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November 28-29, 2025

University of Torino
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table of contents

Proceedings of the 35th National Conference of the Italian Group for the Study of Neuromorphology "Gruppo Italiano per lo Studio della Neuromorfologia" G.I.S.N.

MAIN LECTURES

HOW DO THE CYTOSKELETON AND ITS BINDING PARTNERS CONTRIBUTE TO THE ESTABLISHMENT OF THE BRAIN MORPHOLOGY AND ITS FUNCTION?

Prof. Makoto Sato[1,2,3]

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With the ultimate goal of elucidating the brain mechanisms underlying intelligence, I have been tackling this question by investigating the mechanisms that underlie the formation of brain structures during development. Cell migration and completion of neurotransmission are crucial events that support the development. Because the cytoskeleton is essential for these processes, I have been studying these themes from a cytoskeletal perspective. Periventricular nodular heterotopia is a genetic disorder characterized by the presence of a second cortex (a cluster of neurons) around the ventricles, known as a double cortex. One of the characteristics of this disorder is intractable epilepsy. This disease is believed to be caused by mutations in the actin-binding protein filamin A, which is located on the X chromosome. It has been suggested that filamin A plays a crucial role in cell migration from the ventricular region during cortical development. We have identified and are currently studying a novel molecule, FILIP (filamin A-interacting protein), which promotes the degradation of filamin A. Recently, mutations in FILIP (FILIP1 in humans) have been reported to cause arthrogryposis multiplex congenita, intellectual disability, and encephalocele in humans (FILIP1 disease). At first, I will introduce our research on FILIP and its regulatory factors. Next, we will present our data on how other cytoskeletal molecules contribute to brain development and maturation. On the other hand, when we look at neural circuits, we notice that function and circuit formation are intricately intertwined to enable the brain to function. From this perspective, we have also taken an approach to unravel cells and their associated circuits that are specific to higher animals. We will cite examples of such research and introduce our current approach.

PRENATAL N-ACETYL-CYSTEINE PREVENTS NEURONAL, EMOTIONAL AND METABOLIC IMPAIRMENTS IN AN ANIMAL MODEL OF ADVERSE EXPERIENCES

Dr. Francesca Cirulli

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Adverse maternal conditions during pregnancy increase the risk of neuropsychiatric disorders in the offspring, although the underlying mechanisms remain poorly understood. We have shown that two distinct maternal insults – prenatal stress (PNS) and a high-fat diet (mHFD) – elevate inflammation and oxidative stress in the brains of adolescent female mice. Building on these findings, we focused on mHFD. We demonstrated that its consumption during pregnancy increases maternal oxidative stress and circulating corticosterone levels while reducing the activity of placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2). This impairment compromises the placental barrier, exposing the fetus to dysregulated levels of glucocorticoids that are detrimental to brain development. In adulthood, offspring exposed to mHFD in utero displayed long-term negative effects on emotional, neuroendocrine, and metabolic phenotypes. We next investigated whether restoring maternal redox balance through antioxidant treatment with N-acetyl-cysteine (NAC) could mitigate these effects. Maternal NAC administration prevented anxiety-like behaviors in offspring and rescued hippocampal expression of brain-derived neurotrophic factor (BDNF), a key regulator of neuronal plasticity. *In vitro* experiments confirmed that NAC counteracts the negative effects of glucocorticoids on neuronal plasticity via a BDNF-mediated mechanism. Specifically, inhibition of the TrkB signaling pathway abolished NAC's restorative effects on neuronal morphology, including maximal dendritic length, primary dendrite number, and soma area – supporting *in vivo* observations. Together, these findings highlight a critical role of oxidative stress in fetal brain vulnerability to maternal high-fat diet and suggest that rebalancing redox status may be a promising strategy to protect neurodevelopment under early adverse conditions.

SESSION I
NEURAL DISORDERS

VGF AS BIOMARKER OF PRE-SYMPTOMATIC PHASE OF PARKINSON'S DISEASE

Manai AL[1], Modi A[2], Noli B[1], Choudhary SM[2], Ruda-Kucerova J[3], Dražanová E[3,4], Khairnar A[2,5] and Cocco C[1]

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Parkinson's disease (PD) is a progressive neurodegenerative disorder whose early detection remains a major challenge. The Vgf gene, encoding the VGF neuroprotein, has been implicated in PD, with previous studies reporting reduced GF C-terminal peptide levels in the plasma of drug-naïve PD patients and in the substantia nigra (SN) of advanced PD models. However, similar studies, are lacking in progressive mouse models of PD. In this study, we developed a progressive and early PD mouse model using intranasal rotenone administration, based on the hypothesis that PD pathology begins in the olfactory bulb (OB) and progresses to the midbrain over time (Braak's hypothesis). The aim of this study was to validate VGF C-terminal peptide levels as presymptomatic biomarkers of PD. Male BALB/c mice were divided into three cohorts based on the time of rotenone treatment, *i.e.*, at 2, 3, and 6 months. Behavioral tests included the butyric acid avoidance to assess olfactory dysfunction and inverted grid and pole tests to assess motor impairment. At each time point, the mice were sacrificed, and brain and plasma were collected for q-PCR, immunohistochemistry (IHC) and ELISA. The total RNA was extracted from the isolated OB to perform q-PCR for the following genes: Vgf, Th (tyrosine hydroxylase), and Snca (synuclein alpha) while IHC was performed in OB and SN for TH, alpha-synuclein, and VGF C-terminal peptides. VGF C-terminal levels were measured using ELISA in plasma and OB extracted samples. Behavioral study showed significant olfactory deficits starting from 3-month time point and was persistent throughout the study, whereas motor impairments appeared only after 6 months of rotenone treatment. At 3 months, concurrent with olfactory deficits, using the OB samples, the VGF C-terminus levels were significantly reduced along with the increased immunoreactivity for alpha-synuclein, and decrease of TH. Same results for alpha-synuclein, TH and VGF were observed in the SN along with a decrease for VGF C-terminus levels in plasma. All these changes persisted even after 6 months, at which point gene alterations were observed for the first time. Indeed, the q-PCR results at 6 months showed increased Snca and decreased Th expression in the rotenone treated mice OB while Vgf mRNA levels remained unchanged in all brain regions suggesting a post-translational VGF changes. In conclusion, a role of VGF C-terminal peptides as a potential biomarker of pre-symptomatic PD is suggested.

STRESS GRANULES AND α-SYNUCLEIN: INTERPLAY BETWEEN CELLULAR STRESS AND PROTEIN AGGREGATION IN PARKINSON'S DISEASE

Zanchi G[1], Novello C[1], Calogero AM[2], Mazzetti S[2], Bonaldo B[1], Gomez MCL[1], Rolando C[1], Calandrella D[2,3], Pezzoli G[2] and Cappelletti G[1]

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Stress granules (SGs) are membraneless organelles that are constituted primarily of untranslated messenger ribonucleoproteins, whose assembly occurs via liquid-liquid phase separation (LLPS) in response to various cellular stressors and that play a critical role in maintaining proteostasis. Increasing evidence indicates that chronic stress can disrupt the tightly regulated dynamics of SGs assembly and disassembly, leading to their persistence. These pathological SGs potentially serve as nucleation sites for aberrant protein aggregation in several neurodegenerative disorders (NDDs)[2]. Focusing on Parkinson's disease (PD), current knowledge on the dysregulation of SGs assembly and disassembly in relation to α-Synuclein (α-Syn) aggregation is only based on cellular models, with no evidence from human brain tissue. It is well established that, under specific conditions, α-Syn can form condensates regulated by microtubule dynamics, promoting amyloid fibril formation *via* LLPS, and that α-Syn interacts with several RNA-binding proteins (RBPs) involved in SGs formation. Based on this we aim to determine whether: *i)* α-Syn pathology and SGs are associated in *post-mortem* human brains obtained from PD patients and *ii)* α-Syn aggregates within SGs under conditions of acute and chronic stress in a neuronal cell model (SH-SY5Y cells) and whether this process affects SGs homeostasis. Immunohistochemical analysis on *post-mortem* PD brains highlighted a characteristic pattern of small, round RBPs-positive granules in neuronal cell bodies, which also accumulated alongside α-Syn oligomers. Interestingly, different RBPs were found to be involved in Lewy body formation, and quantitative analyses showed a significant RBPs increase in mature compared to undefined aggregates. This suggests that SGs may act as an intermediate for α-Syn aggregation in PD brains. Simultaneously, SH-SY5Y cells exposed to both acute and chronic treatment with the oxidative stress inducer sodium arsenite showed an increase in intracellular aggregates positive for both SGs markers and α-Syn, indicating enhanced recruitment or association of α-Syn with SGs. Future work will aim to explore the impact of both α-Syn aggregation and microtubule dynamics on the homeostasis of SGs in PD pathology, providing further insight into the molecular mechanisms of the disease.

THE ROLE OF RETINAL mTOR IN RETINAL DEGENERATION

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Retinal neurons are highly susceptible to oxidative stress produced by light-derived reactive oxygen species (ROS) and the metabolic demand generated by photo-transduction. This mostly occurs within retinal pigment epithelium (RPE), which sustains the metabolism of photo-transduction, and removes oxidized proteins, lipids and sugars from the outer segment of photoreceptors. Thus, RPE critically depends on mitochondrial turnover and removal of oxidized species by effective lysosomes. The present study, provides light and ultrastructural evidence about mTOR-dependency of retinal integrity with an emphasis on RPE cells. The mTOR activator 3-MA generates cell loss, which is prevented by the classic mTOR inhibitor rapamycin or the phytochemical curcumin, which possesses strong mTOR inhibiting effects. The loss of cell viability is associated with deficient mitochondrial turnover and accumulation of lipid droplets and glycogen granules. In these conditions typical proteins which form gap junctions (ZO1, occludin, RPE65) are suppressed and their low expression is confined to the perinuclear zone. This is relevant considering the role of these proteins in fostering the blood-retina barrier, endocytosis of photoreceptors, and phenotype of RPE. Moreover, the loss of these proteins, along with a damage to RPE cells and photoreceptors is a hallmark of early stages of age-related macular degeneration (AMD). Degeneration of RPE occurs early in AMD and further progresses towards inner retina involving bipolar, amacrine and ganglion cells. Inhibition of mTOR within RPE is critical to prevent the onset of degenerative changes. In this way, subsequent alterations such as the epithelial-mesenchymal transition, which mature later in AMD, is counteracted by mTOR suppression. Retinal protection induced by mTOR inhibition is concomitant with beclin1 expression. This may occur either as a consequence of pro-autophagy effects of mTOR inhibition or/and non-canonical autophagy-dependent effects of beclin1 activity. Retinal mTOR modulation appears a promising target to counteract retinal degeneration by preventing mitochondrial damage, lipid and glycogen accumulation.

SESSION II
INSIGHTS INTO GLIAL AND VASCULAR NETWORKS

NEUROBIOLOGY OF SUICIDE: MORPHOLOGICAL ASSESSMENT OF MICROGLIAL NETWORK IN HUMAN POST-MORTEM PREFRONTAL CORTEX

Tambuzzi S[1], Bertolotto N[2], Massari S[2], Madaschi L[3], Alini M[2], Brandolese D[2], Ubezio MC[1], Cattaneo C[1] and Amadeo A[2]

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Neuroinflammation is increasingly recognized as a key factor in the pathophysiology of suicidal behavior. This study investigated the morphological profiles of cortical microglia in depressed individuals who died by suicide, compared to healthy controls. Post-mortem samples from the dorsolateral prefrontal cortex (dlPFC) were examined using immunohistochemistry and immunofluorescence with different microglial markers, such as Ionized calcium-Binding Adapter molecule 1 (IBA1), Cluster of Differentiation 68 (CD68) and Purinergic Receptor P2Y, G-Protein Coupled, 12 (P2RY12). To better analyze the gliovascular network, we also used the lectin Lycopersicon esculentum Agglutinin (LEA), which recognizes both microglial and vascular endothelial cells. Microglia predominantly exhibited a ramified morphology ($IBA1^+$, $P2RY12^+$), indicative of a surveillance state, but amoeboid $CD68^+$ cells, revealing activation, were also observed. Preliminary observations suggested how CD68+ activation could be prevalent in female subjects if compared with male ones. Also LEA/IBA1 colocalization analysis revealed sex-specific patterns: in male suicide cases, colocalization was reduced in dlPFC compared to controls, possibly reflecting a functional disconnection between microglia and vasculature. In contrast, female suicide cases showed increased colocalization, suggesting a more pronounced neuroinflammatory response. Densitometric analysis supported this trend, with reduced IBA1 signal intensity in male suicides and increased intensity in females. Although statistical analyses did not yield significant group differences, the observed trends point to a sex-dimorphic microglial response in suicide, potentially modulated by neuroendocrine and immune factors. On the other hand, some clarified samples for 3D analysis showed that LEA+ vessels exhibited a statistically significant reduction in cerebral vascularization in suicide cases compared to controls, with a 52.8% decrease, suggesting a potential role of vascular network dysfunction in the pathophysiology of suicide. The combined use of cellular markers and quantitative approaches provided a multilayered view of glio-vascular interactions in the human cortex, supporting the hypothesis of microglial involvement in suicide vulnerability characterized by sex differences and highlighting the importance of further investigations in larger cohorts to elucidate the neuroinflammatory mechanisms underlying affective disorders.

GLIAL INFLAMMASOME ACTIVATION AND BBB BREAKDOWN IN AGE-RELATED NEURODEGENERATION

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Blood-brain barrier (BBB) breakdown, characterized by the disruption of endothelial tight junctions (TJs), is an early pathogenic event in aging-related neurodegenerative diseases, where neuroinflammation plays a key role. Activation of the NLRP3 inflammasome in glial cells represents a major inflammatory hub contributing to this process. However, its precise role in BBB dysfunction and its potential as a therapeutic target remain to be fully elucidated. In the present study, senescence-accelerated SAMP8 mice and SAMR1 controls were used as models of BBB impairment. Confocal immunofluorescence analysis of brain sections was performed to assess the tight junction proteins occludin and claudin-5, as well as the inflammasome components NLRP3 and ASC in $GFAP^+$ astrocytes and $IBA1^+$ microglia. The effects of two immunomodulatory treatments–the selective NLRP3 inhibitor MCC950 and interferon-gamma (IFN-γ) – were subsequently evaluated. SAMP8 mice exhibited downregulation and a discontinuous staining pattern of occludin and claudin-5, paralleled by inflammasome activation, including upregulation of NLRP3 and ASC in both reactive astrocytes and microglia. Both MCC950 and IFN-γ treatments partially restored BBB integrity, improving TJ staining pattern and protein expression in both microvessels and larger vessels. Structural recovery of TJs correlated with a significant reduction in NLRP3 expression and ASC speck formation in glial cells. These findings demonstrate that glial inflammasome activation is a key driver of BBB structural failure in this aging model. The partial restoration of TJ architecture following inflammasome inhibition with MCC950 or immunomodulation with IFN-γ confirms the link between neuroinflammation and vascular pathology. Overall, this study identifies the NLRP3 inflammasome pathway as a critical therapeutic target to preserve BBB integrity in age-related neurodegeneration.

STANDARDIZING LIGHT SHEET IMAGING AND 3D IMAGE ANALYSIS FOR WHOLE BRAIN VASCULAR NET QUANTIFICATION

Zanella L[1], Moretti M[1], Quadalti C[2], Giardino L[1,3,4], Calzà L[2,3,4], **Baldassarro VA**[1,4] and Lorenzini L[1,4]

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Neuronal function relies on a complex network of blood vessels that deliver oxygen and nutrients essential for metabolism. However, our understanding of the intricate capillary network allowing substance and metabolite exchange remains incomplete. While structural cerebral vasculature and molecular analyses hold promise for offering crucial insights into cerebral circulation and cerebrovascular diseases, technical limitations make difficult to obtain detailed 3D structural information on vascular networks, from large vessels to capillaries. Light sheet microscopy (LSM) coupled with tissue clarification, high calculation power and computer vision algorithms, has emerged as a powerful tool in neuroscience research, offering unique advantages for imaging biological large samples at cellular resolution. Despite the vast potential, the pivotal challenge lies in achieving reliable quantitative imaging. To tackle this challenge, we addressed three methodological hurdles: to optimize antibody-stained section imaging employing non-toxic clearing protocols, to set-up quantitative measure using young-C57BL/6 brain tissues, and to test the quantification pipeline on extremely challenging samples, comparing old-C57BL/6 and old-TG2576 mice (a model of Alzheimer's disease) brain vasculature. To optimize the vessel visualization, we set-up two different stainings in young-C57BL/6 mice: anti-CD31 VioB515 immunostaining and perfusion with dextran-FITC. We used the clearing protocol of Miltenyi MACS® Clearing Kit and Miltenyi UltraMicroscope Blaze™ for imaging, while Miltenyi stitcher software was used for large areas sampling. Three-dimensional, voxel-based images were analyzed using IMARIS software (v. 9.6.2; Oxford Instruments), by the «surface» algorithms to construct the 3D vascular network. We translated the acquisition and 3D reconstruction protocol designed on young-C57BL/6 tissues modifying the algorithm to optimize the analysis on extremely challenging samples: old-derived brains from WT and TG2576 animals. The protocol included a fine study of the fluorescence intensity curve in each sample and experimental group and a mathematic-based approach to correct the inter- and intra-experiment differences. While LSM offers great advantages in imaging large volumes of tissue with high resolution and minimal photobleaching, standardized and optimized protocols for clearing, imaging, and post-processing analysis are a crucial step toward the use LSM for image quantification.

