronal precursors, regulating cell migration and adhesion. Consequently, Reelin plays a crucial role in organizing cortical structure, determining the final positioning of differentiating neural precursors. Constitutive Reelin knock-out murine models have elucidated major aspects of its function, but broad deletion masks region-specific effects. To overcome this limitation, we took advantage of the Cre-lox system to generate conditional knock-out, enabling spatially and temporally restricted Reelin deletion in the telencephalon. In a mouse model where Reelin is deleted in the dorsal telencephalon only starting from E11, we observed only minimal defects in cortical layer-specific markers expression. This is likely due to the timing of Cre activation and compensatory effect of Reelin secretion by cells migrating from the ventral telencephalon. In contrast, when Reelin is deleted in the whole telencephalon starting as early as E9.5, we observed a significant decrease in Reelin expression associated to an evident structural disorganization, and even a reversal of cortical layering. To unravel Reelin functions in species with a non laminated telencephalic structure homologous to mammalian dorsal pallium, we studied Reelin expression in the teleost fish zebrafish. Early findings, using a transgenic line Tg(reln:Gal4;UAS:RFP), indicate that Reelin is expressed in differentiated neurons of the dorsal pallium and specific regions of the subpallium during larval development, suggesting it may play an ancestral role in guiding neuronal migration. These results emphasize the spatial and temporal specific role of Reelin in modulating cortical development and suggest for Reelin a conserved role, yet diversified, in vertebrate brain evolution.

POSTER ID: 1.L.12 – Methylxanthines Alter Neurodevelopment in Zebrafish and Impact on Locomotor Activity

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Methylxanthines are molecules contained in common foods and drinks. From a pharmacological point of view, methylxanthines cause various positive effects, including stimulation of the central nervous system with an increase in the state of wakefulness and attention, relaxation of the bronchial smooth muscles and an increase in cardiac output and work, actions that can be also exploited as pharmacological effects. However, methylxanthines can also cause important side effects. The aim of this work was to evaluate the impact of some methylxanthines during neurodevelopment. To this end, the zebrafish animal model was used. In particular, zebrafish larvae were exposed to increasing concentrations of caffeine, theophylline, doxofylline and pentoxifylline. Dose curves were carried out to establish the toxicity of the various methylxanthines (FET test) and to exclude toxic doses.

Then, the expression of specific markers (Gfap1, Neurod1, Neurog1) by in situ hybridization and quantitative real time PCR were studied. The results showed that treatment with methylxanthines causes a reduction in neuronal markers and in parallel an increase in the levels of glial markers. Furthermore, regarding behavior, three of the methylxanthines tested (all except theophylline) cause an increase in locomotor activity when administered at low doses and a reduction in locomotor activity when administered at high doses. This demonstrates that methylxanthines have a significant effect on neurodevelopment, both regarding cell fate choice and cell differentiation, and on behavior.

POSTER ID: 1.L.13 – Untangling Sotos Syndrome Exploiting Multiple CRISPR/Cas Approaches in Zebrafish

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NSD1 (Nuclear receptor SET Domain-containing 1) gene encodes for a N-methyltransferase involved in methylation of lysine 36 on histone H3 (H3K36). Genetic alterations in NSD1 gene are the leading cause of Sotos syndrome: a neurodevelopmental disorder characterized by prenatal and infantile overgrowth, facial dysmorphism, intellectual impairment as well as cardiac malformation and autistic traits. In order to characterize the pathological mechanisms underlying NSD1 haploinsufficiency, we used the teleost zebrafish. First, we evidenced the maternal origin of both orthologs *nsd1a* and *nsd1b* transcripts by RT-qPCR, even if *nsd1b* seems to be expressed at lower level compared to *nsd1a*, as confirmed by in situ hybridization analysis. Interestingly, the stable *nsd1a* mutant larvae generated by CRISPR/Cas9 technique are characterized by craniofacial malformations as well as reduced levels of methylated H3K36, the target of human NSD1 enzyme, highlighting the potentiality of this animal model to resemble human pathology. We are also investigating possible cardiac defects in zebrafish mutant. In parallel, to modulate the levels of mRNA in the early stages of zebrafish embryonic development, we are setting up an approach based on the CRISPR/Cas13d technique. We already performed proof of concept experiments targeting *tbxta* mRNA that generates a tailless phenotype. In addition, to provide a more patient-relied model, we are setting up the CRISPR/Cas9-derived base editing strategy to establish patient avatar and highlight the impact of NSD1 variants in humans. After validating the functioning of the base editor targeting tyrosinase gene as a proof of concept, the system will be used to target sequences in *nsd1a* and *nsd1b* genes, already identified through in silico selection of NSD1 mutations that can be replicated in zebrafish. In conclusion, integrating different CRISPR/Cas-based approaches using zebrafish as a disease model would aid in understanding Sotos syndrome's molecular mechanisms and identifying personalized therapeutic approaches based on patients' genetic characteristics.

POSTER ID: 1.L.14 – Wnt Signalling Ensures Neurovascular Integrity in Zebrafish Hindbrain Development

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The development of the central nervous system (CNS) vasculature relies on the precise coordination of neural progenitors, astrocytes, microglia, neurons, and neuronal activity. Disruption of this fine-tuned interaction compromises the neurovascular unit and the blood-brain barrier (BBB), impairing neuronal homeostasis, activity, and protection, and potentially leading to neurovascular pathologies in the adult. Although many vascular development-related genes and pathways have been implicated, the complex cellular interactions and molecular mechanisms governing CNS angiogenesis and BBB formation remain incompletely understood. Among these, Wnt/ β -catenin signalling is pivotal in CNS vascularisation and BBB establishment. Using zebrafish models carrying vascular and neuronal reporters, combined with in vivo imaging, we investigate the spatiotemporal regulation of Wnt/ β -catenin signalling during hindbrain angiogenesis. We identified a loss-of-function mutation in the Wnt antagonist *wif1*, leading to benign vascular malformations specifically confined to the hindbrain, reminiscent of cerebral cavernous malformations (CCMs). Our results demonstrate that precise tuning of Wnt/ β -catenin signalling is critical for neurovascular patterning and may act as a sensitising factor for CCM development. Moreover, we uncover a novel compensatory crosstalk among Wnt inhibitors, ensuring tissue robustness, with broader implications for human neurovascular diseases.

POSTER ID: 1.L.15 – Characterizing Retinal Degeneration in a Zebrafish IMPG2-Linked Retinitis Pigmentosa Model

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Inherited retinal dystrophies, such as Retinitis Pigmentosa (RP), are a genetically heterogeneous group of disorders characterized by the gradual degeneration of photoreceptor cells, leading to vision loss and blindness. One gene involved in RP is the interphotoreceptor matrix proteoglycan 2 (IMPG2) gene, which encodes a proteoglycan essential for maintaining retinal homeostasis within the interphotoreceptor matrix (IPM). Disruption of the IPM has been linked to several forms of retinal degeneration, including RP and vitelliform macular dystrophy. To investigate the role of IMPG2 in retinal degeneration we use zebrafish, as its retina is highly similar to the human one. Zebrafish possess two IMPG2 paralogues, *imp2a* and *imp2b*. Using CRISPR/Cas9 technology, we generated mutant lines for both genes, which display a reduction in photoreceptor number and remarkable morphological abnormalities. Both mutant lines exhibit dysregulation of key cellular pathways associated with neurodegeneration. In particular, we observed signs of mitochondrial dysfunction and oxidative stress, both hallmarks of compromised photoreceptor metabolism. In parallel, the altered expression of endoplasmic reticulum stress markers suggests disrupted

protein homeostasis, which could exacerbate cellular damage. Furthermore, we observed upregulation of inflammatory markers and microglial activation, suggesting that neuroinflammation may further contribute to retinal damage. These findings establish zebrafish as a valuable model for investigating IMPG2-related retinal diseases. Understanding the interplay between cellular stress, inflammation, and photoreceptor degeneration in the IPM is crucial for developing therapeutic strategies to preserve or restore vision.

POSTER ID: 1.L.16 – Neurosteroid Pregnenolone Protects HMC3 Microglial Cells Against LPS-Induced Neuroinflammation

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Epilepsy is a neurological disorder in which a person has two or more unprovoked seizures that occur more than 24 hours apart. Almost 30% of patients with epilepsy cannot be efficiently controlled with available antiepileptic drugs so newer pharmacological treatment options are strongly needed. Different studies have demonstrated that status epilepticus induces fluctuations in neurosteroids (NSs) levels in the CNS. NSs are a family of steroid hormones, synthesized in the brain by microglial and astrocyte cells. Among them, pregnenolone (P5) could antagonize neuroinflammation, a well-recognized factor involved in the progression of epilepsy. In this context, it is interesting exploring the potential of P5 as an add-on therapy for the treatment of drug-resistant epilepsy. To elucidate P5 mechanisms of action, this study aimed to investigate: i) the ability of P5 to modulate inflammatory pathways in microglia; and ii) its neuroprotective and anti-inflammatory effects in neurons. The research has been performed in vitro using bacterial lipopolysaccharide (LPS) at a concentration of 100 ng/mL to mimic the proinflammatory effects of stress in human HMC3 microglia, and P5 at a concentration of 10 μ M for 3 hours. Treatment groups were: (1) vehicle; (2) P5 alone; (3) LPS alone; and (4) a combination of LPS and P5. The effect of P5 in reducing the inflammatory state in HMC3 cells was evaluated by quantifying the mRNA and protein levels of selected cytokines (IL-1 β , TNF- α , IL-6, iNOS, Caspase-1, NLRP3, IL-18, TLR4) using Real-Time PCR and Western Blot analysis. The results suggest that P5 is capable of modulating the neuroinflammatory phenotype of HMC3 cells by inhibiting the production of proinflammatory cytokines. This evidence of the anti-inflammatory and neuroprotective efficacy of P5 could open a new frontier toward the identification of more effective therapies for patients with drug-resistant epilepsy.

POSTER ID: 1.R.01 – Exosomes from Presynaptic Terminals: Potential Markers of Age-Related Synaptic Dysfunction

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Exosomes are small extracellular vesicles that mediate non-conventional intercellular communication within the central nervous system (CNS), contributing both to its physiological functions and to the progression of neurological disorders and aging-related changes. Neuron-derived exosomes (NDEs) have been shown to influence synaptic transmission by carrying proteins crucial for synaptic function but also implicated in synaptic deterioration. We previously demonstrated that isolated mouse cortical presynaptic terminals (synaptosomes) release exosomes in a depolarization-evoked, calcium-dependent manner, supporting their active role in synaptic regulation. Building on this evidence, our study explores the hypothesis that age-dependent alterations in the protein content of presynaptic exosomes might contribute to synaptic aging and dysfunction. Our aim was to analyze the protein cargo of exosomes released from cortical synaptosomes of mice at different ages, specifically young (3 months), middle-aged (12 months), and old (18 months), in order to identify changes in the expression of proteins associated with synaptic function and dysfunction. Exosomes released following a depolarizing stimulus were isolated and characterized by dynamic light scattering and western blot analysis. Western blot analysis revealed the presence of classical exosomal markers (CD9, CD63, TSG101), along with key synaptic proteins including SNARE components (syntaxin 1-A, SNAP-25), glutamate receptor subunits (GluA2/3, GluN2B), and complement protein C1q in exosomes from young mice. Comparative analysis showed that the expression of these proteins in exosomes changed significantly with age. These findings indicate that presynaptic exosomes serve as carriers of synaptic proteins whose composition is modified during aging. Such alterations may reflect, or even contribute to, age-related synaptic impairment. Understanding the molecular changes in exosomal cargo during aging may open new avenues for identifying biomarkers and therapeutic targets for neurodegenerative conditions.

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POSTER ID: 1.R.02 – Rabphilin3A as Potential Positive Tag for Potentiated Synapses

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Rabphilin 3A (Rph3A) is a synaptic protein essential for regulating exo- and endocytosis processes at presynaptic terminals and dendritic spines. It promotes the stabilization of the NMDA-type glutamate receptors (NMDARs) at the cell surface, forming a ternary complex with the PSD-95 and GluN2A subunit of the receptor. This complex is needed for long-term potentiation (LTP) induction and NMDAR-dependent hippocampal behaviors, such as spatial learning. Under physiological conditions, Rph3A is present in approximately 50% of hippocampal spines. Notably, Rph3A positive (Rph3A+) spines exhibit enlarged spine head

and postsynaptic density size, indicating enhanced synaptic stability. LTP induction increases both the number of Rph3A+ spines and NMDAR synaptic retention, thus indicating that plasticity promotes the postsynaptic localization of the protein. Importantly, the disruption of Rph3A/NMDAR complex impairs GluN2A accumulation at postsynaptic membrane, preventing LTP induction. Our group has also previously demonstrated that overexpression of Rph3A in primary hippocampal neurons is sufficient to occlude the LTP-induced formation of new dendritic spines, confirming a key role for the Rph3A/GluN2A pathway in these processes. Given these findings, we are currently investigating whether Rph3A can be considered as positive tag for potentiated synapses. Performing calcium imaging experiments in primary hippocampal neurons overexpressing Rph3A we found that postsynaptic calcium influx is strongly increased. To further examine the role of Rph3A in the potentiation of individual spines, we conducted single-spine glutamate uncaging experiments in live neurons. In addition, we are currently studying with live imaging approaches whether overexpression of Rph3A influences NMDARs dynamic and mobility. Collectively, these results suggest that Rph3A overexpression is associated with a potentiated synaptic state.

POSTER ID: 1.R.03 – Cdkl5 loss potentiates GABAergic neurotransmission via non-L-type calcium channels

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CDKL5 Deficiency Disorder (CDD) is a severe X-linked neurodevelopmental disorder, caused by mutations in the CDKL5 gene, that is characterized by severe cognitive and somatosensory impairments, language deficits, and autistic traits. While it has been suggested that CDD may be a channelopathy altering neuronal excitability, very little knowledge about the neuronal and synaptic substrates underlying the role of the CDKL5 protein kinase in regulating channels function and localization is available. By using current- and voltage-clamp recordings in autaptic cultures of the cerebral cortex of prenatal CDKL5-KO and WT mice, in this study we investigated the electrophysiological properties of GABAergic cortical interneurons (INs), a cell type still poorly characterized in the CDD context. We found that in the absence of CDKL5 inhibitory synaptic transmission resulted enhanced, the readily releasable pool of synaptic vesicles increased, and asynchronous GABA release elevated. This overall inhibitory synaptic potentiation was accompanied by an increased excitability of Cdkl5-/- INs, characterized by higher firing rates and altered action potential shape. Mechanistically, we found that this potentiated excitability was sustained by an augmented calcium influx via R-type voltage-gated calcium channels (VGCCs). Furthermore, we identified distinct contributions of P/Q- and N-type VGCCs in the synaptic release of parvalbumin (PV)- and cholecystokinin (CCK)-expressing INs, respectively. In both INs subtypes, neurotransmitter release was significantly more dependent on these channels in the absence of CDKL5, suggesting an increased presynaptic localization of non-L-type VGCCs. These findings were supported by immunofluorescence experiments on autaptic INs, which showed a higher number of VGAT+ puncta and increased density of N- and P/Q-type channels at presynaptic terminals. These results reveal the mechanism by which the CDKL5 regulates inhibitory neurotransmission and

interneuron excitability, provide new knowledge into the synaptic alterations underlying CDD and identify potential therapeutic targets.

POSTER ID: 1.R.04 – Purkinje cells as gatekeepers of seizure susceptibility: insights from optogenetics

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Purkinje cells (PCs), the principal output neurons of the cerebellar cortex, are essential for motor coordination through their inhibitory projections to the deep cerebellar nuclei, which relay cerebellar output to other brain regions. Beyond their role in movement, emerging evidence implicates PCs in the modulation of seizure susceptibility. Seizures arise from disrupted excitation-inhibition balance, leading to excessive neuronal firing. Exploring the role of PCs in this context could clarify mechanisms underlying both motor and epileptic dysfunction, features common to many neurodegenerative diseases. To investigate this, we employed optogenetic tools in zebrafish larvae, taking advantage of the UAS/Gal4 system to express light sensitive ion channels in PCs. Channelrhodopsin-2 (ChR2) was used to activate, and anion channelrhodopsin-2 (ACR2) to inhibit PC activity. We conducted high throughput behavioral assays to assess the impact of PCs modulation on locomotion at 5 days post-fertilization, and local field potentials (LFPs) were recorded to monitor seizure-like activity, particularly under proconvulsant treatment. Our results demonstrate that modulating Purkinje cell activity mediates both motor behavior and seizure susceptibility. Notably, optogenetic inhibition of PCs significantly increased the duration and power of seizure-like events, whereas PCs activation led to a marked reduction in these parameters in the presence of a convulsant agent. Currently, preliminary data from a zebrafish model of neuronal ceroid lipofuscinosis type 5 (CLN5), a progressive myoclonic epilepsy, indicate that optogenetic activation of PCs via ChR2 reduces seizure-like events, further supporting the therapeutic potential of modulating cerebellar circuits in epilepsy. These findings highlight a critical role for PCs in suppressing epileptic activity. Our double transgenic zebrafish lines provide a valuable model to dissect cerebellar contributions to neural circuit dynamics in both physiological and pathological states.

