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# Proceedings of the 34th National Conference of the Italian Group for the Study of Neuromorphology "Gruppo Italiano per lo Studio della Neuromorfologia" G.I.S.N.

November 22-23, 2024

*University of Catania*
*Catania - Italy*

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# *European Journal of Histochemistry*
## *a journal of functional cytology*

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# Proceedings of the 34th National Conference of the Italian Group for the Study of Neuromorphology "Gruppo Italiano per lo Studio della Neuromorfologia" G.I.S.N.




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# MAIN LECTURES

## STARS FOR BRAIN PLASTICITY AND BEHAVIOR

**Rouach N**

*Neuroglial Interactions in Cerebral Physiology and Pathologies laboratory Center for Interdisciplinary Research in Biology, Collège de France*

Our laboratory investigates whether and how the underexplored astrocytes, which are the very abundant non-neuronal, but yet active cells of the brain, play a direct role in information processing. We particularly explore the molecular modalities and functional outcomes of astrocyte-neuron interactions in physiological and pathological contexts focusing *ex vivo* or *in vivo* on neuronal excitability, synaptic transmission, plasticity, synchronization and cognitive functions. To do so, we use a multidisciplinary approach combining electrophysiology, imaging, behavioral testing, mathematical modeling and molecular tools targeting selectively astrocytes in situ and *in vivo* in mice and human tissues. Using this strategy, we performed in the last years mostly fundamental research on role of astrocytes in synaptic transmission, plasticity, network activity and behavior in normal and pathological conditions. We uncovered several major astroglial properties regulating physiological and pathological neuronal activities that I will present. In particular, we have unraveled many ways the connexins control neuronal wiring, activity and behavior via regulation of the extracellular matrix, ion homeostasis, gliotransmitter release or astroglial synapse coverage in various physiological contexts such as critical period plasticity or maternal behavior. Our work thus fuels the emerging concept of neuroglial networks, in which astrocytes actively participate to the formation, activity and plasticity of local neuronal networks.

## NEURODEGENERATION: A MATTER OF GUT?

Gries M[1], Christmann A[1], Rommel S[1], Saha T[1], Puhl HR[1], Schulte S[1], Martin M[1], **Schäfer KH**[1]

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Parkinsons disease is one of the most prominent neurodegenerative diseases we actually face. It is based upon the loss of dopaminergic neurons in the brain, thus leading to severe losses of motoric functions. There is increasing evidence that Parkinson's disease (PD) might start in the gut, thus involving and compromising also the enteric nervous system (ENS). It is highly probable that the ENS is affected by a compromised microbiome or mucosal barrier failure, as well as by changes of the local immune system. At the clinical onset of the disease the majority of dopaminergic neurons in the midbrain is already destroyed, so that there is a huge need for early biomarkers, which allow the timely start of therapeutic approaches. We used a combination of transgenic A30P-α-synuclein-overexpressing PD mouse model experiments with *in vitro* approaches to identify appropriate candidate markers in the gut before hallmark symptoms begin to manifest. A retarded gut motility and very early molecular dysregulations were found in the myenteric plexus of psA30P mice. We found that i.e., neurofilament light chain, vesicle-associated membrane protein 2 and calbindin 2, together with the miRNAs that regulate them, are significantly altered in the psA30P, thus representing potential biomarkers for early PD. Many of the dysregulated miRNAs found in the psA30P mice are reported to be changed in PD patients as well, either in blood, cerebrospinal fluid or brain tissue. Interestingly, the *in vitro* approaches delivered similar changes in the ENS cultures as seen in the transgenic animals, thus confirming the data from the mouse model. Moreover, the mucosaö properties in the PD mice also seem to be altered. Taken all these evidences together, we presume that the gut will be the most suitable target for early diagnosis and treatment of PD.

# SESSION I
# NEURODEGENERATION – SYNUCLEINOPATHIES

## THE NORADRENERGIC SYSTEM AND POST-HYPOXIC EPILEPSY

**Scotto M**[1,2], Galgani A[1], D'Amora M[2], Tantussi F[2], Bartolini E[3], Bosco P[3], Raffa V[4], De Angelis F[2], Tosetti M[3], Giorgi FS[1,3]

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The scientific background and most of the protocol of the study "*TRANSCENDE (TRANslational Study of CEntral Noradrenerenergic system Degeneration in infantile Epilepsy)*" will be illustrated in detail. Perinatal post-anoxic injury (PHI) is a primary cause of secondary epilepsy (PHI-E) in infants, yet the underlying pathophysiological processes of the latter in those patients lacking clear structural brain damage after PHI still remains poorly understood. This study aims to investigate, both in experimental model and in humans, the potential mechanisms involved in PHI-E, particularly focusing on the Locus Coeruleus (LC) and its vulnerability to damage. LC is the main noradrenergic nucleus in the brain and strongly modulates seizures in several epilepsy models. It will be tested the hypothesis that LC alterations occurring following perinatal hypoxia (already reported in post-mortem studies in PHI), could significantly contribute to the onset of PHI-E. Concerning the study in mice: a) pups are exposed to low oxygen concentration (PHI group), or normal $O_2$ levels (negative controls) or to jatrogenic LC lesion (positive controls); b) seizures will be assessed both behaviorally and through EEG: spontaneous electrical seizures and interictal activity will be assessed by chronic EEG Telemetry, while the threshold to seizures will be assessed by acute EEG after sub-threshold Kainate; c) after sacrifice the brain will be analyzed by morphological and molecular investigations aimed at assessing markers of neuroinflammaton, cell damage and neurovascular unit integrity. The integrity of LC, neocortex and specific hippocampal subregions (CA1-3, hilus and dentate gyrus), will be assessed through unbiased stereological analysis. The clinical part of the study aims to explore *in vivo* LC integrity as a potential non-invasive biomarker for epilepsy predisposition in PHI patients. LC features (LC-MRI) in children with PHI who develop epilepsy will be assessed using specific 3 Tesla MRI sequences and ad-hoc post-acquisition analysis; LC-MRI will be correlated with the clinical history, EEG and cognitive data of each subject. A pilot study profiting of Ultra-High MRI (7 Tesla) will be performed in a subgroup of children. Clarifying the effects of hypoxic damage on LC and its role in epilepsy in children with PHI could lead to new therapies targeting the noradrenergic system to prevent E-PHI development.

*Funding: This study is funded by the "Fondazione Pisa" (project 303/22)*

## ALPHA-SYNUCLEIN PATHOLOGY OF THE PERIPHERAL NERVOUS SYSTEM IN PARKINSON'S DISEASE: A PROMISING BIOMARKER

Campagnolo M[1,2], Tushevski A[1,3], Russo FP[2,4], Carecchio M[1,2], Macchi V[2,3], De Caro R[2,3], Parchi P[5,6], Porzionato A[2,3], Antonini A[1,2] and **Emmi A**[1,2,3]

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The role of the peripheral nervous system has been recently highlighted as a major contributor to Parkinson's Disease (PD) pathophysiology, with numerous studies investigating bidirectional transmission of pathological alpha-synuclein (αSyn). However, the extent and the characteristics of pathology in the peripheral nervous system have not been fully investigated. We characterized αSyn pathology in the enteric nervous system (duodenum and stomach) and in the skin, via conformation-specific antibodies and histopathology, and evaluated seeding activity via Real-Time Quaking Induced Conversion (RT-QuIC) assay. We examined 20 patients with advanced PD, 6 untreated patients with early PD, as well as 18 matched healthy controls undergoing routine diagnostic endoscopy. Immunohistochemistry was performed for anti-aggregated αSyn (5G4), enteric glial markers (GFAP, SOX10, S100B), immune-population markers, and nerve fiber markers (PGP9.5, Beta-III-tubulin and neurofilament heavy chain) followed by morphometrical semi-quantitative analysis. RT-QuIC analyses were performed to evaluate alpha-synuclein seeding activity. Immunoreactvity for aggregated αSyn, presenting a typical thread-like pattern, was identified in all PD patients (early and advanced) and colocalized with neuronal markers (beta-III-tubulin, PGP9.5); statistically-significant quantitative differences between early and advanced PD patients were also detected. Evaluation of enteric glial cells revealed increased size and density when compared to controls, suggesting reactive gliosis. Similarly, increased T- and B-lymphocyte densities, as well as higher expression of HLA-DR, was detected in the gut of PD patients. The accuracy of αSyn RT-QuIC was 87.7% in skin, 67.4% in duodenum, 80.0% in gastric biopsies, with significantly higher sensitivity in advanced PD (skin: 81.8%; gastric: 88.9%; duodenal 58.8%). Misfolded αSyn was detected with higher sensitivity in advanced PD across all matrices, likely reflecting the progression of αSyn pathology. The seeding activity was lower in the duodenal than in the gastric wall, indicating differences in αSyn burden. In conclusion, we found evidence of αSyn pathology in the peripheral nervous system of PD patients, including the ENS, which was accurate in discerning PD patients from healthy controls. Future studies are required to evaluate how early in the disease process peripheral nervous system pathology occurs, and whether it plays any role in mediating dopamine treatment efficacy in advanced patients.