SESSION III
ADVANCES IN CONNECTOMICS

WHOLE-BRAIN CATECHOLAMINERGIC CONNECTOMICS IN ALZHEIMER'S DISEASE

Bellomi FE[1], Loffredo G[2], Petrocchi F[1], Ullman NS[1], Caturano C[1], Cauzzi E[2], Cognini F[3], Mittoni F[4], LaBarbera L[2], Soda P[5], D'Amelio M[2] and Vivacqua G[1]

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Increasing evidence indicates that catecholaminergic degeneration, especially in the ventral tegmental area (VTA) and locus coeruleus (LC), precedes classical Alzheimer's disease (AD) pathology, a finding confirmed by structural and functional imaging in patient cohorts with amnestic MCI and AD. Given the central role of these nuclei in shaping motivation, arousal and memory, we developed a dedicated pipeline to reconstruct the catecholaminergic connectome at the whole-brain level. Tg2576 mice overexpressing human APP695 with the Swedish mutation were employed. Brains were cleared, immunolabeled for tyrosine hydroxylase (TH), and imaged using volumetric light-sheet microscopy. Tiles were stitched with BigStitcher, and Arivis Pro U-Net models segmented soma, axon hillocks, dendrites, and nuclei. 3D reconstruction and automated tracing were performed with Vaa3D APP2. Reconstructions were registered to the Allen Brain Atlas. NetworkX was used for data analysis, and in MATLAB the physiological laws of CNS impulse transmission were applied to the networks to perform simulations. The pipeline generated a whole-brain catecholaminergic connectomic model for Tg2576 and controls. A decrease in TH^+ neuron counts was observed in both VTA and LC, leading to marked denervation of the hippocampus, amygdala, medial prefrontal cortex, and the entire limbic lobe. Alterations were also noted within and in between catecholaminergic nuclei, with changes in their intrinsic functional circuit units, modifications of dendritic arborizations, and a reduction in reverberant circuits that normally sustain continuous output activity. conclusions: This represents the first connectomic model of catecholaminergic architecture in both healthy and AD brains. TH^+ fibers normally synapse onto GABAergic neurons; their denervation drives excitotoxicity and degeneration in target areas. These alterations may underlie prodromal psychiatric symptoms and, through hippocampal and cortical denervation, may be the cause of pathological alterations and subsequent cognitive decline.

CONNNECTOME-BASED BRAIN FINGERPRINTS PREDICT ART THERAPY RESPONSE IN PARKINSON'S DISEASE

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Art therapy has emerged as a promising non-pharmacological, complementary approach in Parkinson's Disease (PD), engaging motor, cognitive, and emotional systems and promoting neuroplasticity. However, individual responses to art therapy are highly variable, and predictors of therapeutic efficacy remain largely unknown. Here, we propose a novel framework combining brain fingerprinting and machine learning to predict art therapy outcomes in PD. We mapped functional connectomes from resting-state functional MRI (fMRI) of PD patients before and after art therapy, assessed individual connectome-based fingerprints, and examined their spatial specificity. First, we found that functional connectomes derived from resting-state fMRI retained high levels of identifiability in both healthy controls and patients with PD, indicating that stable and subject-specific brain fingerprints are preserved even in the context of neurodegeneration. Second, we identified associations between fingerprint topography and cognitive domains relevant to PD. Although mean edge-wise reliability was comparable across conditions, the topography of reliability differed by group and session. In PD, reliable edges shifted toward posterior midline and occipital territories, with fewer stable connections in several associative systems. This pattern suggests a shift in reliability toward sensory-perceptual hubs and away from networks that support flexible control. Finally, leveraging network fingerprints, we computed subject-wise topology measures which served as input to a supervised classification framework designed to predict clinical responder versus non-responder status, based on changes in UPDRS-III scores. Among the tested classifiers, tree-based methods provided the most robust predictive performance, with random forest achieving the highest performance, with an accuracy of 0.83 and a ROC-AUC of 0.80. Our results demonstrate that brain fingerprint-informed network measures capture interindividual variability in art therapy response, offering a data-driven, personalized approach to rehabilitation. This study provides the first evidence that functional connectome fingerprints can guide art therapy interventions, thus opening a new precision medicine framework in PD.

SESSION IV
PERIPHERAL SYSTEM AND GUT

A PRECLINICAL STUDY ON THE MODULATION OF THE MICROBIOTA–GUT–BRAIN AXIS BY A FUNCTIONAL SNACK PROMOTING HEALTHY AGING

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Clinical and preclinical studies increasingly report alterations in gut microbial composition and reduced microbial diversity in patients with Alzheimer's disease (AD), suggesting the gut ecosystem may influence disease onset and progression. Preclinical studies in transgenic AD mouse models indicate that gut dysbiosis could be correlated with impaired intestinal barrier function, local deposition of amyloid-β (Aβ) in the gastrointestinal tract, and regional and systemic inflammatory changes. These alterations have been proposed to accelerate central Aβ accumulation and neuroinflammation through immune and metabolic pathways. Together, these findings support a model in which gut microbiota and mucosal barrier modulation contribute to reinforce the cascade that may promote neuropathology in AD. However, although causal links in humans remain to be fully established. A novel engineered health-promoting cookie formulated with red lentil prebiotics and coated in multi-strain probiotic (SLAB51®)-fortified chocolate was tested in 3xTg-AD mice. At eight weeks of age, mice were divided into experimental groups and subjected to a four-month supplementation protocol. The groups received a functional cookie containing the prebiotic and probiotic formulation, a prebiotic cookie without SLAB51®, a standard recipe cookie, or SLAB51® administered in drinking water. The possible effects on intestinal mucosa morphology, secretion and integrity, neuroplasticity in the enteric and central nervous system, and neuroinflammation were evaluated with biochemical and immunohistochemical approaches. Experimental groups receiving the functional snack exhibited well-preserved intestinal wall architecture and a restoration of epithelial homeostasis. These effects were associated with enhanced mucus layer stability and improved barrier integrity, linked by attenuated pro-inflammatory microenvironment consequent to the decrease in enteric glial cells activation. Furthermore, the intake of this symbiotic formulation preserved cognitive function and was associated with decreased amyloid load, oxidative stress, gliosis, and neuroinflammation in brain areas including the hippocampal region. This symbiotic formulation represents a novel dietary vehicle that may provide a nutritional approach to mitigate AD–related microbiota–gut–brain axis disturbances and promote healthy aging.

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SENSORY NEURON TOXICITY TRIGGERED BY ANTICANCER DRUGS IS COUNTERACTED BY EXTRACELLULAR VESICLES DERIVED FROM ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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Chemotherapy-induced peripheral neuropathy (CIPN) is a frequent and disabling adverse effect of several anticancer drugs, characterized by paraesthesia, numbness, and dysesthesia, often leading to dose reduction or therapy discontinuation. Despite extensive research, no neuroprotective strategy has yet demonstrated definitive clinical efficacy. However, *in vitro* evidence suggests the therapeutic potential of Adipose-derived mesenchymal Stem Cell Extracellular Vesicles (ASC-EVs). EVs function as key mediators of intercellular communication, transporting proteins, RNAs, and lipids, and ASC-EVs in particular have been implicated in cellular repair mechanisms. In this study, we employed primary cultures of dorsal root ganglion (DRG) neurons isolated from rat embryos, a well-established model for investigating neurotoxicity, neuroprotection, and post-mitotic neuronal development. We specifically investigated the effects of two clinically relevant chemotherapeutic agents – cisplatin (CDDP, 6 μM) and bortezomib (BTZ, 20 nM) – administered either alone or in combination with a single dose of extracellular vesicles (EVs) for 24 and 48 h. Neuronal viability was assessed by a cellular count at bright-field microscopy, quantifying live cells based on the presence of a birefringent outline. Treatment with CDDP resulted in a pronounced and time-dependent decline in neuronal viability at both 24 and 48 h. Co-administration with ASC-EVs significantly mitigated this effect, indicating a strong neuroprotective action. In contrast, BTZ induced a distinct neurotoxic profile, characterized by milder alterations at earlier time points – likely reflecting differences in its mechanisms of action compared with CDDP. Despite these differences, ASC-EVs were also able to counteract BTZ-induced toxicity, further reinforcing their neuroprotective potential. Collectively, these results indicate that the neuroprotective efficacy of ASC-EVs is influenced by both the pharmacological properties of the chemotherapeutic agent and the duration of exposure, suggesting a dynamic, time-dependent interplay between EV- mediated mechanisms and drug-induced neuronal stress. The observed protection is plausibly linked to the modulation of oxidative stress and apoptotic pathways, which are currently being explored. Future investigations will aim to identify the specific molecular mechanism within ASC-EVs responsible for mediating these neuroprotective effects.

SESSION V
CNS/PNS DISORDERS

LOCUS COERULEUS STRUCTURAL INTEGRITY IS ASSOCIATED WITH CIRCULATING SOLUBLE AXL IN ALZHEIMER'S DISEASE

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The locus coeruleus (LC) is among the earliest brain regions affected in Alzheimer's disease (AD), exhibiting neuronal loss and accumulation of hyperphosphorylated tau decades before the onset of symptoms. At the same time, growing evidence highlights neuroinflammation as a central contributor to AD pathogenesis, even at prodromal stages. The TAM (Tyro3, Axl, and MerTK) receptor family plays a crucial role in regulating immune homeostasis and inflammatory signaling. However, a potential link between LC degeneration and peripheral inflammatory markers has not been demonstrated to date. We studied a cohort of 102 participants for whom both high-resolution MRI and blood samples were available. LC integrity was quantified using MRI-based contrast measures of LC signal intensity, focusing on rostral and caudal subregions. Plasma concentrations of soluble TAM receptors (Tyro3, Axl, and MerTK) were determined by enzyme-linked immunosorbent assay (ELISA). Participants were stratified into AD+ (amyloid-positive) and AD– groups based on clinical and biomarker criteria. Across the entire cohort, plasma soluble Axl (sAxl) levels showed a significant negative correlation with rostral LC integrity ($p=0.007$), indicating that higher circulating sAxl levels are associated with more pronounced LC degeneration. This relationship remained significant within the AD+ group ($p=0.017$) but not in the AD – group, suggesting a disease-specific association. No significant correlations were observed for Tyro3 or MerTK. Our findings identify a novel association between peripheral soluble Axl levels and central noradrenergic degeneration in AD. This is the first time, to our knowledge, that an *in vivo* link between neuroinflammation and LC degeneration has been detected in patients. These results provide new insight into the interplay between immune activation and early brainstem vulnerability in Alzheimer's disease and may pave the way toward new therapeutic targets.

SODIUM-CALCIUM EXCHANGER PIVOTAL ROLE IN OXALIPLATIN-INDUCED ALTERATIONS LEADING TO PERIPHERAL NEUROTOXICITY, AXONAL DAMAGE AND NECROPTOSIS

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Oxaliplatin (OHP) is the cornerstone drug for Colorectal Cancer treatment, but is hampered by OHP-induced Peripheral Neurotoxicity (OIPN), which comprises an acute and chronic form. Acute OIPN consists of a precocious axonal hyperexcitability syndrome, caused by OHP-induced functional alterations of Voltage-gated Sodium Channels (NaV). Prolonged OHP treatment, instead, leads to a chronic OIPN, mainly characterized by a sensory neuropathy with a potentially persistent axonal damage (AxD). The Sodium-Calcium exchanger (NCX) might play a role in AxD development, consequent to a NaV physiological dysfunction. NCX is, in fact, highly co-expressed with NaV. Indeed, OHP-induced NaV prolonged opening may cause an unbalance in Na^+ and Ca^{2+} neuronal levels: the excessive Na^+ intake can lead to NCX switching to *reverse-mode*, which in turn causes Ca^{2+} abundant intake to rebalance Na^+, thus leading to Ca^{2+}-related AxD. We aimed to investigate OHP-induced morphological and physiological changes and to clarify the potential role of NCX in AxD development. For this purpose, we performed multiple toxicological studies on primary Dorsal Root Ganglia (DRG) neuronal culture of adult mice (male C57BL/6 mice; 8-10 weeks) using primarily the Nanolive CX-A 3D holotomographic microscope, a state-of-art live-imaging platform. OHP-exposed neurons (25 µM, 48h) showed an overall neurite fragmentation and a high-reduced viability, preceded by autophagic stress and necroptosis. Moreover, chronic exposure (48h) of DRG neurons to different OHP concentrations (7.5, 15, 25, 50 µM) highlighted alterations in both viability and neurites elongation in a dose and time-dependent manner, that can be efficiently counteracted by SEA0400 low-dose pretreatment (1 µM, 3h before OHP-incubation), a potent and selective NCX inhibitor, confirming the pivotal role of NCX in AxD development. In conclusion, we observed that OIPN is related to both morphological and functional changes in NaV and NCX, paving the way to potential new treatment strategies to prevent AxD. Furthermore, for the first time, we were able to follow AxD leading events longitudinally by exploiting such an advanced approach as holotomographic live-imaging.

CONTRIBUTION OF MAGNETIC RESONANCE IMAGING ASSOCIATED WITH MACHINE LEARNING TO AN EARLY ASSESSMENT OF THE CONVERSION OF MILD COGNITIVE IMPAIRMENT IN OVERT DEMENTIA

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Mild Cognitive Impairment (MCI) is a medical condition characterized by noticeable but relatively mild changes in cognitive abilities (memory, thinking, and reasoning) that are greater than expected with normal aging but not severe enough to interfere significantly with daily life or independent function. MCI is broadly classified into amnestic and non-amnestic. Amnestic MCI affects primarily memory and is often considered as a risk factor for Alzheimer's disease. Non-amnestic MCI affects other thinking skills and may lead to other types of dementia, like frontotemporal dementia or Lewy body dementia. While not all individuals with MCI progress to dementia, identifying those who will convert remains a critical challenge in neurodegenerative research and clinical practice. Magnetic Resonance Imaging (MRI) provides high-resolution structural and functional information about the brain, making it a valuable tool for detecting subtle neuroanatomical changes that precede clinical dementia. However, the complexity and high dimensionality of MRI data limit traditional statistical approaches. Recent advances in machine learning (ML) offer the potential to uncover nonlinear relationships and subtle imaging patterns that can predict disease progression more accurately and earlier than standard diagnostic criteria. We have analyzed MRI findings in 60 subjects aged 76±5 years suffering from amnestic MCI that were examined for 12 months. Subjects were evaluated with a combination of clinical assessment, cognitive testing and imaging tests. Clinical and cognitive testing did not show significant changes along the course of the study. Brain regions analyzed by voxel morphometry included cerebral ventricles, cerebral cortex grey and white matter, temporal cortex, right and left hippocampus and right and left amygdala. Linear mixed models were used to compare within-person rates of change as predictors of MRI biomarkers. Images were pre-processed by skull stripping, normalization, segmentation and structural features were then extracted. This kind of analysis allowed tracking of the rate of brain atrophy over time, earlier than clinical decline and cognitive testing results indicating that MRI combined with ML can enhance early detection of dementia risk among MCI patients.