POSTER ID: 1.R.05 – Basal Forebrain Control of Large-Scale fMRI Networks

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Resting-state functional magnetic resonance imaging (rs-fMRI) enables the identification of brain regions that exhibit synchronous fluctuations in spontaneous BOLD signals. Extensive research has shown that the resulting patterns of synchronous activity (often referred to as “resting-state networks (RSNs)”) are evolutionarily conserved, and display high spatiotemporal structure, dynamically reconfiguring as a function of brain state. However, the mechanisms orchestrating RSNs organization remain elusive. Neuromodulatory systems

such as the basal forebrain (BF) - a major source of cortical acetylcholine and a key regulator of arousal - are strong candidates for controlling RSNs dynamics. Yet, whether and how BF activity causally organizes large-scale RSNs is unclear. Here, we addressed this question by employing targeted manipulations of the BF in awake, head-fixed mice. To probe the effect of global BF output on RSNs dynamics, pan-neuronal BF lesions were induced by local expression of diphtheria toxin. In a subsequent set of investigations, we used chemogenetics to probe the contribution of cortically projecting BF parvalbumin-positive (Pv+) neurons to controlling RSNs organization. Chronic BF ablation caused a dramatic reconfiguration of brain-wide connectivity, with robustly strengthened integration within the default mode network (DMN), and a prominent reduction in connectivity between cortico-cortical systems. In contrast, activation of Pv+ neurons strongly increased cortico-cortical coupling, resulting in enhanced connectivity between the DMN and somatomotor networks. These effects were brain state dependent, as they were strongly attenuated and qualitatively different in states of low arousal produced by sedation. This indicates that arousal critically gates how the BF modulates brain network dynamics. These findings suggest that the BF exerts a causal, arousal-dependent influence on RSNs. Moreover, distinct BF cell populations, such as Pv+ neurons, appear to differentially affect functional connectivity and brain network dynamics. Ongoing studies are exploring the roles of additional BF neuronal subtypes in orchestrating RSNs configuration.

POSTER ID: 1.R.06 – The Neurotoxic Threat of PFOS: Implications for Human Health

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In recent years, the scientific literature has grown to recognize the potential health risks environmental contaminants pose. Among the emerging pollutants, endocrine disruptors (EDs) are notable for their ability to interfere with hormones, leading to toxicity in various systems, including the Central Nervous System (CNS), even at low concentrations. These include perfluoralkyl substances (PFAS), a class of chemical characterized by their high persistence in the environment; in fact, they are also known as “eternal contaminants”. Among them one of the most environmentally present is perfluorooctanesulfonic acid (PFOS). This study focuses on the identification of cellular pathways disrupted by subtoxic concentrations (1 μ M, and 0.1 μ M) of PFOS and explore the role of differentially expressed miRNAs leading to cellular’s homeostasis dysregulation. Differentiated SH-SY5Y human neuronal cells were treated with PFOS for 48h to determine conditions that did not alter the cellular redox state or cell viability. miRNA evaluation revealed significant changes in hsa-miR-133, hsa-miR-200a-3p, and hsa-miR-653-5p, which are linked to neurotoxicity. Computational analysis identified target genes involved in the Ras and PI3K/Akt/mTOR pathways, which regulate cell proliferation, survival, and apoptosis. Gene modulation was confirmed via Real-Time PCR, showing significant upregulation of EGFR, IGF1R, AREG, BTG2 and SH3BP4. Western Blotting further validated these findings, showing upregulation of EGFR, and Akt and downregulation of ERK. Although preliminary, these results indicate a correlation between PFOS exposure and the activation of pathways that promote cell proliferation, potentially

increasing susceptibility to neurotoxicity. Further studies need to be carried out to fully understand the human health implications posed by prolonged PFOS exposure.

POSTER ID: 1.R.07 – Expansion-Assisted Light-sheet Microscopy: Illuminating Neuronal Circuits in Volumetric Samples

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Understanding the functional architecture of neuronal circuits across the entire brain requires imaging tools capable of resolving connectivity at both macro- and microscales while preserving three-dimensional spatial context. To date the knowledge of how the whole brain works is based on the aggregation of multiple studies carried out in individual areas and merged, after the analysis, or those that try to map single projections across the whole sample which are labor-intensive, unwieldy and time consuming. To address this problem, we optimized an innovative imaging platform that combines expansion microscopy (ExM) and light sheet microscopy (LSM) to enable high-resolution, volumetric mapping of neural circuits in intact, centimetre-scale whole mouse brain samples. This method allows for whole-brain imaging at neuronal-scale resolution, capturing intricate circuit topographies and long-range projections in their native volumetric arrangement, overcoming the traditional trade-offs between spatial resolution, sample size and time required for imaging. By bridging histological detail with systems-level anatomy, this tool represents a critical step toward comprehensive, multiscale brain mapping capable of resolving connectomics on an organism-scale level, allowing the understanding of neurological disorders, such as aberrant circuit remodelling in neurodegeneration or trauma disorders.

POSTER ID: 1.R.08 – Alterations in NMDAR-Related Amino Acids in Parkinson's Disease

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Gender is known to influence the clinical presentation and progression of Parkinson's disease (PD), yet its impact on glutamatergic neurotransmission and the homeostasis of NMDAR-related amino acids remains poorly understood. In this study, we investigated serum levels of NMDAR-associated amino acids and their precursors in PD patients and healthy controls (HC), with a particular focus on sex differences. Using high-performance liquid chromatography (HPLC), we initially analyzed serum samples from 83 PD patients and 41 HCs, revealing a selective increase in D-serine (D-Ser) levels in female PD patients. Adjusting for levodopa

equivalent daily dose (LEDD), D-Ser levels were significantly elevated in PD patients compared to controls ($\Delta\%$ = 38.7%), while levels of L-serine, the D-/total serine ratio, and other excitatory amino acids remained unchanged. To validate and expand these findings, we analyzed a larger cohort (336 PD patients and 203 HCs) stratified by sex. Strikingly, we observed drastic changes in L-glutamate, L-aspartate, glycine, L-serine, L-glutamine, and L-asparagine in male PD patients. These findings indicate a sex-specific and age-dependent disruption in the peripheral regulation of NMDAR-related amino acids. Importantly, serum D-Ser emerged as a potential sex-specific biomarker, elevated exclusively in female PD patients. Given the known modulatory effects of estrogen on glutamatergic transmission, we hypothesize that sex hormones may underlie this differential regulation. Our findings also suggest that dopaminergic treatment downregulates D-Ser and other excitatory amino acids in a dose-dependent, sex-specific manner. These results underscore the importance of considering sex as a biological variable in PD research and treatment. Future clinical trials targeting the NMDAR glycine site should account for sex and dopaminergic therapy interactions to optimize therapeutic strategies."

POSTER ID: 1.R.09 – Optimized and Standardized Culture Conditions for iGluNeurons

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Advancements in human brain in vitro models represent a cornerstone in neurological and psychiatric research, overcoming limitations posed by species-specific differences in animal models. This study focuses on standardizing and optimizing the generation of iPSC-derived glutamatergic neurons through inducible overexpression of Neurogenin-2 (NGN2). Key improvements include rigorous screening for genomic rearrangements following fibroblast reprogramming, selection of a homogenous population with integrated NGN2 cassettes, and the introduction of an intermediate step during neuronal differentiation to store neuronal progenitors. The resulting neural cultures demonstrate a high degree of neuronal maturation and consistency, as evidenced by single-cell and network electrophysiological recordings. These enhancements aim to provide more reliable tools for disease modelling and drug screening in neurological disorders. In parallel, we wanted to investigate, among various culture parameters, three factors that critically influence neuronal differentiation efficiency and functional maturation: (1) coating, (2) plating density, and (3) culture medium. Variations in these parameters significantly affect neuronal morphology, electrophysiological properties, and ultimately the interpretation of experimental outcomes. To systematically address these variables, we compared eight different combinations of coating, density, and medium conditions, evaluating their effects on neuronal morphology, proteomic expression, and functional development. Our goal is to identify the culture conditions and specific days in

vitro at which neuronal cultures reach functional maturity, alongside characterizing their morphological features and protein expression profiles. This comprehensive and standardized framework will enhance the physiological relevance of in vitro neuronal models, facilitating more accurate disease modelling and efficient drug screening for neurological disorders.

POSTER ID: 1.R.10 – A Cre-lox Technique to Address Olfactory Dopaminergic Cell Functional Heterogeneity

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The main olfactory bulb (OB) is the first and only relay structure for odors before reaching the olfactory cortex and hosts the most numerous dopaminergic (DA) population of the forebrain. These inhibitory interneurons are typically identified by tyrosine hydroxylase (TH) expression and at least two main subpopulations can be recognized based on their soma size, in vitro and in vivo electrophysiological properties, and generation time. Although increasing evidence places the role of these cells in the early stages of sensory processing, their exact function remains unclear. Moreover, their high heterogeneity challenges the study of these cells and thus a specific method to target the two subpopulations differentially is required to study their properties and roles separately. To this aim, we validated a virally delivered, time-controlled Cre-lox technique that allows to express either morphological or functional reporters in the OB TH-positive cells selectively. The specificity of this labelling is comparable to that of the TH-GFP transgenic mouse line and can target TH-positive subpopulations enriched in either small or large cells whether it is performed in adult mice or in embryos and newborns, respectively. By expressing GCaMP indicators in TH-positive cells, we were able to perform in vivo two-photon functional calcium imaging experiments in anesthetized, head-fixed adult mice. This allowed the assessment of their spontaneous activity as well as their evoked responses to opposite-sex urine. Since we previously observed a specific TH-positive cell recruitment in the posterior OB of female mice exposed to male urine, our goal is to focus on this area while observing TH-positive cell function using our labelling strategy. Taking advantage of a multidisciplinary approach based on this OB DA cell targeting technique, our main aim consists in dissecting a possible differential role for the two DA subpopulations in processing ethologically relevant odors, specifically those associated to sexual behaviors.

POSTER ID: 1.R.11 – NETRIN-1 Participates to Motor Units' Selective Vulnerability to ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that leads to relentless loss of almost all motor units. Interestingly, extraocular muscles (EOMs) remain relatively spared until the final stages of the disease. Several lines of evidence have suggested a tight link between ALS pathophysiology and axon guidance pathways. Among them, Netrin-1 emerged as one of the most significant. Indeed, Netrin-1 can act as both an attractant and repellent axon guidance signal, influencing axonal projections and pathways during nervous system development and regeneration. It can bind to receptors like DCC and Neogenin to attract axons, or to UNC5 receptors to repel them. Our work aimed to investigate and characterize the possible role of Netrin-1's pathway in neuromuscular junction's differential susceptibility to ALS. To this purpose, the expression of Netrin-1 and its receptors UNC5A and DCC was evaluated in EOMs, gastrocnemius and soleus muscles of G93A-SOD1 mice by means of qRT-PCR and Western Blot analysis, in pre- and late-symptomatic stages of the disease. Our data showed an up-regulation of Netrin-1 in EOMs of ALS mice in a presymptomatic stage. Additionally, while we didn't observe variations in UNC5A receptor's levels, DCC was exclusively expressed by EOMs and not by vulnerable limb muscles. Ongoing experiments are being carried out in the attempt to assess the hypothesis of Netrin-1's implication in muscle responsiveness to disease. To this aim, Netrin-1 has been silenced or overexpressed in the C2C12 myoblast cell line and in the NSC-34 motor neuron-like hybrid cell line exposed to L-BMAA, a reported ALS-like stimulus. Incucyte system, MTT and LDH analysis will be used to evaluate cell proliferation and cell viability in the different experimental conditions. Our results may pave the way for the pathophysiological characterization of Netrin-1's pathway in ALS skeletal muscles and its influence on muscle-motor neurons communication.

POSTER ID: 1.R.12 – Nusinersen Rescues Cerebrospinal Fluid L-Arginine Deficiency in Severe SMA

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Spinal Muscular Atrophy (SMA) is a progressive neuromuscular disorder caused by homozygous loss of the survival motor neuron 1 (SMN1) gene, leading to reduced SMN protein expression. Increasing evidence implicates neurotransmission deficits in the pathophysiology of SMA. In particular, alterations in neuroactive amino acids involved in glutamatergic neurotransmission have recently been identified in both the cerebrospinal fluid (CSF) of SMA1 patients and the spinal cord of SMNΔ7 mouse models. L-arginine, a precursor of nitric oxide, plays a critical role in glutamatergic receptor signaling, influencing neurotransmitter release, synaptic plasticity, and neuroprotection. However, it remains unclear whether SMN deficiency affects L-arginine metabolism in SMA patients and animal models. To address this, we used high-performance liquid chromatography to investigate whether SMN deficiency alters L-arginine homeostasis in the central nervous system of SMNΔ7 mice and in the CSF of SMA patients with varying disease severity, both before and after treatment with the SMN-inducing therapy Nusinersen. Notably, we observed significantly reduced L-arginine levels in the brainstem and spinal cord of symptomatic SMA mice compared to age-matched wild-type littermates. Consistent with these findings, we revealed abnormally lower L-arginine levels in the CSF of severe SMA1 patients compared to milder SMA2 and SMA3 patients and healthy controls. Importantly, Nusinersen-mediated SMN upregulation fully restored L-arginine homeostasis in the CSF of severe SMA1. In conclusion, our results demonstrate a dysregulation of L-arginine in SMA and highlight a role for SMN-enhancing therapies in restoring neurochemical alterations observed in severe patients with this neurodegenerative disease.

POSTER ID: 1.R.13 – Context-Dependent Neural Fingerprints in the Primate Premotor Cortex

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Decoding the dynamics of voluntary motor control requires understanding how neural populations in the motor cortex adapt to varying behavioral constraints. In this study, we analyzed large-scale local field potential (LFP) recordings obtained from multiple 32-channel floating microelectrode arrays (FMA) chronically implanted in the premotor and primary motor cortices of both hemispheres of two macaques. Recordings were collected while the animals performed manual (i.e. grasping) and mouth (i.e. sucking and biting) actions under three distinct conditions in each session: classical head-fixed condition in a primate chair, head-free in a primate chair, and freely moving with no constraint. Using unsupervised clustering of time–frequency neural activities, we identified groups of recording sites that shared similar spectral changes during motor behavior. These clusters were then examined across actions and behavioral contexts to assess how consistently they appeared and whether their activity patterns reflected changes in motor control. In the beta band, we observed marked condition-dependent modulation, including strong suppression during head-fixed actions, while in freely moving conditions, different clusters either maintained suppression or showed sustained or increased beta power. Additionally, measures of phase-locking (PLV) and phase–amplitude coupling (PAC) between frequency bands revealed shifts in synchrony and signal coordination across arrays. While these interactions were preserved in the recorded region, their strength and timing varied with action and movement freedom, suggesting that local population dynamics are flexibly tuned. These preliminary results suggest that consistent neural motifs underlie goal-directed actions, yet their temporal and spectral expression flexibly reorganizes depending on action demands and motor freedom.

POSTER ID: 1.R.14 – Laminar distribution pattern and size of crossed-corticostriatal neurons in macaques

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Recent data in macaques showed that the laminar distribution pattern of corticostriatal neurons in the ipsilateral hemisphere largely varied across different areas, independently from density and projecting area, but likely according to the target striatal zone (Borra et al., *JNeurosci* 2021, 41:1455–69). Furthermore, other data showed that in macaque there are robust crossed corticostriatal projections to the caudate and the putamen, up to 30% of total (ipsi+contra) corticostriatal labeled cells, and that the origin of the direct and the crossed corticostriatal projections is not symmetrical (Borra et al. *JNeurosci* 2022, 42:7060–76). In the present study, we qualitatively and quantitatively compared the distribution pattern and size of crossed vs ipsilateral corticostriatal neurons in four macaques in which neural tracers were injected in motor putamen (2 cases), precommissural putamen, and caudate head. The results showed that, as in the ipsilateral hemisphere, the pattern was multilaminar, involving at a variable extent layers III, V, and VI. However, in areas F1, F3, and 24c after injections in the motor putamen and in areas 9, 24, and 32 after injections in the caudate head, the percentage of labeled cells in layer III was significantly lower ($p < 0.001$) in the contra- vs ipsilateral hemisphere. The dimension (area of the soma) of the neurons in layers V and VI was not different between ipsilateral and contralateral labeled areas (F3 and F1). In other areas, such

as F6, F7, and 45B, the laminar distribution pattern was relatively similar. These observations add complexity to the architecture of the striatal input and suggest more complex modes of information processing in the basal ganglia for different motor and nonmotor functions.