### CLUSTERIN AND ALPHA-SYNUCLEIN ASSOCIATION IN PARKINSON'S DISEASE

**Zanchi G**[1], Pizzi S[1,2], Calogero AM[1,3], Mazzetti S[3], Triggiani T[1], Rolando C[1], Corti C[2], Pezzoli G[3], Russo I[4], Cappelletti G[1]

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Clusterin (CLU) is a ubiquitous chaperone involved in various biological functions, including clearance of misfolded proteins. CLU has been implicated in neurodegeneration, since variations in its gene are the third major risk factor for Alzheimer's disease and it is involved in amyloid-β aggregation and clearance. Few studies, in Parkinson's disease (PD) cell models, have reported that CLU also modulated alpha-Synuclein (α-Syn) aggregation and clearance, but whether this modulation is neuroprotective or neurotoxic is still controversial. Moreover, data regarding the association between CLU and α-Syn in PD human brains are currently lacking. This study in PD *post-mortem* human brain aims to: *i)* analyze whether CLU is differently associated to α-Syn in *Substantia Nigra pars compacta* (SNpc) of patient brain compared to control individuals', *ii)* define whether and how CLU is involved in the aggregation process of α-Syn in patients. First, performing Western blot, we highlighted a positive correlation between CLU and α-Syn levels in both Triton soluble and insoluble fractions of PD patients. Then, we investigated the co-localization levels between CLU and α-Syn in fixed brain slices, observing no differences between controls and PD. However, since α-Syn redistribution in the neuronal compartments occurs during pathology, we decided to investigate the association between the two proteins in different subcellular compartments of the controls and PD patients using Proximity Ligation Assay (PLA). This led to revealing higher levels of association between CLU and α-Syn in neurons that were highly affected by α-Syn pathology. Moving forward to the second aim of the project, we previously observed the presence of CLU in 100% of brainstem Lewy bodies. Moreover, our preliminary data show that CLU exhibited a different distribution in undefined and ring-shaped Lewy bodies, that are aggregates at a diverse stage of maturation. These data suggest that CLU could be involved in the maturation process of Lewy bodies. Consequently, we decided to analyze, in PD patients, the association between CLU and α-Syn in the staging of Lewy bodies formation, a model previously proposed by our laboratory. Our findings strongly suggest the association between CLU and PD pathophysiology and pave the way to mechanistic studies aimed at unravelling the role of CLU in the modulation of α-Syn aggregation, that would be important in the perspective of the development of a mechanism-based therapeutic strategy.

*This work was supported by "The Michael J. Fox Foundation for Parkinson's disease", and by "Fondazione Pezzoli per la Malattia di Parkinson" (Italian "5 x 1000" funding).*

## SESSION II - BRAIN TUMORS

### TRACT DENSITY FOR SURVIVAL STRATIFICATION IN GLIOBLASTOMA

**Falcó-Roget J**[1], Basile GA[2], Milardi D[2] and Cacciola A[2]

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Understanding survival heterogeneity of glioblastoma multiforme (GBM) remains elusive despite its increasing prevalence. While biomarkers like MGMT show promise, predictive models struggle with median survival stratification. Recent studies have focused on interactions between white matter fibers and tumors using diffusion-weighted MRI, revealing potential spreading pathways. Herein, we analyzed overall survival (OS) and median survival times in a cohort of GBM patients (n=395) using normative tractograms and MRI lesion masks delineating tumor core (necrotic), enhancing, and non-enhancing (edema) tissues. Imaging data were non-linearly warped to standard space, and a normative tractogram was constructed from 985 subjects in the Human Connectome Project, comprising ~12 million unique streamlines. We extracted streamlines traversing the whole tumor, core, enhancing, core + enhancing, and non-enhancing tissues to compute a "lesion" tract density (TD) map, estimating the density of streamlines interacting with identifiable tumor tissues. A lesion TD index was then obtained by averaging the aforementioned maps for each subject and tissue type. We correlated OS times with lesion TD indices, finding the strongest association with the enhancing lesion TD index ($\rho$ = -0.157, p = 0.0017, two-sided exact test). Hence, we split the cohort based on the distribution of lesion TD values ($50^{th}$ percentile) and conducted Kaplan-Meier analysis, revealing significant OS differences between low and high indices for enhancing ($\chi^2$=11.25, p=0.0008, two-sided log-rank test) and core+enhancing lesions ($\chi^2$=6.85, p=0.009, two-sided log-rank test). Patients with lower lesion TD indices had significantly higher median survival times (p=0.0003 for enhancing; p=0.002 for enhancing+core; two-sided Mann-Whitney test). Similar results held true for more aggressive percentiles (e.g., from 40-60% up to 25-75%). We also found a significant association between OS times and enhancing tumor volumes ($\chi^2$=10.9169, p = 0.001, two-sided log-rank test). These results indicate that the specific characteristics of the enhancing or active part of tumoral tissue, together with its interaction with peritumoral white matter carry essential prognostic value for an accurate prediction of survival rates in GBM. These preliminary but very promising findings extend recent work both in terms of normative and disconnection paradigms setting strong foundations for a *connectomic* description of brain tumors.

## OF MICE, HUMANS, FISH: THE GLIOBLASTOMA SPREADING

**Virtuoso A**[1], Milior G[2], Moulard J[2], Evstratova A[2], De Luca C[1], Cirillo G[1], De Angelis F[1], Huberfeld G[2], Rouach N[2] and Papa M[1]

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Glioblastoma multiforme (GBM) is a highly invasive, central nervous system (CNS) cancer with no cure. Invading tumor cells evade treatments, limiting the efficacy of the current standard therapies. GBM spreads into the CNS via several anatomical routes, including white matter tracts, the perivascular compartment, and gray matter, suggesting a functional integration into the brain rather than a restricted local expansion. The GBM infiltration into the CNS relies on electrical and molecular connections. Among the molecules which may play a role in GBM spreading, connexins (Cx) have been explored. Interestingly, Cx43 seems to be a marker for GBM in mouse models. Understanding the underlying mechanisms that support GBM spreading in human models may allow for the generation of novel GBM therapies. To characterize the role of Cx43 in the human neuroglial network, we used acute and organotypic slices from the human peritumoral cortex deriving from patients affected by glioma. Human primary GBM cells were tagged using lentiviral transduction and injected into organotypic slices. GBM cells were tracked until DIV 7 to test the tumor progression as well as their response to Gap19, a selective blocker of the Cx43 hemichannels. Astrocytes, microglia/ macrophages, and extracellular matrix were studied using morpho-molecular techniques. The injected GBM cells integrated into the neuroglial network and invaded the peritumoral tissue, inducing functional modifications. Connections within the peritumoral tissue of patients with astrocytoma led to a major molecular remodeling. The blockade of Cx43 hemichannels resulted in further alterations of the peritumoral microenvironment, accompanied by a reduction of the epileptic discharges and polarization of GBM cells. The morphology and the direction of single GBM cells changed in a synchronized manner, as it occurs for schooling fishes fleeing from turbulent water. Cx43 hemichannels may contribute to the spread, and recurrence of the tumor, revealing a complex dynamic. The present evidence prompts a paradigm shift, challenging the perception of GBM as a stranger within the brain. Accordingly, extra-CNS metastasis remains a rare clinical entity.

## NAVIGATING THE BLOOD-BRAIN-TUMOR BARRIER TRANSITION

**Mora A**[1], d'Amati A[1], Girolamo F[1], Errede M[1], Marzullo A[2], De Giorgis M[1], Signorelli F[3], Ingravallo G[2], Virgintino D[1]

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Adult-type diffuse gliomas are aggressive, malignant CNS tumors characterized by high vascularity and classified according to the CNS WHO Classification of Tumors (5th edition, 2021). These gliomas, identified by distinct genetic and molecular profiles, along with integrated histological criteria, encompass five primary histotypes: glioblastoma, IDH-wildtype, CNS WHO grade 4 (GB); astrocytoma, IDH-mutant, CNS WHO grade 4 (ASTR-4); astrocytoma, IDH-mutant, CNS WHO grade 3 (ASTR-3); oligodendroglioma, IDH-mutant, 1p/19q co-deleted, CNS WHO grade 3 (OLIG-3); oligodendroglioma, IDH-mutant, 1p/19q co-deleted, CNS WHO grade 2 (OLIG-2). Among the histological markers employed by the WHO grading system, microvascular proliferations (MVPs) are the hallmark sign for GB, ASTR-4, and OLIG-3 and appear frequently associated with a defective function of the blood-brain barrier (BBB). Although blood vessel proliferation is known to play important roles in tumor biological behaviour, little data is available concerning the molecular profile of MVP cell types - endothelial cells, pericytes, and astrocytes - involved in the BBB to Brain-Tumor Barrier (BTB) transition. To unveil key phenotypical changes that may contribute to BBB breakdown and BBB-BTB transition in adult-type diffuse gliomas, this study employed microscopic and morphometric techniques applied to the combined analysis of two molecules, both implicated in cancer development and progression: the BBB-specific efflux transporter P-glycoprotein (P-gp) and the signalling receptor and marker of cell stemness CD146. The parallel histochemical and morphometric analysis of P-gp and CD146 on the vascular and tumoral compartments allowed to identify distinct profiles for each of the different glioma histotypes. According to the obtained data, at one end is OLIG-2, characterized by high expression of endothelial P-gp and absence of the transporter in cancer cells; on the contrary, it is GB, which shows remarkably reduced levels of endothelial P-gp and high P-gp and CD146 expression on cancer cells. These results improve our understanding of BBB-BTB transition and suggest the observed shifting of P-gp and CD146 on endothelial and cancer cells, as an additional, reliable histological marker of adult diffuse glioma histotypes.

# SESSION III
# NEURODEGENERATION AND NEUROPROTECTION

## THE NEUROTOXIN MPP+ DAMAGES CHOLINERGIC NEURONS

**Lenzi P**, Lazzeri G[1], Ferrucci M[1], Scotto M[1], Binda P[1] and Fornai F[1,2]

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The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was discovered in the early 80's to produce Parkinson's disease (PD) in humans. The administration of MPTP in various animal species including rodents and primates mimics most of the features of PD. This is due to its active metabolite 1-methyl-4-phenyl pyridinium ($MPP^+$), which is selectively taken up by dopamine (DA) and norepinephrine (NE) neurons through the specific transporters (DAT and NET, respectively). The mechanisms of $MPP^+$ toxicity are due to inhibition of the complex I within mitochondrial respiratory chain. In PD, a number of symptoms are present and cognitive impairment up to dementia is frequent. It is debated whether this depends on a damage to NE systems, mesolimbic DA systems or it may be produce by a loss of neurons in the septo-hippocampal pathway. A recent paper reports that MPTP destroys cholinergic neurons of the pro-encephalon. Therefore, in the present study we profited from cholinergic cells (Ach) SN56 isolated from medial septal nucleus, to document the potential effect on the source of the septo-hippocampal pathways and the subcellular mechanisms, which operate in the cells of the medial septum under the influence of $MPP^+$. The cholinergic cell line SN56 was administered various doses of $MPP^+$ (0.1 μM, 1 μM, 10 μM, 100 μM and 500 μM) for 72h. In the first set of experiments, the effects of $MPP^+$ on cell viability were assessed through staining with Hematoxylin and Eosin, and Trypan Blue. In the second set ultrastructure analysis of mitochondria in these Ach cells was carried out. We found that $MPP^+$ produces dose-dependent cell death, which starts from a dose of 1 μM. This is quite surprising since the sensitivity of cholinergic neurons is one-hundred-folds higher compared with that of classic nigral DA neurons (100 μM). Within spared cells, mitochondrial ultrastructure was markedly affected. Mitochondrial alterations were already evident at sub-lethal $MPP^+$ doses and are detailed in this presentation. These data show a higher sensitivity of Ach neurons compared with DA neurons to parkinsonism inducing neurotoxins, despite similar subcellular targets. The present findings indicate that cognitive alterations in PD are likely to be sustained by a specific damage in the limbic system.