SESSION VI
DEDICATED TO "GIANCARLO PANZICA"

INSIGHTS INTO THE EVOLUTION OF DOPAMINERGIC CONTROL OF REPRODUCTION: THE BRAIN- PITUITARY-GONADAL AXIS OF THE SMALL-SPOTTED CATSHARK *SCYLIORHINUS CANICULA*

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In all vertebrates, reproduction is controlled by the brain–pituitary-gonadal (BPG) axis, which integrates environmental cues such as photoperiod and temperature with neural and gonadal functions to regulate gametogenesis and gamete maturation. This axis has become a key topic in conservation biology for its relevance to species adaptability and ecosystem health, consistent with the "One Health" concept. Despite its importance, the mechanisms underlying BPG axis regulation remain largely unclear across vertebrate lineages. In cartilaginous fishes, the neuroendocrine regulation of reproduction through the BPG axis remains poorly explored, although these organisms occupy a pivotal position at the base of the gnathostome evolutionary lineage. The small-spotted catshark, *Scyliorhinus canicula*, represents a reference model within this group, in which the Gonadotropin-Releasing Hormone, released from specific brain areas, plays a central role in gonadal development and maturation. Our study revealed that in females of *S. canicula*, the preoptic nucleus contains tyrosine hydroxylase-immunoreactive neurons whose morphometry and abundance vary with sexual maturity, being more numerous in subadult females than in adults. This feature suggests a possible dopaminergic inhibition of the reproductive axis, a role known for other vertebrate groups. Moreover, the presence of dopamine receptors D1 and D2, identified in brain areas associated with the BPG system, supports the potential involvement of dopamine in the neuroendocrine regulation of reproductive activity.

NEUROACTIVE STEROIDS PROTECT FROM BORTEZOMIB-INDUCED TOXICITY: EVIDENCE FROM *IN VITRO* MODELS

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Chemotherapy has significantly improved patient survival but has also increased the incidence of chemotherapy-induced peripheral neuropathy (CIPN), a common side effect that affects the patients' quality of life and limits treatment efficacy. Among the agents associated with CIPN development, bortezomib (BTZ) - a proteasome inhibitor used in the treatment of multiple myeloma - is known to induce painful peripheral neuropathy (BIPN), for which analgesic options remain limited. Recent evidence suggests that neuroactive steroids (NAS), cholesterol derivatives with already proven beneficial effects towards both the central and the peripheral nervous system, may promote neuroprotection in CIPN models. For our study, we selected a palette of different NAS for screening and dose-finding purposes. First, we tested them (alone and in combination with BTZ) in two different cell lines: F11, a somatic cell hybrid of a rat embryonic dorsal root ganglion (DRG) and mouse neuroblastoma cell line N18TG2, and MSC80, a mouse Schwann cell line. To assess the effects of these treatments, cell viability in F11 and MSC80 cell lines was evaluated via crystal violet assay after 24 h of treatment with BTZ (10 nM) alone or in combination with the respective NAS (100 nM). Based on the results, we selected three NAS for further experiments: pregnenolone (PREG) and dihydroprogesterone (DHP), that partially but significantly prevented BTZ-induced cytotoxicity in F11 and MSC80, respectively, as well as allopregnanolone (ALLO), that was mildly protective in both cellular models. Subsequently, we decided to test the abovementioned combinations (BTZ + PREG/DHP/ALLO) in a more complex *in vitro* model, using organotypic cultures from embryonic rat DRGs. DRGs from E15 rat embryos were treated with a toxic dose of BTZ (5 nM) for 24 or 48 h. The neurotoxic effect was assessed by measuring neurite length of DRG explants. ALLO and PREG were able to protect from the toxicity induced by BTZ exposure at different degrees in both time points, demonstrating their neuroprotective action, whereas DHP was ineffective at any time point. Taken together, our results highlight PREG and ALLO as promising neuroprotective agents in BIPN *in vitro* models, paving the way for future experiments aimed at testing their efficacy in BIPN *in vivo* models as well as addressing their specific mechanism of action in counteracting BTZ-induced toxicity.

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EX VIVO MAPPING OF NIGRO-THALAMIC DOPAMINERGIC TERMINALS REVEALS REGIONAL SPECIALIZATION AND SYNAPTIC MARKERS IN THE HUMAN THALAMUS

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The thalamus plays a crucial role in integrating subcortical inputs and relaying them to cortical circuits, yet the extent and specificity of dopaminergic innervation to human thalamic nuclei remain incompletely understood. Recent tractography-based studies suggest the existence of direct nigro-thalamic pathways, but direct histological evidence is still limited. In this study, we provide an *ex vivo* mapping of dopaminergic projections to the human thalamus using high-resolution immunohistochemistry and confocal microscopy. Human brain specimens were processed to detect multiple dopaminergic markers, including tyrosine hydroxylase (TH), vesicular monoamine transporter 2 (VMAT2), and aromatic L-amino acid decarboxylase (AADC), across anatomically defined thalamic nuclei. Multilabel immunofluorescence and 3D reconstruction allowed precise identification of axonal arborization and synaptic varicosities within the mediodorsal (MD), and ventral anterior (VA)/ventral lateral (VL) nuclei. Quantitative analysis revealed a significant expression of TH+/VMAT2+ fibers and D2R staining in the MD/VA/VL nuclei, supporting the notion of region-specific dopaminergic input. These results provide the first immunohistological confirmation of selective dopaminergic innervation of the human thalamus. This structural evidence complements prior neuroimaging findings and suggests a potential role for the nigro- thalamic pathway in modulating thalamo-cortical circuits involved in executive and cognitive functions.

SESSION VII
NEUROPROTECTIVE STRATEGIES

NEUROPROTECTIVE POTENTIAL OF ADIPOSE MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES ON DAMAGED MOTONEURONS

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Intranasal delivery represents a non-invasive and promising route to directly target the central nervous system (CNS), as it enables therapeutic agents to bypass the blood–brain barrier and allows repeated administration with low invasiveness. In this context, extracellular vesicles derived from adipose mesenchymal stem cells (ASC-EVs) are gaining increasing attention for their intrinsic neuroprotective and immunomodulatory properties, making them attractive candidates for the treatment of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). In this study, we established an *in vitro* epithelial barrier model using RPMI 2650 cells (derived from nasal epithelium carcinoma) cultured on transwell system and coupled with motor neuron-like NSC-34 cells treated with hydrogen peroxide to mimic oxidative stress-induced injury. This setup was designed to investigate the ability of ASC-EVs to cross the epithelial layer, representing the first step of access to the CNS following intranasal administration, and to reach damaged motor neurons. The study also explores the mechanisms underlying epithelial transport by evaluating both transcellular and paracellular routes through the use of selective pathway inhibitors and by monitoring changes in transepithelial electrical resistance (TEER) and junctional integrity. Our findings demonstrate that ASC-EVs are able to traverse the epithelial barrier and reach damaged motor neurons, where they are internalized and exert significant neuroprotective effects. These results provide a mechanistic basis supporting the potential of intranasally delivered ASC-EVs as a non-invasive therapeutic approach for ALS and other neurodegenerative disorders.

ADNP EXPRESSION IS REGIONALLY UPREGULATED BY MODERATE AEROBIC EXERCISE IN THE RAT BRAIN

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Engaging in moderate aerobic exercise has been shown to significantly enhance cognitive performance and support overall brain health. Physical activity promotes adult neurogenesis and increases synaptic plasticity, particularly in brain regions involved in learning and memory, such as the hippocampus and those related to motor coordination, like the cerebellum. Activity-dependent neuroprotective protein (ADNP) plays a crucial role in neurodevelopment, brain maturation, and synaptic plasticity. However, the influence of moderate exercise on ADNP expression and its distribution within the rat brain has not yet been explored. This study aimed to examine how moderate exercise affects ADNP levels and neuronal activity, the latter being assessed through the expression of the microtubule-associated protein β-Tubulin III. A total of 24 rats were randomly assigned to one of two groups: a sedentary control group and a group subjected to a 12-week treadmill-based moderate exercise regimen. Our findings demonstrated that moderate aerobic activity led to increased expression of both ADNP and β-Tubulin III in the dentate gyrus of the hippocampus and in the cerebellum. Additionally, we observed co-localization of ADNP and β-Tubulin III in these regions, indicating a possible direct relationship between ADNP and exercise-induced neuronal activation.
In summary, these results suggest that the beneficial effects of moderate physical activity may, in part, be attributed to the upregulation and interaction of ADNP with β-Tubulin III in specific areas of the brain.

KAEMPFEROL TARGETS LIPID BALANCE, ENDOCANNABINOID PATHWAYS, AND PPARA IN THE RAT CEREBRAL CORTEX AFTER BCCAO/R

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Transient bilateral common carotid artery occlusion and reperfusion (BCCAO/R) has previously been established as an effective model for replicating early brain inflammation triggered by acute hypoperfusion and subsequent reperfusion events. Recognizing the significant role of diet and nutrition in shaping brain neuroplasticity, this investigation explored the neuroprotective impact of kaempferol (KAM), a dietary flavonoid, within a rat BCCAO/R paradigm. Adult Wistar rats received a single oral dose of KAM (40 mg) 6 h prior to surgery. Extensive lipidomic and molecular assessments were performed on frontal and temporal-occipital cortical tissues in addition to plasma samples. In the frontal cortex, KAM treatment led to increased concentrations of anti-inflammatory N-acylethanolamines – namely palmitoylethanolamide (PEA), oleoylethanolamide (OEA), and docosahexaenoylethanolamide (DHAEA) – while diminishing levels of oxidized arachidonic acid derivatives. KAM further suppressed cyclooxygenase-2 (COX-2) protein and selectively reduced the endocannabinoid 2-arachidonoylglycerol (2-AG), reflecting alterations in arachidonic acid metabolism. These molecular effects were accompanied by elevated peroxisome proliferator-activated receptor alpha (PPARα) and cannabinoid receptors CB1R and CB2R, supporting activation of anti-inflammatory pathways at both nuclear and membrane levels. No marked changes emerged in the temporal-occipital cortex. In plasma, DHAEA levels increased in parallel with cortical findings, whereas PEA and OEA elevations were restricted to sham-operated KAM-treated subjects, implying potential central redistribution during hypoperfusion/reperfusion stress. Collectively, these results indicate that KAM confers anti-inflammatory protection by both suppressing COX-2- mediated prostanoid production and enhancing PPARα-dependent lipid signaling. This dual mechanism underscores KAM's promise as a dietary strategy to mitigate neuroinflammation following hypoperfusion–reperfusion injury.

MODULATION OF EXTRACELLULAR SIGNAL-RELATED KINASE, CYCLIN D1, GLIAL FIBRILLARY ACIDIC PROTEIN, AND VIMENTIN EXPRESSION IN ESTRADIOL-PRETREATED ASTROCYTE CULTURES TREATED WITH COMPETENCE AND PROGRESSION GROWTH FACTORS

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The present study seeks to elucidate the interactions between the "competence" growth factor basic fibroblast growth factor (bFGF) and/or estrogen 17b-estradiol and the "progression" growth factors epidermal growth factor (EGF), insulin-like growth factor-I (IGF-I), and insulin (INS) on DNA labeling and also cyclin D1, extracellular signal- related kinase 1/2 (ERK1/2), glial fibrillary acidic protein (GFAP), and vimentin expression in astroglial cultures under different experimental conditions. Pretreatment for 24 hr with bFGF and subsequent exposure for 36 hr to estradiol (E2) and EGF, IGF-I, or INS stimulated DNA labeling in the last 12 hr, especially when the cultures were treated with progression growth factors. bFGF pretreatment and subsequent treatment with E2 for 36 hr stimulated DNA labeling. The 36-hr E2 treatment alone did not significantly decrease DNA labeling, but contemporary addition of E2 with two or three growth factors stimulated DNA labeling remarkably. When E2 was coadded with growth factors, a significantly increased DNA labeling was observed, demonstrating an astroglial synergistic mitogenic effect evoked by contemporary treatment with growth factors in the presence of estrogens. Cyclin D1 expression was markedly increased when astrocyte cultures were pretreated for 36 hr with E2 and subsequently treated with two or three competence and progression growth factors. A highly significant increase of ERK1/2 expression was observed after all the treatments (EGF, bFGF, INS, IGF-I alone or in combination with two or three growth factors). GFAP and vimentin expression was markedly increased when the cultures were treated with two or three growth factors. In conclusion, our data demonstrate estradiol growth factor crosstalk during astroglial cell proliferation and differentiation in culture.

SESSION VIII
NEURO-ONCOLOGY

UNRAVELING GLIAL REMODELING IN SPACE AND TIME DURING GLIOBLASTOMA EVOLUTION

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Glioblastoma (GBM) is the most aggressive primary malignant tumor of the central nervous system (CNS), characterized by marked molecular heterogeneity, rapid proliferation, extensive invasiveness, and strong resistance to conventional therapies. Despite current standard treatments–surgery, radiotherapy, and temozolomide–median patient survival remains below 15 months. Key contributors to GBM progression include activated glial cells and remodeling of the extracellular matrix. In this study, we investigated the cellular and molecular dynamics of the tumor microenvironment, focusing on astrocytic and microglial responses, and on the modulation of glial markers such as GFAP, Iba1, Connexin 43 (Cx43), and the purinergic receptor P2X4R. Acute and organotypic brain slice cultures were prepared from C57BL/6J mice, into which GL261 glioblastoma cells were injected into the cortex. Tissues were collected 3 and 7 days post-injection to analyze tumor progression. GBM growth was monitored through the proliferation marker Ki67. Histological analysis, confocal immunofluorescence, and western blotting were performed to assess marker expression and localization. GL261 cells engaged with the surrounding neuroglial network, migrating in fascicle-like patterns that extended even into the contralateral hemisphere. This dynamic infiltration was associated with pronounced peritumoral astrocyte activation (increased GFAP) and a robust microglial response, evidenced by morphological changes in Iba1-positive cells. Cx43 expression was altered by tumor invasion, suggesting disrupted intercellular communication, while P2X4R expression dynamics indicated modulation of the local immune response. Consistently, elevated Ki67 levels confirmed the highly proliferative and aggressive behavior of the tumor. Organotypic slice cultures represent a fast and reliable *ex vivo* platform to investigate GBM invasiveness, migration, and neuroglial interactions, revealing spatiotemporal remodeling of the tumor microenvironment during disease progression. Region-specific glial activation and time-dependent modulation of Cx43 and P2X4R highlight the complex and evolving nature of glial–tumor crosstalk. These findings may help identify potential therapeutic windows and support the development of molecularly targeted treatment strategies.

PURKINJE CELL INTEGRITY IS PRESERVED BY EXERCISE IN PARANEOPLASTIC CEREBELLAR DEGENERATION: EVIDENCE FROM A CANCER CACHEXIA MOUSE MODEL

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Paraneoplastic syndromes are secondary conditions to the systemic effects of cancer. Among these, paraneoplastic cerebellar degeneration (PCD) is one of the most common neurological forms. PCD is characterized by immune-mediated damage to Purkinje cells and progressive cerebellar atrophy. Exercise has already been suggested as a non-pharmacological strategy to counteract cachexia, one of the most prevalent paraneoplastic syndromes. However, the impact of physical training on PCD has not yet been investigated. In this study, male BALB/c mice were assigned to four experimental groups. Two groups (T^+) were inoculated with C26 tumor fragments and either underwent a 4-week endurance training protocol (TR) or remained sedentary (SED). The remaining two groups (T^-) were subjected to the same training or sedentary conditions. Tumor groups were sacrificed at the onset of cachexia to assess the impact of proactive endurance training. Cachexia onset was confirmed during the third week through body weight loss and reduced grip strength analyses. Preliminary histological analysis revealed that the overall cerebellar area was comparable across groups. Interestingly, the Purkinje cell body circularity index and layer thickness were significantly reduced in SED T^+ mice and restored in TR T^+ animals. Among cerebellar regions, the flocculonodular lobe -crucial for postural and oculomotor regulation- was the most impacted, yet also the one where training provided the strongest effect in preserving Purkinje cell soma size. Since ZIC4 is one of the transcription factors targeted by the autoimmune response in PCD, we evaluated its expression and localization using both immunohistochemistry and immunofluorescence. ZIC4 expression was reduced in the Purkinje cells of SED T^+ mice, but training was able to protect Purkinje cells from any autoimmune attack during tumour development. We also assessed the expression of PSD95, a common marker used for the immunolabeling of the post-synaptic side of excitatory synapses. The very low expression of the PSD95 protein in SED T^+ mice suggested that the tumour mass affected the synaptic plasticity, while endurance training again preserved synaptic integrity. Altogether, these results suggest an important role of training in the protection of cerebellum damage. Further studies are needed to clarify the role of exercise in protecting the stimulating synapses of the efferent fibers of the cerebellar cortex.