POSTER ID: 1.R.15 – Maternal S-Adenosylmethionine Partially Mitigates Valproic Acid-Induced Behavioral Abnormalities in Mice

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Adverse environmental factors, such as maternal exposure to the antiepileptic drug valproic acid (VPA), significantly increase the risk of neurodevelopmental disorders, including autism spectrum disorder (ASD), in offspring. Among its various pharmacological actions, VPA has gained considerable attention for its potent activity as a histone deacetylase inhibitor. It is hypothesized that VPA-induced epigenetic modifications during critical developmental windows may result in persistent alterations in gene expression, thereby influencing key neurodevelopmental processes. In this study, we investigated whether prenatal supplementation with S-adenosylmethionine (SAM), a potent methyl donor involved in epigenetic regulation, could ameliorate ASD-like behavioral abnormalities in offspring exposed to VPA. To achieve this, pregnant CD-1 mice received a single intraperitoneal injection of VPA (500 mg/kg) or saline (as control) on gestational day (GD) 12.5. Subsequently, they were administered SAM (30 mg/kg/day) or phosphate buffered saline (as control) via oral gavage on GD 13.5, 14.5, and 15.5. Offspring of both sexes were assigned to two cohorts and subjected to behavioral testing tailored to their developmental stages (i.e., neonatal and adolescent). Data indicate that neonatal mice of both sexes prenatally exposed to VPA emitted significantly fewer ultrasonic vocalizations during a brief maternal separation, a hallmark of early socio-communicative deficits, an effect not rescued by SAM treatment. However, SAM supplementation reduced the latency to reach the area containing nest bedding in the Homing test in VPA-exposed pups, suggesting a partial amelioration of nest-seeking behavior. In adolescence, male offspring exposed to VPA displayed increased repetitive behavior, as indicated by a higher number of marbles buried in the Marble burying test. Notably, prenatal SAM supplementation effectively reduced this abnormal behavior. These findings suggest that SAM may partially attenuate specific behavioral effects of prenatal VPA exposure and highlight the potential of epigenetically-targeted interventions in mitigating ASD-related phenotypes.

POSTER ID: 1.R.16 – Glial Dysregulation in a Rat Model of Fragile X Syndrome

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Astrocytes and microglia have emerged as essential regulators of brain functions and immune defense through complex interactions with neuronal cells. In recent years, there has been growing evidence suggesting that glia dysregulation may play a critical role in the pathophysiology of neurodevelopmental disorders, including Autism Spectrum Disorder (ASD) and Fragile X Syndrome (FXS). Based on these premises, the present study aimed to investigate the morpho-functional, proliferative and biochemical alterations in the hippocampus of Fmr1-Δexon 8 rats, a recently validated rat model of FXS (that is also a genetic rat model of ASD), in both adolescence (PND 35) and adulthood (PND 75). We first performed immunostaining assays and morphological evaluations of glia cells on brain tissue from Fmr1-Δexon 8 and wild-type (WT) adult animals, using GFAP and Iba1 antibodies as markers of astroglia and microglia activation, respectively. In parallel, we performed a multiplex assay on hippocampal and plasma samples to assess the peripheral and central inflammatory state of these animals compared to WT controls. Finally, we examined the activation of the NF-κB pathway, a transcription factor that plays a critical role in regulating the immune response and inflammation. Our results show that Fmrp deficiency can induce local changes in density and morphology of glia cells, likely altering neurodevelopmental trajectories and tissue homeostasis and contributing to the pathogenesis of FXS. Further research is needed to better understand the role of immune dysregulation in neurodevelopmental disorders and potentially facilitate the development of new pharmacological treatments aimed at reducing reactive glial toxicity.

POSTER ID: 1.R.17 – MacroH2A1.1 Deletion Partially Mimics Neurodevelopmental Effects of Prenatal Valproate Exposure

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MacroH2A1 (mH2A1) is a H2A histone variant, with two isoforms: mH2A1.1 and mH2A1.2. Although mH2A1 has been implicated in cellular differentiation and development, its role in atypical neurodevelopmental conditions, such as autism spectrum disorder (ASD), remains largely unexplored. This study investigated the contribution of mH2A1.1 to neurobehavioral development in mice prenatally exposed to valproate (VPA), an environmental risk factor for ASD. Pregnant heterozygous (HT) mH2A1.1 female mice, mated with HT mH2A1.1 males, received a single injection of VPA (500 mg/kg, i.p.) or vehicle (VEH) on gestational day 12.5. Offspring of both sexes were evaluated during the neonatal and juvenile stages for: (i) ASD-

like behavioral phenotype, (ii) hippocampal synaptic plasticity, and (iii) neurotransmitter levels. Neonatal behavioral responses were unaffected by either mH2A1.1 deletion or VPA exposure. However, in the juvenile stage, knock-out (KO) males exhibited increased repetitive behaviors, while KO females displayed heightened anxiety-like behavior compared to their sex-matched wild-type (WT) counterparts. While mH2A1.1 deletion did not impact social interaction, VPA treatment induced social deficits exclusively in males (both WT and KO), who spent less time interacting with a conspecific and emitted fewer ultrasonic vocalizations. VPA-exposed WT mice displayed impaired hippocampal long-term depression in the neonatal stage and a significant reduction in serotonin levels in the hippocampus and striatum across developmental phases. Interestingly, similar neurochemical alterations were observed in VEH-exposed KO mice. These findings suggest a role for mH2A1.1 in shaping brain and behavioral development, potentially modulating susceptibility to neuropsychiatric disorders. Its deletion appears to partially mimic the effects of prenatal VPA exposure, pointing to a potential epigenetic mechanism underlying ASD-related phenotypes.

POSTER ID: 1.R.18 – Behavioral and Molecular Characterization of Pten T167N Mouse Model

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Phosphatase and tensin homologue (PTEN) is a dual-specificity phosphatase that suppresses cell growth and survival by negatively regulating the PI3K/PDK1/AKT/mTOR pathway through dephosphorylation of PIP3 to PIP2. PTEN mutations have been linked to both cancer and neurodevelopmental disorders. In this study, we investigated a novel knockin mouse model carrying a heterozygous (Het) point mutation (T167N) in the Pten gene. This de novo mutation was originally identified in 8-year-old girl diagnosed with Autism spectrum disorder (ASD). Notably, the mutation does not appear to promote tumor formation, as neither the patient nor mice developed tumors, even at advanced ages. To assess autism-related behaviors, we performed core behavioral tests in 4–6-month-old mutant and wild-type mice. Interestingly, a sex-specific stratification emerged: Pten T167N/+ mice exhibited several ASD-like behaviors compared to WT littermates. Het females displayed reduced exploration of the central area in the open field test, suggesting increased anxiety; Het males showed reduced sociability in the social habituation/dishabituation test. Moreover, a trend toward increased self-grooming behavior was observed in Het mice, a behavior frequently reported in other ASD mouse models. To investigate the underlying molecular mechanisms, we performed DAB immunostaining for phosphorylated Akt (pAkt) and ERK (pERK), key effectors in the PTEN pathway. Current quantification focuses on areas (prefrontal cortex, hippocampus, hypothalamus, olfactory regions) implicated in social behavior and sensory regulation. Preliminary findings suggest a region-specific dysregulation. Future directions include single-cell transcriptomic profiling of the prefrontal cortex to uncover cell-type specific molecular alterations and pathways that may contribute to the observed behavioral phenotypes. In addition, functional MRI will be used to map brain activity and connectivity, offering crucial

insights into how this mutation affects large-scale neural circuits linked to ASD behaviors. Taken together, our findings support a role for the PTEN T167N mutation in driving autistic-like behaviors, with notable sex-specific differences in phenotype expression.

POSTER ID: 2.L.01 – Neuroplasticity and Behavioral Changes in Fmr1KO Mice Across Postnatal Development

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Background Fragile X syndrome (FXS), caused by inactivation of the Fmr1 gene, is the most common cause of intellectual disability and autism spectrum disorder. Fmr1 knockout mice display a disorganization of perineuronal nets (PNNs), extracellular matrix structures that preferentially form around parvalbumin-positive interneurons (PVIs) during postnatal development, limiting plasticity. PNN and PVI expression is altered in various neuropsychiatric disorders, and their manipulation in specific brain areas has proven to reverse related deficits; despite that, a systematic study in Fmr1KO mice has never been conducted. **Methods** We performed a brain-wide analysis in adult Fmr1KO and control (WT) male mice to study FXS-dependent alterations of PNNs and PVIs affecting different brain areas. To this aim, we developed a customized method for whole-brain 3D reconstruction of histologically stained sections, followed by high-throughput automated image analysis. **Results** We found a general reduction of PNN and PVI expression in FXS mice compared to WT, greatly affecting the cortex, amygdala, hippocampus, and striatum. PNNs were dramatically decreased in the isocortex, more markedly in primary areas than in association areas, whereas PVI density was significantly decreased in the amygdala. The whole-brain analysis allowed us to define a section sampling distance sufficient to robustly identify the PNN reduction in Fmr1KO mice. By combining this sampling strategy with our customized method, we were able to extend the analysis of PNNs and PVIs to different developmental stages in both male and female mice. **Conclusions** Thanks to our new customized biocomputational method, we precisely described, for the first time, the expression of PNNs and PVIs in the FXS model. This comprehensive study enable the analysis of PNN and PVI expression in the brain of Fmr1KO mice during different developmental stages, paving the way to identify specific brain areas and corresponding time windows of intervention that could be therapeutically relevant for FXS.

POSTER ID: 2.L.02 – Mitochondrial Abnormalities and Oxinflammatory Mechanisms in Rett Syndrome

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Rett Syndrome (RTT) is a neurodevelopmental disorder primarily affecting girls, characterized by an initial period of normal development followed by a progressive decline in cognitive and motor function. Mutations in the MECP2 gene, which encodes a crucial protein involved in gene regulation, are the primary cause of RTT. While the genetic basis is understood, the precise molecular mechanisms driving the devastating neurological consequences remain elusive. Growing evidence points to a critical role for mitochondrial dysfunction, oxidative stress, and inflammation in the pathogenesis of RTT. This study investigates the intricate molecular pathways involved in the transition from the presymptomatic to symptomatic phases of RTT. Utilizing in vitro and ex vivo models, such as MeCP2 knockout primary neurons and brains from MeCP2 knockout mice, we comprehensively analyzed alterations in mitochondrial dynamics, morphology, and function. Specifically, we examined key genes and proteins involved in critical mitochondrial processes, including mitochondrial biogenesis and mitophagy, to elucidate the underlying mechanisms contributing to mitochondrial dysfunction. Our preliminary findings reveal mitochondrial deficits in MeCP2-null neurons at all pathological stages (6, 12, 18 DIV), including impaired mitochondrial dynamics and morphology and altered mitochondrial membrane potential. These mitochondrial impairments are hypothesized to contribute to increased production of reactive oxygen species (ROS), leading to oxidative stress and subsequent activation of inflammatory pathways. These findings provide crucial insights into the pathophysiology of RTT, highlighting mitochondrial dysfunction as a key driver of disease progression. Understanding these complex molecular mechanisms opens new avenues for developing novel therapeutic strategies. By targeting mitochondrial dysfunction, reducing oxidative stress, and modulating inflammation, we aim to develop innovative interventions that can delay disease progression, alleviate symptoms, and ultimately improve the quality of life for individuals with RTT.

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POSTER ID: 2.L.03 – Evaluation of the Early Sensory-Motor Development in Rett Syndrome

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Rett Syndrome (RTT) is a rare, incurable neurological disorder affecting primarily females carrying de novo mutations in the gene encoding methyl-CpG-binding protein 2 (MECP2), located on the X-chromosome. A wide spectrum of MECP2 mutations can cause RTT, resulting in miscellaneous phenotypical manifestations mainly affecting motor, cognitive and social skills. Patients exhibit a peculiar clinical progression: after an apparently normal prenatal and perinatal period, a regression of acquired skills occurs. Due to the late onset of overt symptoms, the diagnosis occurs late in infancy, preventing early interventions that could mitigate the onset and progression of the symptomatology and thus guarantee a better prognosis for RTT patients. Much evidence, however, points to the presence of subtle

alterations early in development, although clear clinical evidence is limited due to the condition's rarity and lack of heritability. Exploiting the availability of highly validated RTT murine models carrying the most frequent RTT-causing MECP2 mutations, the present study aimed to perform a standardized characterization of the early sensory motor development during the first two postnatal weeks. We provide evidence that early subtle and transient alterations preceding the onset of overt symptomatology can be detected in RTT mouse models well before the onset of overt symptoms. However, an high variability among RTT models was observed in the developmental profile, confirming that the type and localization of the MeCP2 mutation affect the phenotypic manifestation, even in early development. The alterations identified in this study could provide insight into potential early clinical signs, leading to an anticipation of the diagnosis and intervention in RTT.

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POSTER ID: 2.L.04 – CDKL5: a novel regulator of the inhibitory post-synaptic complex

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Mutations in the cyclin-dependent kinase-like 5 gene (CDKL5) are responsible for a severe neurodevelopmental disorder, namely CDKL5 deficiency disorder (CDD), characterized by early-onset epileptic encephalopathy, intellectual disability, and intractable seizures. So far, the role of CDKL5 in excitatory synapses has been widely explored; conversely, more has still to be investigated regarding its influence on the inhibitory compartment. Our recent data showed that CDKL5 loss impacts the number of synaptic GABAA receptors, which may be explained by its interaction with the postsynaptic scaffolding complex containing gephyrin, collybistin (CB) and neuroligin 2 (NL2). Gephyrin is the core scaffolding protein of the inhibitory synapse, while CB is a brain specific GEF, involved in recruiting gephyrin to postsynaptic sites. CB is retained in a folded-inactive conformation by its auto-inhibitory SH3 domain; when this domain interacts with other proteins, as NL2, CB switches to the open-active conformation. Considering all the above, this project aims at exploring the potential role of CDKL5 in the inhibitory synapse, focusing the attention on the aforementioned scaffolding complex. Through co-immunoprecipitation and immunofluorescence approaches, we found that CDKL5 can release collybistin from its inactive conformation thus promoting the distribution of gephyrin to sub-membranous sites. Moreover, through biochemical approaches we have found that CDKL5 regulates NL2 levels making us hypothesize a role of CDKL5 in regulating the interaction between collybistin-neuroligin. Altogether, these results seem to place CDKL5 as a novel key regulator of the postsynaptic scaffolding complex of inhibitory synapses and the translational potential of our findings is underscored by reduced levels of synaptic GABAARs in hippocampi of post-mortem brains from two patients with CDD. Elucidating synaptic networks regulated by CDKL5 may pave the way to comprehend the pathogenic mechanisms of CDD and to develop novel therapeutic approaches."

POSTER ID: 2.L.05 – Exploring Extracellular Vesicles as Molecular Biomarkers for CDKL5 Deficiency Disorder

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Mutations in the CDKL5 gene cause CDKL5 Deficiency Disorder (CDD), a rare neurodevelopmental disease (NDs) characterized by a broad constellation of clinical manifestations, including early-onset seizures, cognitive, sensorimotor, and neurological impairments. These complex symptoms have challenged the development of effective treatments and routinely manageable biomarkers for CDD. Mounting evidence show that identifying the content of extracellular vesicles (EVs), membrane-enclosed nanoparticles secreted by all cells, represents a promising strategy to reveal biomarkers and molecular causes of neurological conditions, including NDs. To assess the biomarker potential of EVs, we analyzed miRNomic profiles of salivary EVs collected from >20 CDD patients and neurotypical controls. Differential expression analysis with stringent statistical thresholds, followed by the exclusion of miRNAs with identifiers above 500 (e.g., mir-500 and higher), was used to detect only biologically relevant miRNA families. This led to the identification of 17 differentially expressed miRNAs (DEmiRNAs) in CDD patients, many of which are brain-enriched and linked to pathogenic processes such as autism and epilepsy, that are currently under investigation as biomarkers. Because the transport of EVs cargoes is a form of communication that is crucial for cerebral cortex development through still unidentified molecular mechanisms, we next investigated the role of CDKL5 in this process. EVs were thus isolated from cultured cerebral cortical neurons of both CDKL5-KO (KO-NDEVs) and WT (WT-NDEVs) mice. Intriguingly, 2-days treatment with WT-NDEVs rescued neurotransmission and synaptic defects shown by CDKL5-KO neurons, while KO-NDEVs produced severe synaptic alterations in WT neurons. Finally, by miRNomic profiling we identified 16 DEmiRNAs previously unreported carried in KO-NDEVs that were associated with neuronal organization, synaptic development and function pathways by GO enrichment analyses. Thus, our findings disclosed that: CDKL5 regulates synaptic-relevant EV-mediated neuron-to-neuron communication; several miRNAs may be future therapeutic targets for CDD; EV-derived miRNAs hold promise as accessible, non-invasive, molecular biomarkers for this disease.

POSTER ID: 2.L.06 – Uncovering understudied developmental roles of TCF4 to investigate Pitt-Hopkins Syndrome

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TCF4 loss-of-function variants cause Pitt-Hopkins syndrome (PTHS), a rare neurodevelopmental disorder characterised by intellectual disability, distinctive facial features, intermittent hyperventilation followed by apnoea, motor incoordination and intestinal constipation. This plethora of symptoms reflects the widespread distribution of TCF4 in different embryonic cell types. Previous studies have focused on TCF4 function in a subset of brain areas involved in cognition, while its role in several other brain regions remains unexplored. Similarly, the biological causes of craniofacial dysmorphism, muscle hypotonia, motor incoordination and gastrointestinal constipation are unknown. Elucidating these aspects is essential to understand the requirement for TCF4 during development and to plan interventions that could have a significant impact on patients' quality of life. To this end, we have recently generated a new model of PTHS in zebrafish using the CRISPR/Cas9 approach. *Tcf4*^{+/-} embryos were studied at different developmental stages to analyse craniofacial morphogenesis, central and peripheral nervous system development, neuro-muscular development and enteric nervous system development. Characterisation of heterozygous embryos revealed phenotypic impairments that may resemble the characteristics of Pitt-Hopkins patients. To assess functional impairment in *tcf4*^{+/-} embryos, we also perform behavioural tests to assess larval motor activity and investigate enteric nervous system functionality by visualising and quantifying *in vivo* gut peristalsis and gut mobility. A transcriptomic analysis of *tcf4*^{+/-} embryos and embryos overexpressing *tcf4* is underway to reveal novel tissue-specific *tcf4* targets. We hope that this project will disclose new developmental roles for *tcf4* and pave the way for planning new intervention strategies to improve the quality of life of PTHS patients.