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## DRUG REPOSITIONING FOR SPINAL MUSCULAR ATROPHY

**Boido M[1]**, Menduti G[1], Rasà DM[1,2], Stanga S[1], Artero R[3], Martinat C[4], Di Schiavi E[5] and Vercelli A[1]

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Spinal Muscular Atrophy (SMA) is a neuromuscular disease affecting children, caused by the deletion/mutation of the Survival Motor Neuron 1 (*SMN1*) gene. The disease severity is modulated by the copy number of *SMN2* gene, which mainly undergoes an alternative splicing. The consequent lack of SMN protein determines motor neuron (MN) degeneration, skeletal muscle atrophy and premature death. Fundamental limitations of current therapies still drive the need for new approaches aimed at increasing functional SMN production. Drug repositioning (DR) for SMA treatment represents a reliable tool to address significant unmet therapeutic needs. Here we show two DR approaches that allowed the identification of SMN-dependent and –independent promising therapeutic candidates for SMA. Through a Drosophila-based screening, we identified GT5 (code name) that has been tested *in vivo*, on delta7 mice (a severe murine model of SMA). The SMN expression, the neuroprotective and anti-inflammatory effects have been evaluated by immunofluorescence reactions, morphometric analyses and WB assays. Moreover, we assessed the behavioral performance and survival in treated and untreated mice. We demonstrated the SMN-dependent efficacy of GT5 *in vivo*, as we revealed delayed MN degeneration, lower neuroinflammation, improved motor performance. These data were further confirmed *in vitro*, on patient's iPSCs-derived-MNs and primary SMA fibroblasts and myoblasts. Moreover, thought a drug screening on a *C. elegans* SMA model, we identified two FDA-approved drugs capable of rescuing neurodegeneration in affected worms. We validated their efficacy *in vitro*, on primary SMA cortical neurons. Morphometric analyses have been performed by Neurolucida software, to evaluate soma size, neurite length and branching of SMI31-positive neurons. We also analyzed the signal intensity and distribution of synapsin labelling. Compared to untreated cells, both candidate compounds were able to significantly improve the general phenotype of SMA cortical neurons, and to recover a uniform vesicle distribution throughout the entire cell. Moreover, both drugs did not modulate the SMN expression, suggesting a SMN-independent mechanism of action. Overall, these results strengthen the value of the DR strategy for discovering new therapies for rare diseases.

## A ROLE FOR DYSFUNCTIONAL MITOCHONDRIA IN ALS

**Stanga S**[1], Lall D[2], Diaz F[2,3], Di Lullo E[4], Mezzanotte M[1], Chicote J[1], Hernandez S[2], Sansa A[2], Prochiantz A[4] and Svendsen CN[2]

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Motor neuron diseases (MNDs) are progressive and multifactorial pathologies defined by loss and death of both upper and lower motor neurons (MNs), with consequent muscle weakness and wasting, loss of skeletal muscle movements, and spastic paralysis. Because of high energy requirements, neuronal and muscle cells are enriched in mitochondria which play a central role in the maintenance of cellular homeostasis. Amyotrophic Lateral Sclerosis (ALS), the most common adult-onset MND, currently has no effective treatment. Extensive research on mitochondria is ongoing in the field, since mitochondria dysfunctions may be one trigger for the neuronal decline. Indeed, mitochondrial oxidative stress and defective axonal transport of mitochondria are some of the earliest neuropathological features observed in ALS. However, how mitochondrial dysfunctions promote neuronal degeneration is not clear from a mechanistic standpoint. This study aims to improve the knowledge of pathological mechanisms related to mitochondria dysfunctions to facilitate the recognition of early disease manifestations and ultimately identify new therapies. We used biochemical and imaging approaches to study human post-mortem brains as well as cortical and spinal MNs derived from induced pluripotent stem cells of ALS patients and controls. We aim to understand the heterogeneity in ALS to define mitochondria-related mechanisms of neurodegeneration and test the capability of a diverse pharmacological arsenal (homeoproteins, antioxidants, iron supplementation) to recover mitochondrial functionality and mobility. Preliminary data show that the ALS motor cortex is enriched in iron deposits. Further, we show that healthy control iPSC-derived cortical neurons are resistant to high concentrations of iron, while ALS-patient derived MNs show a clear dysfunction at the level of mitochondria. We have shown that the human recombinant homeoprotein Engrailed 1 is able to promote survival following toxic insult to the MN cultures. Collectively, this data indicates that mitochondria dysfunction is involved with MNDs and represent a promising therapeutic target.

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## ANALYSIS OF THE MODULATION AND ABLATION OF S100B GENE ON GLIAL CELLS: EFFECTS ON MULTIPLE SCLEROSIS

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It has been demonstrated that S100b actively participates in neuroinflammatory processes of different diseases of central nervous system (CNS) (1), such as Experimental Autoimmune Encephalomyelitis (EAE), a recognized animal model for Multiple Sclerosis (MS). We previously showed that the inhibition of S100B activity using pentamidine and of S100B astrocytic synthesis using arundic acid determined an amelioration of clinical and pathologic parameters of the disease: the symptoms were milder and delayed (2-4). This study further goes in detail on the role of S100B, and of astrocytic S100B in these neuroinflammatory processes. To this aim we have purchased S100B KO mice. EAE induction on this mouse strain resulted in an amelioration of clinical and pathological parameters. To dissect the potential mechanisms that could explain the role of S100B in the development of EAE we sorted, cultured and compared neural subpopulations (astrocytes, microglia and oligodendrocytes) deriving from S100B KO and wild type mice, through flow cytometric panels and ELISA. Neural cells were analyzed for proinflammatory molecules showing a significant reduction of TNFα protein in mice where S100B was silenced. As expected, S100B protein levels were significantly lower in this strain. To dissect the role of S100B in MS we cultured astrocytes and microglial cells sorted and enriched from the brains of EAE affected animals, both from KO and wild type animals. The silencing as the usage of S100B inhibitors both demonstrate the direct impact of these molecules on specific subpopulation of neural cells, such as Astrocytes and microglia. The present results further individuate astrocytic S100B as a key factor and as a potential therapeutic target for EAE neuroinflammatory processes, possibly contributing to reducing the inflammatory features and to the maintenance of sustained remission.

## CB1 RECEPTORS IN THE HIPPOCAMPUS OF TWO LINES OF MALE RATS DIFFERING IN THEIR RESPONSE TO STRESSORS

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The present study was undertaken to investigate the impact of acute forced swimming (FS), an intense stressor, on the expression of CB1 cannabinoid receptors (CB1R) in the hippocampus (HC) of the outbred Roman High- (RHA) and Low-Avoidance (RLA) rat lines, one of the most validated genetic models for the study of behavior related to fear/anxiety and stress-induced depression, by means of Western blot (WB) and immunohistochemistry (IHC) assays. The distinct responses to FS confirmed the different behavioral strategies of the two phenotypes when exposed to stressors, with RLA and RHA rats displaying a reactive vs. a proactive coping, respectively. The WB analysis, in baseline conditions, showed lower CB1R relative levels in RLA rats than in their RHA counterparts. After FS, RLA rats showed increment of CB1R in the dorsal HC (dHC) vs. no change in the ventral HC (vHC), while RHA rats displayed no change in the dHC vs. a decrease in the vHC. In the dHC tissue sections, FS elicited an increment of CB1R-like immunoreactivity (LI) in the CA1 and CA3 sectors of the Ammon's horn of RLA rats, while in RHA rats the CB1R-LI increased only in the CA1 sector. In vHC tissue sections, FS caused an increase over the control values of CB1R-LI only in the CA1 sector of the RLA rats and a decrement of the CB1R-LI in the CA1 sector and the dentate gyrus of the control RHA rats. This study shows for the first time that FS induces rapid and distinct changes in the expression of CB1R in the HC of Roman rat lines with a different distribution along the septo-temporal extension of the HC and that the FS induces rapid and distinct changes in the hippocampal expression of CB1R of RLA vs. RLA rats, in keeping with the view that endocannabinoid signaling may contribute to the molecular mechanisms that regulate the different responses of the dHC vs. the vHC to aversive situations in male Roman rats. Data obtained also support the involvement of CB1R in mechanisms that regulate the different susceptibility to stress-induced depression of RLA and RHA rats.