POSTER SESSION

GENERATION AND CHARACTERIZATION OF A KNOCK-IN MOUSE MODEL FOR FLVCR1-RELATED SENSORY NEUROPATHY

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FLVCR1 (Feline-Leukemia-Virus-subgroup-C-Receptor-1) encodes a plasma membrane choline/ethanolamine importer involved in intracellular heme homeostasis and mitochondrial calcium handling. Mutations in FLVCR1 cause complex sensory neuropathies featuring retinitis pigmentosa, sensory ataxia, pain insensitivity, and motor deficits, but the mechanisms underlying neuronal dysfunction remain elusive. To address this, we generated mouse models carrying the pathogenic FLVCR1-P221S mutation in homozygosity ($FLVCR1^{P221S/P221S}$) and compound heterozygosity ($FLVCR1^{P221S/del}$), and established primary cultures of dorsal root ganglia (DRG) sensory neurons. Behavioral testing revealed preserved motor coordination up to 15 months, although some animals developed late-onset motor symptoms. In nociceptive assays, $FLVCR1^{P221S/P221S}$ mice displayed delayed thermal responses, whereas $FLVCR1^{P221S/del}$ mutants showed reduced mechanical sensitivity and thermal hypersensitivity. Morphological analysis of DRG neurons revealed reduced soma area and axon length in both genotypes, indicating intrinsic structural vulnerability. However, metabolic profiling of DRG, brain, liver, and muscle showed no major alterations, suggesting compensatory adaptations *in vivo*. These findings establish a reliable model for FLVCR1-linked sensory neuropathies, highlighting the morphological and functional consequences of FLVCR1 dysfunction in sensory neurons. This model provides a valuable tool to investigate both *in vivo* and *in vitro* on primary sensory neurons coltures how impaired choline transport and calcium signaling contribute to neuronal vulnerability and to develop targeted therapeutic strategies to preserve sensory neuron integrity.

HIGH RESOLUTION MRI TO ASSESS AXONAL DAMAGE IN NEUROPATHY MODELS

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Peripheral neuropathies are common conditions whose treatment, in most cases, is still lacking due to an incomplete pathogenetic knowledge; therefore, preclinical *in vivo* models are crucial, but highly translational outcome measures are still needed in this field. High resolution diffusion MRI could be a surrogate, translational, biomarker to characterise early morphological changes as neuropathy ensues, creating a virtuous link between bench and bedside. We tested this approach in a *proof-of-concep*t and a *feasibility* setting. We aimed at characterising MRI changes in a robust model of axonopathy that ensues after repetitive administration of paclitaxel (PTX). We compared 2 groups (n=12 each) of female Wistar rats: control (CTRL, vehicle treated, iv) and PTX (10mg/kg, 1qwx4, iv). At the end of treatment, neuropathy development was verified via Dynamic test, nerve conduction studies (NCS) and light microscopy of the caudal nerve. 7T MRI was performed on whole rat tails (collected after sacrifice and formalin-fixed, n=3/group) to study caudal nerves and the anatomical relationship with surrounding structures. High resolution anatomical images were acquired by means of a T1w sequence with a voxel size of 50x50x50 μm^3. Diffusion weighted images were acquired in five b-shells: b of 500, 2000, 4500, 6000, 8000 sec*mm-2 with 15, 24, 33, 42, 51 isotropically distributed gradient directions and a voxel size of 125x125x125 μm^3. Diffusion data were fitted with the Diffusion Tensor Imaging (DTI) classical model and Fractional Anisotropy (FA), Axial, Radial and Mean Diffusitivity (AD, RD and MD) were computed. All selected outcome measures (dynamic test, NCS and morphological assessment of caudal nerves) demonstrated that nerve damage induction was satisfactory and consistent with a moderate-severe axonal polyneuropathy, making our cohort ideal to test 7T MRI implementation. Neuroimaging showed a decrease of FA (by 5%) and an increase of diffusivity, being the more relevant variation in RD (by 15%), in the PTX group if compared to control. These preliminary results may sustain the hypothesis of axonal damage leading to an increased water diffusivity in the PTX tissue microstructure. In summary, we provided preliminary promising data of the 7T MRI exploitation to study axonal damage in the preclinical setting.

MYELIN MAINTENANCE AND CELL VIABILITY: INVESTIGATING BECLIN 1 *IN VIVO* AND *IN VITRO*

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Schwann cells (SCs) of the peripheral nervous system (PNS) ensure axonal integrity and form the myelin sheath essential for nerve conduction. Autophagy is a key basic process in cell homeostasis that is involved in SC differentiation and cytoplasm reabsorption upon myelination as well as in myelin clearance (myelinophagy), following nerve injury. In this context, SCs revert to a repair phenotype: they de-differentiate, clear myelin debris, release cytokines that promote immune cell recruitment, and produce trophic factors supporting axonal regrowth. We recently demonstrated that Beclin 1, a core component of autophagy and endosomal trafficking, is crucial for Schwann cell maturation and peripheral nerve development, and its dysfunction induces in mice a severe demyelinating phenotype. Besides its role during development, Beclin 1 might be critical for both myelin maintenance and nerve regeneration in adulthood. To investigate the role of Beclin-1 in SCs in adulthood, an inducible, SC-specific knockout mouse line was generated by crossing *Becn1$^{f/f}$* mice with *P0-CreERT2* animals. Sciatic nerves were collected 4, 8, and 12 weeks after tamoxifen (TAM) or vehicle (VEH) administration. *Becn1$^{f/f}$* and VEH-treated mice were used as controls. While no major histological differences were detected at 4 and 8 weeks, ultrastructural analyses at 8 weeks already revealed SC alterations. By 12 weeks, *Becn1*-deficient nerves exhibited reduced axonal and fiber diameters, thinner myelin sheaths, and increased overall nerve area. Myelinated fibers appeared disorganized, with enlarged interstitial spaces. Transmission electron microscopy confirmed the presence of autophagic vacuoles, vesiculation, cytoplasmic enlargement, and signs of myelin degradation. *In vitro*, rat SCs were transfected with *Becn1* siRNAs. Western blot analysis confirmed Beclin 1 knockdown. Live-cell imaging showed reduced proliferation and Cytotox Dyes revealed increased cell death. Beclin 1 emerges as a key determinant of SC viability, proliferation, and myelin maintenance. Its loss causes early ultrastructural changes, later morphological defects and myelin degradation. Complementary *in vitro* findings confirm that Beclin 1 deficiency compromises SC growth and survival. Altogether, these results identify Beclin 1–regulated pathways as pivotal mechanisms in peripheral nerve homeostasis.

SHORT-TERM EXPOSURE TO PARTICULATE MATTER TRIGGERS SELECTIVE MOLECULAR AND BEHAVIORAL ALTERATIONS IN A MOUSE MODEL OF MULTIPLE SCLEROSIS

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Multiple Sclerosis (MS) is a chronic disease of the Central Nervous System (CNS), where neuroinflammation and autoimmune response against myelin lead to functional impairments and psychiatric symptoms. Exposure to peaks of air pollution – and in particular of particulate matter (PM) - has been associated with increased hospitalizations for MS onset and relapses, pointing to persons with MS (or predisposed to develop MS) as a PM-vulnerable cohort. With the aim to disclose the biological substrate of such vulnerability, and to understand whether individuals primed to develop autoimmunity (as it occurs in MS and in the experimental autoimmune encephalomyelitis - EAE - animal model of MS) respond differently to PM, we firstly characterized plasmatic extracellular vesicles (EVs) and their microRNA (miRNA) cargo in healthy and presymptomatic EAE mice after exposure to PM10, compared to unexposed healthy and EAE mice. The response of EAE mice to PM10 did not differ in terms of EV number or source compared to that of healthy mice. Remarkable differences existed instead in the identity of deregulated EV-associated miRNAs, which in EAE mice were predicted to target several MS-relevant biological processes and nervous system-, immune- and inflammation-related pathways, possibly contributing to disease worsening. To validate our findings, we monitored EAE pathological course. PM10 exposure in the presymptomatic phase did not modify EAE course, as assessed by clinical and neuropathological analyses. Yet, in PM-exposed EAE mice we observed selective behavioral alterations - *i.e.* increased disinhibited, risk-taking and novelty-seeking behaviors - compatible with neurotransmitter imbalances linked with the detected EV-packaged miRNA deregulation. Overall, these data show a selective vulnerability of immunologically primed mice toward the effects of PM, detected at molecular and behavioral levels as early as in the pre-symptomatic stages of the pathology.

THE DUAL EFFECT OF CADMIUM IN ASTROCYTE PHYSIOLOGY: FROM ADAPTIVE ACTIVATION TO POTENTIAL NEUROTOXICITY

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Environmental pollutants, and in particular heavy metals, pose a serious risk to human health due to their persistence, bioaccumulation, and toxic effects on various organs and systems. Among them, cadmium (Cd) is of particular concern for its widespread presence in industrial and consumer products, including pesticides, plastics, pigments, and cigarette smoke. However, the early effects of low-dose Cd exposure on human astrocytes remain poorly defined. Astrocytes play a crucial role in maintaining central nervous system (CNS) homeostasis, providing metabolic and structural support to neurons, regulating synaptic activity, and preserving blood–brain barrier (BBB) integrity. In this study, we investigated the impact of Cd acetate (CdAc) on astrocyte viability, migration and activation. The human astrocytic SVGp12 cell line was exposed to increasing concentrations of CdAc (0.1-100 µM) for increasing time 6-48 h. Preliminary results showed that 24 h exposure to 1 µM CdAc induced a significant 10% increase in cell viability compared to untreated astrocytes. In addition, a 30% increase in cell migration was also observed in CdAc-treated cells (1 µM, 24 h) compared to the control, as revealed by wound healing assay. In accordance, CdAc exposure (1 µM, 24 h) induced cytoskeletal rearrangement consistent with a motile phenotype, as demonstrated by F-actin immuno-fluorescence analysis. Moreover, the expression of the astrocyte markers GFAP and S100β was also significantly increased after CdAc exposure (1 µM, 24 h), indicating an early astrocyte activation. In contrast, higher CdAc concentrations (≥5 µM) reduced viability, impaired migration and induced morphological features of cytotoxicity in human astrocytes. In conclusion, our findings indicate that subtoxic Cd exposure may induce an early astrogliosis, affecting migratory ability, viability and activation of human astrocytes. This reactive state may contribute to neuroinflammatory processes and BBB dysfunction, highlighting the potential impact of low environmental Cd levels on CNS homeostasis.

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MULTI SCAFFOLD 3D-BIOPRINTED MODEL OF SPINAL CORD ARCHITECTURE *IN VITRO*

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Recreating physiologically relevant spinal cord models *in vitro* requires not only appropriate cell types but also structural and topographical cues that mimic native tissue. While 2D cultures fail to reproduce the spatial complexity of the central nervous system, 3D bioprinting strategies enable more realistic neural microenvironments. We aim to develop a 3D spinal cord model by combining murine cell lines or human stem cell–derived neural progenitors with a multi-material scaffold system. Murine neuronal/astrocytic (NE-4C) and motor neuron-like (NSC-34) cells were embedded in gelatin methacrylate (GelMA) hydrogels and layered on aligned polycaprolactone (PCL) microfibers produced by Melt Electrowriting via extrusion-based bioprinting. The matrix supported long-term viability, proliferation, and differentiation: neuronal (MAP2, βIII-tubulin) and glial (GFAP) markers increased progressively over 14-21-28 days *in vitro* (DIV), confirmed by immunofluorescence and western blot, and spontaneous network activity was detected through calcium imaging. In parallel, human neural progenitor cells (NPCs) derived from induced pluripotent stem cells (iPSCs) were successfully differentiated and later cultured within a composite hydrogel composed of GelMA and Geltrex for 7, 10, and 14 DIV. The NPCs expressed SOX2 and nestin, confirming their neural progenitor identity, and exhibited a characteristic rosette- like morphological organization, indicative of early neuroepithelial architecture. Over time, they showed high viability and progressive neuronal maturation, with MAP2, βIII-tubulin, and synapsin expression suggesting neuronal interconnection and emerging network formation. This preliminary 3D system might represent a versatile and biomimetic platform for modeling spinal cord structure and function, with translational potential for studies on spinal cord injury, neurodegeneration, and physiologically relevant drug screening.

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A NOVEL DRUG TARGETTING THE INFLAMMASOME AMELYORATES AD PATHOLOGY IN THE MURINE MODEL 5xFAD

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Alzheimer's disease (AD) is a progressive neurological disorder commonly associated with aging and the main widespread form of dementia. It is known that, concomitant with plaques and tangles accumulation, patients undergo a severe neuroinflammatory and iron accumulation processes that contributes to neuronal loss during AD progression. Currently, available pharmacological treatments are unable to resolve the disease, therefore the development of novel drugs able to prevent neuroinflammation before symptoms are fully present may provide an alternative approach. In this study, 5xFAD mice, a murine model with five familial human AD mutations inducing constitutive amyloid accumulation, have been treated orally from weaning until 5 months of age with INF235. This novel compound is able to inhibit the NLRP3 inflammasome, a multiprotein complex involved in the inflammatory process that plays an active role in the pathogenesis of AD. INF235 treated mice have been compared to non-treated mice and mice treated with MCC950, a well-known NLRP3 inflammasome inhibitor. At the end of the treatment, we conducted a battery of behavioral tests to evaluate the cognitive status of 5xFAD mice and histological analysis of brain tissue. Results showed that, in the asymptomatic phase of AD, mice treated with MCC950 and INF235 have similar behaviour in terms of general cognitive improvement compared to non-treated mice. Moreover, histological staining of the main areas affected by AD (cortex, hippocampus, third ventricle, striatum) revealed a significant reduction in the amount of iron deposits in INF235-treated 5xFAD brains; and inflammatory markers, IBA- 1 and GFAP, showing a significant reduction in the state of activation of glial cells. This preliminary work shows how the novel inflammasome inhibitor, INF235, is able to reduce iron accumulation and the activation state of important players in neurodegeneration offering a novel strategy to deepen into the prevention of not only neuroinflammation but also iron dyshomeostasis during presimptomatic AD.

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THE NEUROPROTECTIVE PEPTIDES PACAP AND ADNP RESCUE CEREBELLAR GRANULE CELLS FROM APOPTOSIS

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Neuronal fate is determined by signals that promote either survival or apoptotic death. These signals are essential for the proper development of the central nervous system and the maintenance of homeostasis in adulthood; however, their dysregulation can contribute to the onset of neurological disorders. Through genomic screening and a reverse engineering approach, we previously characterized the transcriptional program regulating apoptosis and survival in cerebellar granule cells (CGCs) deprived of neurotrophic factors. Among the genes analyzed, we identified two transcription factors – Homeobox D9 (Hoxd9) and Nuclear Receptor 4A1 (Nr4a1) – as potential master regulators of the apoptotic process in CGCs cultured under serum deprivation and low potassium conditions (5 mM KCl). In the present study, we investigated the neuroprotective effects of the peptides PACAP and ADNP to assess whether they can prevent neuronal death by modulating the expression of these transcription factors. Using a continuous CGC cell line, we defined the temporal window of neuroprotection for both peptides and evaluated their impact on Hoxd9 and Nr4a1 expression levels. Our findings suggest that Hoxd9 and Nr4a1 may play a pivotal role in the genetic program underlying neuronal survival and represent potential targets for neuroprotective strategies.