[*This work was supported by Fondazione Telethon, Grant n. GMR22T1071, "Modeling Pitt-Hopkins syndrome and new pathogenetic variants of TCF4 by gene editing: a step forward toward personalized medicine (HOPEFOR)".]

POSTER ID: 2.L.07 – NR2F1 Deficiency Impairs Mitochondria in Mouse and Human iPSC-Derived Neurons

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The Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS; OMIM#615722), is a rare neurodevelopmental disorder caused by mutations in the NR2F1 gene, a transcriptional regulator playing pleiotropic roles in brain development and function. Most NR2F1 pathological variants are deletions and/or mutations leading to haploinsufficiency or dominant negative effects, thus compromising NR2F1 transcriptional regulatory activity. As the vast majority of neurodevelopmental disorders, BBSOAS is characterized by a wide clinical spectrum where symptoms are very heterogeneous, including moderate to severe intellectual disability, developmental delay, optic atrophy, autistic traits and hypotonia. By combining *in silico* and *in vivo* approaches, our recent study (Bonzano et al., 2023) revealed a novel regulatory role for NR2F1 on mitochondria. We demonstrated that NR2F1 regulates a

network of nuclear-encoded mitochondrial genes by influencing mitochondrial mass and shape, and pointing to mitochondrial dysfunctions in neural tissue as a potential key pathological mechanism in BBSOAS. Accordingly, Nr2f1-heterozygous mice, a validated BBSOAS animal model, display dysregulation of several nuclear-encoded mitochondrial genes and downregulation of key mitochondrial proteins in the brain. However, how NR2F1-deficiency might lead to mitochondria-related functional defects in human neurons is still completely unknown. We are currently translating our findings to human iPSCs lines to further characterize the cellular and molecular mechanisms underlying mitochondrial activity in the absence of NR2F1 function. We established a differentiation protocol to derive human iPSC lines into a telencephalic cortical phenotype, generating a population of neural progenitors and neurons expressing key regional markers such as PAX6 and FOXG1. By means of advanced imaging techniques, preliminary data are suggesting an increase in mitochondrial fragmentation in human iPSC-derived neurons in NR2F1-HET and NR2F1-KO clones. Our findings highlight NR2F1 as an active regulator of mitochondrial gene expression and function in the brain, paving the way for further investigations into mitochondria-related mechanisms and new perspectives for targeted therapeutic strategies.

POSTER ID: 2.L.08 – Involvement of Autophagy and Mitophagy in Epileptogenesis

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A strategy for secondary prevention of temporal lobe epilepsy (TLE) may be the use of therapies that intervene early after an insult to limit brain damage or to interrupt epileptogenesis. We have to better understand the complex molecular and cellular mechanisms of epileptogenesis. In particular, this study aims to explore the involvement of autophagy during epileptogenesis using in vitro (organotypic hippocampal slice cultures, OHSC) and in vivo (the rat pilocarpine model) models. We are evaluating cell viability, astrogliosis and markers of autophagic flux at different timepoints at days in vitro (DIV)3 to 28 during epileptogenesis in OHSC. Moreover, we performed a systematic evaluation of the expression profile of autophagy markers at multiple time points during epileptogenesis in the rat pilocarpine model: early and late latency (seizure-free period) and in chronic period (spontaneous recurrent seizure onset). After pilocarpine injection, rats underwent status epilepticus (SE) and then were monitored for seizures occurrence, using video-EEG recording; at different timepoints, animals were sacrificed and markers of the autophagic flux were measured in the hippocampus. The protein levels of LC3 and p62 revealed significant modulation of autophagy both in vitro and in vivo. Preliminary data on OHSC outlined an autophagy blockage at DIV3. Moreover, in pilocarpine model we obtained a higher level of autophagy markers during early latency (4-5 days after SE) compared to late latency (9-10 days after SE). Our findings suggest a block of the autophagy flux, implicating that this may have an active role in epileptogenesis. Our data need further investigation to confirm the blockade of autophagy pathways in the early phase of the epileptogenic process. These

findings could identify a new target for repurposed drugs able to activate autophagy, which would have a preventive, disease-modifying effect.

POSTER ID: 2.L.09 – Trilostane Ameliorates Epileptogenesis, Reducing Seizure Frequency in Stressed Kainic-Acid Model

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Background: Stress contributes to the onset and progression of epilepsy, exacerbating comorbidities like depression and anxiety. Neurosteroids (NSs), synthesized in the brain, modulate neuronal excitability through GABA receptors and exhibit anti-neuroinflammatory effects. This study aims to investigate the effects of trilostane on epileptogenesis and epilepsy progression in a stress-exacerbated temporal lobe epilepsy model by modulating NSs levels in the brain. **Methods:** Self-limited status epilepticus (SE) was induced in Sprague-Dawley rats using 15 mg/kg kainic acid intraperitoneally, confirmed by video-electrocorticographic recordings. Rats were assigned to two groups: Group 1 (n=7) received sesame oil (vehicle, s.c.), and Group 2 (n=12) received trilostane (50 mg/kg/day, s.c.) for 21 days. Stress was induced through social isolation and daily injections. Seizure frequency, stage, and duration were monitored using Video-EcOG. **Results:** During the 21-day treatment period, all animals in the vehicle group developed spontaneous recurrent seizures (SRS), with non-convulsive seizures starting on day 5 $\pm$ 1 and convulsive seizures on day 11 $\pm$ 0.8. In contrast, only 4 animals in the trilostane group developed SRS, with non-convulsive seizures starting on day 10 $\pm$ 0.9 and convulsive seizures on day 13 $\pm$ 0.3. Statistical analysis (Fisher's exact test) revealed a significant difference in the number of epileptic rats between the groups ($P < 0.05$). We monitored five rats from the trilostane group for one month after treatment discontinuation. Only two of these rats developed recurrent seizures two weeks after discontinuation. The total number of SRSs differed significantly between the groups during treatment ($P < 0.001$, T-test), but no significant difference in seizure duration was observed. **Conclusion:** This study suggests that trilostane, by modulating NSs levels in the hippocampus and neocortex, partially prevents epilepsy and reduces seizure frequency in a stress-exacerbated temporal lobe epilepsy model. These effects were evident during trilostane administration and were partially sustained following treatment discontinuation.

POSTER ID: 2.L.10 – Dissecting Dimethyl Fumarate Repurposing in Alpha-Synuclein-Induced Parkinson's Disease Mouse Model

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Drug repurposing strategy has increased the possibility of proposing new therapeutic approaches for brain disorders. Preclinical drug repurposing studies should rely on state-of-the-art models that allow the translation from animals to humans. This opportunity is crucial for the treatment of neurodegenerative diseases, given the urgent need to identify new pharmacological approaches capable of, at least, slowing the progression of the disease. Here, we aim to repurpose dimethyl fumarate (DMF) to counteract alpha-synuclein-induced detrimental effects in a mouse model of Parkinson's disease. DMF is a marketed molecule used for the treatment of multiple sclerosis. We use the model of bilateral intrastratial injections of alpha-synuclein preformed fibrils (α -syn-PFF), which mimics early phases of the disease before the onset of neurodegeneration. Specifically, to study the progression of α -syn-PFF-induced alterations, we use a time-dependent approach that also allows us to correlate them with the potential rescuing effects of DMF treatment. We set two different time points: at 6 weeks (6 wpi) and 12 weeks (12 wpi) after injections of α -syn-PFF or PBS, as control. While 6 wpi α -syn-PFF-injected mice do not show the motor deficits observed at 12 wpi, cognitive symptoms and early synaptic electrophysiological and molecular changes are already present at this early disease stage. Notably, chronic treatment of 12 wpi α -syn-PFF mice with DMF leads to protective effects at behavioural, electrophysiological and molecular levels. The combination of biochemistry and molecular biology is employed to dissect DMF's mechanisms of action. Although DMF is known to act on several cellular pathways—including the Nrf2 signalling pathway—none has yet been explored in the context of α -syn-PFF-induced pathology. Overall, this study provides novel insights into early α -synucleinopathy and potentially supports the translational of DMF as a disease-modifying treatment in Parkinson's disease.

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POSTER ID: 2.L.11 – Poly- γ -Glutamic Acid Modulates α -Synuclein Pathology and Astrocyte Inflammatory Response

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α -Synuclein misfolding and spreading are key features of Parkinson's disease (PD) and highlight the importance of regulating the clearance of extracellular aggregates. In this context, astrocytes, alongside microglia, play a significant role in this process, by actively contributing to clearance. However, when overwhelmed, astrocytes can trigger an inflammatory response that worsens neurodegeneration. Therefore, strategies aimed at regulating the uptake of extracellular α -Synuclein aggregates by astrocytes and mitigating inflammation could hold therapeutic promise. In this sense, we investigated the potential protective role of poly-gamma-glutamic acid (γ -PGA), a biodegradable, non-toxic, and non-immunogenic biopolymer, that is produced through fermentation by *Bacillus* species. Known for its gut-stabilizing properties and widely consumed in Japan, γ -PGA has also been suggested to possess antioxidant and anti-inflammatory effects, potentially alleviating neuronal cell death and memory deficits. Murine primary astrocytes were isolated from the cortex of P1-P2 mice and treated with α -Synuclein pre-formed fibrils (PFFs) and γ -PGA. MTT assay was carried out to evaluate γ -PGA and PFFs effect on astrocytes viability. Additionally, high-resolution imaging and 3D reconstruction were used to examine the interplay between γ -PGA and PFFs, as well as the polymer's impact on PFF-induced inflammation. Lastly, RT-QuIC analyses was carried out to evaluate γ -PGA ability to interfere with α -Synuclein aggregation in vitro. Treatment with γ -PGA significantly improved cell viability in astrocytes exposed to PFFs. Moreover, γ -PGA colocalizes with PFFs leading to a reduction in the uptake of these aggregates by astrocytes and a subsequent decrease in their inflammatory response. Lastly, γ -PGA was able to significantly delay α -Synuclein aggregation in vitro. γ -PGA showed potential as a neuroprotective agent by modulating α -Synuclein aggregation, uptake and inflammation in astrocytes, indicating that it may have a buffering activity. These findings place γ -PGA as a promising candidate for therapeutic intervention in PD.

POSTER ID: 2.L.12 – SIAH2 and Mitochondrial Impairment in Models of Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative disorder in which the accumulation of α -synuclein (α Syn) and mitochondrial dysfunction play a key role in dopaminergic neurodegeneration. However, the molecular mechanisms underlying α Syn and mitochondrial dynamics, as well as intracellular mitochondrial trafficking, remain debated. The E3 ubiquitin

ligase Seven in Absentia Homolog 2 (Siah2) may have a role, as evidence suggests it is involved in modulating α Syn aggregation. Furthermore, Siah2 is capable of ubiquitinating outer mitochondrial membrane proteins, thus supporting its potential involvement in mitochondrial dysfunction. This study explores the relationship between Siah2 and mitochondrial impairment in PD, by using SH-SY5Y cells treated with rotenone for 24 hrs, to mimic the sporadic form of PD, and A53T transgenic mice, representative of familial form of PD. In vitro results show that Siah2 protein expression increases along with LC3-II, marker of mitophagy, in cells exposed to rotenone. Conversely, TFAM, marker of mitochondrial biogenesis, decreases. These effects are associated with alterations in mitochondrial membrane potential and mitochondrial calcium levels $[Ca^{2+}]_m$, measured by confocal microscopy, thus confirming that in a sporadic model of PD, changes in the expression of Siah2 might be related to mitochondrial dysfunction and mitophagy. Parallel in vivo experiments confirm this hypothesis since in 4-month-old A53T transgenic mice, the upregulation of Siah2, α Syn and LC3-II occurs in midbrain and striatum. To understand whether Siah2-induced mitophagy in PD models might have a role in regulating mitochondrial trafficking, further experiments were performed in vivo by investigating the expression of Miro1, a mitochondrial Rho GTPase essential for mitochondrial trafficking, and KIF5A, a microtubule-associated kinesin motor protein involved in mitochondrial transport. The results obtained in the midbrain and striatum of 4-month-old A53T transgenic mice reveal an upregulation of Miro1 and a reduction in KIF5A protein levels, thus suggesting a potential relationship between the Siah2 increase and mitochondrial trafficking.

POSTER ID: 2.L.13 – Modulation of RIT2 Levels as Therapeutic Strategy in Parkinson's Disease

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Parkinson's Disease (PD) is a neurodegenerative disorder characterized by the accumulation of phosphorylated α -Synuclein (pS129- α Syn) in the surviving Substantia Nigra dopaminergic neurons. The major unmet clinical need in PD is the lack of disease-modifying treatments. The small GTPase RIT2 has been identified as a risk factor for PD via genome-wide association studies. RIT2 mRNA is downregulated in the brains of idiopathic PD patients. Increasing RIT2 in PD cellular and murine models, overexpressing G2019S-LRRK2 or A53T- α Syn, reduces pS129-LRRK2 phosphorylation and pS129- α Syn-positive inclusions, attenuates lysosomal dysfunction, and prevents neurodegeneration, suggesting that restoring RIT2 levels is likely therapeutically beneficial. We employ SINEUP, a long non-coding RNA-based technology to moderately increase endogenous RIT2 protein levels. The molecule includes a sequence for

target specificity by antisense pairing with the target mRNA and an effector domain that fine-tunes the endogenous translation, avoiding overexpression- and ectopic-related toxicity. We designed five SINEUP-Rit2 molecules and delivered them into murine Neuro2A cells. We observed increased RIT2 protein without transcriptional changes for two molecules. Furthermore, we show that increased RIT2 levels led to a trend of increase in p38-MAPK phosphorylation, a downstream effector of RIT2, suggesting that SINEUP-dependent RIT2 translational increase has functional effects. We are now testing the most promising SINEUP-RIT2 molecules in LRRK2-G2019S overexpressing SH-SY5Y human cells, which display pS129- α Syn inclusions, increased pS1292-LRRK2 and lysosomal dysfunction. Preliminary observations showed that these RIT2-targeted SINEUP molecules reduced the number of pS129- α Syn inclusions, recapitulating RIT2 overexpression. Experiments are ongoing to prove that SINEUP-RIT2 can also counteract other pathological features. Furthermore, we use a proteomics approach to evaluate alterations in the proteomic landscape associated with RIT2 modulation, aiming to identify previously uncharacterized downstream effectors involved in the RIT2 signaling pathway. Thus, our data highlights the feasibility of modulating the expression of RIT2 via SINEUP technology as a potential neuroprotective strategy for PD.

POSTER ID: 2.L.14 – Unveiling ITPKB's Role in Synucleinopathies: Implications for PD and MSA

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Synucleinopathies are neurodegenerative diseases characterized by the accumulation of α -synuclein aggregates in neurons or glial cells. This group includes Parkinson's disease (PD) and Multiple System Atrophy (MSA), where α -synuclein aggregates are primarily found in dopaminergic neurons and oligodendrocytes, respectively. Currently no treatments are capable of halting or slowing the progression of these diseases. Key pathological features associated with α -synuclein aggregation are the disruption of calcium (Ca^{2+}) signaling pathways and mitochondrial dysfunction. The gene encoding for Inositol-trisphosphate 3-kinase B (ITPKB) has been linked with neurodegenerative diseases, such as PD. ITPKB converts inositol 1,4,5-trisphosphate (IP3) into inositol 1,3,4,5-tetrakisphosphate (IP4), reducing IP3-stimulated Ca^{2+} release from the endoplasmic reticulum (ER) into the cytoplasm. We are analyzing the functional role of ITPKB across cellular, pathological and clinical models. We observed that increased ITPKB protein levels correlate with α -synuclein augmentation in the cortex and striatum of a mouse model of MSA. In ITPKB-overexpressing cells, we are monitoring its impact on intracellular Ca^{2+} homeostasis, mitochondrial network and function. In these cells, inhibiting ITPKB restores histamine-dependent ER Ca^{2+} release dynamics, comparable to control cells. We have also observed that higher expression of ITPKB correlates with mitochondrial network disruption. We are currently investigating whether ITPKB increased expression alters mitochondrial respiration. In parallel, we are conducting a

comprehensive multi-omics analysis to uncover ITPKB's molecular mechanisms in PD and MSA. This involves integrating genetic, transcriptomic, and proteomic data. Analysis of brain spatial transcriptomics datasets will further reveal the spatial distribution and functional implications of ITPKB in these diseases. Given that increased levels of ITPKB are common features in both preclinical models of PD and MSA, we aim to rescue altered phenotypes by reducing the kinase activity. Our study will determine whether the inhibition of ITPKB could represent a therapeutic strategy for preventing and slowing the progression of PD and/or MSA.