## NORADRENERGIC DENERVATION ON MOUSE BRAIN AGEING

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The early degeneration of the Locus Coeruleus (LC) may play a critical role in Alzheimer's Disease (AD) pathogenesis. Experimental studies suggest that the acute lesion of noradrenergic (NA) terminals can increase amyloid accumulation and exacerbate neuroinflammatory and neurovascular changes in the brain of middle-aged transgenic AD mice. However, no studies have explored how chronic LC impairment alone may influence physiological brain aging, an experimental condition that may better mimic non-genetic AD. This study aims to investigate whether chronic NA denervation induces AD-like pathological changes in the brain of wild-type mice. C57Bl/6J mice were monitored until 18 months of age. To induce LC degeneration, they were administered with the specific neurotoxin DSP-4 every four months. Cognitive assessments, including the Open Field test and Novel Object Recognition test, were conducted before and after each injection and before sacrifice. Brains were fixed for optical microscopy, and samples of the prefrontal cortex were used for transmission electron microscopy (TEM). Neuroinflammatory activation and amyloid accumulation were investigate through immunohistochemical staining for the markers GFAP, IBA1 and 4G8, respectively. Neurovascular integrity was assessed using TEM. Stereological counts of tyrosine hydroxylase (TH)-positive populations in the LC and the hippocampal areas CA1, CA2, CA3, and dentate gyrus (DG) were performed. DSP-4-treated mice exhibited increased astrogliosis, microglial changes, and ultrastructural changes in brain capillaries. Chronic DSP-4 induced a marked reduction of NA terminals in target areas, in parallel with significantly lower TH+ neuronal count in the LC compared to controls, as confirmed by stereological assessment. Neuronal density was reduced in hippocampal areas CA1, CA2, and DG in DSP-4-treated mice. Our analysis confirmed that we successfully obtained an animal model of chronic NA denervation and LC degeneration. LC-lesioned mice showed pathological alterations consistent with accelerated brain aging, even in the absence of other harmful factors. This preliminary evidence supports the hypothesis that LC disruption may play a key role in the earliest stages of AD pathogenesis.

## SESSION IV - NEURAL DISORDERS

### SEXUALLY DIMORPHIC ORGANIZATIONAL ROLE OF ERs ON ANXIETY AND 5-HT SYSTEM

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Estradiol (or 17β-estradiol, $E_2$) is the best known of the gonadal estrogen hormones for its physiological relevance, playing a key role in sexual differentiation and the cognitive sphere, the literature has also demonstrated its importance in the regulation of anxiety, not only in the activational but also at the organizational level, through its interaction with estrogen receptors: two nuclear receptors, ERα and ERβ, and one membrane receptor, GPR30. The serotonin (5-HT) system plays a crucial role in the regulation of anxiety-like behaviors and is estrogen-sensitive, 5-HT neurons distributed in the Raphe nucleus co-express estrogen receptors. In this work we studied which estrogen receptor acts its organizational effect on anxiety behavior and consequently on the development of the distribution of 5HT+ neurons in the dorsal Raphe nucleus (DRN) and the median Raphe nucleus (MRN). Male and female CD1 mice were treated during the delicate postnatal hormonal window with subcutaneous injections from postnatal day 5 (PND5) to PND12 of $E_2$ alone or with the addition of the different selective estrogen receptor antagonists (MPP, PHTPP, and G15, respectively ERα, ERβ and GPR30 inhibitors). In adulthood, mice were subjected to several behavioral tests, the Open Field test (OF) and the Elevated Plus Maze test (EPM), to assess their anxiety state and immunohistochemical analysis of the 5-HT system in the Raphe Nucleus. The results show that the effect of estrogen treatment alone is sexually dimorphic; indeed, females treated postnatally with E2 report masculinized anxious behavior, as does the distribution of 5HT cells in the Dorsal Raphe. These alterations are due to the nuclear receptor ERα, which therefore has an organizing role in controlling the anxiety state and the 5HT system in females only. In males, on the other hand, the membrane receptor GPR30 seems to be particularly involved; in fact, its antagonization leads not only to behavioral alteration but also to the expression of 5HT cells in the Median Raphe. In conclusion, the data demonstrate that estrogens exert their organizing role on anxiety behavior and the serotonin system that governs it by acting on different estrogen receptors in the two sexes.

### ALTERED MATERNAL NUTRITION AFFECTS THE STRESS AXIS OF THE MOTHER AND OFFSPRING

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Offspring health can be affected by maternal nutrition during pregnancy. For instance, a low-protein diet may pose a greater risk to their physical and neurological development. Therefore, dietary supplements such as soybeans, which are rich in phytoestrogens, particularly Genistein (GEN), are recommended to address deficiencies in maternal diets during pregnancy. Phytoestrogens, particularly Genistein (GEN), are classified as endocrine disruptors due to their ability to bind to estrogen receptors, affecting various estrogen-sensitive neural systems, including the stress axis. In this study, we examined the effects on the HPA axis both directly of the mothers and in the offspring in Sprague Dawley rats of a chronic maternal diet low in protein (8%) with and without GEN. Molecular analysis by RT-PCR of the brains of mothers sacrificed at the end of lactation demonstrate a strong alteration of the stress axis, mainly with reduced expression of glucocorticoid receptors at both hypothalamic and hippocampal levels in all treated groups. The brain is particularly sensitive to changes in energy production that occur during sustained stress signaling, analysis by WB revealed alterations in ATP production within the treated groups in the hypothalamus and hippocampus. Moreover, analysis of breast milk revealed lower protein and fat content in two experimental groups, pups born to these mothers from birth are smaller and have never resumed normal development, remaining consistently underweight. The HPA axis analysis at PND1 of these pups demonstrated a sexually dimorphic effect with altered stress axis, especially in females, as well as reduced energy production. In adulthood, these animals were subjected to behavioral tests (Open Field and Elevated Plus Maze), and the females showed anxiety-like behaviors, particularly in females born to mothers on a low-protein diet supplemented with GEN. In conclusion, these results show that maternal diet is essential to preserve in a delicate phase such as pregnancy the stress axis. In fact, the diet if inadequate could turn into a chronic maternal stressor that if enriched with endocrine interferents can lead to sexually dimorphic alterations in the neurodevelopment of the offspring.

## ASTROCYTES IN SUICIDE AND PSYCHIATRIC DISORDERS

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Suicide is a significant global health concern and one of the leading causes of death worldwide, claiming approximately 700,000 lives annually. There are numerous risk factors, but psychiatric disorders are certainly one of the most important. In this context, suicide prevention constitutes a major health priority. Recently, mental distress and suicidal behaviour have been associated with glial cell dysfunction. These changes included alterations in astrocyte density, morphology and protein expression. Therefore, our study was conducted on postmortem brain with the aim of investigating astrocyte patterning. For this purpose, six men who died by suicide and suffered from major depressive disorder and six matched control cases (natural deaths in healthy individuals) were selected. Immunohistochemical analyses were performed in the white and grey matter regions of the dorsolateral prefrontal cortex (dlPFC), which plays a role primarily in the control of motivation and emotions and in mental illness, and in the somatosensory cortex (SS), which is less involved in behavioural control. The basal ganglia and corpus callosum have also been studied as examples of subcortical structures and white matter, respectively. Cell counts and colocalizations analysis with two well-known astrocytic markers, glial fibrillary acidic protein (GFAP) and S100β, were performed for all selected cases and the above-mentioned brain regions. In addition, preliminary colocalization analyses of aquaporin 4 (AQP4) with GFAP were performed for a subsample (three study cases and three matched controls) and only in the dlPFC and SS regions. The preliminary results displayed an increasing trend in the total number of GFAP+ and S100β+ cells in the dlPFC of the suicides, while they decreased in the SS of the same cases. On the other hand, colocalization coefficients showed a general increasing trend in all areas studied. Regarding AQP4/GFAP colocalization, an interesting decreasing trend was observed only in the dlPFC. Finally, a tissue clarification protocol was developed for future studies that would allow 3D analysis of the glial network in the human brain. Our results are promising for the identification of astrocytic alterations by the combined use of different markers and shed light on possible neurochemical imbalances in the human brain related to suicide risk.

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## CHRONIC MILD STRESS IN A PARKINSON'S DISEASE MODEL

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In this study, in order to characterize the effects of chronic mild stress (CMS) on neuroinflammation activation and its involvement in the progression of Parkinson's disease (PD), mice were subjected to CMS and, at the end of treatment, received 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP, after systemic administration, causes the selective degeneration of the *Substantia Nigra* dopaminergic neurons, that is the main pathological feature of PD. Initially mice were randomly assigned to control or CMS group: CMS was carried out by exposing mice every day for 24 weeks to a random stimulus including Isolation, Social stress, Damp bedding, Removal of bedding, Cage tilting at 45°, Restraint Stress Loading, Alteration of light/dark cycle, Intermittent illumination, Food deprivation, Water deprivation, Tail Suspension Test, Forced swimming test. Afterwards half mice of each group were treated with MPTP. Inflammation activation was evaluated by GFAP (for astrocytes) and IBA1 (for microglia) expression; the synthesis and release of dopamine within the nigrostriatal pathway was evaluated by TH and DAT immunoreactivity analysis. MPTP treatment caused a significant reduction of TH immunoreactivity. In particular, the expression was reduced in dopaminergic neurons of stressed animals subject to MPTP treatment respect to non-stressed counterparts. A significant reduction of DAT immunoreactivity in MPTP vs CTR and STRESS-MPTP vs STRESS groups was found. Moreover, it was reduced in *Striatum* of stressed animals respect to non-stressed ones. As expected, MPTP treatment caused a strong astrocyte activation. Moreover, GFAP expression was also increased in *Striatum* of stressed animals respect to non-stressed counterparts. In MPTP treated animals microglia showed a pro-inflammatory, activated phenotype. Also, the number of reactive microglial cells was slightly increased in *Striatum* of stressed animals respect to non-stressed counterparts. A preliminary characterization of inflammation activation was carried out by TLR4 and IL1-β expression analysis. MPTP treatment induced a significative increase of TLR4 expression, as well as stress, although in a less intense manner. IL-1β expression was also increased by MPTP and CMS: higher levels were found in animals subjected to both CMS and MPTP treatment.