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CRAFTING THE MICROVASCULAR CANVAS: BIOENGENEERED MATERIALS OR NATIVE ECM FOR THE CENTRAL NERVOUS SYSTEM

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The THOR project (European Innovation Council-Pathfinder Programme, Grant Agreement number 101099719) addresses the fundamental challenge of developing a durable, working microvasculature that accurately reflects the crucial neurovascular unit (NVU) for CNS vitality and regenerative therapies. This work aims to reconstruct the complex natural process of vessel invasion into the CNS extracellular matrix (ECM) seen during embryogenesis. We utilized an innovative technique to spin and precisely deposit individual, 10-20 μm high-performance functionalized silk fibers in tailored configurations, serving as biomimetic scaffolds. We report on experiments where C57BL/6 Mouse Embryonic Brain Endothelial Cells were successfully seeded on silk scaffolds and tubes modified with either vascular endothelial growth factor (VEGF) or the IKVAV peptide from the laminin alpha-1 chain. We observed successful vascular invasion of both scaffold types and the formation of tube-like structures expressing markers for the blood-brain barrier (claudin−5) and associated pericytes (CD13, PDGFRβ). Critically, we confronted the results obtained on the functionalized silk scaffolds with those obtained using decellularized ECM derived from adult brain slices. This comparison validated the effectiveness of the engineered silk in guiding cell organization. The resulting endothelium secreted basal lamina proteins (collagen-IV, laminin) with alignments that were directed by the silk fibers (parallel, perpendicular, or anchored). The resulting biohybrid endothelium has been cultivated within a sophisticated microfluidic system featuring a pressurized lid, parallel channels, and integrated flow sensors to maintain precise culturing conditions. Our findings indicate that diverse peptides linked to the silk and the advanced microfluidics can effectively direct embryonic cell self-organization within this engineered framework to support brain tissue, acting as an engineered substitute for the complex cues of the native embryonic ECM. This work represents a significant advance toward constructing intricate microvascular networks and progressing regenerative therapies for the CNS.

MICROBIAL INFLUENCE ON PERIPHERAL NERVOUS SYSTEM: INSIGHTS FROM GERM-FREE AND RECOLONIZED MICE

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Emerging evidence links gut microbiota to nervous system development and repair. We investigated how different microbial states affect peripheral nerve morphology and gene expression. Peripheral nerves and dorsal root ganglia (DRG) were collected from four murine models: complex gut microbiota (CGM, control), gnotobiotic (OMM12, mice stably colonized with 12 specifically defined bacterial strains), germ-free (GF, mice bred in sterile environment to prevent microbial exposure) and ex-germ-free (EX-GF, mice which were initially maintained in a germ-free environment but later exposed to environmental microbes) mice. Fecal communities were profiled (species- level relative abundance, α/β diversity). Sciatic nerve and DRG transcriptomes were analysed by bulk RNA-seq. Ultrastructure was examined by transmission electron microscopy to assess myelination, and nodal features. Cohousing recolonized EX-GF mice, but did not reconstitute CGM complexity: CGM samples showed significantly higher α-diversity and separated from EX-GF by β-diversity. RNA-seq revealed a distinct transcriptional program in GF, OMM12 and EX-GF compared to CGM. TEM showed hypermyelination in GF and EX-GF mice: increased myelin thickness reflected a greater number of myelin lamellae rather than altered lamellar spacing. Aberrant Schwann cell-axon interactions (myelin outfoldings, inclusions, sheath splitting) were observed in OMM12, GF and EX-GF groups. Nodal length frequency distribution was similar in CGM and OMM12, and broader in GF and EX-GF, pointing to a longer node of Ranvier. DRG soma and nuclear areas were largely unchanged, in spite of that, injury-associated genes were differentially regulated across microbiota conditions. These results showed that partial microbiota reconstitution in EX-GF mice does not restore CGM-like peripheral nerve transcriptomes or morphology. Microbiota composition strongly influences myelination patterns, suggesting microbial control of nerve development and repair.

HUMAN IPSC-DERIVED CEREBRAL ORGANOIDS AS A MODEL FOR C9ORF72-LINKED FRONTOTEMPORAL DEMENTIA: INSIGHTS INTO NEURONAL AND GLIAL DIFFERENTIATION DYNAMICS

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Frontotemporal Dementia (FTD) is a progressive neurodegenerative disorder characterized by selective degeneration of the frontal and temporal lobes, leading to profound alterations in personality, behavior, and language. Among its genetic causes, the hexanucleotide repeat expansion in the C9ORF72 gene represents one of the most frequent mutations, also implicated in Amyotrophic Lateral Sclerosis (ALS). In this study, we explored the potential of cerebral organoids derived from human induced pluripotent stem cells (hiPSCs) as a dynamic and physiologically relevant model to investigate FTD pathophysiology associated with C9ORF72 mutations. Organoids were generated from hiPSCs obtained from both healthy donors and FTD patients and cultured up to 120 days *in vitro* (DIV). Their development was monitored at 30, 60, 90, and 120 DIV through morphometric and immunohistochemical analyses targeting key neural and glial markers. The neural markers SOX2 and Ki67 were employed to assess stemness and proliferation, PAX6 to identify neural progenitors, and TBR1, CTIP2, SATB2, and MAP2 to evaluate neuronal differentiation and cortical organization. In parallel, the astrocytic markers GFAP and S100B were analyzed to investigate glial maturation and temporal dynamics of differentiation. Preliminary results demonstrated a time-dependent growth for both control and FTD organoids, with diameters increasing from approximately 400 µm at early stages to over 2 mm by day 90. Marker expression analysis revealed an expected progressive transition from progenitor to neuronal identity: $SOX2^+$ cells decreased from ~50% to 30%, and $Ki67^+$ cells from ~30% to <10%, indicating reduced proliferative activity. SATB2 expression, associated with upper-layer cortical neurons, increased significantly between day 60 and 90, reaching ~30% in control organoids, but was markedly reduced or absent in FTD-derived organoids, suggesting altered cortical layer formation. Furthermore, analysis of glial markers revealed consistent differentiation of astrocytic populations after approximately four months in culture in both control and FTD organoids, with clear expression of GFAP and S100B indicating astrocyte maturation. This temporal emergence of glial identity complements neuronal findings, underscoring the complex cellular interplay underlying cortical development. Overall, these results highlight the potential of cerebral organoids as a robust and versatile platform for modeling FTD, enabling the investigation of disease mechanisms and the testing of novel therapeutic strategies beyond traditional animal models.

OVEREXPRESSION OF ANOSMIN-1 ALTERS THE MYELINATION PATTERN AND THE MORPHOLOGY OF AXONAL COMPONENTS IN CEREBELLAR PURKINJE CELLS

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Anosmin-1 (A1) is an extracellular matrix glycoprotein that regulates FGF, Wnt, BMP, and VEGF signaling pathways during nervous system development, and is widely expressed throughout the central nervous system, including the cerebellum. Mutations in the ANOS1 gene (formerly KAL1), which encodes A1, cause Kallmann syndrome (KS), a rare congenital disorder characterized by hypogonadotropic hypogonadism and anosmia. Given that the A1-overexpressing mouse model exhibits ataxia-like motor deficits, we hypothesized that these abnormalities might result from cerebellar dysfunction. Alterations in myelin formation and in the structural components of axons are major contributors to both the generation and conduction velocity of action potentials and may represent a key mechanism underlying the motor impairments observed in A1-overexpressing mice. To investigate this possibility, we examined the maturation dynamics of the axon initial segment (AIS) and the overall myelination pattern. Immunostaining of Ankyrin-g, a reliable marker of the AIS, revealed distinct morphological modifications in Purkinje cell axons of A1-overexpressing mice. Specifically, AISs in mutant mice are significantly longer and located further away from the soma compared to wild-type (WT) controls at adult stages. To assess potential structural modifications in the nodal and paranodal regions, we measured the length of nodes of Ranvier and found that, at postnatal day 60, mutant mice displayed longer nodes compared to WT. Moreover, during development, A1-overexpressing mice exhibited an altered timeline of myelination with evidence that the deposition occurs earlier compared to WT. Yet, in the mutants myelination reaches normal levels at adult stages. These data indicate that A1 regulates both the timing of myelin deposition and the structural organization of axonal domains, thereby participating in the neuromorphological shaping of Purkinje cell axons essential for efficient signal transmission.

CALRETININ IMMUNOREACTIVITY IN NERVE CELLS OF THE HUMAN MIDBRAIN AND THEIR POTENTIAL ROLE IN NEURODEGENERATIVE DISORDERS

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Calretinin (CR) is an intracellular calcium binding protein of the EF-hand family, firstly described in the chick retina. CR is composed by 261-271 amino acids, with a MW of 29 kDa .CR and calbindin D-28k present 58% of identical amino acids residues. CR is widely expressed in the neuronal cell bodies and processes and in synaptic specializations on spines, dendrites and cell bodies. Although, studies suggest a role of CR in midbrain dopamine-related disorders (*e.g.* schizophrenia, autism spectrum disorders, Parkinson's disease), currently not exist in the human midbrain a morphofunctional analyses of the nerve cells (neurons and glia) immunoreactive to CR. Therefore, the aim of this study is to carry out, using an immunohistochemical approach, a detailed analysis of CR immunoreactivity (ir) in the adult human midbrain. The study was carried out on postmortem human midbrain fragments taken from human brains with no clinical history of neuropsychiatric disorders. The midbrain were fixed in neutral buffered formalin, embedded in paraffin, cut into 4 μm sections and subjected to light microscopic immunohistochemistry with CR mouse polyclonal antibody. For CR positive controls were used fragments of human mesothelioma subjected to the same experimental procedure. In the midbrain, CR immunoreactivity (ir) in the neuropil (puncta and tracts of processes) and in neuronal cell bodies and processes of the substantia nigra pars compacta and (SNpc), and pars reticulata (SNpr), ventral tegmental area (VTA), in the reticular formation and in other areas has been detected. In addition, CR-ir in cell bodies and processes of glial cells (astrocytes; oligodendrocytes, microgliocytes) has been also detected. Therefore, in the midbrain CR is not only involved in excitatory and inhibitory neurotransmission mechanisms but, also in gliotransmission and in immunitary mechanisms. Furthermore, in previous studies the absence in the midbrain of others calcium binding proteins (*e.g.* calbindin D-28k) in vulnerability for dopamine-related brain disorders has been suggested. In addition, we do not exclude the pathophysiological role of CR in the midbrain dopamine-related disorders. Moreover, although these results overall constitute a novelty, we subsequently aim to compare CR data between normal and pathological midbrain. Finally, based on CR functions do not exclude the preparation of therapies, by means of a combined use of pharmacological approach and transcranial stimulation.

MORPHOLOGICAL AND TRANSCRIPTOMIC ANALYSES HIGHLIGHT DIFFERENTIAL REGENERATION KINETICS IN IMMEDIATE VERSUS DELAYED NERVE REPAIR

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Peripheral nerve injuries represent a significant clinical challenge due to their limited regenerative capacity. Although immediate repair is ideal, delayed intervention is often unavoidable. Understanding the mechanisms underlying both conditions is essential for optimizing functional recovery. Thus, this study aimed to uncover key pathways in nerve regeneration through the first transcriptomic analysis of regenerating nerves within a conduit, also comparing immediate and delayed nerve repair over time in a rat model. Immediately after injury, or following a delay of three months, microsurgical intervention with a chitosan tube was performed to repair an 8-mm median nerve gap, and regenerated nerves inside the conduit were collected at 14- and 21-days for morphometric analysis, and at 7-, 14-, and 21-days post-repair for RNA sequencing. Morphometric analysis based on absolute values showed a significant reduction in Schwann cell and axonal areas at 14-days, along with a decreased number of blood vessels and an overall smaller section area at 21-days in the delayed group. However, when normalized to the total section area to assess the relative proportions occupied by Schwann cells, axons, and vessels, no significant differences were observed between immediate and delayed groups at 21-days. To correlate morphometric data with transcriptomic profiles, RNA sequencing was conducted. Approximately 25,000 genes were differentially expressed in regenerating nerves compared to healthy controls, mainly related to inflammatory response, phagocytosis, cell signalling, and response to lipoprotein particles. Only 137 genes differed between delayed and immediate repair. Gene ontology analysis showed that the most enriched pathways were involved in angiogenesis, especially at 7-days, in accordance with the higher density of vessel area observed at 14-days. Overall, the comparison between the experimental groups indicated that immediate repair initiated a more rapid regenerative response, while delayed repair followed a slower, but ultimately convergent, trajectory highlighting that regeneration is postponed and partially impaired. Indeed, it is noteworthy that the nerve calibre was hindered in the delayed compared to the immediate repair. These findings underscore the importance of early intervention, but also suggest that, over time, delayed repair might achieve similar regenerative outcomes, especially if novel therapies will be developed to further enhance recovery.

CHEMOTHERAPY-INDUCED REMODELING OF CORTICAL PYRAMIDAL NEURONS: MORPHOLOGICAL EFFECTS OF OXALIPLATIN AND PACLITAXEL

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Chemotherapy-induced neurotoxicity represents a limitation in cancer treatment, often leading to long-lasting sensory and cognitive disturbances. Oxaliplatin (OHP) and Paclitaxel (PTX) are two commonly used agents known for inducing peripheral neuropathy, yet growing evidence suggests they may also affect central neuronal circuits. This study investigates the impact of these drugs on cortical pyramidal neurons, aiming to identify structural correlates of chemotherapy-related cognitive impairment. Adult male Balb/c mice received intravenous injections of OHP (7 mg/kg/week, 8 weeks) and PTX at three different doses (5-7,5-10 mg/kg, 8 weeks), compared to vehicle-treated controls. Golgi-Cox staining was performed on brain tissue, and morphometric analyses were carried out on layer V pyramidal neurons in the somatosensory and prefrontal cortices using Neurolucida software. At the end of OHP treatment (T1), both cortical areas displayed reduced average dendritic length and branching, with preserved dendrite number. Spine density was maintained in the somatosensory cortex but shifted toward immature types, while the prefrontal cortex exhibited a reduction in spine number and mature (mushroom-type) forms. After 4 weeks of follow-up (T2), decrease in dendritic complexity and persistent spine immaturity were evident in both regions. Ongoing analyses on PTX-treated animals aim to define the dose-dependent effects of this drug on cortical morphology. Preliminary observations suggest similar trends in dendritic and spine alterations, potentially with distinct regional or dose-related patterns. Overall, these findings indicate that both OHP and PTX induce structural remodeling of cortical pyramidal neurons, providing morphological evidence for central neurotoxicity associated with chemotherapy.

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THERAPEUTIC VERSUS PREVENTIVE ADMINISTRATION OF NEUROACTIVE STEROIDS TO TREAT BORTEZOMIB-INDUCED PAINFUL NEUROPATHY

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Chemotherapy-induced peripheral neuropathy (CIPN) is a disabling condition resulting from antineoplastic treatment. The proteasome inhibitor bortezomib (BTZ) can cause painful peripheral neuropathy (BIPN) with a negative impact on cancer survivors' quality of life. Although reducing pain is often a main focus of BIPN treatment, remarkably few analgesics have been tested. A growing number of reports suggest that CIPN can be attenuated by the concomitant use of neuroactive steroids (NAS), cholesterol derivatives with proven neuroprotective effects in several *in vivo* models of peripheral neuropathy. However, an important factor in the development of neuroprotective intervention is whether to adopt a therapeutic or a preventive approach. Therefore, we tested the analgesic effect of two NAS, allopregnanolone (ALLO) and pregnenolone (PREG), in two rodent models of BIPN. Female Wistar rats were intravenously treated with BTZ (0.2 mg/kg, 3qw) for 4 weeks. To study the therapeutic effect, co-administrations of BTZ and ALLO (3 mg/kg/every 2 days) or PREG (6 mg/kg/every 2 days) were performed subcutaneously for another 4 weeks. Instead, in the preventive schedule, ALLO or PREG were co-administered for 4 weeks with BTZ from the beginning of the study. Here, we tested the protective effects of the two NAS using a battery of behavioral and neurophysiological tests as well as morphological and morphometrical analyses of myelinated nerves and intraepidermal small unmyelinated fibers (IENF) densities. Treatment with BTZ induced significant mechanical allodynia and thermal hyperalgesia, as well as a reduction of sensory action potential amplitude in peripheral nerves already at 4 weeks, with severe neuropathic symptoms at 8 weeks. NAS administration alleviated BTZ-induced behavioral alterations and partially prevented neurophysiological symptoms. In addition, BTZ treatment induced a significant loss of both myelinated and unmyelinated fibers in the caudal nerves and skin, respectively. A protective effect was observed after NAS treatment on IENF. Taken together, our results suggest that since NAS counteracted painful symptoms induced by BTZ, they could be used to alleviate BIPN neurotoxic manifestations. Moreover, having knowledge of the precise timing of therapeutic intervention is paramount to boost any neuroprotective treatment efficacy.