POSTER ID: 2.L.15 – Pomalidomide Counters Neuroinflammation-Induced Synaptic Dysfunction in Parkinson's Model

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by both motor and non-motor symptoms. Among the latter, cognitive impairment is particularly debilitating, often preceding motor deficits and significantly affecting quality of life (Backstrom 2018; Garcia-Ptacek 2016). Recent evidence implicates pathological α -synuclein (α -Syn) in triggering microglial reactivity and promoting neuroinflammation, which may drive synaptic dysfunction and cognitive decline (Palmas 2022). To investigate these mechanisms and evaluate therapeutic strategies, we employed a PD rat model based on the intranigral infusion of human α -Syn oligomers (H- α SynOs). Three months post-infusion, H- α SynOs-infused rats exhibited cognitive deficits despite no overt neuronal loss in the anterior cingulate cortex (ACC). Histological analysis revealed marked neuroinflammation, with increased microglial TNF- α expression, and mitochondrial dysfunction, evidenced by reduced neuronal COX4 levels. Notably, immunofluorescence analyses demonstrated a significant increase in microglial phagocytosis of presynaptic Synapsin, indicating aberrant synaptic pruning. This microglia-mediated engulfment correlated with compromised synaptic integrity, as shown by decreased synaptic proteins PSD-95 and Synapsin, reduced synapse number, lower dendritic spine density, and a shift toward immature spine morphology. To address these alterations, animals were treated with pomalidomide (POM), an immunomodulatory imide drug that can modulate microglial states. Chronic POM administration significantly improved cognitive performance and restored key synaptic and neuronal markers. Synapsin expression was fully normalized, COX4 levels partially recovered, and dendritic spine density markedly increased. Importantly, these beneficial effects of POM were associated with reduced microglial TNF- α expression and Synapsin engulfment, indicating a robust attenuation of neuroinflammation and microglia-mediated synaptic loss. Taken together, these findings identify microglia-driven synaptic dysfunction as a key contributor to early cognitive decline in PD and support immunomodulation with POM as a promising therapeutic strategy targeting microglial activity.

POSTER ID: 2.L.16 – Insights into NF- κ B/c-Rel and O-GlcNAcylation Dysregulation in Parkinson's Disease

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterised by the selective degeneration of dopaminergic neurons in the substantia nigra (SN) pars compacta and the accumulation of Lewy body, which are primarily composed of α -synuclein (α -syn) aggregates. While the etiology of PD remains unknown, the identification of signalling pathways governed by PD-associated gene defects has provided insight into PD pathogenesis. Increasing evidence suggests that the transcription factor NF- κ B plays a pivotal role in maintaining neuronal health, and its dysregulation contributes significantly to the molecular mechanisms driving neurodegeneration. We previously demonstrated that male mice lacking the NF- κ B/c-Rel subunit (c-rel/- mice) develop a PD-like phenotype with aging, recapitulating key features of the human disease, including motor symptoms, synucleinopathy and nigrostriatal degeneration. Notably, these mice exhibit progressive accumulation of fibrillar α -syn aggregates and, by 18 months, c-Rel/- mice exhibit dopaminergic neuron loss in the SN, reduced striatal dopamine levels and the onset of motor deficits such as bradykinesia and gait disturbances. Recently, we demonstrated that NF- κ B/c-Rel DNA-binding activity is significantly reduced in both post-mortem SN tissue and peripheral blood mononuclear cells (PBMCs) from PD patients compared to healthy controls. Apparently, this DNA-binding reduction was evident despite unchanged NF- κ B/c-Rel protein content. Since NF- κ B/c-Rel DNA-binding has been shown to depend on post-translational modifications of c-Rel, we explored the role of O-linked- β -N-acetylglucosamine glycosylation (O-GlcNAcylation) a modification influenced by diet and known to enhance NF- κ B/c-Rel activity. Strikingly, western blot analysis and immunoprecipitation assay revealed a reduction in both global and NF- κ B/c-Rel O-GlcNAcylation levels in PBMCs isolated from fed PD patients. These data highlight the involvement of NF- κ B/c-Rel dysregulation in PD pathophysiology and the critical role of O-GlcNAcylation in regulating its DNA-binding activity. This suggests that O-GlcNAcylation of NF- κ B/c-Rel as well as of other potential targets could serve as novel biomarker for PD diagnosis.

POSTER ID: 2.R.01 – The Chaperone Clusterin: a Player in Parkinson's Disease Human Brain?

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OBJECTIVES: Clusterin (CLU) is a ubiquitous chaperone, involved in the clearance of misfolded proteins and implicated in neurodegeneration. Focusing on Parkinson's disease (PD), few studies reported a modulatory role of CLU on α -Synuclein (α -Syn) aggregation and clearance. This issue remains controversial and almost nothing is known about the interaction of CLU and α -Syn in human brain. Therefore, this study, performed in PD post-mortem human brain, aims to: i) analyze whether CLU is differently associated to α -Syn in Substantia Nigra pars compacta of patients compared to controls; ii) define whether CLU is involved in Lewy body (LB) formation. **METHODS:** Western Blots were performed on frozen post-mortem human brain to analyze Triton soluble and insoluble fractions, which contain monomeric and aggregated α -Syn, respectively. Immunofluorescence, Proximity Ligation Assay (PLA), and 3D reconstruction were used to analyze fixed brain slices. **RESULTS:** Biochemical analysis highlighted a positive correlation between CLU and α -Syn levels in all the fractions. Moving to fixed brain slices, no differences between controls and patients were observed in the co-localization of CLU with α -Syn. However, PLA analysis in different subcellular compartments revealed higher association between CLU and α -Syn in the somata showing α -Syn pathology. Interestingly, 100% of midbrain LBs were positive for CLU. Moreover, we evaluated how the distribution and association of CLU with α -Syn change during the LBs maturation process previously proposed in a model by our laboratory. **CONCLUSIONS:** Our findings strongly suggest CLU as a possible player in LB formation, paving the way to mechanistic studies aimed at unravelling the role of CLU in the modulation of α -Syn aggregation. This could place CLU as a promising target for a mechanism-based therapeutic strategy.

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POSTER ID: 2.R.02 – Therapeutic Potential of Intranasally Delivered Painless NGF in Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc), motor impairments, and chronic neuroinflammation. Growing evidence points to dysfunctional microglia as central drivers of disease progression, impacting both neuronal survival and synaptic architecture. Moreover, disruptions in gamma-band oscillations—partly orchestrated by parvalbumin-positive (PV) interneurons—have been increasingly linked to PD-related motor deficits. Compounding these alterations, PD is associated with an imbalance

in dopamine-acetylcholine (ACh) signaling and reduced levels of nerve growth factor (NGF), contributing to further neuronal vulnerability. We propose a novel therapeutic approach using human painless NGF (hNGFp), a mutated form of NGF with reduced nociceptive activity, administered intranasally for non-invasive delivery. In a 6-hydroxydopamine (6-OHDA) mouse model of PD, we investigate the effects of hNGFp on multiple pathological hallmarks, including microglial activation, DA neuron integrity, and PV interneuron function. Preliminary observations suggest that hNGFp may promote a shift in microglial phenotype toward a homeostatic, neuroprotective state. Wide-field calcium imaging revealed lesion-induced disruptions in PV-interneuronal connectivity, which we hypothesize may be sensitive to anti-inflammatory modulation by hNGFp. Additionally, by acting on NGF-responsive cholinergic neurons, hNGFp may indirectly restore ACh/DA balance. Although behavioral improvements are limited at this stage, our ongoing analyses aim to link cellular and circuit-level changes to functional outcomes. We propose that targeting microglial dysfunction—together with restoring synaptic and cholinergic balance—could represent a promising multi-modal strategy for early-stage PD intervention.

POSTER ID: 2.R.03 – Nusinersen Rescues Taurine Deficiency in Severe Spinal Muscular Atrophy

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Keywords: Spinal muscular atrophy; Cerebrospinal fluid; Nusinersen; Central nervous system; SMNΔ7 mouse model; taurine

Background: Spinal muscular atrophy (SMA), a leading genetic cause of infant mortality resulting from ubiquitous SMN deficiency, disrupts key biological processes such as neurotransmission, oxidative stress, and inflammation, all of which may be modulated by the neurotransmitter taurine. However, it remains uncertain whether a connection exists between SMN deficiency and the regulation of taurine homeostasis within the central nervous system (CNS). **Methods:** We used high-performance liquid chromatography (HPLC)

to quantify taurine in the spinal cord, brainstem, cortex, and cerebellum in SMN Δ 7 mice, during postnatal development. We then translate our observation into the clinic by measuring taurine concentrations in the cerebrospinal fluid (CSF) from control individuals (n = 7) and SMA patients of varying disease severity (n = 37) before and after therapy with the SMN-inducing drug Nusinersen. Results: Our data show a downregulation of taurine levels in the brainstem of SMN Δ 7 mice at late symptomatic stage relative to control littermates. Furthermore, we highlight a taurine reduction in the CSF of naïve SMA type 1 patients compared to controls. Importantly, Nusinersen treatment restored the taurine deficit in these SMA patients. Conclusions: These findings demonstrate that SMN deficiency dysregulates taurine homeostasis in the CNS of overt symptomatic mouse models and SMA patients affected by the most severe form of the disease. They also reveal the therapeutic efficacy of Nusinersen treatment in correcting this amino acid metabolism deficit. However, further research is needed to determine the mechanisms by which SMN deficiency causes taurine dysregulation and its potential contribution to SMA pathology.

POSTER ID: 2.R.04 – Neuroprotective Role of Pomegranate and Ellagic Acid Microdispersions in EAE

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Multiple sclerosis (MS) is a progressive, chronic neuroinflammatory disease characterized by immune-mediated demyelination, synaptic dysfunction, and central nervous system (CNS) infiltration by activated immune cells. Despite advances in disease-modifying therapies, there is a need for safer and more accessible neuroprotective strategies. Pomegranate extract (PE), particularly rich in ellagitannins and ellagic acid (EA), has demonstrated anti-inflammatory and antioxidant properties. However, EA's poor water solubility and limited bioavailability restrict its therapeutic potential. In this study, we assessed the efficacy of a novel pomegranate peel extract microdispersion (PEm), enriched in EA and punicalagin and obtained via Pulsed Ultrasound-Assisted Extraction (PUAE), in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS. We compared its effects to an ellagic acid microdispersion (EAm), both titrated to deliver 50 mg/kg EA. Female C57BL/6 mice were immunized with myelin oligodendrocyte glycoprotein (MOG_{35–55}) to induce EAE and treated from day 10 post-immunization with PEm or EAm dissolved in drinking water for 14 days. Clinical symptoms were assessed daily until day 23 $\pm$ 1. Postmortem spinal cord tissues were analyzed for inflammatory infiltration (CD45), microgliosis (Iba-1), astrogliosis (GFAP), and demyelination using histochemistry and Western blot. Both PEm and EAm significantly reduced clinical scores ($p < 0.01$), without impacting body weight or fluid intake. While demyelination showed partial, non-significant improvement, both treatments markedly reduced microglial and astrocytic activation, particularly in the spinal gray matter ($p < 0.05$). Furthermore, computational docking and in vitro assays revealed that EA may modulate α 2A/C adrenergic receptors, which are upregulated in EAE, suggesting a novel mechanism of action. Overall, these findings highlight the therapeutic potential of PEm and EAm in

mitigating neuroinflammation and glial activation in MS, offering promising candidates for future dietary supplementation or adjunctive therapy in MS management.

POSTER ID: 2.R.05 – Complement Aids Glutamate Release, Contributing to MS-Related Synaptic Damage

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Background: Complement proteins are key mediators of neuroinflammation in multiple sclerosis (MS), yet their impact on glutamate regulation and synaptic integrity remains unclear. Glutamate dysregulation contributes to excitotoxicity and disease progression in MS. **Objective:** To investigate the role of complement components C1q and C3 in modulating glutamate release and synaptic transporter function in a mouse model of MS. **Methods:** Experimental autoimmune encephalomyelitis (EAE) was induced in mice using MOG_{35–55} peptide. Cortical synaptosomes and gliosomes were isolated by Percoll® gradient centrifugation. Ultrastructural features were examined using transmission electron microscopy. Protein levels of C1q, C3 and glutamate transporters EAAT1 and EAAT2 were analyzed via western blotting. Glutamate release was assessed using [³H]D-Aspartate superfusion assays. Data (n ≥ 5) were analyzed using ANOVA with post hoc Tukey's or Dunnett's tests, and Student's t test for direct comparisons. **Results:** In EAE mice, synaptosomes showed decreased EAAT2 expression (p < 0.05), while gliosomes exhibited increased EAAT1 levels (p < 0.05). Complement-evoked glutamate release was reduced in synaptosomes and increased in gliosomes (p < 0.05). Pharmacological inhibition of EAAT1/2 with DL-tBOA significantly reduced complement-induced glutamate release in both synaptosomes and gliosomes (p < 0.05). Depletion of either C1q or C3 led to a significant reduction in glutamate release (p < 0.05), indicating their cooperative role in modulating transporter reversal. Elevated levels of C1q and C3 were confirmed in EAE mice compared to controls (p < 0.05). **Conclusion:** Complement proteins C1q and C3 disrupt glutamate transporter function, contributing to excitatory imbalance in the EAE model. This dysregulation may underlie synaptic dysfunction observed in MS and highlights complement modulation as a potential therapeutic strategy.

POSTER ID: 2.R.06 – Sex-Dependent Role of Autophagy in Glial Cells in ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that affects men and women differently, both clinically and at the cellular level. Glial cells—astrocytes and microglia—are key players in disease progression. One protective mechanism in these cells is chaperone-assisted selective autophagy (CASA), which helps clear misfolded proteins. However, whether CASA is regulated differently by sex in ALS remains unclear. To explore this, we analyzed spinal cord astrocytes from adult SOD1-G93A ALS mice. RNA sequencing revealed 620 differentially expressed genes (DEGs) in females and 187 in males, including a reduction in HSPB8 and HSPB6 in female ALS astrocytes. Conversely, qPCR showed increased expression of HSPB8 and BAG3 in male ALS astrocytes, while females showed a decrease. No differences were observed in astrocytes isolated at postnatal day 2 (P2), suggesting age-related changes. Autophagy markers confirmed these trends: p62 body counts were higher in wild-type (WT) males than in G93A, and NH₄Cl treatment (blocking autophagy) enhanced this difference. In females, G93A astrocytes showed significantly more p62 bodies than WT, indicating impaired protein clearance. Female WT astrocytes also had fewer p62 bodies than male WT, hinting at more efficient baseline autophagy. LC3 puncta, representing autophagosomes, were reduced in G93A versus WT astrocytes in males, but unchanged in females. In microglial cultures activated with cytokines, LC3 mRNA decreased in both sexes in G93A samples, while BAG3 protein expression dropped specifically in females, regardless of genotype. Overall, male astrocytes appear to compensate for ALS-related stress by upregulating CASA components. Female astrocytes, although more autophagy-competent at baseline, seem more susceptible to ALS-induced dysfunction. These findings highlight the importance of considering sex as a biological variable in ALS research and therapeutic design.

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POSTER ID: 2.R.07 – Sigma-1R: a Key Protein Modulating Ca²⁺ Organelles Interplay in Neurodegeneration

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Amyotrophic lateral sclerosis (ALS) is one of the most aggressive neurodegenerative diseases characterized by upper and lower motor neurons degeneration. Unfortunately, there is no adequate pharmacological treatment for this devastating neurodegenerative disease. Calcium (Ca²⁺) dyshomeostasis, with its complex machinery, is considered as one of the major pathomechanisms involved in motor neuronal loss and, therefore, as a putative target. Small organized subcellular compartments, including mitochondria, endoplasmic reticulum (ER), and lysosomes, are known to interact both physically and functionally in order to maintain inter-organelle Ca²⁺ balance and cellular homeostasis. Accordingly, aberrant communication

between organelles can significantly impact intracellular environment accelerating the course of the disease. In this context an important role is played by Sigma-1 receptor (Sigma-1R), a protein expressed at the mitochondria associated-endoplasmic reticulum membrane (MAM). It helps to locate ER Ca²⁺ channels, regulates Ca²⁺ homeostasis and acts as a molecular chaperone to control cell fate and signal transduction. Therefore, in our study we investigated the role of Sigma-1R in the interplay among intracellular organelles in preclinical models of ALS represented by NSC-34 motor neurons exposed to the neurotoxin L-BMAA (beta-methylamino-L-alanine), reproducing the Guamanian form of the disease (ALS/PDC), and SOD1G93A transgenic mice, a widely recognized model of the disease. Preliminary results showed that Sigma-1R was significantly downregulated in these preclinical models of the disease. In particular, ALS symptomatic animals showed decreased Sigma-1R expression in both brainstem and cortex, two brain areas mainly involved in the disease pathogenesis. Furthermore, several markers of ER stress and organellar suffering significantly increased in these brain regions in which Sigma-1R was downregulated, indicating a significant repercussion on organellar function. Based on these early findings, we suggest that organelle crosstalk and Sigma-1R could be considered as new druggable targets in ALS therapy.