# SESSION V
# PERIPHERAL SYSTEM AND GUT-BRAIN AXIS

## MORPHOLOGICAL AND METABOLOMIC CHANGES IN PIPN

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Chemotherapy-induced peripheral neurotoxicity (CIPN) is one of the most common dose-limiting side effects of paclitaxel (PTX) treatment. Many age-related changes have been hypothesized to underlie nerve damage. The results of these studies, however, are inconclusive and other potential biomarkers of nerve impairment need to be investigated. Twenty-four young (2-months) and 24 adult (9-months) Wistar male rats were randomized to either PTX treatment (10 mg/kg i.v. once/week for 4 weeks) or vehicle administration. Neurophysiological and behavioral tests were performed at baseline, after 4 weeks of treatment and 2-week follow-up. Intraepidermal nerve fiber density and nerve morphology/morphometry were analysed. Blood and liver samples were collected for targeted metabolomics analysis. At the end of treatment, the neurophysiological studies revealed a reduction in sensory nerve action potential amplitude ($p<0.05$) in the caudal nerve of young PTX-animals, and in both the digital and caudal nerve of adult PTX-animals ($p<0.05$). A significant decrease in the mechanical threshold was observed only in young PTX-animals ($p<0.001$), but not in adult PTX-ones. Nevertheless, both young and adult PTX-rats had reduced IENF density ($p<0.0001$), which persisted at the end of follow-up period. Targeted metabolomics analysis showed significant differences in the plasma metabolite profiles between PTX-animals and age-matched controls, with triglycerides, diglycerides, acylcarnitines, carnosine, long chain ceramides, sphingolipids, and bile acids playing a major role in the response to PTX administration. Our study identifies for the first time multiple related metabolic axes involved in PTX-induced peripheral neurotoxicity, and suggests age-related differences in CIPN manifestations and in the metabolic profile.

## MALADAPTIVE PLASTICITY OF THE NEUROVASCULAR UNIT

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Peripheral nerve injury (PNI) offers a unique model for studying spinal cord plasticity without directly affecting the central nervous system (CNS). The sciatic nerve lesion with sural sparing (SNI) combines motor axon damage and sensory nerve fiber loss, impacting ventral and dorsal horn neurons. The model represents a simultaneous axotomy of motor neurons in the gray matter of the spinal cord and a lesion of the peripheral processes of pseudounipolar neurons in the dorsal root ganglia (DRG). We focused on the neurovascular unit (NVU) within the lumbar spinal cord, examining early changes following SNI in rats. Our results reveal a complex interplay between the coagulation protein thrombin, its receptor PAR-1, and matrix metalloproteinase 9 (MMP9). PAR-1 is initially expressed on neurons and perivascular cells. After injury, it clusters near astrocytic endfeet, where MMP9 can cleave and activate it. MMP9 also alters the spinal extracellular matrix (ECM), particularly the basal lamina, contributing to maladaptive plasticity. Our analysis using immunohistochemistry, RNA sequencing, and RNA scope demonstrates the timely upregulation of MMP9 and its targets. Additionally, we observed changes in tight junctions and channel proteins, primarily at the protein level. Astrocytic water channel aquaporin 4 (AQP4) and gap junction protein connexin 43 (Cx43) become redistributed, and microglia/macrophages infiltrate the spinal cord. The dorsal and ventral horns exhibit distinct responses to the injury. Our findings expand our understanding of the NVU's role in spinal cord damage and highlight the importance of vascular factors in maladaptive plasticity.

## *IN VIVO* STUDY OF CARFILZOMIB-INDUCED NEUROPATHY

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Chemotherapy-induced peripheral neuropathy (CIPN) is a common side effect of cancer treatment with chemotherapeutic agents. 20S proteasome inhibitors such as Bortezomib (BTZ) and Carfilzomib (CFZ) have been approved for treatment of multiple myeloma and other liquid tumors, also include CIPN among their side effects. To date, no effective treatment for this condition has been developed. Observations in patients treated with these drugs showed that BTZ induces a worse neuropathic phenotype when compared with ones treated with CFZ. While the strong BTZ-induced neuropathic symptoms have been replicated in a preclinical setting, there is still no preclinical animal model of CFZ-

induced neuropathy. Our aim is to investigate the behavioral and morphological differences between BTZ and CFZ in terms of nerve damage and fiber loss. Therefore, we first selected a CFZ dose able to guarantee a level of anti-neoplastic activity comparable to that of BTZ in terms of proteasome inhibition as well as good tolerability for the model animals. Then, we treated the animals via intravenous administration of 0.8 mg/kg 2qwx4 of BTZ and 2 mg/kg 2qwx4 of CFZ. Here, we evaluated general toxicity over time as well as the insurgence of neuropathy and neuropathic pain using conduction velocity analyses, dynamic Von Frey tests, and evaluation of intraepidermal fibers density (IENF): all these tests show clear neuropathy developing as early as 2 weeks after the beginning of treatment with BTZ, whereas mice treated with CFZ show only mild symptoms throughout. We next sought to dissect any difference in the morphological and morphometrical features in peripheral nerves between the two treatments. We observed a clear degeneration and loss of axonal fibers in both the caudal and sciatic nerves of BTZ-treated animals, that is already evident at half-treatment (2 weeks), whereas the impact on the CFZ-treated cohort is much less severe and becomes significant only at the end of treatment (4 weeks). Taken together, these results show a clear difference in the neurotoxic symptoms between the two drugs, which reflects in the morphological and functional discrepancies. Therefore, these models are able to reproduce the clinical aspects of CIPN and pave the way for the investigation of the molecular mechanisms that underlie these differences.

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## dECM HYDROGELS FOR SUPPORTING NERVE REGENERATION

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Peripheral nerve injury is a clinical condition that severely reduces patient quality of life by damaging sensory and motor functions. In the last decades, tissue engineering has developed a variety of materials that can promote the regeneration of peripheral nerves in case of severe nerve damage. Among these, due to their ability to preserve tissues native environment, stimulate the proliferation and migration of Schwann cells (SC), and provide cues for nerve regeneration, Extracellular matrix (ECM) hydrogels could be a significant advancement in nerve regeneration support systems.
The aim of the present study is to define the possible role of a human decellularized extracellular matrix (dECM) in sustaining peripheral nerve regeneration *in vitro* and then *ex vivo*, in order to develop an innovative strategy in the field of nerve repair. The dECM tested in this study is derived from cadaver human skin and underwent a decellularization protocol to obtain a dECM hydrogel. It was tested *in vitro* on neuronal (NSC34) and glial (RT4-D62PT) cell lines and on primary Schwann cell culture. Proliferation assay was performed on RT4-D62PT SC cell line, using dECM in solution, while primary SC have been cultured to analyze its role in promoting migration, with promising results. To study the interactions of neurons with the extracellular molecules and to evaluate neurite orientation and outgrowth, NSC34 cells were seeded on coverslips coated with dECM, differentiated after 3 days of culture in order to quantify the neurites number and length. The preliminary results showed that this matrix has a significant impact on the proliferation and migration of glial cells, and on axonal sprouting and elongation of motor neurons. The dECM hydrogel will be tested also *ex vivo* on dorsal root ganglia (DRG) and autonomic explants to obtain a multicellular structure that provides a closer approximation to *in vivo* conditions. Further investigations are underway to deepen the effect of the dECM in the activation of molecular pathways related to peripheral nerve regeneration.

## THE ROLE OF GUT-MICROBIOTA ON PERIPHERAL NERVES

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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, leading to the definition of "gut-organ-axes". We have recently demonstrated that also the somatic peripheral nerves and the neuromuscular system depend on the presence of a well-balanced gut microbiota for proper development; indeed, we showed that germ-free mice (GF, mice bred in sterile environment to prevent microbial exposure) and gnotobiotic mice (OMM12, mice stably colonized with 12 specifically defined bacterial strains) have smaller diameter and hypermyelinated axons, together with a dysregulation of pathways critical to development and myelination, compared to control mice (CGM). We therefore analysed peripheral nerves of GF mice colonized with donor complex microbiota at weaning (EX-GF) with the aim to reveal a possible rescue effect. Interestingly, data from stereological and morphometrical analysis showed a significantly thicker myelin sheath and g-ratio of EX-GF nerves in comparison to all other groups. These findings prompted us to further investigate whether gut microbiota impacts other fibre features, such as internodal length and nodes of Ranvier. Preliminary results show an increase in internodal length of GF mice compared to CGM, OMM12 and EX-GF, and longer nodes of Ranvier. Moreover, through RNA-seq analysis, we investigated the gene expression of those genes express in the different regions of the node of Ranvier (node, paranode, juxtaparanode and internode) and we found that several genes are altered in GF, OMM12 and EX-GF, compared to CGM. These preliminary results show that the lack of microbiota leads to morphological and biomolecular alterations in the peripheral nerves, and colonization of GF mice at weaning seems to result in an over compensatory response in the myelination of peripheral axons. Further studies need to be carried out in order to

elucidate the mechanism underlying these effects and to understand which bacterial strains and metabolites modify peripheral myelination.

## A FUNCTIONAL COOKIE MODULATES THE GUT WALL AND ENS IN 3xTg-AD MICE

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Alzheimer's disease (AD) is a progressive neurodegenerative disease considered the most common type of dementia in elderly people. AD showed multifactorial pathogenesis including not only neuronal death induced by beta-amyloid (Ab) plaques, but also dysbiosis, dysfunction of intestinal epithelial barrier and disorders of the gut-brain axis. Maintaining the gut microbiota biodiversity is crucial for healthy aging and represents a further approach in delaying neurodegeneration. Dietary patterns rich in prebiotics and probiotics can counteract gut dysbiosis, modulate intestinal permeability and reduce inflammatory metabolites. A hypocaloric cookie with prebiotic-rich ingredients (red lentils) coated with a multi-strain probiotic (SLAB51®) enriched chocolate was developed. The objective was to evaluate the protective effects of this functional cookie on the colonic mucosa and enteric nervous system (ENS) of 3xTg-AD mice. 8-week-old mice were divided into experimental groups according to the different 4-months supplementation of the functional cookie or the prebiotic cookie without SLAB51®. Mice supplemented with a classic cookie or SLAB51® dissolved in water have also been used. Histochemical and western blot analysis were performed on colon samples to assess the colonic mucosa morphology, the mucus secretion, the intestinal barrier integrity, the neurodegeneration of myenteric plexus, and oxidative stress status. The colon of the different experimental groups showed a normal morphology of the colonic wall, with the mucosa presenting enterocytes and goblet cells organized in colonic crypt without presence of inflammatory infiltrate and fibrosis. The SLAB51® administered in water and the functional prebiotic enriched cookies red lentils based with and without SLAB51® appeared to be useful to reduce mucus secretion. Moreover, functional cookies seemed to enhance the intestinal barrier integrity modulating the expression of tight junctions' proteins such as zonula occludens-1 and claudin-5. On the ENS, functional cookies decreased gliosis, without a modulation of the neuronal markers of the enteric cholinergic and nitrergic neurons and the oxidative stress conditions. Although it is difficult to establish the mechanism and the synergistic effects of this symbiotic association, the data, in addition to previous evidence, supports the hypothesis of a healthy innovative cookie as a simple dietary supplementation to prevent the onset of AD-related gut alterations.