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BEYOND THE SPINAL CORD: MORPHOLOGICAL ALTERATIONS OF CORTICAL PROJECTION NEURON IN SPINAL MUSCULAR ATROPHY

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Spinal Muscular Atrophy (SMA) is a life-threatening childhood-onset neurodegenerative disease, caused by loss-of-function mutations in the *SMN1* (*Survival Motor Neuron 1*) gene. While the lack of functional SMN protein is known to cause lower motor neuron (MN) degeneration, growing evidence suggests a broader pathological impact involving also other organs, including the brain. Nonetheless, the involvement of the sensorimotor cortex in SMA pathology, remains unclear. To determine the effect of SMN reduction on the survival and morphology of cortical projection neurons, we examined the somatosensory cortex of SMAΔ7 mice, a severe SMA model, at early (P5) and late (P11) symptomatic stages. Our findings indicate a selective vulnerability in cortical projection neurons: indeed at P11, we observed a 50% reduction in corticospinal neurons and a 36% reduction in callosal neurons. Interestingly, corticothalamic neurons show no sign of degeneration. Moreover, in SMA condition, corticospinal and callosal neurons exhibit alterations in morphological traits: this includes a significant decrease in soma size (by -32%% and -17.8%, respectively), alongside a reduction in basal dendrite length (-41% and -40%) and complexity (-64% and -46%). Furthermore, these neuron populations display a less mature dendritic spine phenotype, characterized by an increase in filopodia and a decrease in mushroom spines compared to wild-type controls. In marked contrast, corticothalamic neurons appeared resistant, showing no signs of degeneration or evident morphological changes. These data suggest that specific populations of cortical projection neurons are uniquely sensitive to SMN absence. Interestingly, while the most severe cell death was observed at P11, the morphological changes detailed above in cortical projection neurons were already evident at the early stage of P5, coinciding with the onset of spinal MN degeneration. The identification of these early morphological markers of either vulnerability or resistance across distinct cortical cell types is essential to advancing our understanding of SMA progression and may reveal novel, selective therapeutic targets to preserve upper motor circuit function.

EXPERIMENTAL PARKINSONISM RECRUITS CHOLINERGIC MEDIAL SEPTAL NEURONS

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The neurotoxin 1-methyl-4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) induces Parkinsonism (PD) in humans and various animal species through its MAO-B-dependent, active metabolite, 1-methyl-4-phenyl pyridinium (MPP^+). The toxic effects of MPP^+ are based on the quite selective uptake within neurons where it inhibits complex I of the mitochondrial respiratory chain. Apart from movement disorder due to a dopamine (DA) nigrostriatal damage, PD patients frequently suffer from cognitive deficits up to dementia. This is variably interpreted concerning the anatomical substrate(s). Concerning MPTP/MPP^+ Only a few studies analysed whether this neurotoxin may overlap for cognitive symptoms. Since PD patients may feature neuronal damage affecting forebrain cholinergic neurons, it is intriguing to assess whether a cholinergic neuronal damage may occur following MPTP/MPP^+. This is mostly critical considering learning and memory promoted by the cholinergic medial septal nucleus Therefore, in the present study, cholinergic cells (Ach) derived from the medial septal nucleus were used to assess MPP^+ toxicity. Once neural loss was documented, the cellular targets of MPP^+ toxicity were investigated. These include autophagy, which was impaired following MPP^+. Therefore, MPP^+-induced damage was challenged by using autophagy activators. These effects were analysed by light and electron microscopy. To evaluate the impact of MPP^+ in combination with autophagy activators on cell viability, H&E staining along with Trypan Blue and Fluoro Jade-B were carried out along with ultrastructural analysis. Here we show that, the sensitivity of cholinergic neurons to MPTP/MPP^+ was remarkably higher compared with DA cells. In fact, Ach damage was one hundred-folds more severe, than that of conventional nigral DA neurons. Although subcellular targets are similar in both neuronal phenotypes. Similarly, just like observed for DA neurons the recruitment of autophagy through the phytochemical curcumin protects Ach cells. Mitochondrial alterations appear at sub-toxic MPP^+ concentrations and are counteracted as well. Altogether, the present data suggest that an experimental model of DA damage may also serve to reproduce Ach cell loss thereby mimicking cognitive dysfunction in PD in addition to movement disorder, which is modulated by the phytochemical curcumin.

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GAIT CHARACTERIZATION IN A MOUSE MODEL OF CHRONIC MILD STRESS- AND MPTP-INDUCED NEUROINFLAMMATION

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Chronic mild stress (CMS) is an etiological risk factor for neuroinflammation that, in turn, is involved in the pathogenesis and progression of Parkinson's disease (PD). To study the relationship between CMS, neuroinflammation and PD, we set up an *in vivo* model in which mice were subjected to CMS and, at the end of treatment, received 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) that causes the selective degeneration of the substantia nigra dopaminergic neurons, that is the main pathological feature of PD. In patients, disturbances in gait are symptomatic of PD. In mice, the administration of MPTP results in PD symptoms, including gait alteration. Several studies in mouse models of PD have characterized gait by estimating stride parameters by painting animals' paws. Therefore, to compare the effects CMS and MPTP on mice gait, from the analysis footprint patterns four step parameters were measured: stride length, hind-base width, front-base width and overlap between forepaw and hindpaw placement. Control and stressed mice exhibited comparable stride lengths for both forepaws and hindpaws. By contrast, MPTP-treated mice displayed a significantly shorter stride length compared with control mice ($p<0.05$), as well as MPTP-treated stressed mice compared to the ones subjected only to CMS ($p<0.05$). The frontbase width did not differ significantly in mice from all experimental conditions; in contrast, the hindbase width of MPTP-treated mice was broader than that shown by controls ($p<0.05$); also hindbase width of MPTP-treated stressed mice resulted significantly higher compared to the one of mice subjected only to CMS ($p<0.05$). Stressed mice displayed reduced hindbase width compared to control one. The front/hind footprint overlap provides an indication of the accuracy of foot placement and of the uniformity of step alternation: surprisingly, MPTP-treated mice displayed a footprint overlap similar to control one whereas, as expected, overlap in MPTP-treated stressed mice resulted significantly lower compared to the ones subjected only to CMS ($p<0.05$). Although stressed mice displayed better step overlap compared to control one, the variability of the distances measured resulted higher. Overall, these data suggest that MPTP induced in both unstressed and stressed mice a significative gait alteration compared to respective controls, while the effects of CMS on motor behaviour were limited to the alteration of the uniformity of step alternation.

THE LINK BETWEEN NEURONAL BRANCHING AND PAIN

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In the human body, axons can extend up to a meter in length, making efficient intracellular transport essential for maintaining neuronal function and growth. During development or after injury, neurons tend to elongate rapidly, forming long axons with limited branching. As neurons mature, axonal growth slows while branching increases, promoting the formation of complex neural networks. However, the efficiency of a neuron that continues to grow and branch depends on its ability to support local protein synthesis and long-distance signaling, both of which rely on retrograde transport mechanisms. In this study, we investigated how neuronal branching efficiency changes with aging and injury, and how these processes relate to pain sensitivity. Using *importin α3* knockout (KO) mice, we examined the role of retrograde transport in axonal branching. *Importin α3* KO mice display reduced pain perception in both young and aged animals. Analysis of primary DRG neurons cultured from young and old wild-type (WT) males revealed increased branching in aged WT neurons. In contrast, aged *Imp α3* KO neurons exhibited significantly reduced neurite outgrowth and branching. These findings suggest that enhanced branching in aged WT neurons may contribute to increased pain sensitivity, possibly through heightened synaptic connectivity or excitability. Interestingly, in young animals, KO neurons showed increased axonal outgrowth and branching, particularly after *in vitro* injury compared to WT neurons. This indicates that growth capacity is higher in young neurons but not necessarily associated with increased pain perception. In conclusion, young and aged neurons exhibit distinct capacities for axonal plasticity and pain perception, which can be modulated by impairing retrograde signaling transport. These findings suggest that targeting retrograde transport pathways, such as importin α3-mediated signaling, may represent a potential strategy to modulate neuronal growth and pain sensitivity across aging.

MORPHOLOGICAL AND SYNAPTIC CHANGES OF THE CORTICAL GABAERGIC INHIBITORY SYSTEM IN SPINAL MUSCULAR ATROPHY

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Spinal Muscular Atrophy (SMA) is a neuromuscular disease due to the lack of Survival Motor Neuron (SMN) protein, characterized by lower motor neuron (MN) degeneration and muscle atrophy. Moreover, evidence shows motor cortex (CRTX) morpho-functional abnormalities in SMA patients, suggesting altered maturation and maladaptive plasticity. Given limited access to patient samples, preclinical models are mandatory to clarify motor cortical involvement and underlying mechanisms in SMA pathogenesis. Building on our previous observation of upper MN vulnerability in SMA mice, we dissected cortical inhibitory GABAergic signalling, metabolism, and interneuron (IN) function in the sensorimotor (SM) CRTX and in primary neuron-astrocyte co-cultures from a severe SMA mouse model, compared to WT controls. Bioinformatic and biochemical analyses revealed stage-specific alterations in GABAergic pathways and metabolite profiles, particularly at late stage (P12), indicating a critical window of cortical network vulnerability. Imaging and molecular studies showed $GABA^+$ neuron loss (-38%) and impaired GABA synthesis (GAD65/67). Moreover, confocal analysis revealed marked morphological alterations of parvalbumin $(PV)^+$ INs in the SM CRTX of SMA mice, characterized by smaller somas and reduced dendritic dendritic complexity compared to controls. To determine whether these changes affected specific cortical layers, we quantified PV^+ IN distribution and found a pronounced reduction in layer 5 of the motor CRTX (M1: -33.1%; S1: -25.7%). Consistently, the density of inhibitory synapses, assessed by gephyrin/GAD puncta, was markedly decreased in the SMA motor CRTX, particularly in layer 5 compared to WT controls (≤50%). Electrophysiological characterizations further confirmed decreased inhibitory neurotransmission onto layer 5 pyramidal neurons. Subsequent analyses showed that SMN loss impairs GABA release and reuptake by altering astrocytic transporter expression (SNAT5: -45%; GAT3: +29%), leading to reduced neuronal GABA and its accumulation in astrocytes. This imbalance leads to cortical GABAergic dysfunction, contributing to SMA pathology and highlighting SMN's critical role in neurotransmitter regulation. Our data support a mechanistic model in which SMN deficiency impairs cortical GABAergic networks, expanding our understanding of cortical mechanisms in SMA and providing a framework for cortical-targeted therapeutic strategies.

INVESTIGATION OF STRUCTURAL PRESERVATION OF PERIPHERAL NERVE EXTRACELLULAR MATRIX FOLLOWING *EX VIVO* LIMB PERFUSION

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Peripheral nerve injuries (PNI) represent a significant clinical challenge, frequently resulting in permanent sensory and motor defects. Traumatic injuries can severely alter the peripheral nerve's extracellular matrix (ECM), which is fundamental in guiding Schwann cell (SC) and neuronal responses during regeneration. Given its importance, there is growing interest in understanding how traumatic injuries (amputations) and post-lesion treatments, such as *ex vivo* perfusion, affect ECM structure and composition. The study aims to evaluate ECM integrity following traumatic injury of peripheral nerves and post-lesion treatment. Amputated porcine limbs were perfused *ex vivo* with Perfadex® solution for 6 h, after which tibial and common fibular nerves were harvested. ECM components were analyzed using histological stainings: Wallart and Houette's Trichrome for collagen, Verhoeff's Van Gieson for elastin, and Alcian blue for glycosaminoglycans. Laminin and fibronectin distribution were assessed by immunofluorescence and ultrastructural features were examined by transmission electron microscopy (TEM). This work is expected to provide insights into ECM remodeling after peripheral nerve trauma and to clarify whether *ex vivo* perfusion contributes to preserving its structural and molecular organization. Such knowledge may support the development of improved strategies for peripheral nerve repair.

FLVCR1a REGULATES CORTICAL INTERNEURON DEVELOPMENT AND MIGRATION IN THE EMBRYONIC CORTEX

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FLVCR1 (feline leukemia virus subgroup C receptor 1) is a solute carrier protein implicated in choline and ethanolamine transport, highly expressed in the developing brain. Biallelic pathogenic variants in FLVCR1 are associated with a broad spectrum of neurological disorders, including congenital hydrocephalus, microcephaly, developmental delay, epileptic encephalopathy, and adult-onset neurodegeneration. While Flvcr1a deletion in neural progenitor cells (NPCs) disrupts mitochondrial calcium homeostasis and energy metabolism leading to defective neurogenesis and congenital hydrocephalus, this does not fully account for the cognitive symptoms observed in patients. Here, we explore the contribution of FLVCR1a to cortical interneuron development, as these GABAergic neurons are essential for the formation of neuronal networks. Using a conditional knockout mouse model lacking Flvcr1a in NPCs, we observed altered interneuron migration, characterized by reduced interneuron migration rates, shortened leading processes, and aberrant direction changes, along with altered morphology *in vitro*. Additionally, explants of medial ganglionic eminence (MGE) from mutant embryos displayed migration deficits compared to wild-type littermates. To dissect the molecular mechanism, we investigated the FLVCR1a protein interactome and identified DOCK7, a Rac1 guanine nucleotide exchange factor, and CRAD, an inhibitor of F-actin capping proteins, as FLVCR1a interactors. Additionally, by using a FRET biosensor, we reported that FLVCR1a modulates Rac1 activity a key regulator of cytoskeletal organization during migration. These findings link FLVCR1a to the regulation of cytoskeletal dynamics and cell polarity in migrating interneurons. These findings suggest that FLVCR1a deficiency disrupts cellular migration and morphology during development, likely through the modulation of cell bioenergetics and cytoskeletal dynamics. This study contributes to a deeper understanding of neurodevelopmental disorders linked to FLVCR1a mutations.

5XFAD MOUSE MODEL: INVESTIGATING BRAIN IRON IMBALANCE AND MITOCHONDRIAL FEATURES' ALTERATION

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In the brain iron supports neuronal activity and neurotransmitter synthesis. The primary site of iron utilization is mitochondria where it is required for the formation of cofactors essential to redox reactions and energy metabolism, such as ATP production *via* the electron transport chain (OXPHOS). We previously showed that iron dyshomeostasis occurs with aging, and evidence links disrupted iron balance to mitochondrial dysfunction, energy loss and neuronal death in neurodegenerative diseases like Alzheimer's disease (AD). The role of brain iron-driven mitochondrial impairment in AD remains unclear. Here, we examined iron homeostasis during the pre-symptomatic phase in 5xFAD mice, carrying five familial human AD mutations. At 2 months, 5xFAD mice showed marked brain iron accumulation in subcortical and striatal areas as revealed by Prussian blue Perl's staining along with early intracellular amyloid-beta deposition. Due to mitochondria's key role in iron trafficking and utilization, enriched mitochondrial fractions isolated from 5xFAD brains showed increased expression of mitochondrial number verified by TOM20, mitochondrial aconitase and OXPHOS complexes. Interestingly, we found a significant increase of mitochondrial ferritin, the iron stock within mitochondria, indicating altered iron handling. Moreover, the increased mitochondrial number is not due to *de novo* biogenesis, as shown by unchanged expression of Cytochrome b, NADH dehydrogenase 1, and PGC-1α; instead, it results from mitochondrial accumulation. Indeed, we found a marked reduction in markers of autophagy and of mitophagy, indicating compromised autophagy in 5xFAD mice and sustaining the previous results of mitochondria accumulation. Furthermore, accumulated mitochondria showed reduced ATP production and respiration while oxidative stress markers were increased. These findings reveal early disruptions in brain iron metabolism and mitochondrial turnover, highlighting mitochondrial iron as a potential target for Alzheimer's disease.

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REDUCING PACLITAXEL-INDUCED PERIPHERAL NEUROTOXICITY *VIA* LIPOSOMAL DRUG TARGETING

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Paclitaxel (PTX) is a highly effective chemotherapeutic agent widely used in breast cancer treatment, but its clinical application is significantly limited by peripheral neurotoxicity. Innovative drug delivery systems are needed to selectively release PTX at the tumor site while minimizing neuronal damage. In this proof-of-concept study, we developed and characterized PTX-loaded liposomes functionalized with a metalloproteinase-sensitive lipopeptide (MSLP), exploiting the overexpression of metalloproteinases in the tumor microenvironment to achieve site-specific drug release. Liposomes were prepared *via* thin-film hydration followed by extrusion, resulting in four formulations: PTX-loaded liposomes (LipoPTX), metalloproteinase-sensitive functionalized PTX liposomes (MSLP-LipoPTX), and unloaded liposomes (functionalized or not). Antitumor activity was evaluated in MCF-7 breast cancer cells using the MTT assay. Neurotoxicity was assessed both *in vitro*, using primary cultures of adult mouse sensory neurons, and *in vivo*, in transgenic zebrafish embryos (Tg(isl2b:GFP)zb7), analyzing morphological, behavioral, and molecular endpoints. Our results demonstrated that both LipoPTX and MSLP-LipoPTX preserved the anticancer activity of free PTX while significantly reducing its neurotoxic effects. In sensory neuron cultures, liposomal formulations induced only mild neurite shortening compared with free PTX. In zebrafish embryos, treatment with LipoPTX and MSLP-LipoPTX was associated with lower mortality, fewer caudal fin abnormalities, and improved responsiveness to mechanical stimuli. Overall, these findings suggest that functionalized liposomes can effectively deliver PTX while mitigating peripheral neurotoxicity in both *in vitro* and *in vivo* models. This strategy offers a promising approach to enhance the therapeutic index of PTX and supports further investigations in zebrafish xenograft models of human breast cancer to validate its translational potential.