POSTER ID: 2.R.08 – Therapeutic Effects of MSC-Derived EVs Intranasally Administered on ALS Mice

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by death of upper and lower motor neurons (MNs) with no effective therapies. Our previous results revealed that a single intravenous administration of mesenchymal stem cells (MSCs) prolonged survival probability and ameliorated pathological features, including gliosis and neuroinflammation, in the SOD1G93A mouse model of ALS. We postulated that the beneficial effect could be mediated by the paracrine activity of extracellular vesicles (EVs) secreted by MSCs shuttling microRNA. Subsequently, we demonstrated in-vitro the activity of MSCs-derived EVs on mouse and human ALS astrocytes in modulating the aberrant reactive phenotype, which in turn reduced the pathological release of pro-inflammatory factors and the accumulation of reactive oxygen species. Moreover, the exposure to MSCs-derived EVs significantly reduced the neurotoxicity of mouse and human ALS astrocytes towards co-cultured MNs. As a translational step, we here investigated the in-vivo efficacy of the chronic administration of MSC-derived EVs in symptomatic SOD1G93A. Male and female animals were intranasally administered, twice a week, with 10e9 MSC-derived EVs or vehicle solution, starting at the early symptomatic stage of the disease (80 days of life). Two-photon microscopy images demonstrated that MSC-derived EVs efficiently get into the CNS after

intranasal administration. The behavioral studies confirmed that MSC-derived EVs administration significantly ameliorates motor function scores and survival probability. While ex-vivo histological studies showed no significant effects on glial reactivity and neuroinflammatory parameters, EVs-treated animals demonstrated spinal cord MNS preservation and improved antioxidant response compared to control mice. The NFL serum levels, monitored at the end stage, were only partially reduced by EVs treatments. Overall, our data pave the way for the application of MSCs-derived EVs as a promising therapeutic strategy for ALS cure.

POSTER ID: 2.R.09 – The Role of HDAC11/STAT3/GSDMD Pathway in SOD1-G93A Skeletal Muscle Toxicity

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by the progressive degeneration of motor neurons and severe skeletal muscle atrophy. In muscle atrophy conditions such as ALS, muscular dystrophy, and cachexia, histone deacetylases (HDACs) contribute to increased protein degradation and impaired muscle regeneration. However, while the neuronal aspects of ALS have been extensively studied, the molecular mechanisms underlying muscle toxicity still remain poorly understood. Herein, we show that transient transfection with the human SOD1-G93A construct, followed by oxidative stress (H_2O_2), leads to a significant reduction in C2C12 cell viability. Notably, among the HDAC inhibitors tested, only the class IV HDAC inhibitor Mocetinostat prevented cell death. Furthermore, silencing HDAC11 significantly improved the survival of H_2O_2 -treated SOD1-G93A cells. HDAC11 protein levels were preferentially increased in the nucleus of SOD1-G93A cells following oxidative stress, underscoring its key role in SOD1-G93A-induced toxicity. We also found that in SOD1-G93A+ H_2O_2 -treated cells, Gasdermin D (GSDMD) -a key executor of pyroptosis-was significantly upregulated at both the transcriptional and translational levels, and that HDAC11 silencing (siHDAC11) counteracted the H_2O_2 -induced GSDMD upregulation. Moreover, Co-IP revealed that the signal transducer and activator of transcription 3 (STAT3) physically interacted with HDAC11 following H_2O_2 exposure. Indeed, siHDAC11 increased STAT3 acetylation and reversed the H_2O_2 -induced elevation of STAT3 protein levels. Lastly, chromatin immunoprecipitation (ChIP) assays revealed that STAT3, but not HDAC11, binds to the GSDMD promoter. Collectively, these findings uncover a novel HDAC11/STAT3/GSDMD axis regulating pyroptosis in ALS-associated muscle atrophy, suggesting that targeting HDAC11 may offer a new therapeutic strategy to preserve muscle integrity and function.

POSTER ID: 2.R.10 – Novel GlyT2 Mutation Impairs Transporter Function and Induces Hyperekplexia Phenotype

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Hyperekplexia, also known as startle disease (OMIM 149400), is a rare neurological disorder characterized by increased muscle tone, spasticity, and exaggerated startle responses to minor sensory stimuli. In severe cases, life-threatening apnea episodes may occur during the neonatal period. The condition arises from a disruption of inhibitory glycinergic neurotransmission, either partial or complete. The second most frequent genetic cause of hyperekplexia involves loss-of-function mutations in the SLC6A5 gene, which encodes the presynaptic neuronal glycine transporter GlyT2. Here we characterize a previously undescribed single-nucleotide point substitution of the GlyT2 gene, serendipitously generated by random mutagenesis in a mouse model (C57BL/6J *Slc6a5*^{trsl/Grsl}), which reproduces the hyperekplexia symptoms including tremor with stiff legged walk. This novel mutation results in the substitution of methionine at position 276 with isoleucine (Met276Ile). Despite the substitution being conservative, this recessive mutation has been predicted to be "probably damaging" by the bioinformatics tool PolyPhen-2. To understand the pathogenic mechanism of the mutation, we have constructed the GlyT2 mutant through site-directed mutagenesis and expressed the transporter in heterologous expression systems. Transporter function was analyzed by radiolabeled glycine uptake, and trafficking properties were assessed through biochemical and immunochemical techniques. The mutant showed residual uptake activity compared to the wild-type and even if it was predominantly retained in the endoplasmic reticulum. Using two-electrode voltage clamp (TEVC) in *Xenopus laevis* oocytes, the transport-associated currents were recorded, confirming the preserved substrate-coupled ion transport. These results indicate that the mutant retains residual activity which may be susceptible to enhancement by chemical chaperones.

POSTER ID: 2.R.11 – Cerebral Irisin and Its Role in Post-Stroke Neuroinflammation and Neurogenesis

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Physical exercise is being considered as a novel strategy to improve neurodegenerative disease management owing to the boost of myokine production that can stimulate a wide range of biological processes into the brain. Particularly, Irisin, a circulating myokine derived from the cleavage of the cell membrane protein FNDC5 (Fibronectin Type III Domain-Containing Protein 5), can cross the blood-brain barrier and modulate both neuroinflammation and neurogenesis. Consequently, this evidence shed light on the potential role of physical exercise and myokines to facilitate the restoration of brain damage and

achieve enhanced outcomes following ischemic strokes. In this context, our study aimed to: (1) establish a mild physical exercise protocol able to stimulate, in ischemic mice, FNDC5 expression in muscle tissue, and (2) investigate the role of irisin in the ischemic brain. Interestingly, our results, obtained in in vitro and in vivo models of brain ischemia, demonstrate that not only FNDC5 is physiologically produced within brain cells, but also that its expression significantly increased following exposure to Oxygen and Glucose Deprivation (OGD) in BV-2 mouse microglial cells, thus supporting the involvement of irisin in post-stroke neuroinflammation. Surprisingly, immunofluorescence analysis revealed the presence of irisin receptors on the plasmamembrane of microglial cells. This finding suggests an autocrine mechanism and expands the initial hypothesis of a simple paracrine effect. Furthermore, the colocalization of FNDC5 with neural progenitor cell markers within neurogenic niches supports a potential role for FNDC5/irisin not only in neuroinflammation but also in neurogenesis. Together, these findings support the development of non-invasive and innovative pharmacological strategies for the treatment of ischemic stroke by stimulating irisin release and irisin-mediated pathways to promote neurogenesis.

POSTER ID: 2.R.12 – Investigating HTT Somatic Instability in Huntington’s Disease Brain Organoids

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Huntington’s Disease (HD) is a neurodegenerative disorder caused by the expansion of CAG repeats in the HTT gene. The number of CAG repeats inversely correlates with the age of onset: adult-onset HD typically presents with 36–55 repeats, while juvenile cases exceed 60, in the germline. This trinucleotide tract is particularly unstable in post-mitotic neurons of the striatum and cortex, leading to somatic mosaicism that accelerates selective neuronal degeneration. Although recent GWAS have uncovered both trans- and cis-acting modifiers of HTT CAG instability and symptom onset, it remains unclear which factors contribute to somatic instability, how they interact, and why their effects are cell-type specific. The full spectrum of genetic modifiers, the role of repeat length and sequence purity, and the transcriptional context of vulnerable neurons is still unexplored. To address this, we have optimized a long-term 3D neuronal differentiation protocol using the CAGinSTEM human embryonic stem cell platform, enabling the generation of HD-relevant neuronal subtypes within ventral-dorsal organoids. This system faithfully models key aspects of HD pathology and provides a robust in vitro platform to study somatic repeat instability. Building on this, we are developing an integrated molecular and computational pipeline that combines single-nucleus HTT CAG sizing with matched transcriptomic profiling. This multimodal approach will allow us to link CAG repeat heterogeneity to gene expression at the single-cell level, offering unprecedented insights into how somatic instability drives HD progression in a cell-type-specific manner. Ultimately, this study will provide a powerful tool to dissect the temporal and spatial dynamics of HD pathogenesis, with broader implications for understanding other repeat expansion disorders and for developing therapeutic strategies aimed at rescuing somatic instability.

POSTER ID: 2.R.13 – Chromatin Disruption in Huntington's Disease Alters Development and Precedes Neuropathology

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Huntington's Disease (HD) is a neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the HTT gene, leading to an abnormally long polyglutamine (polyQ) tract in mutant huntingtin (muHTT). Although clinical symptoms typically emerge in adulthood, increasing evidence indicates that the mutation exerts effects during early development, potentially initiating pathological processes decades before symptom onset. Among the affected processes, muHTT interferes with chromatin organization and gene regulation. In vitro studies have shown that muHTT alters the deposition of key histone modifications—H3K4me3, associated with active transcription, and H3K27me3, linked to repression—thereby disrupting euchromatin and heterochromatin compartmentalization. Notably, these two marks can co-occur at the same transcription start sites, forming bivalent domains crucial for developmental gene regulation. To further understand these early alterations, we investigated the role of Polycomb group (PcG) proteins, focusing on the PRC1 and PRC2 complexes, through both in vitro and in vivo models. Our analyses reveal abnormalities in PcG bodies number and size, suggesting that chromatin organization is impaired at early developmental stages in HD. These findings are supported by ChIP-seq data, which demonstrate dysregulation of facultative heterochromatin and euchromatin compartmentalization. Overall, our data indicate that muHTT-driven chromatin disorganization precedes neuropathology and may contribute to early disruption of neuronal pathways involved in vesicle trafficking, synaptic architecture, and neurite projection. These insights reinforce the concept of HD as a neurodevelopmental disorder with epigenetic underpinnings.

POSTER ID: 2.R.14 – Terpenes and Alzheimer's Disease: in Vitro Neuroprotective Effects

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Alzheimer's disease (AD) is the primary cause of dementia during ageing. Although its pathological mechanisms are partly understood, disease-modifying treatments remain

unavailable. Accordingly, there is growing interest in exploring lifestyle interventions that could aid in preventing and alleviating symptoms. In this context, we investigated the potential benefits of nature exposure, particularly through the inhalation of terpenes, with a focus on pinene. Therefore, we set up two stem cell models of AD. First, we used NE-4C cells, a murine neural stem cell line that upon retinoic acid stimulation differentiates at 7 days in neurons and at 14 days in a mixed population of neurons and astrocytes. In this model, a 48h administration of A β 1-42 monomers (0.1-5 μ M) to both undifferentiated and differentiated cells led to increased cell death (assessed through MTS assays and Incucyte live cell imaging). Interestingly, it was partially prevented by pinene (250 μ M) co-administration. Moreover, A β 1-42 oligomers (whose assembly has been verified through WB) seem to be more toxic to NE-4C cells, compared to monomers. Following monomers or oligomers treatment, we also evaluated apoptotic pathway activation through C-Caspase3 staining, whose levels were significantly decreased following pinene treatment in both neurons (-40%) and astrocytes (-37%). Finally, through Ellman's assay, we demonstrated that pinene is able to partially inhibit acetylcholinesterase activity (-30%). As a second model, we differentiated cortical neurons from a healthy donor line of human induced Pluripotent Stem Cells (iPSCs). Upon A β 1-42 oligomer treatment, preliminary data confirm results similar to those observed in murine cells. This study may help to clarify the mechanisms behind the benefits of green exposure, providing a scientific foundation to support the adoption of lifestyle interventions for the prevention and treatment of AD.

POSTER ID: 2.R.15 – Pharmacological Modulation of Mitochondrial Metabolism to Mitigate Alzheimer's Disease Neurodegeneration

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, memory loss, and neuronal death. Mitochondrial dysfunction is a hallmark of AD, contributing to impaired energy production, increased oxidative stress, and disrupted ionic homeostasis. Therefore, our therapeutic approach involves the pharmacological modulation of mitochondrial metabolism through the activation of glutamate dehydrogenase (GDH). GDH, an enzyme involved in the oxidative deamination of glutamate to α -ketoglutarate, plays a crucial role in mitochondrial metabolism by facilitating the tricarboxylic acid (TCA) cycle and, therefore, ATP production. In our experimental model the pharmacological activation of GDH by the allosteric activator 2-Aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) enhanced mitochondrial ATP production, thereby improving cellular bioenergetics and reducing oxidative stress, ultimately promoting neuronal survival. Ionic dysfunctions in AD are linked to disrupted calcium homeostasis and impaired ion channel function. Additionally, mitochondria play a pivotal role in regulating intracellular calcium levels. In this context GDH activation enhanced mitochondrial calcium buffering capacity. By refilling the TCA cycle and improving cellular bioenergetics, GDH activation helped restore calcium homeostasis, potentially maintaining neuronal excitability and synaptic function,

which are critical for cognitive processes. Another major contributor to AD pathology is oxidative stress, driven by mitochondrial ROS production. GDH activation mitigated oxidative stress by improving mitochondrial respiration and enhancing antioxidant defenses. In the context of AD, excessive accumulation of amyloid-beta (A β 1-42) and phosphorylated tau (pTau) contributes to neurodegeneration. The present data suggests that by enhancing mitochondrial energy metabolism and reducing oxidative stress, the activation of GDH by BCH can reduce the pathological buildup of these biomarkers, thereby promoting neuronal survival. In conclusion, the pharmacological modulation of mitochondrial metabolism through GDH activation presents a promising avenue for mitigating neurodegeneration, ionic dysfunctions, and oxidative stress in AD. This approach, targeting core metabolic deficits in AD, potentially offers a novel therapeutic strategy to slow disease progression.

POSTER ID: 2.R.16 – Targeting NSC Metabolism to Promote Neurogenesis in Alzheimer’s Disease

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Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterised by several factors including impaired glutamate neurotransmission, which results in sustained receptor activation, chronic neuronal depolarization and excitotoxic neuronal death. Neural stem cells (NSCs) are present in the adult brains of all mammalian species and contribute to the continuous generation of neural cells throughout life. They have multilineage potential being able to differentiate into either neuronal or glial cell. However, in AD, NSCs function is impaired, potentially due to metabolic dysregulation within the neurogenic niche. This research examines how glutamate utilization in adult NSCs could be dysfunctional during AD progression, emphasizing changes in glutamate metabolism, mitochondrial function, and reactive oxygen species (ROS) production as putative druggable targets. In this work, NSCs were isolated from both triple-transgenic (3xTg-AD) mice, recapitulating key features of human AD, and age-matched wild-type (WT) controls. In vitro studies revealed that 3xTg-AD NSCs had a reduced proliferation and viability, along with an increase in glutamate transporters and glutamine synthetase, which indicated altered glutamate handling. Interestingly, there was no decrease in glutamate dehydrogenase expression, which is typical in AD brains, suggesting a possible adaptive shift toward glutamate-dependent energy metabolism. The results suggest that early AD-related metabolic stress could lead to compensatory mechanisms in NSCs to sustain mitochondrial function and energy production through glutamatergic pathways. On this ground, interventions that sustain the metabolic use of glutamate may simultaneously limit the excitotoxicity burden, restore mitochondrial bioenergetic functions thereby improving the regenerative potential of NSCs into both neuronal and glial cells in AD. Therefore, modulating the glutamatergic system could enhance cellular resilience, promote neurogenesis, and contribute to neural repair in the early stages of disease progression.

POSTER ID: 2.R.17 – Isolation and Characterization of Brain-Derived Extracellular Vesicles in 5XFAD Mice

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Extracellular vesicles (EVs) are nanosized membranous particles secreted by cells into the extracellular space containing a variety of functional cargoes, including lipids, proteins, mRNAs and miRNAs. EVs represent a physiological mechanism of intercellular communication but also a key player in the development of several neurodegenerative disorders, such as Alzheimer's Disease (AD). Recently, EVs have been efficiently isolated from cerebrospinal fluid and blood of AD patients and transgenic AD mouse models demonstrating their capability to transport pathological cargoes, such as amyloid β , pro-inflammatory cytokines and miRNAs. In addition, brain-derived EVs are capable to cross the blood-brain barrier, allowing detection in peripheral body fluids. This characteristic opens up opportunities for their potential use as disease biomarkers, to assess a differentiation of AD subtypes at early stage and follow-up monitoring. In order to identify and validate useful EV-derived AD biomarkers at pre-clinical level, we optimized a novel protocol for isolating vesicles from mouse brain areas, obtaining sufficient yield for ex-vivo analysis. Firstly, we compared four methods to isolate EVs from three specific mouse brain regions, namely cortex, hippocampus and hypothalamus; then, isolated EVs were quantified and morphologically characterized by means of Nanoparticle Tracking Analysis. Subsequently, the expression of specific membranous EV-markers was measured using an untargeted proteomic approach. Finally, a miRNome analysis was performed via Next-Generation Sequencing and qPCR in the cortex and hippocampus of wild type and 5XFAD -a transgenic model of AD- mice, in order to identify differentially expressed miRNAs across different time points of AD progression (2, 4 and 8 months of age). In this study we offer a standardized technical protocol to efficiently isolate EVs from specific brain areas; in addition, the analysis of their content provides new insights into the potential role of EVs in early AD pathogenesis, opening the way for future biomarker discovery and therapeutic strategies.