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## ENDOTHELIAL TP DEPLETION LEADS TO ENS ALTERATIONS

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The relationship between vascular and nervous systems is essential for gastrointestinal (GI) function. Thymidine phosphorylase (TP), expressed by endothelial cells, regulates angiogenesis. In mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), a genetic disorder leading to severe GI dysmotility and caused by mutations in TP's encoding gene TYMP, TP depletion is associated with neuronal degeneration and vascular abnormalities. Our preliminary data suggest that a similar scenario occurs in cases of idiopathic severe GI dysmotility, highlighting the potential role of endothelial-derived TP in maintaining enteric neural integrity. Thus, this study investigated how TP depletion affects enteric neurons and neural network morphology using an *in vitro* model where primary myenteric plexus cultures exposed to TP-silenced endothelial cell supernatants were combined with electrophysiological stimulations on multielectrode arrays. Primary enteric neuronal cultures exposed to supernatants from TP-silenced endothelial cells showed disrupted neural networks, with increased glial cell numbers and fibroblasts proliferation. Neuronal swelling, elevated nNOS expression, high pro-inflammatory mediators' levels and neuronal hyper-activity following stimulation of enteric neuronal networks suggest a stress response. On the other hand, chronic exposure up to 24h led to the loss of neuronal activity, similar to a complete "burnout" of neuronal function. Finally, an ancillary test performed on enteric organoids suggests that TP depletion might also affect epithelial regenerative potential. These findings highlight the critical role of endothelial-derived TP in maintaining neural integrity, suggesting TP levels as a new research target to address vascular-related GI dysmotility.

# SESSION VI
# NEUROMORPHOLOGY AND METABOLISM

## INTRANASAL DELIVERY OF EXTRACELLULAR VESICLES FROM ADIPOSE MESENCHYMAL STEM CELLS AS A NOVEL THERAPY FOR MULTIPLE SCLEROSIS

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Adipose mesenchymal stem cells (ASCs) represent a promising therapeutic approach in several neurological disorders such as multiple sclerosis (MS). ASCs display an intrinsic ability to target damaged central nervous system (CNS) sites and promote neuroprotection and immunomodulation. Most of the biological activities of ASCs are mediated by the release of soluble factors in small extracellular vesicles (EVs). It has been previously shown that the intravenous injection of ASC-EVs was able to ameliorate clinical and pathological aspects of experimental autoimmune encephalomyelitis (EAE), an experimental mouse model of MS. In order to identify an efficient strategy to deliver ASC-EVs directly to the CNS, in view of a future translational use, we focused on the intranasal route of administration. Intranasal delivery represents a safe and non-invasive route for the treatment of CNS disorders, bypassing the blood brain barrier and allowing ASC-EVs to reach damaged areas. To validate the intranasal ASC-EVs delivery, primarily, we verified the capability of ASC-EVs to migrate through an epithelial barrier and reach their cellular target. To this end, we developed an *in vitro* model of nasal-derived epithelial cells, evaluating the neuroprotective effect of ASC-EVs following their passage through the epithelium on damaged neurons. The results showed that ASC-EVs are able to rescue damaged cells after their passage through the epithelium. Moreover, starting from MRI evidences, which showed ASC-EVs able to migrate towards lesion sites, we assessed their therapeutic efficacy in EAE affected mice. The intranasal injection of ASC-EVs in EAE mice (starting from the onset of the disease and repeated every 4 days), showed a significant improvement in the clinical outcome of affected mice together with a reduction in the spinal cords of T lymphocytes infiltrates, a reduction of the demyelinated areas and a decrease of microglial reaction. The morphological effects were accompanied by a decrease of several cytokines and chemokines related to the inflammatory response, in both spinal cords and brain of injected EAE mice. Our results promote the intranasal administration of ASC-EVs as a novel, non-invasive potential strategy for future clinical applications to treat neurological disorders and in particular MS.

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## NEURAL DIFFERENTIATION OF ADIPOSE STEM CELLS BY MELATONIN

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In the last decades, multipotent differentiation ability of adipose-derived mesenchymal stem cells (ASCs) has been widely explored in order to develop therapeutic strategies to treat a variety of human pathologies, including nervous system diseases. In fact, other than differentiate into cells of mesodermal origin, under appropriate condition, they are able to transdifferentiate into epithelial and nerve cells. In this study, a neural-like differentiation was tested by growing human ASCs in conditioned media (CM) from Olfactory Ensheathing Cells (OEC-CM) or Schwann cells (SC-CM). In samples of each culture condition, 1 μM melatonin was added and, in a further set of samples, the effects of Melatonin alone were tested. ASC neural differentiation was assessed by investigating the expression modifications of typical neural markers, such as Nestin, glial fibrillary acidic protein (GFAP), microtubule associated protein 2 (MAP2), and Synapsin I. To this purpose, fluorescent immunocytochemistry and flow cytometry techniques were used. Results show that CM treatments were able to increase ASC immunocytochemical expression of Nestin, GFAP, MAP2, and Synapsin I. These increases were differently modulated when melatonin was also present: a further increase of Nestin, MAP2, and Synapsin I was obtained, whereas an attenuation of GFAP expression was observed. On the other hand, no significant modifications were detectable after the melatonin treatment alone. Overall, it can be concluded that melatonin may exerted a synergistic effect within an appropriate environment, mainly addressing a neuronal differentiation rather than a glial one. Therefore, this combined strategy provides a further method to develop therapeutical protocols in the field of cell-bases medicine, to be applied for the treatment of neurodegenerative disorders.

## GLYMPHATIC SYSTEM ALTERATION IN AN ANIMAL MODEL OF METABOLIC SYNDROME

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Metabolic syndrome (MetS) is a clustering of metabolic and cardiovascular factors responsible for an increased risk of developing

cardiovascular diseases and type 2 diabetes. MetS is also characterized by a chronic low-grade inflammatory status that could contribute to neuroinflammation and neurodegeneration, but the underlying mechanism is still unclear. Recently, an association between MetS, glymphatic dysfunction, and cognitive decline has been described. In the brain, metabolic waste removal is performed by the glymphatic system involving the astroglia water-channel protein aquaporin-4 (AQP4). Here, we evaluated glymphatic dysfunction associated with MetS-induced neuroinflammation using a high-fat diet (HFD) rabbit model of MetS and focusing on the hypothalamus, a brain area crucially regulating energy homeostasis and metabolism. Using immunohistochemical analysis, we confirmed the onset of hypothalamic inflammation in HFD rabbits, as demonstrated by the activation of resident immune cells (microglia) and astrocytes, as well as by the presence of RAM11-positive macrophage infiltrate and the significant induction of pro-inflammatory genes (COX2, IL-6, and CD68). Interestingly, immunohistochemistry analysis showed that AQP4 expression was significantly increased in HFD hypothalamic sections compared to controls, also showing increased colocalization at the astrocytic endfeet surrounding blood vessels. Accordingly, AQP4 vascular polarization was observed in the hypothalamus of the HFD group compared to controls. By ex vivo magnetic resonance imaging (MRI), we also analyzed structural morphological changes in the brains of HFD rabbits. Preliminary data showed no significant differences in total brain structure measurements. Overall, our data demonstrate an increase in glymphatic activity during Mets-induced neuroinflammation into the hypothalamus, suggesting a prior neuroprotective mechanism to maintain brain homeostasis. The lack of significant morphological and structural changes in brain areas suggests a precocious stage of MetS-related neuroinflammation not yet associated with neurodegenerative consequences.

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## SEX-BASED REGULATION OF VESSEL-ASSOCIATED MICROGLIA

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Loss-of-function variants of TREM2 (Triggering receptor expressed on myeloid cells 2) are risk factors for neurodegenerative disorders such as Alzheimer's disease (AD) and Cerebral Amyloid Angiopathy. TREM2 controls microglial metabolic fitness during stress events, and its absence results in vessel calcification and hemorrhagic events, resembling age-associated vascular dysfunctions. Interestingly, the TREM2-dependent microglia response is affected by sex: genetic lack of TREM2 in the AD mouse model APP/PS1 accelerates plaque load in 6-7-month-old female mice, the R47H AD variant impairs spatial learning in adult females, and 5xFAD mice with APOE3 knock-in results in low TREM2 expression in plaque-associated microglia in female brains. Sex-based differences in these contexts are not due to uneven Aβ or tau burden, but rather to an altered microglial response to that burden. Still, the underlying molecular mechanisms are unknown. This study aims to investigate the sex-specific morphological and functional interplay between brain vessels and microglia cells in response to AD-related risk factor stimuli. Our data show a TREM2-dependent, sex-specific regulation of vessel-associated microglia in healthy mice, as well as under exposure to AD-promoting factors such as sleep deprivation and lipopolysaccharide-mediated inflammation. The sex-specific study of TREM2 in the regulation of vessel-associated microglia may offer novel insights into the prevention and treatment management of dementia in the context of gender-personalized medicine.