EXPLORING NEURON–GLIOMA SIGNALING THROUGH *IN VITRO* CO-CULTURES

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The tumor microenvironment (TME) represents a highly dynamic and multifaceted ecosystem composed of both cellular and non-cellular components that collectively modulate tumor initiation, progression, and metastatic potential. Within this intricate milieu, neuron-tumor interactions have recently emerged as critical determinants of cancer behavior, especially in malignancies of the nervous system, where neuronal activity appears to sustain and even accelerate tumor growth. Increasing evidence supports the existence of a bidirectional communication between neurons and cancer cells, involving the exchange of soluble factors, neurotransmitters, and vesicular signals that influence cell survival, proliferation, and morphology. To dissect the molecular mechanisms underlying this neuron-tumor crosstalk, we established and optimized a series of *in vitro* co-culture models. Primary cortical neurons derived from C57BL/6 mice and neurons differentiated from human induced pluripotent stem cells (hiPSCs) were co-cultured with U87 glioblastoma cells to reproduce the neural-tumor interface. Both transwell-based co-culture systems and conditioned media (CM) approaches were implemented to evaluate the impact of glioma-secreted components on neuronal viability and morphology. Neuronal dynamics were monitored in real time using the Incucyte live-cell imaging system equipped with the Neurotrack module. Exposure to glioma-conditioned medium led to pronounced neurotoxic effects in murine cells, including a 36% reduction in neurite length ($p=0.0055$), a 44% decrease in cell number ($p=0.0024$), and a significant increase in cytotoxicity after 48 h ($p=0.0116$). These findings highlight the detrimental influence of glioblastoma-derived soluble factors on neuronal structure and function. Complementary Western blot analyses confirmed this phenotype, revealing a marked decrease in βIII-tubulin (-60%) and synapsin (-80%) expression in CM-treated murine neurons, indicating impaired cytoskeletal stability and synaptic integrity. Current efforts focus on extending these observations to hiPSC-derived neuronal cultures and enhancing the physiological relevance of the model by integrating advanced microfluidic co-culture systems. Such platforms will allow spatiotemporal control of cellular interactions, providing a more faithful *in vitro* representation of the brain tumor microenvironment and enabling the identification of molecular targets capable of disrupting the pro-tumor neuronal support network.

EFFECTS OF MATERNAL LOW-PROTEIN AND PHYTOESTROGEN-ENRICHED DIETS ON THE CORRELATION BETWEEN EATING BEHAVIORS AND ANXIETY IN OFFSPRING

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Maternal nutrition during pregnancy and lactation represents a crucial determinant of offspring health, with long-term effects on metabolic and neurobehavioral development. This study examined the effects of altered diets during pregnancy and lactation on adult rat pups that received a standard postnatal diet. Since in often altered low-protein diets proteins are supplemented with foods rich in phytoestrogens, such as soy, four groups were compared: low-protein maternal diet (LOW), genistein-enriched diet (GEN), a combination of both (LOWGIN), and a control group (CON). Maternal milk composition and various offspring parameters were analyzed, including metabolic measures, (body weight and intake), behavioral assessments (EPM and OF tests), and neurochemical markers of the hypothalamic POMC system and serotonergic systems (immunohistochemistry for 5-HT in the Raphe, and POMC, 5-HT, and 5-HT_2C in the arcuate nucleus of the hypothalamus). Maternal protein restriction significantly reduced milk protein and fat content, hindered neonatal growth, and decreased POMC expression, while also increasing 5-HT_2C receptors levels, making it more difficult for the neonate to adapt to changes and new stimuli. The phytoestrogen Genistein, increased milk lipid content and neonatal weight, suggesting a potential obesogenic effect in female pups. The long-term effects of genistein were sex-specific: males exhibited enhanced serotonergic sensitivity, characterized by increased expression of the 5-HT system, and an anxiolytic behavioral profile. In contrast, females showed serotonergic hyperactivity. The combination of the two diets displayed milk composition and metabolic parameters similar to those of the LOW group, suggesting a predominant effect of protein restriction that overrides the potential benefits of genistein. Behaviorally, profiles were sexually dimorphic: males showed anxiety, whereas females displayed an anxiolytic profile similar to GEN females, highlighting greater female sensitivity to genistein's estrogenic effects. Overall, the results demonstrate that early nutritional programming has a stable influence on the development of metabolic and emotional circuits in a sex-specific manner, underscoring the importance of adequate protein intake during pregnancy and lactation, as well as the cautious evaluation of phytoestrogen consumption during critical periods, when maternal dietary choices can have lasting consequences for offspring health.

SCHWANN CELLS BEHAVIOR ON SMART ELECTROCONDUCTIVE BIOMATERIALS FOR PERIPHERAL NERVE REGENERATION

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Functional recovery following peripheral nervous system (PNS) injuries remains a significant challenge for clinical research. The PNS exhibits intrinsic regenerative capacity in which Schwann cells play a pivotal role, with their remarkable phenotypic plasticity, capable of modulating several processes to achieve axonal regeneration. Despite this intrinsic potential, when the injury is severe, with a gap exceeding 5 mm, or the surgical repair is delayed, recovery is often unsatisfactory, resulting in the formation of a non-functional nerve. The current clinical gold standard is autograft, however, it is associated with notable limitations, such as the sacrifice of healthy nerve tissue and potential surgical complication. Among the strategies aimed at promoting functional PNS regeneration, biomaterials have emerged as a promising alternative, thanks to their ability to provide mechanical support and recreate an extracellular environment that promotes regenerative responses. In the present study biocompatible and biomimetic biomaterials based of chitosan/carboxymethylcellulose were developed (Ch70CMC30 scaffolds). Reduced graphene oxide (RGO), an electroconductive biomaterial, was incorporated into these starting biomaterials, both in bulk form at different concentrations (0.3%, 0.6%, 1.0%) and as surface striped micropatterns with two distinct dimensions (100 μm and 200 μm). The glial cell behaviour to the films was assessed in terms of proliferation, morphological responce and protein expression. In the initial experimental phase, the RT4-D6P2T glial cell line derived, from Schwannoma, was used to conduct the experiments. Morphological analysis was then also performed with primary culture of rat Schwann cells, to evaluate a model more representative of the regenerative environment. The scaffolds demonstrated efficacy in supporting consistent cell growth and stable expression of markers associated with cell survival and proliferation, confirming their cytocompatibility. In particular, in the case of RGO micropatterns, morphological analysis revealed preferential cell adhesion to RGO coated regions and a pronounced alignment along the pattern. These findings highlight the potential of RGO-functionalized scaffolds in modulating cell growth and migration, laying the foundation for future studies pointing at developing advanced regenerative strategies.

DISTRIBUTION, DENSITY, AND PHYLOGENETIC VARIATION OF CORTICAL IMMATURE NEURONS IN MACAQUES (RHESUS MONKEY)

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Brain structural plasticity, especially neurogenic processes, vary across species, anatomical regions, and ages. In addition to stem cell-driven adult neurogenesis, a form of "neurogenesis without division" has been recently shown, involving "immature" or "dormant" neurons in the cortical layer II (cortical immature neurons, cINs). These cells are prenatally generated and remain in a state of "arrested maturation", retaining the ability to resume maturation at different life stages and functional integrate into pre-existing circuits. Previous quantification of cINs in widely different mammals showed high abundance of these cells in large-brained species with respect to rodents, and covariation with brain size and neocortical expansion. Here, using the same approach (immunocytochemical detection of the cytoskeletal protein doublecortin, after establishing correspondent neuroanatomical levels in each species), we characterized and quantified the cINs in macaques (Rhesus monkey), a primate with human-like brain anatomy, phylogenetics, and cognitive functions. First, we studied the cIN density in 4 young adult macaques (7-10 years) to understand their position in the phylogenetic variation. Our results place them among the gyrencephalic species and confirm the covariance between cIN density and both increased brain size and neocortical expansion. Then, we used 11 middle-aged macaques (23 years) to quantify the cIN density across 16 neuroanatomically defined cortical regions in search for possible regional variation. Significant differences were observed, with the highest presence in the medial and inferior temporal cortex. Overall, it is confirmed that cINs follow an evolutionary trend in mammals, involving the entire cortical mantle in gyrencephalic species, yet, with regional differences. Previous studies reported high amount of doublecortin-positive cells in the temporal lobe of humans, thus supporting the value of macaque as a good translational model for studying cINs.

THE EFFECT OF ANTIBIOTIC-INDUCED DYSBIOSIS ON PERIPHERAL NERVE REPAIR

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Human gut microbiota is the dynamic and complex population of microorganisms (bacteria, fungi, protozoa and viruses), which contributes to tissue homeostasis through a series of physiological functions. The gut microbiota influences not only the gastrointestinal tract, but also a growing list of other organs, impacting on function such as barrier integrity, metabolism, hematopoiesis and inflammation, leading to its consideration as a 'vital organ'. Recently, our laboratory identified a connection between the gut microbiota and the peripheral nervous system (PNS). To study the impact of microbiota composition on peripheral nerve regeneration, we administered a cocktail of antibiotics (vancomycin 0.25 mg/mL, ampicillin 0.5 mg/mL, metronidazole 0.5 mg/mL) to mice *via* drinking water. Animals were divided into four groups: Group 1 (Treated with antibiotics before and after the surgery), Group 2 (Treated with antibiotics before the surgery), Group 3 (Treated with antibiotics after the surgery) and Group 4 (Not treated with antibiotics). Mice were subjected to peripheral nerve injury (transection or crush) and, after 2, 7 or 28 days different tissues/organs were harvested for the analysis: median and ulnar nerve, flexor digitorum superficialis and flexor digitorum profundus, spleen, lymphnodes, serum, fecal pellet, DRGs, and forepaw. To assess functional recovery, we performed the grasping test. Antibiotic treatment did not cause weight loss or evident behavioral alterations. Microbiota analysis revealed distinct patterns across groups/treatments and timepoints. Overall, antibiotic treatment induced dysbiosis, with the extent and nature of the disruption varying according to treatment duration. The rescue group (treated only before surgery) demonstrated partial recovery of the microbiota, particularly at later timepoints. The grasping test revealed that mice treated 10 days before median nerve injury showed reduced strength in the injured forelimb compared to untreated animals until day 10 post-injury. This difference progressively diminished, with similar performance among groups by day 28. Additional analyses are ongoing. Our findings indicate that microbiota depletion leads to functional alterations in the PNS. Further analyses are ongoing to elucidate mechanisms underlying these effects.

THE ESTABLISHMENT OF A hiPSC-DERIVED *IN VITRO* MODEL TO STUDY DIABETIC PERIPHERAL NEUROPATHY

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Diabetic peripheral neuropathy (DPN) is the most frequent complication of diabetes, involving progressive damage to neurons, Schwann cells, and microvasculature. It results in altered pain perception, either heightened sensitivity or complete sensory loss, raising the risk of injury. Due to the structural complexity of peripheral nerves, the pathogenic mechanisms of DPN remain poorly understood, limiting therapeutic development. Human induced pluripotent stem cell (hiPSC)-based models are emerging as promising tools to recreate the cellular diversity of peripheral nerves *in vitro* and study DPN pathogenesis. To model DPN *in vitro*, we first established a rat-based system using primary Schwann cells and primary dorsal root ganglion (DRG) neurons cultures. Schwann cells were then cultured under high-glucose and hypoxic conditions to mimic diabetic stress, showing altered morphology, reduced proliferation, increased oxidative stress, and decreased expression of mature Schwann cell key markers. In parallel, dissociated rat DRG neurons exposed to the same conditions showed reduced neurite complexity, with fewer branches and junctions per cell. These findings confirm the induction of key DPN-like features in our primary cell cultures model. Building on this foundation, we differentiated human induced pluripotent stem cells (hiPSCs) into Sensory Neurons and Schwann cells following previously established protocols. We successfully obtained both iNOCs (induced-nociceptors) and iSCs (induced-Schwann cells) and cultured them under hyperglycemic conditions on the basis of preliminary data obtained on primary cell cultures. These foundational steps are critical for our ultimate goal of integrating both cell types into a multicellular system, fully human-based *in vitro* model of DPN. Moreover, by validating cellular responses to diabetic stress in both rat and human-derived components, we are laying the groundwork for a translational platform that will enable deeper mechanistic insights into the pathophysiology of DPN and support future personalized and therapeutic development.

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MICROFLUIDIC PLATFORM TO UNRAVEL GLIOMA-SECRETOME INTERACTION

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Diffuse infiltrating gliomas (DIGs) account for approximately 80% of all malignant gliomas, making them the second most common primary tumor of the central nervous system (CNS). Among them, glioblastoma (GBM) is the most aggressive form, associated with poor prognosis. These features are attributed to the interplay between clonogenic glioblastoma stem cells (GSCs) and the tumor microenvironment (TME). In fact, the secretion of growth factors and cytokines supports GSCs, collectively altering the tumor secretome that maintains cancer cell pluripotency, drives invasiveness, facilitates intra-brain tumor dissemination, and conferrers resistance to chemotherapy. The goal of this study is to develop a novel approach to investigate the interplay between GSCs and secretome through an *in vitro* microfluidic system, through the MIVO platform organ-on-chip. To this aim, tumor spheres of different glioma cell lines were cultured using the hanging drop method and moved to the upper chamber of MIVO support: a compartment divided from the fluidic channel by a porous membrane, simulating the microcirculation of secreted cells in the TME. A volume of 150-200 µL of medium was added into the chamber to balance the liquid level inside and outside the insert, to ensure proper flow. The plate with the inserts was placed in the specific support, then the cartridge was secured inside the pump head. To validate the platform's potential, deferoxamine (DFX, a hypoxia-mimetic agent) was added to the flow of the microfluidic system, worsening the tumor microenvironment. In this contest, DFX effects on the secretome were evaluated by ELISA assay. Preliminary results revealed a significant increase of vascular endothelial growth factor (VEGF) and interleukin-1β (IL-1β) in glioma secretomes following DFX exposure, observing some differences among glioma cell lines. These findings support our hypothesis by this microfluidic platform is able to offer a valuable tool for studying GSC behavior during therapeutic interventions. Furthermore, it holds promise for use in preliminary screening of therapeutic agents targeting DIGs, potentially accelerating drug discovery and improving treatment strategies.

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ENDOCRINE DISRUPTOR EXPOSURE ACROSS GESTATION AND LACTATION MODIFIES MATERNAL AND ANXIETY-RELATED BEHAVIORAL PATTERNS

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Endocrine disruptors (EDs) are compounds that affect the endocrine system by interfering with hormone synthesis and action, altering organ development, reproductive and metabolic processes. Bisphenols and perfluoroalkyl compounds are key EDs studied, used in industrial processes to produce everyday products, i.e. plastics for food packaging. This creates a direct pathway for human exposure, with potential risks for pregnant women and their offspring, about which little is known. This study examined how maternal exposure to bisphenol A (BPA), bisphenol S (BPS), and perfluorooctanesulfonic acid (PFOS) affects mothers' spontaneous behavior and anxiety levels. CD1 mouse mothers were orally treated during pregnancy and lactation and divided into four groups: 1) control, 2) BPA (0.2 ng/kg), 3) BPS (0.2 ng/kg), and 4) PFOS (0.7 ng/kg). The mothers were subsequently tested for maternal and anxiety-related behaviors. Feeding behavior indicated an increase in food intake among PFOS-treated mothers, while their body weight remained unchanged. The pups from these mothers were significantly heavier than the controls, whereas the pups exposed to BPA were smaller. These characteristics persisted throughout development. Behavioral tests (elevated plus maze and open field) revealed increased anxiety in PFOS mothers, while BPA mothers showed hyperactivity. Maternal care analysis from PND2 to PND8 revealed that PFOS mothers exhibited fewer maternal behaviors, spending less time nursing and more time outside the nest. Additionally, immunohistochemical analysis of vasopressin immunoreactivity revealed altered vasopressin content in the paraventricular nucleus of PFOS-treated mothers, suggesting a neuroendocrine basis for behavioral changes. In conclusion, BPA, BPS, and PFOS exposure during pregnancy and lactation resulted in diverse behavioral and metabolic effects. Future analyses will focus on the sexual and anxiety behavior of adult puppies and neural markers linked to these topics.