POSTER ID: 2.R.18 – Extracellular Vesicles Derived from Preconditioned Stem Cells Against Alzheimer's Disease

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Alzheimer's disease (AD) is the most common form of age-related dementia. While no single causative factor has been identified, the role of the immune system in AD pathogenesis is becoming increasingly evident, thus highlighting the need to identify novel strategies to target glial cells in the brain, namely astrocytes and microglia. In this perspective, mesenchymal stem cells (MSCs) could represent an important ally, as they can secrete extracellular vesicles

(EVs) with relevant paracrine anti-inflammatory effects. In our study, we tested the role of pro-inflammatory cytokine-preconditioned EVs and oxygen-glucose deprivation-preconditioned EVs, which were intranasally injected in wild-type animals as well as two distinct murine models of AD: the 5xFAD model, which exhibits amyloid pathology, and the hTau model, which mimics AD tauopathy. Our findings suggest improved performance during behavioural tests in the WT group, suggesting an intrinsic trophic effect of MSC-EVs, however they seem unable to revert cognitive decline in the transgenic animals. Despite this, molecular data reveal a reduction in astrogliosis and amyloid plaque burden in 5xFAD, as well as a significant decrease in the accumulation of levels of tau phosphorylation in the hippocampus of hTau mice. A deeper investigation is required to understand how EVs modulate glial activity and which downstream processes are activated to improve AD pathology. Nevertheless, these preliminary data suggest that MSC-derived EVs play a crucial role in modulating AD-related pathology, potentially offering a novel therapeutic approach for neuroinflammation and disease progression.

POSTER ID: 2.R.19 – Age-Dependent Increase of Senescence Markers in an Alzheimer’s Mouse Model

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Cellular senescence is a state of irreversible cell cycle arrest triggered by various stressors such as DNA damage, oxidative stress, and chronic inflammation. Accumulation of cellular senescence is one of the major drivers of aging and play an important role in age-related diseases. Senescent cells are characterized by a senescence-associated secretory phenotype (SASP), marked by increased expression of cell cycle inhibitors (p16INK4, p21, p53), secretion of pro-inflammatory cytokines (e.g., IL-6, IL-1 β , TNF α), elevated levels of reactive oxygen species (ROS), enhanced activity of senescence-associated β -galactosidase (SA- β -Gal), impaired phagocytosis and morphological alterations. In Alzheimer’s disease (AD), it is hypothesized that microglia, astrocytes, and neurons become senescent in response to amyloid- β accumulation, primarily due to oxidative stress, chronic inflammation, and DNA damage. This study aims to investigate the onset and progression of a senescence state in the Alzheimer’s mouse model AppNL-G-F. The levels of the senescence markers p16INK4, p21 and p53 were evaluated at 4, 12, and 24 months of age through western blot analysis; the positivity for these markers was also assessed in microglia, astrocytes and neurons by means of immunofluorescence analysis. SA- β -GAL enzymatic activity and gene expression of p16INK4, p21, p53, IL-1 β and IL-6 were also determined in 12-month-old mice. No significant differences in senescence markers were observed between 4-month-old WT and AppNL-G-F mice. Interestingly, at 12 months of age, AppNL-G-F mice showed higher levels of senescence markers both at the protein and gene expression level, corresponding also to a higher percentage of microglia and astrocytes positive for the markers and to a higher SA- β -GAL enzymatic activity. These data were consistent also for the 24-month-old AppNL-G-F mice. Overall, this study suggests an age-dependent increase of senescence markers in AppNL-G-F

mice, suggesting the possible role of this mouse model in further senescence studies and for the development of senotherapeutics.

POSTER ID: 2.R.20 – Behavioral, Molecular, and Device-Based Gene Therapy Insights in FTD/GRN Model

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Progranulin-related frontotemporal lobe dementia (FTD/GRN) is an inherited form of FTD caused by decreased levels of progranulin (PGRN). FTD/GRN results from mutations on the GRN gene, which encodes for PGRN, a key regulator of neuronal survival and lysosomal function. PGRN haploinsufficiency caused by heterozygous GRN mutations leads to impaired glucocerebrosidase (GCase) activity, a lysosomal enzyme essential for glucosylsphingosine and bis(monoacylglycerol)phosphate (BMP) metabolism. To restore brain PGRN signaling addresses the root cause of the disease, although is challenging to achieve from a treatment perspective. We exploited our grn knockout mouse model and a gene therapy device (GTD) platform to deliver therapeutic factors directly into the brain of grn^{-/-} and grn^{+/-} mice. Behavioral assessments, at both pre-symptomatic and advanced disease stages, revealed increased aggression and significant deficits in spatial memory in mutant mice, while no detectable motor impairments or altered pheromone perception were observed. We also evaluated social decision-making abilities during the symptomatic phase. Moreover, molecular analyses indicated alterations of ionotropic glutamate receptors at postsynaptic sites. ELISA analyses confirmed reduced plasma PGRN levels in grn^{+/-} and complete absence in grn^{-/-} mice. Enzymatic assay revealed decreased GCase activity in the mutant mice's central nervous system (CNS) and liver. Implanting for 8 weeks the GTD-PGRN or -PGRN/PSAP significantly reduced aggressive behavior and enhanced GCase activity within the CNS, while ongoing experiments are assessing whether the GTD platform can also restore glucosylsphingosine and BMP levels. Combined, these experiments deliver preclinical proof-of-concept data, a key milestone in the development of novel therapies for FTD/GRN.

POSTER ID: 3.L.01 – Modeling and Characterization of Frontotemporal Dementia via Cerebral Organoids

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Frontotemporal Dementia (FTD) is a progressive neurodegenerative disorder characterized by the degeneration of the frontal and temporal lobes, leading to profound alterations in personality, behavior, and language. This project explores the potential of cerebral organoids derived from human induced pluripotent stem cells (hiPSCs) to model the pathophysiology of FTD, with a particular focus on the C9ORF72 mutation—one of the most prevalent genetic

causes of both FTD and amyotrophic lateral sclerosis (ALS). Organoids were generated from hiPSCs obtained from both healthy individuals and FTD patients and their developmental trajectory was assessed at 30, 60, and 90 days in vitro (DIV) using a combination of morphometric measurements and immunohistochemical analysis of neural markers. Specifically, SOX2 and Ki67 were used to evaluate stemness and cell proliferation; PAX6 was analyzed as a marker of neural progenitors; and neuronal maturation was confirmed through the expression of TBR1, CTIP2, SATB2, and MAP2. Initial findings indicate robust and progressive organoid development over time. Notably, organoids derived from FTD patients exhibited signs of accelerated neuronal maturation compared to controls. Morphometric analysis revealed a consistent increase in organoid size—from approximately 400 μ m at early stages to over 1,5 mm of diameter by day 90—with significant morphological divergence between healthy and FTD-derived organoids emerging over time. Future investigations will focus on pathological hallmarks associated with the C9ORF72 mutation, including the formation of RNA foci and the accumulation of dipeptide repeat proteins (DPRs). Particular attention will be directed toward Golgi apparatus fragmentation, a possible consequence of DPR-related toxicity in neurodegenerative disease. Overall, this study supports the use of cerebral organoids as a reliable and dynamic in vitro model for FTD, providing a valuable platform for mechanistic studies and therapeutic development, while potentially reducing the dependence on animal models.

POSTER ID: 3.L.02 – The Transcriptional Repressor REST/NRSF in the Experimental Autoimmune Encephalomyelitis

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Neuroinflammation causes neuronal dysfunctions that contribute to the pathogenesis of multiple sclerosis and its mouse model, the experimental autoimmune encephalomyelitis (EAE). Neuroinflammation is also associated with the upregulation of the transcriptional repressor REST (RE1-silencing transcription factor), a master regulator of neuron-specific genes. Its contribution to promoting or protecting from the pathology remains to be clarified. Here, we found that REST is upregulated in neurons in the spinal cord during EAE, which is associated with a consistent downregulation of REST-repressed genes. We then knocked out REST in neurons of the lower central nervous system by injecting AAV vectors expressing CRE or its recombinase-defective variant (deltaCRE) intra cisterna magna at birth, and induced EAE after 8 weeks with myelin-oligodendrocyte glycoprotein peptide 35-55 (MOG35-55). We observed that REST depletion is associated with an experience-dependent decrease in the latency-to-fall time on the Rotarod compared to deltaCRE-treated EAE mice and with lower walking performance in the open field. Although the treatment did not significantly affect

clinical score and weight changes in female EAE mice, in males it was associated with a higher peak score and trends towards a larger relative weight loss and a higher disease incidence. Our data show that, during EAE, REST is upregulated in neurons, suggesting that this may protect from EAE-induced motor impairments.

POSTER ID: 3.L.03 – Biallelic FASTKD5 Mutations Are Associated with COX Deficiency and Leigh-Syndrome

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The mitochondrial genome is transcribed as two polycistronic transcripts that must be processed to release the 13 mRNAs, 2 rRNAs and 22 tRNAs in its coding sequence. Most open reading frames are flanked by tRNAs, and their maturation is accomplished by RNase P and RNase Z. However, a few mRNAs are not flanked by tRNAs, and their maturation requires the activity of FASTKD5, a member of the FASTK family of proteins, all of which are associated with RNA transactions. Using exome sequencing we identified compound heterozygous variants of unknown significance in FASTKD5 in three subjects with Leigh syndrome, a

progressive neurodegenerative disease characterized by lesions in the brainstem and basal ganglia. Among the three subjects we identified three missense variants and two frameshift variants leading to a premature stop codon. Analysis of fibroblasts from two subjects showed reduced steady-state levels of FASTKD5 protein by immunoblot, reduced translation of the cytochrome c oxidase subunit 1, impaired assembly of complex IV, and a consequent decrease in cytochrome c oxidase enzymatic activity. The extent of these deficiencies correlated with the severity of the clinical phenotype. Expression of a wild-type FASTKD5 cDNA, but not cDNAs expressing the missense mutations, rescued all the molecular defects in the subjects' fibroblasts, demonstrating that the alleles are pathogenic. Two of the three identified missense mutations resulted in near complete loss of function while one was hypomorphic resulting from impaired protein stability. These are the first reported cases of mitochondrial disease associated with biallelic variants in FASTKD5, adding to a growing list of primary genetic mutations causing Leigh syndrome associated with complex IV deficiency.

POSTER ID: 3.L.04 – Pharmacological Autophagy Stimulation Rescues Proteinopathy and Cognitive Decline in LSDs

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Lysosomal storage disorders (LSD) are a group of rare inherited metabolic disorders caused by defects in lysosomal function. Among these, Mucopolysaccharidoses (MPS) are characterized by the loss of function of lysosomal enzymes responsible for the degradation of glycosaminoglycans (GAGs). When the undegraded GAG is heparan sulfate (HS), such as in MPS type IIIA, children manifest neurodegeneration and dementia. Defective degradation of HS is associated to the accumulation of secondary storages, made of beta-amyloid, tau and alpha-synuclein. Promoting the degradation of secondary storages is one of the most promising therapeutic strategies to prevent neurodegeneration. Genetic overexpression of the transcription factor EB (TFEB), which controls genes involved in the autophagy/lysosomal degradation process, seems to promote the degradation of protein aggregates in animal models of neurodegeneration. However, there are still few synthetic drugs capable of stimulating TFEB and crossing the blood-brain barrier. In the current study, using validated animal and cellular models of MPS, we are testing a compound that has been shown to promote TFEB-mediated autophagy and lysosomal biogenesis in wild-type/control animals. Our in vitro analysis demonstrated that the drug is effective in patients' cell lines of various types of MPS, shedding light on the mechanism by which it activates autophagic flux in the context of disease. Analyses conducted in a validated animal model of MPS-IIIA have shown that the drug improves the cognitive deficits and favours the clearance of secondary storages

composed of beta-amyloid. These results provide proof-of-concept evidence on a novel therapeutic compound for the treatment of LSDs.

POSTER ID: 3.L.05 – Investigating in Vitro the Neuroinflammatory Processes in Opioid Side Effects

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Substance use disorder is a mental disorder affecting behavior and leading to the inability to control the use of addictive substances. Opioid use disorder can develop after the chronic use of opioid drugs, which represent the gold standard for the therapeutic treatment of moderate-to-severe pain. Their use can lead to numerous side effects, including tolerance, dependence, and hyperalgesia. In this respect, alternative opioid drugs such as the bifunctional MOR/NOP receptor ligands cebranopadol and BU08028 are currently under investigation as promising analgesic agents provided with a lower abuse liability profile. Recent studies indicate that microglial activation and proinflammatory processes in the central nervous system may contribute to different opioid side effects including analgesic tolerance. For this reason, the aim of this study was to evaluate if cebranopadol and BU08028, that seem to induce less tolerance and dependence than morphine, were able or not to alter inflammatory pathways in vitro, using neuronal and microglial cell lines. In addition, since the prolonged opioid exposure may interfere with oligodendrocyte precursor cells, differentiation and myelination processes, a primary culture of neural stem cells-derived oligodendrocyte precursor cells was set up in order to investigate their role in opioid side effects. Preliminary data indicate that the treatment with cebranopadol and BU08028 (1 μ M) leads to a reduction in the neuronal expression of Toll-like receptor 4 (TLR4) and Protein Kinase C epsilon (PKC ϵ) compared to morphine (10 μ M), suggesting that these compounds may attenuate this signal transduction pathway. Moreover, a significant decrease in interleukin-6 (IL-6) expression was observed in a microglial cell line, supporting the hypothesis that cebranopadol and BU08028 may induce lesser microglial activation than morphine. These results may be helpful for a better understanding of the involvement of neuroinflammatory processes in opioid side effects, including the development of analgesic tolerance.

POSTER ID: 3.L.06 – Evaluation of the Anti-Glycation and Neuroprotective Potential of Plant-Based Extracts

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Advanced glycation end-products (AGEs) play a crucial role in the progression of neurodegenerative diseases by promoting oxidative stress, protein aggregation and chronic inflammation. In our preclinical studies, we previously demonstrated the neurotoxic and degenerative effects of methylglyoxal (MGO), a precursor to AGEs, which was shown to impair

cognitive function and induce neuronal loss, following systemic inflammation observed both in plasma and at the intestinal level. Thus, it became imperative to identify novel druggable targets able to prevent the detrimental effect of AGEs. Plant kingdom is a powerful source of antiglycating compounds, so, in this study, we investigated the antiglycation and neuroprotective effects of endemic and rural plant-based extracts in SH-SY5Y human neuroblastoma cells. After optimizing the anti-glycation assay, we incorporated fluorescence-based AGE quantification and Thioflavin-T aggregation assays to more comprehensively assess antiglycation activity. *Lavandula angustifolia*, *Pistacia vera*, *Zingiber officinale*, *Ilex paraguariensis*, *Vaccinium myrtillus* L., *Oryza sativa* and *Brassica sprout* extract significantly inhibited AGE formation and reduced protein aggregation. To further screen their neuroprotective efficacy, SH-SY5Y cells were exposed to glycated-BSA or MGO-induced stress in the presence or absence of plant extracts. Cell viability, ROS levels, and neuronal markers were assessed. Extracts with high antiglycation capacity also demonstrated significant neuroprotective effects. Additionally, we explored the use of an electrochemical biosensor as a rapid, non-fluorescent method to detect AGEs and differentiate native from glycated proteins, showing promising preliminary results for future screening applications. Our findings support the hypothesis that selected natural extracts possess dual antiglycation and neuroprotective properties. These results underscore the potential of plant-derived compounds as complementary tools for preventing AGE-related neuronal damage, paving the way for further investigations in models of neurodegenerative diseases. In future studies, we plan to perform phytochemical analyses to identify the major bioactive components responsible for these effects and to further disclose their mechanism of action.