# SESSION VII
# NEW FINDINGS AND TECHNIQUES IN NEUROSCIENCE

## IMAGING THE LYSOSOME IN CELLULAR SYSTEMS

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Lysosomal storage disorders (LSDs) are a group of rare diseases characterized by a genetic-derived lysosomal metabolism error leading to an excess of substrate accumulation. Within the complex spectrum of symptoms, the LSDs strongly affect the central nervous system (CNS). However, currently available treatments include enzymatic replacement therapies directed to peripherical symptoms only, mainly for the inability of the proteins to pass the blood-brain barrier. In the last years, in fact, innovative therapies are formulated to deliver the drug to the CNS, leading to the emerging need of comprehensive reliable testing systems.
We set up an *in vitro* platform using peripheral cells, i.e. primary human fibroblasts from patients affected by two model pathologies (alpha-mannosidosis, aMAN; Niemann-Pick A, NP-A) and healthy subjects. In the second phase of the study, we translated the readouts on neuronal cells, isolating primary neuronal/astrocytes cultures from cerebral cortex of WT mice and transgenic animal models of the two diseases. Lysosomal defects were analyzed by morphological and functional tools, and validation of the readouts was performed assessing the restoration of the lysosomal features by treatment with approved enzyme replacement therapies. LAMP1 staining was set up for the analysis by confocal microscopy. Using the voxel-based IMARIS image analysis software, we reconstructed the volume of each single lysosome in the cell, quantifying the average lysosome volume per cell, also tracking the 3D intracellular distribution. We described how the mutations lead to an increase in number and volume of lysosomes, and the change in distribution, with the lysosomal net accumulating around the nucleus. We than implemented the morphological analysis with functional data using LysoTrackerRed and DQ-BSA staining. The number of LysoTrackerRed stained lysosomes significantly increased in mutated cells, describing an acidification of the lysosomal content. Moreover, the accumulation of DQ-BSA resulted increased as well, demonstrating that mutant lysosomes are not able to properly degrade the BSA. For each readout, scaling to High-Content Screening methodology was considered, to increase the statistical and translational power of the platform. In conclusion, we used two model pathologies to set up an imaging-based *in vitro* platform aimed to recapitulate the morphological and functional lysosomal dysfunction in a dish, efficiently respond to replacement therapies.

## PROTEASOME INHIBITORS: *IN VITRO* MORPHO-FUNCTIONAL CHANGES

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Chemotherapy has significantly increased patient survival rates, but it has also led to a rise in chemotherapy-induced peripheral neuropathy (CIPN), which can severely impact quality of life and may lead to treatment discontinuation. Among chemotherapy drugs, 20S proteasome inhibitors like bortezomib (BTZ) and carfilzomib (CFZ), approved by the U.S. Food and Drug Administration for treating multiple myeloma and some other liquid tumors, have several limitations, including CIPN development. This study aims to investigate the effects of BTZ and CFZ on the cytoskeleton and mitochondria in primary cultures of dorsal root ganglion (DRG) sensory neurons isolated from adult mice. Neurons were treated with BTZ (10 nM) and CFZ (60 nM) for 10 and 24 h. Mitochondrial activity and functionality were assessed using the Seahorse Assay, mitochondrial membrane potential and mitochondrial trafficking measurements, while mitochondrial morphology was analysed using Mitochondrial Network Analysis (MiNA). Then, we examined the drug-induced changes in multiple proteins involved in the cytoskeleton and mitochondrial dynamics using immunoblotting. Our data show that both BTZ and CFZ impact mitochondrial membrane potential at 10 h post-treatment while mitochondrial respiration is affected by both drugs only after 24 h. Regarding mitochondrial trafficking, both BTZ and CFZ appear to affect anterograde transport as early as 12 h post-treatment, but only BTZ causes a significant increase in the number of stationary mitochondria at 24 h. Moreover, BTZ can alter microtubule dynamics by promoting more stable tubulin isoforms, as evidenced by increased MAP2 expression and stable tubulin modifications, such as acetylated and Δ2-tubulin, as early as 10 hours after treatment. Taken together, these results suggest that changes in mitochondrial morphology might represent a common mechanism of cellular toxicity, while the neurotoxic effects of BTZ might be linked to specific cytoskeletal alterations, resulting in significant axonal transport impairment. A deeper understanding of these pathways could help to identify potential therapeutic targets for BTZ-induced CIPN.

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## AN ANATOMICAL STUDY ON THE ATYPICAL SOM+ NEURONS

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The atypical long-range somatostatin projections belong to a subtype of somatostatin-expressing (SOM) inhibitory interneurons in the cerebral cortex. These long-range interneurons are characterized by axon trees that span two or more cortical and sub-cortical regions, providing fast communication between the innervated areas. Little is known about the circuits that extend throughout the brain starting from the anterior cingulate cortex (ACC). The aim of the present study was to characterize the SOM long-range neurons innervated areas, studying the once starting from ACC. To achieve this goal, brains from three SOM-Cre line mice in which the virus AAV5-EF1a-DIO-eYFP.WPRE.hGH was injected in ACC, were processed using immunofluorescence. We performed the rostro-caudal evaluation by processing 40 µm thickness coronal sections of the whole brain and mapping the areas in which the long-range projections spread. We found different innervated nuclei and areas. In general the interested areas were: telencephalon (in particular neocortex, olfactory system, septum, the accumbens nuclei, ventral pallidum, caudate putamen and amygdaloid complex) and diencephalon (in particular hypothalamus). Our results suggest that the SOM long-range neurons reaching these areas can be involved in the modulation of emotional responses and cognitive and homeostatic functions. Further studies are needed to confirm these hypotheses; however, this anatomical study represents the first suitable starting point for future analysis.

## BRIDGING THE GAP BETWEEN WHITE MATTER ANATOMY AND FUNCTION

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White matter pathways represent the anatomical foundation of brain functional organization, facilitating and modulating synchronized activity across distant brain regions. Traditionally, these pathways have been classified on an anatomical basis, and their functional roles have been inferred indirectly by mapping functional deficits from brain lesions onto the underlying structural connections. Herein, we use a hybrid framework combining structural and dynamic functional connectivity to identify spatially independent white matter components that support dynamic changes in functional connectivity. Finally, by integrating results from meta-analytic modeling of task-based functional MRI data, we developed a dedicated pipeline to investigate the contribution of white matter structures to behavior and cognition. We employed structural, diffusion-weighted, and resting-state functional MRI data of 210 subjects from the Human Connectome Project (HCP). Preprocessed imaging data were analyzed using track-weighted dynamic functional connectivity (tw-dFC) paired with independent component analysis (ICA) to identify spatially and temporally coherent components. To functionally decode white matter structures, we applied a spatial dimensionality reduction algorithm to ~6000 meta-analytic term maps derived from task-based functional MRI and computed the spatial similarity to the track-weighted component maps. Spatial ICA of tw-dFC data revealed anatomically meaningful patterns of white matter connectivity. Clustering analysis of the correlated connectivity fluctuations between pairs of components disclosed a hierarchic functional organization of white matter components into associative, sensorimotor, and visual clusters. When applied to well-known association white matter pathways such as the arcuate fasciculus, spatial ICA identified anatomically and functionally distinct segments. The results of the meta-analytic decoding process suggested that independent components of white matter activity may have distinct cognitive, behavioral, and pathophysiological implications. Our findings offer novel evidence for integrating structural and functional connectivity data to enhance our understanding of the structure-function relationship in the human brain.

## EXECUTIVE FUNCTIONS AND DUAL TASK WALKING

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Executive functions (EFs) are neurocognitive processes planning and regulating daily life actions. The basic EFs are working memory, inhibition. The working memory is the ability to keep in mind information while performing complex tasks. The inhibition allows to control thoughts, behavior, and/or emotions by overcoming a strong internal predisposition or external pull. The ability to carry out cognitive tasks while simultaneously walking is one of the most essential skills for daily-life activities. Performance of two simultaneous tasks, requiring the same cognitive resources, lead to a cognitive fatigue. Several studies investigated cognitive-motor task and the interference during walking, highlighting an increasing risk of falls especially in elderly and people with neurological diseases. A few studies instrumentally explored relationship between activation-no-activation of two EFs (working memory and inhibition) and spatial-temporal gait parameters. Aim of our study was to detect activation of inhibition and working memory during progressive difficulty levels of cognitive tasks and spontaneous walking using, respectively, wireless electroencephalography (EEG) and 3D-Gait analysis. Thirteen healthy subjects were recruited. Two cognitive tasks were performed at two levels of difficulty, activating inhibition (Go-NoGo_1 and Go-NoGo_2) and working memory (N-back_1 and N-back_2) during

walking. EEG features (the absolute and relative powers) were extracted by Power Spectral Density (PSD) function of the signal in eight bands for eight active channels. Seven spatial-temporal and nine kinematic parameters were computed. A significant decrease of stride length and an increase of external-rotation of foot progression were found during dual task walking with Go-NoGo. Moreover, a significant correlation was found between the relative power in the delta band at channels Fz, C4 and progressive difficulty levels of Go-NoGo (activating inhibition) during walking, whereas working memory showed no correlation. Evidence of the present study suggested a lower balance control, more instability and revealed specific kinematic adaptations during dual cognitive-motor task with Go-NoGo, reinforcing the hypothesis of the prevalent involvement of inhibition in motor task execution with respect to working memory, and probably revealing an interference of this EF during walking. The foundations for EEG-based monitoring of cognitive processes involved in gait are laid.

## ANALYSIS OF FACE INNERVATION BY ULTRASOUND TECHNOLOGY

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Magnetic Resonance Imaging (MRI) and ultrasound are the imaging modalities of choice for evaluation of the peripheral nerves given their high soft tissue contrast and resolution, respectively. The improved resolution of ultrasounds makes this technology an alternative modality for diagnostic evaluation of peripheral nerves. Advantages of ultrasound include lower costs, dynamic assessment of peripheral nerves, and the possibility to use it in patients in which MRI is contraindicated. The growing demand for minimally invasive aesthetic procedures has increased the size of the aesthetic medicine market. Although the incidence of complications in aesthetic medicine procedures is low, adverse events can be minimized using safe approaches. Ultrasound represents a technology the use of which is increasing and can contribute to a safe aesthetic medicine practice. The present study has investigated the applications of ultrasonography analysis on cadaver faces to identify the nerve supply of the face as a guide for safe aesthetic medical practice. Analysis was done on fresh human cadaver samples. The first stage involved an anatomical dissection of the different areas of the face. Dissection was made paying attention to move and not remove the overlying tissues. After dissection, the anatomical structures of interest were highlighted and prepared for analysis with colored plastic filaments. These structures were then photographed. Anatomical forms were examined using a Clarius 20 MHz ultrasound with HD3 wireless linear probe. Ultrasound images were then digitally acquired, examined and compared with dissection photos. The face was divided into 7 different areas. The great auricular nerve, the temporal branch (frontal), the mandibular, zygomatic and buccal branches of the facial nerve were identified. Supraorbital, supratrochlear, infraorbital and mentalis branches of the trigeminal nerve were also easily identified by ultrasound analysis. Transverse and longitudinal ultrasound demonstrated the normal sonographic appearance of different peripheral nerves supplying the face with hypoechoic fascicles and surrounding echo-genic epineurium. Cadaver analysis can represent the basis for a patient's evaluation. Ultrasound provides a valuable understanding of individual anatomical variations. This is helpful to plan aesthetic surgeries and minimally invasive treatments for improving both safety and aesthetic results respecting each patient's unique anatomical structure.