MITOCHONDRIAL DYSFUNCTION AS A KEY FACTOR OF MOTOR NEURON DEGENERATION: INSIGHTS FROM ALS CELLULAR MODELS

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Motor Neuron Diseases (MNDs) are progressive pathologies defined by loss of both upper and lower motor neurons (MNs). Mitochondrial dysfunction is recognized as a crucial contributor to MNDs pathogenesis, including Spinal Muscular Atrophy (SMA) and Amyotrophic Lateral Sclerosis (ALS). Both disorders, despite differences in onset and genetic causes, share progressive MNs degeneration, in which alterations in mitochondrial morphology, function and transport play a central role. However, how mitochondrial dysfunctions promote neuronal degeneration is not clear from a mechanistic standpoint. This study aims to understand pathological mechanisms related to mitochondrial dysfunctions to facilitate the recognition of early disease manifestations and ultimately identify new therapies for MNDs. Since mitochondrial dysfunction are one of the earliest neuropathological features observed in ALS, ongoing work employs NSC-34 motor neuron cell lines stably transfected with the human wild-type SOD1 gene or the mutated one (G93A), as an *in vitro* model. By using live-cell imaging techniques (Incucyte and Operetta), mitochondrial dynamics and distribution are monitored in real-time and semi-quantitatively, as confirmed by Western Blot. Alongside a comprehensive morphological analysis of SOD1 motor neurons and control cells, we investigated the effects of MitoQ, a mitochondria-targeted antioxidant that might alleviate ALS-related mitochondrial abnormalities. To this aim, the optimal non-toxic yet effective dose was identified. So far, our data indicate that mitochondrial dysfunction represents a promising target for MND investigations.

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A PILOT STUDY TO INVESTIGATE THE ANTIAGING EFFECTS OF THE OLIVE POMACE BY-PRODUCT ON THE BRAIN OF *DROSOPHILA MELANOGASTER*

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Olive pomace (OP), a byproduct of olive oil extraction, retains numerous bioactive compounds (in particular, hydroxytyrosol and tyrosol, but also other beneficial flavonoids, phenols and secoiridoids like luteolin, oleocanthal, and oleuropein) found in extra virgin olive oil, which are recognized for their health-promoting properties, particularly in reducing oxidative stress and inflammation. Recent studies have demonstrated that oleuropein and other polyphenols may exert significant neuroprotective effects contributing to improved cognitive function and reducing neuroinflammation, thereby supporting neuronal health. Furthermore, research suggests that polyphenols can promote neurogenesis and synaptic plasticity, essential processes for maintaining brain function during aging. Dietary supplementation (DS) with OP compounds may positively influence gut microbiota, which interacts with the central nervous system, highlighting the intricate relationship between diet and brain health. Following our observation in a previous study that dietary supplementation with OP significantly increased the half-life in both female and male flies, in the present pilot study, using one of the two OP batches previously employed, we evaluated the effects of dietary supplementation with OP on motor abilities and on the maintenance of brain tissue morphological integrity during aging in female flies. Climbing ability, brain size, and quantitative analyses of vacuole-like degeneration associated with specific brain regions were utilized as indicators of brain functional integrity and neurodegeneration during the aging process. Dietary supplementation commenced at 30 of flies age (*w1118* strain), and climbing ability was assessed at the beginning (T0) and after 10 days of OP DS (T1). Brain tissue collections were conducted immediately after the T1 climbing assay. Female flies supplemented with OP exhibited significantly improved climbing activity at T1 ($p<0.0188$), a trend toward larger brain size ($p=0.053$) along with a significantly reduced total volume of brain vacuoles compared to flies without DS ($p<0.05$). The gut microbiota modulation by bioactive compounds has been linked to various health benefits through multiple mechanisms. Understanding how OP-derived polyphenols specifically influence these microbial populations and their functional outputs would provide valuable insights into the neuroprotective and longevity-promoting effects observed in our observations.

CHANGE IN AQP4 EXPRESSION AND LOCALIZATION DURING AMYOTROPHIC LATERAL SCLEROSIS AND SPINAL MUSCULAR ATROPHY PROGRESSION

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The glymphatic system is a glial-dependent perivascular network responsible for clearing waste and neurotoxic substances from the central nervous system. Aquaporin-4 (AQP4) water channels, expressed on the astrocyte endfeet and localized around blood vessels, are key components of this system. Dysfunction of the glymphatic pathway has been associated with several neurodegenerative diseases, including Alzheimer's disease and Amyotrophic Lateral Sclerosis (ALS). Our purpose was to evaluate if the alterations in AQP4 expression may also contribute to neurodegeneration in ALS and Spinal Muscular Atrophy (SMA), two different motor neuron diseases. We investigated AQP4 expression in the ventral horns of spinal cord in delta7SMN and $SOD1^{G93A}$ mice, respectively SMA and ALS models. Three time points were selected to represent the presymptomatic, early symptomatic, and late disease stages. The lumbar spinal cord was collected, sectioned at 40 μm, and processed for immunofluorescence analysis using antibodies against AQP4, GFAP (astroglial marker) and ChAT (motor neuron marker). Images were acquired with a Zeiss Apotome microscope and analyzed with ImageJ. We observed a significant increase in AQP4 expression in $SOD1^{G93A}$ mice at both early and late disease stages compared to healthy controls: specifically, during these stages, AQP4 levels in the peri-motoneuronal region increased by 89.8% and 45.6%, respectively, in ALS mice relative to controls. In addition, an increasing trend in AQP4 expression was detected between the presymptomatic and early disease stages. Conversely, AQP4 levels decreased at the late stage, possibly due to neurodegeneration. Comparable expression patterns were observed in SMA mice, in which the increase between the presymptomatic and early stages reached approximately 36%. Our study highlights a potential contribution of the glymphatic system in the pathogenesis of motor neuron diseases and suggests AQP4 as a potential therapeutic target to delay neurodegeneration.

ALPHA SYNUCLEIN REGULATION IN BRAIN AND BONE MARROW IS RELETED TO THE DIFFERENTIAL EXPRESSION OF GATA1 TRANSCRIPTION FACTOR

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Alpha-synuclein (α-syn), is a protein abundantly expressed in the central nervous system and in the erythrocytes, playing a pivotal role in the pathogenesis of Parkinson's disease and other synucleinopathies. Among the GATA family transcription factors (TFs), GATA1 and GATA2 regulate the meg-erythrocytic differentiation starting from the hematopoietic stem cell. In erythropoiesis, the GATA1-2 switching, is known to regulate the α-syn gene (SNCA) expression, which is essential for iron metabolism and membrane stability. Abnormalities in α-syn regulation alter erythrocytic function, possibly contributing to pathological mechanisms of different synucleinopathies. Due to this potential role for GATA1 in synucleinopathies we aimed to underline the effects of GATA1 down-regulation on aging and α-syn expression. To this aim we explored the contribution of hematological alteration in the development of neurodegenerative disorder by analyzing the different organs from the Gata1^{low} mice, as model of aging and Myelofibrosis. Bone marrow and brain section from young and aged Gata1^{low} mice showed significant differences in the α-syn expression compared to their relative controls, suggesting a trend in α-syn aggregation that increases with aging. In the brain, the GATA1 expression was reduced in aged Gata1^{low} mice and resulted in shrinker neurons with mitochondrial alterations. The bone marrow from aged Gata1^{low} mice was characterized by increased level of inflammatory cytokine as TGF-β, that parallel the different expression and the aggregation of α-syn. Moreover, morphological determinations revealed that α-syn expression was related to cells resembling the most immature myeloid phenotype (*i.e.* Reticulocytes). These results suggest the pivotal role of GATA1 TF in the regulation of α-syn expression and aggregation, highlighting the potential role of GATA1 and bone marrow in the pathogenesis of synucleinopathies.

GENETIC AND MORPHOLOGICAL FACTORS IN CORTICAL FOLDING: STUDIES ON THE ROLANDIC FISSURE

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Human cortical folding plays a crucial role in neurodevelopment and cognition. In this study, we analyzed several features of the cerebral sulci that describe their morphology, investigated their genetic correlations, and explored typical patterns of the central sulcus. The genetic and morphological factors underlying variations in sulcal shape remain poorly understood. To address this issue, for simplicity's sake, we focused on the central fissure of Rolando. This sulcus was chosen because it appears around the 20th week of gestation; this means that, in the case of early alterations during gestation or in the perinatal period, it may present long-lasting developmental anomalies. Furthermore, the morphology of the central sulcus has been associated with neurological and neurodevelopmental disorders, including stroke, cerebral palsy, and amyotrophic lateral sclerosis (ALS). Since 1995, attempts have been made to morphologically classify the central sulcus, specifically the Hand Motor Cortex (HMC), following the model of Yousry *et al.,* who defined the «omega» shape (Ω) and the «epsilon» shape (ε). Recent studies by Caulo *et al.* and Wu *et al.* have identified three new morphological variants of the HMC: medially asymmetric epsilon (mae), laterally asymmetric epsilon (lae), and null (n). The goal of this work was to validate Caulo *et al*.'s morphological classification by applying it to the ABCD study population (children aged 9 to 10 years) using MRI data, thus expanding the reference sample. We manually classified a subset of 1,193 individuals, including both right- and left-handed individuals. Subsequently, the central fissure was automatically segmented using BrainVISA software, ensuring standardized sulcus identification across all subjects. Since the most frequent morphological categories were the «omega» and «epsilon», we focused on these two. A Random Forest model was used to automatically classify the remaining 10,000 subjects. The most frequent shape in both hemispheres was the «ω» category, followed by «ε». The «n», «mae», and «lae» variants were less common. These results suggest the predominance of specific morphological configurations of the central sulcus among individuals. Furthermore, in our study, we used SOLAR-Eclipse to estimate the heritability of different shapes of the central sulcus by analyzing the genetic architecture of this structure. We tested the heritability of parameters such as width, length, depth, and surface area of the central sulcus, as well as the total surface area of the cortex and its mean thickness. The results showed that the mean cortical surface area had the highest heritability value, while the parameter with the lowest estimate was the length of the central fissure. Sulcus-based heritability analysis showed significant values in multiple sulcal regions, with the sulcal width and surface area exhibit greater heritability. We found that the morphology of the central sulcus, particularly in the right hemisphere, has a modest but significant genetic basis. The close correlation between brain structure and genetic factors may contribute to genetic susceptibility to diseases that compromise the integrity of the cortex.

CONNEXIN43 HEMICHANNELS CAN DRIVE THE GLIOBLASTOMA SPREADING

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Glioblastoma (GBM) is the most common and aggressive primary tumor of the central nervous system (CNS) among high-grade gliomas (HGG). Patients with GBM often have a poor prognosis. Glioblastoma cells have a remarkable ability to infiltrate the CNS and migrate long distances from the tumor core, making complete surgical resection impossible. GBM infiltration relies on electrical and molecular connections with nervous tissue. Among molecular interactions, connexin 43 (Cx43) appears to be a marker for HGG in mouse models. Cx43 is a multifunctional protein, and is found on the plasma membranes of glial cells – particularly astrocytes – in the form of gap junctions or hemichannels, ensuring cell-to-cell or cell-to-extracellular space communication, respectively. Cx43 can be expressed by transformed astrocytes, i.e., GBM cells. Its abnormal expression can alter the electrical state of the system, resulting in epileptic events. To study the dynamics of GBM spread in humans, we characterized tumor and peritumoral tissue from glioma patients. Using confocal microscopy and functional assays, we assessed the state of the neuroglial network in fresh brain sections. Electrical activity, Cx43 density, and glial reactivity were correlated with the degree of tumor infiltration. In parallel, primary human glioblastoma cells were labeled by lentiviral transduction and injected into organotypic tissue sections from the same patients. The glioblastoma cells were monitored until day seven in culture by time-lapse microscopy to follow tumor progression and their response to Gap19, a selective blocker of Cx43 hemichannels. Tumor tissue has a different architecture than peritumoral tissue, which is reflected in specific electrical and morphological profiles. In peritumoral tissue, implanted GBM cells spread into the neuroglial network, and blockade of Cx43 hemichannels results in tissue remodeling and GBM cell polarization, which may correspond to a migratory pattern. This study reveals that tumor progression is not an isolated phenomenon but it is driven by interactions with the surrounding environment in living brain tissue. In particular, Cx43 hemichannels emerge as key players in tumor spread and recurrence, revealing a more complex dynamic than expected. This finding challenges the traditional idea of GBM as an entity external to the brain, especially considering the rarity of its metastases.

AN IPSC MODEL OF FLVCR1-RELATED NEUROPATHY UNCOVERS MITOCHONDRIAL AND MEMBRANE DISORGANIZATION IN SENSORY NEURONS

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FLVCR1-related sensory neuropathies are rare childhood-onset disorders characterized by the progressive degeneration of sensory neurons and/or photoreceptors, leading to sensory ataxia, pain insensitivity, and retinitis pigmentosa. Mutations in the Feline Leukemia Virus Subgroup C Receptor 1 (FLVCR1) gene disrupt a multifunctional transporter that mediates choline and ethanolamine fluxes and interacts with the IP3R3–VDAC complex to regulate mitochondrial Ca^{2+} uptake at mitochondria-associated membranes (MAMs). Our findings reveal that patient-derived fibroblasts carrying pathogenic FLVCR1 variants display reduced choline availability, altered membrane lipid composition, and impaired MAM architecture, culminating in defective mitochondrial bioenergetics. To investigate the neuronal consequences of these alterations, we reprogrammed patient fibroblasts into induced pluripotent stem cells (iPSCs) and differentiated them into sensory neurons expressing the lineage-specific marker BRN3A. Strikingly, patient-derived neurons exhibited aberrant morphology, compromised axonal outgrowth, and disrupted network organization. Together, these data point to a dual pathogenic mechanism in which FLVCR1 mutations perturb both mitochondrial function and membrane homeostasis – two processes essential for neurotrophic signaling, axonal transport, and neuronal integrity. Our iPSC-derived model captures the neuromorphological and metabolic vulnerability of sensory neurons in FLVCR1-related neuropathies, providing a valuable platform to dissect disease mechanisms and identify novel therapeutic targets.

MODULATION OF IMMATURE NEURONS IN THE NEOCORTICAL LAYER II OF SHEEP KEPT IN DIFFERENT ENVIRONMENTAL CONDITIONS

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Cortical immature neurons (cINs) are prenatally generated, non-dividing neuronal cells in arrested maturation that can "awake" during adulthood to integrate as new functional neurons in the layer II (neurogenesis without division). While restricted to paleocortex in rodents, the cINs are far more abundant in large-brained, gyrencephalic mammals, also extending into their entire neocortical mantle. Because of this interspecies difference, the possible modulation of these neurons in the neocortex can be studied only in gyrencephalic species. Environmental conditions are known to positively (enriched environment) or negatively (stress) affect the rate of stem cell-driven adult neurogenesis, yet nothing is known regarding the cINs. To address this issue, we investigated three groups of young sheep, including controls (animals within their habitual environment), isolated animals (a condition considered to be perceived as negative in sheep), and animals kept in an enriched environment, across a two month-long experiment. The cINs of the layer II were identified in postmortem fixed brains using immunocytochemistry for the cytoskeletal marker doublecortin and counted to obtain linear densities (number of cells/mm). The counting was performed on a total of 15 brains following the entire layer II perimeter of 180 coronal sections, by considering piriform cortex and neocortex separately. Both conditions resulted in reduced number of immature cells with respect to controls, the drop being significant only in the neocortex. These results show that environmental conditions can influence the availability of neocortical immature neurons, suggesting that detours from routine life can lower their number, likely by accelerating their maturation. On these bases, remarkable structural plasticity might be figured out to occur in the neocortical upper layers of gyrencephalic species as a consequence of lifestyle.