POSTER ID: 3.L.07 – In Vitro Modeling of Neuron-Glioma Interactions Using Co-Culture Systems

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The tumor microenvironment (TME) is a dynamic and multifaceted system composed of cellular and non-cellular elements that significantly influence cancer growth and metastasis. Within this context, neuron-tumor interactions have gained increasing attention, particularly in nervous system tumors, where they appear to play a pivotal role in influencing key processes such as proliferation, survival, and overall tumor progression. Growing evidence indicates that neurons and tumor cells engage in a bidirectional exchange of molecular signals, influencing each other's behavior. To investigate the mechanisms underlying this neuron-tumor crosstalk, we developed and optimized different in vitro co-culture models. We used primary cortical neurons from C57BL/6 mice and human induced pluripotent stem cells (hiPSCs)-derived cortical neurons, cultured with U87 glioblastoma cells. Both transwell-based systems and conditioned media (CM) approaches were employed to assess the influence of glioma-derived factors on neuronal health. In particular, neuronal morphology and viability were monitored in real-time using the Incucyte live-cell imaging system and the Neurotrack module. CM exposure induced a significant 36% reduction in neurite length ($p = 0.0055$), a 44% decrease in cell number ($p = 0.0024$), and increased cytotoxicity after 48 hours ($p = 0.0116$). These results highlight the neurotoxic potential of glioblastoma-secreted

components. Furthermore, preliminary Western Blot analyses indicated a marked decrease in tubulin (–60%) and synapsin (–80%) protein expression in CM-treated neurons, suggesting compromised cytoskeletal integrity and synaptic function. Ongoing efforts aim to replicate these findings using hiPSC-derived neurons and refine co-culture models through advanced systems such as microfluidic devices. These platforms will provide a more physiologically relevant setting to investigate the dynamics of neuron-tumor interactions and identify potential therapeutic targets to disrupt pro-tumor neuronal support.

POSTER ID: 3.L.08 – Manipulating GABAergic Mechanisms to Delay Glioblastoma Invasion of Peritumoral Microenvironment

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The interaction between tumor cells and the main components of the tumor microenvironment (TME) seems to guide the progression of glioma. Several recent studies have highlighted that the synaptic activity of pyramidal neurons potently drives Glioblastoma (GB) proliferation. However, the role played by other neuronal cell types is still poorly understood. Based on our previous findings showing that the optogenetic stimulation of parvalbuminergic (PV)-inhibitory interneurons negatively regulates glioma progression, here we test whether a chemogenetic manipulation of peritumoral PV-interneurons could influence GB growth. To address this issue, AAV-hSyn-DIO-hM3D(Gi)-mCherry or AAV-hSyn-DIO-hM4D(Gq)-mCherry were injected in the motor cortex bilaterally with respect to the GL261 injection site in PV-Cre mice. In order to manipulate PV-interneurons activity, animals were randomized to receive CNO or vehicle intraperitoneally twice a day from day 12 (i.e., the glioma symptomatic stage) to 22 (i.e., the glioma late stage) post tumor induction. As readouts, we: i) evaluated tumor volume using MRI, ii) performed longitudinal motor testing (Grip Strength and Grid Walk), iii) assessed survival, and iv) determined glioma proliferation. Our findings indicate that inactivation of PV-interneurons exacerbates glioma-associated symptoms, impairing motor performance and inducing seizures in ~43% of cases, without affecting tumor volume or survival. In contrast, PV-activation reduced tumor volume and BrdU-positive cell proliferation. Finally, to explore potential pharmacological parallels, we tested the GABA-B receptor agonist baclofen, which did not impact glioma proliferation in vitro but significantly reduced tumor volume and slowed motor decline in vivo in GL261-bearing mice. This effect closely mirrors the results observed with chemogenetic activation of PV-interneurons, supporting the idea that the PV-inhibitory circuitry in the peritumoral region may play a critical role in delaying GB growth, thereby protecting peritumoral tissue from GB proliferation and invasion.

POSTER ID: 3.L.09 – FLASH Radiotherapy in Mice: Preclinical Look into Brain Tissue Response

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Radiotherapy (RT) remains a cornerstone in the treatment of various tumors following surgical resection, including glioblastoma (GBM)—the most common and aggressive primary brain tumor in adults. Despite its central role, RT often encounters a major hurdle in GBM: radioresistance, which significantly contributes to its poor prognosis and high mortality rate. In such context, the development of ultra-high dose-rate irradiation ($\geq 40\text{Gy/s}$), known as FLASH radiotherapy, has been shown experimentally, on animal brain cancer models to allow a remarkable sparing of health tissue in comparison with conventional dose rates, opening a new perspective in the clinical management of GBM. This project aims to investigate the biological mechanisms of the FLASH effect using murine models, including glioma model. By integrating MRI-based tumor monitoring, behavioral assessments, and immunohistochemical analyses at multiple time points, we explored how FLASH irradiation influences both tumor progression and the surrounding brain microenvironment. Following these preliminary results on tumor control, our attention turned to the assessment of normal tissue sparing in the peritumoral area, a critical factor for clinical translation. These findings could strengthen the rationale for translational studies.

POSTER ID: 3.L.10 – ATM KO Human iPSC-Derived NPC: a Cellular Model for Ataxia-Telangiectasia

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Ataxia-Telangiectasia (A-T), an autosomal recessive and multisystem rare disorder, is caused by loss of function mutations in the gene encoding for Ataxia Telangiectasia Mutated (ATM), a serine/threonine kinase. ATM phosphorylates a plethora of substrates involved in many mechanisms. In its most severe form, A-T is characterized by early childhood onset of progressive neurologic manifestations (cerebellar ataxia, cognitive impairment), telangiectasia, immunodeficiency and predisposition to cancer development. Currently there

is no cure for A-T. The role of ATM in the context of DNA damage repair, cell cycle regulation or response to oxidative stress has been widely described, but mechanisms underlying neurodegeneration in A-T still deserve further investigation. Our aim is to better understand the role of ATM in neural progenitor cells and their progeny in the pathophysiology of A-T and to propose new potential targets for pharmacological interventions. Human iPSC-derived Neural Progenitor Cells (hNPCs) were used to generate, via CRISPR/Cas9 genome editing, ATM gene deletion. We demonstrated that ATM KO hNPCs recapitulate defects associated with ATM dysfunction, such as reduced survival rate, inability to repair DNA after damage and higher oxidative stress. Interestingly, we observed that ATM KO hNPCs also display novel and potentially interesting phenotypes, i.e. altered Ca²⁺ signaling, cytoskeletal remodelling and reduced cell fate commitment toward the neuronal lineage. Moreover, by proteomic analysis we identified several proteins and pathways which are deregulated in ATM KO hNPCs. Currently, we are investigating how these altered pathways may functionally affect hNPCs and their progeny and whether these alterations may be pharmacologically corrected.

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POSTER ID: 3.L.11 – The adenosine A2B receptor antagonism prevents cuprizone-induced demyelination

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Myelination is a dynamic and finely regulated process in which both newly generated and preexisting oligodendrocytes contribute to the formation and restoration of myelin sheaths. Oligodendrocytes originate from their precursor (OPCs), a pool of quiescent proliferating cells that, under opportune stimuli, migrate throughout central nervous system (CNS) to provide myelin turnover and repair, a process often impaired in demyelinating pathologies. Adenosine plays a key role as regulator of oligodendrogenesis through the activation of its four metabotropic receptors: A1, A2A, A2B and A3, all expressed by oligodendroglial cells; the involvement of A2B receptors in demyelination is still controversial. The cuprizone (CPZ) model provides a valuable in vivo system for the study of demyelinating diseases. We evaluated the effects of selective adenosine A2B receptor (A2BR) antagonist, PSB-603, in preventing CPZ-induced brain damage. The toxin was added to standard diet of male C57BL/6 mice for 5 weeks and PSB-603 was administered (i.p., 0.05mg/kg) during the last 2 weeks of CPZ intake. At the end of the fifth week, behavioral tests followed by immunohistochemical analysis were performed. CPZ-fed animals exhibited an alteration in motor coordination compared to control animals. This impairment was restored by PSB-603 treatment. Furthermore, a significant reduction in myelin basic protein (MBP) positive area, evaluated by immunofluorescent analysis in different brain areas (corpus callosum, striatum and motor cortex) was found in CPZ-fed animals compared to control mice. Notably, treatment with PSB-

603 effectively counteracted this effect in all examined brain regions. Finally, we demonstrated that the A2BR antagonist also counteracted CPZ-induced upregulation of GFAP and IBA1 expression, typical markers of astrocyte and microglia activation, respectively. In conclusion, A2BR antagonism reduces CPZ-induced demyelination and glial activation in all brain areas examined, suggesting that A2BRs may represent a valid molecular target for the development of new therapeutic options for demyelinating diseases.

POSTER ID: 3.L.12 – Modulation of Glutamate Release by A2A-D2-OTR Receptor Complex in Astrocytes

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Receptor–receptor interaction (RRI) of G protein-coupled receptors (GPCRs) leads to new functional entities fundamentally distinct from the simple addition of signals from the individual receptors. Due to reciprocal allosteric modulation, GPCR complexes operate as highly plastic integrative units, offering a new model for the regulation and integration of extracellular signalling. Focusing on astrocytes, evidence exists for both inhibitory and facilitatory RRIs, including heteromers formed by adenosine A2A and dopamine D2 receptors, A2A and oxytocin receptors (OTR), and D2-OTR heteromers. Here, we provide the first demonstration of the involvement of these receptors in a mosaicism in striatal astrocytes and their processes. By biophysical and functional approaches, we focused on the existence of an A2A-D2-OTR high-order receptor complex and its role in modulating cytosolic calcium levels and endogenous glutamate release, when striatal astrocyte processes were stimulated with 4-aminopyridine. Functional data indicates a permissive role of OTR on dopamine signaling in the regulation of the glutamatergic transmission, and an inhibitory control mediated by A2A on both the D2-mediated signaling and on the OTR-facilitating effect on D2. Imaging, biochemical and bioinformatic evidence confirmed the existence of the A2A-D2-OTR complex and its ternary structure in the membrane. These findings suggest that the D2 receptor could operate as a hotspot in the control of the glutamate release from the striatal astrocytic processes and may contribute to the regulation and integration of different neurotransmitter-mediated signaling in the striatum by the A2A-D2-OTR heterotrimers. Given the role of dysregulated glutamate transmission in neuropsychiatric and neurodegenerative disorders, the A2A-D2-OTR heterotrimer emerges as a selective novel pharmacological target. Further

studies are necessary to elucidate the influence of allosteric D2 receptor modulators on mosaic organization and to better understand the molecular and intracellular pharmacology of OTR, as an individual receptor and within heteromeric or high-order receptor complexes.

POSTER ID: 3.L.13 – Long-Term Effects of 5F-MDMB-PICA IVSA: Neurobehavioral Consequences and Prefrontal Dysfunction

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Synthetic cannabinoids (SC) are the largest group of new psychoactive substances monitored worldwide (EMCDDA, 2022). 5F-MDMB-PICA is a recent SC classified as a potent full agonist at CB1/CB2 receptors able to activate the mesolimbic dopamine (DA) transmission in adolescent but not adult mice (Musa et al, 2020). Here, we have studied its reinforcing effects in adolescent mice and characterized the neurochemical and behavioral effects induced in the same animals at adulthood. First, by self-administering varying doses (1-5 µg/kg/25µl) in an intravenous self-administration (IVSA) paradigm, we observed an inverted U-shaped trend. The dose of 2.5 µg/kg/25ul elicited behavior aimed at obtaining the substance, as measured by different response ratios (FR1-3, PR). Adult mice exposed to 5F-MDMB-PICA during adolescence showed behavioral and neurochemical differences compared to controls, including a propensity for aggression and reduced social interaction, an anhedonic state, and delayed mPFC DA response to olfactory stress as estimated by in vivo brain microdialysis. In addition, the analysis of the excitatory neuronal activity in the mPFC, by using the fiber photometry technique and calcium sensors, revealed diminished responses to aversive stimuli in 5F-MDMB-PICA-exposed mice, compared to controls. These results correlate with the neurochemical analyses indicating disrupted DA signaling. This study provides the first evidence that 5F-MDMB-PICA IVSA is acquired and sustained by adolescent mice at lower doses than that of the prototypical SC JWH-018 (Margiani et al., 2023), confirming a higher abuse liability of this newer SC. Moreover, 5F-MDMB-PICA IVSA during adolescence induced long-term behavioral and neurochemical changes confirming the detrimental consequences related to the use of SC during adolescence.

POSTER ID: 3.L.14 – Cholesterol-Shh Signaling in Lysosomal Storage Disorders: Myriocin's Therapeutic Potential

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Loss-of-function mutations of Niemann Pick C1 intracellular cholesterol transporter (NPC1) cause a rare genetic condition (NPC1 disease, NPCD) in which cholesterol (Chol) and sphingolipids are entrapped in late endosomes/lysosomes and lipid raft integrity is disrupted. Sonic hedgehog (Shh) signaling, which is essential for the development of neurons and glia as well as the formation of synapses, is one of the signaling pathways impacted by this Chol dyshomeostasis. In fact, cholesterolylation and its interaction with the Patched receptor to

control the Chol pool required for the activation of Smoothed and downstream effectors at the primary cilium are part of Shh activation. Interestingly, lysosomal Chol accumulation also causes mTORC1 to become hyperactive. This has been demonstrated to affect Shh signaling in cells lacking Tsc2 by mislocalizing Smoothed and lowering the concentration of Gli2 in primary cilia. We predict that by decreasing sphingomyelin-complexed Chol and raising the metabolically active Chol pool, myriocin, an inhibitor of sphingolipid production, will restore reduced autophagic flux and faulty Shh signaling in Npc1 cellular models. According to our preliminary findings, Npc1 cellular models exhibit increased p62 and LC3II levels, which are suggestive of compromised autophagy and are restored by myriocin therapy. Furthermore, myriocin restores normal Gli1 levels that are decreased in Npc1 KO cells. Given the established role of p62 in modulating mTORC1, our findings point to a complicated interaction between myriocin, cholesterol homeostasis, autophagic flux, and mTORC1 signaling in the context of NPCD. They also suggest that myriocin therapy may be used to restore Shh signaling.

POSTER ID: 3.L.15 – Epigenetic and Microbial Modifications in the Pathophysiology of Anorexia Nervosa

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Anorexia nervosa (AN) is a severe psychiatric disorder with profound physiological and psychological disturbance and distinct biological signatures outside of its clinical phenotype. Recent research has highlighted the complex interplay of epigenetic alterations, gut microbiota composition, and regulatory molecules in AN pathophysiology. Global DNA methylation status, salivary microbiota composition, and microRNA (miRNA) expression were analyzed in AN patients and compared to healthy controls. Saliva samples were collected for exosomal miRNA extraction and genomic DNA extraction. Genome-wide DNA methylation patterns were comprehensively evaluated using reduced representation bisulfite sequencing (RRBS), and salivary microbiota composition was analyzed using 16S rRNA gene sequencing. In addition, miRNA profiling (miRNome) using quantitative PCR was performed for the detection of differentially expressed miRNAs. Our preliminary results revealed distinctive methylation patterns and salivary microbiota compositions in AN patients compared to controls. Several miRNAs were differentially expressed in AN individuals. Integrative analysis of these three data layers implicates the potential interactions between epigenetic marks, microbial signatures, and miRNA expression and the anorexic phenotype. This multi-omic investigation underscores the intricate interplay between epigenetic control, salivary microbiome, and miRNA expression in anorexia nervosa. Our findings strengthen the potential for saliva-based biomarkers to reveal novel molecular mechanisms of AN and guide the development of future non-invasive diagnostic biomarkers and targeted therapies.

POSTER ID: 3.L.16 – Adult Zebrafish Behavioral and Neurotoxic Responses to Environmental Contaminants

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Environmental contamination by chemical substances, including pharmaceuticals, personal care products, and pesticides, poses a growing risk to human and animal health. These contaminants are frequently found in wastewater, groundwater, and surface water, where their persistence and possible ability to cross the blood-brain barrier result in chronic exposure for aquatic organisms. Pesticides are known to disrupt dopaminergic neuronal pathways and induce neurotoxicity, with widespread use leading to environmental accumulation. Psychoactive drugs like benzodiazepines act at low concentrations, yet the effects of environmentally relevant levels remain poorly understood. Penicillin antibiotics, due to their β -lactam ring, are also of interest for potential neuroactive properties. Thus, we explored the neurobehavioral, molecular, and histological effects of environmentally relevant concentrations of selected pharmaceuticals and pesticides on adult zebrafish. As shown in the novel tank test, long-term exposure to the pesticides rotenone and deltamethrin altered locomotor activity and stress responses in zebrafish. Diazepam exposure induced sex-specific behavioral effects, with females and males showing opposite trends. Exposure to amoxicillin and clavulanic acid (AMX/CLAV) led to behavioral changes, showing stress-related and movement alterations accompanied by social impairments. At the molecular levels, diazepam exposure altered *gabra1* gene expression in opposite directions in the two sexes, while AMX/CLAV exposure increased *gabrb2a* and *gad1b* gene expression only in females, suggesting modulation of GABAergic neurotransmission. Histological analyses showed that pesticide exposure increased apoptosis in key brain regions, such as the diencephalon and telencephalon, whereas immunohistochemistry revealed reduced dopaminergic neurons in several brain areas, including the posterior tuberculum. Diazepam exposure upregulated GABA-A receptor $\alpha 1$ subunits in the telencephalon only in females. These findings confirm that environmental contaminants disrupt zebrafish neurological health by affecting behavior, neurotransmission, and neuronal integrity. As a well-established model organism, zebrafish provides valuable insight into the impact of environmental pollution on vertebrate brain health, suggesting potential implications for humans.