## POSTERS SESSION

### SHEAR STRESS-INDUCED BBB ALTERATION: THE ROLE OF OXIDATIVE STRESS

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It is well known that fluctuations in blood pressure have been connected to brain damage and neurodegenerative disorders but the trigger of such a deleterious event is not fully understood. During physiological conditions, the cerebral blood flow is strictly regulated and its increase can lead to shear stress that has been reported to induce oxidative stress. The key cellular compartment in both conditions is the brain endothelial cells that exert their role as a physiological blood-brain barrier (BBB) strictly regulating the exchange of the molecules between blood circulation and brain parenchyma. In the present preliminary study, we evaluated the effect of shear stress on human brain endothelial cells (HBEC-5i), by mimicking the blood flow increase using the LiveBox2 (LB2) instrument. Briefly, the HBEC-5i cells were gently seeded on the coverslip of LB2 chamber system, and allowed to grow for at least for 24 h. When cells were completely attached, the flow was induced by a pump of the LB2, and its rate was set up from 5 to 500 µl/min, and left from 1 to 8h at 37°C, 5% $CO_2$ in a humidified atmosphere. After that the system was opened and the cells were fixed in PFA 1% for 10 min. at room temperature. Immunofluorescent staining for NRF2, a key anti-oxidant regulator, was performed in order to evaluate the role of oxidative stress during flow increase. Our results demonstrated that shear stress induces oxidative stress on brain endothelial cells at an early time point, starting from 2h after fluidic flow induction. In conclusion, the shear stress may exert its deleterious effect on brain endothelial integrity, as previously demonstrated, by the induction of oxidative stress, thus hypothesizing the protective role of antioxidant molecules in ameliorating BBB function and strength.

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### NEUROPROTECTIVE EFFECTS OF POLYPHENOLS AGAINST A-SYNUCLEIN AGGREGATION

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α-Synuclein aggregation is the main pathological feature of Parkinson's disease. The aggregation process follows a first order kinetics, starting from smaller aggregates, namely oligomers, acting as seeds for the formation of greater assembly named fibrils, species that features the intracellular inclusions that characterize the pathology. These aggregates can diffuse within brain areas and their toxicity has been proven in both cellular and animal models. Recent therapeutic strategies have focused on the identification of compounds able to either promote the degradation of pre-existing aggregates or interfere with the aggregation process. In this context, natural-derived polyphenols have been proposed as potential tools against α-synuclein pathology, owing to their capabilities in ameliorating cognitive functions and suspected to be neuroprotective. On these bases, we tested the neuroprotective potential of oleuropein aglycone in multiple models of Parkinson's disease: i) neuroblastoma derived dopaminergic neurons, either overexpressing α-synuclein or under α-synuclein fibrils exposure, and ii) two *C. elegans* strains overexpressing α-synuclein in either dopaminergic neurons or muscle wall. Our results show that the compound was effective in reducing the burden of oligomeric pathology upon α-synuclein overexpression in neuroblastoma cells and neutralizing both the extent and the toxicity of administered pre-formed-fibrils. In nematodes, oleuropein aglycone improved both lifespan and motor features, recovered morphological defects in dopaminergic neurons and reduced the extent of α-synuclein pathology. Overall, these data support the neuroprotective potential of oleuropein aglycone against α-synuclein aggregation and toxicity by clearing pre-existing aggregates. In addition, the compound was able to improve the symptoms related to neuronal damage by recovering the physiological morphology of dopaminergic neurons. This work, strengthened by molecular modeling of the polyphenol/α-synuclein interaction, shed light into the molecular features of these mechanisms, paving the way to further studies to assess the neuroprotective potential of this polyphenol in mammals and humans.

## BaP INHIBITS HUMAN GnRH NEURON MIGRATION BY ALTERING THE RhoA PATHWAY

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Benzo[a]pyrene (BaP) is a widespread pollutant that can act as an endocrine-disrupting chemical, negatively affecting various physiological functions, including reproduction. The central network of reproduction is controlled by gonadotropin-releasing hormone (GnRH) neurons, which originate in the olfactory placode and migrate to the hypothalamus during fetal development. Using human fetal GnRH neuroblasts (FNCB4), we recently demonstrated that BaP (10μM, 24h) interferes with the migratory ability and, therefore, maturation of GnRH neurons. In this study, we used RNA-sequencing (RNA-seq) to clarify the mechanisms by which BaP affects FNCB4 migration. The differential expression analysis identified 585 significant differentially expressed genes (DEGs; FDR<0.05) in BaP-treated (10μM, 24h) compared to untreated cells, including 272 up-regulated and 313 down-regulated genes. According to functional analysis by Gene Ontology (GO) enrichment, BaP specifically altered 86 genes related to cell adhesion, cell migration and extracellular matrix organization processes. Similarly, Reactome enrichment analysis indicated that BaP exposure significantly changed genes involved in cell motility pathways, such as syndecan interactions, extracellular matrix proteoglycans, integrin and non-integrin cell surface interactions. Among these, we found a down-regulation of syndecan-2, syndecan-4 and CD44 which are strictly related to the RhoA pathway, an important signaling implicated in cell adhesion, cytoskeletal remodeling and migration, especially in neurons. To better understand the BaP mechanism of action and confirm the implication of RhoA, we analyzed its subcellular localization in FNCB4. Accordingly, immunofluorescence analysis showed that BaP exposure inhibited RhoA membrane translocation and, therefore, its activation, thus compromising the downstream signaling. In conclusion, our findings suggest the alteration of the RhoA pathway as a possible mechanism through which BaP affects GnRH neuron development.

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## BRAIN IRON AND MITOCHONDRIAL FEATURES IN 5XFAD MOUSE

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Iron is essential for neuronal activity, neurotransmitters' synthesis and energy homeostasis, especially for ATP production by the electron transport chain. Several evidence reported that iron dyshomeostasis impacts mitochondrial function in neurodegenerative diseases, such as Alzheimer's disease (AD), leading to energy failure and contributing to neuronal death. However, the mechanism of iron-induced mitochondrial dysfunction and its contribution to AD are not yet deciphered. Here, we investigated if iron homeostasis in the 5xFAD mouse model, expressing five familial human AD mutations on APP and PSEN1 genes, is altered during the pre-symptomatic phase of the disease. At 1 month of age, there is no brain iron deposition nor extracellular amyloid deposition in 5xFAD mice compared to wild-type mice of the same age, verified by co-staining Prussian blue Perl's and Thioflavin T (ThT). Consistently with the absence of iron deposition, we found no differences between 5xFAD and wild-type mice in iron import, export and storage measured by protein levels of the Transferrin Receptor 1, Ferroportin 1 and light and heavy chain of the iron deposit protein Ferritin, respectively. Interestingly, in 2-month-old 5xFAD we found consistent brain iron deposits in the hippocampal and striatal areas, but still in absence of extracellular amyloid plaques. Being mitochondria an important site for iron trafficking and function, we isolated enriched mitochondrial fractions from 5xFAD brains and we observed a significant increase in the expression of the mitochondrial import receptor subunit Tom20, mitochondrial aconitase and all the complexes of the respiratory chain. Moreover, we found a significant increase of mitochondrial ferritin, the iron stock within mitochondria. However, the increment in mitochondrial content is not due to *de novo* biogenesis, since the expression of Cytochrome b, NADH dehydrogenase 1 and also Peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1alpha mitochondrial genes is not modified in 5xFAD versus wild-type, suggesting that mitochondria increase is due to mitochondrial accumulation. Collectively, our data show early alterations in brain iron metabolism together with perturbations in mitochondria content in 5xFAD mice suggesting a close correlation between these two phenomena and amyloid pathology.

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## NEW MECHANISMS AND STRATEGIES TO PREVENT AD

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Alzheimer's disease (AD) is a progressive neurological disorder mainly associated with aging and one of the most severe forms of dementia with a higher prevalence in older adults. However, no effective treatment to fully restore the health condition of the patients has been yet found. This is why novel drugs that might help to prevent the disease before the symptoms are fully present is a major goal in the field. In this study, 5xFAD mice, a model that replicates the severe process of amyloidosis that patients undergo during the progression of AD due to the constitutive accumulation of amyloid plaques, have been treated orally from weaning until 4.5 months of age with leriglitazone (MIN-102). This compound is an orally bioavailable, blood-brain-barrier (BBB) penetrable, selective peroxisome proliferator-activated receptor (PPAR) subtype gamma agonist that has shown robust preclinical proof-of-concept in animal models of multiple central nervous system (CNS) diseases. At the end of the treatment, we conducted a battery of behavioral tests to evaluate the cognitive status of WT and 5xFAD mice, resulting in initial but not significant differences in learning and memory. Histological analysis of brain tissue by Prussian blue PERL's, DAB and thioflavin-T co-staining from 5xFAD mice revealed a significant and abnormal iron and amyloid accumulation in different regions: cortex, third ventricle, striatum and the hippocampus, the main brain area affected by AD. Interestingly, after 4 months of treatment, a significant reduction in the number of amyloid plaques was found in the hippocampal region of treated 5xFAD. Additional analyses are being performed at a longer treatment period. Also, inflammatory markers, such as IBA-1 and GFAP, were analyzed by immunofluorescence and morphological investigation on the glial activation status is ongoing. Although AD is widely characterized by the accumulation of different protein aggregates in the form of amyloid plaques and fibrillary tangles, iron deposition has gained attention as it could be playing an important role in the development of the disease. These data suggest a correlation between the two main types of deposits and stress the relevance of iron accumulation during the amyloidosis process prior to cognitive deterioration. We point these evidences as a novel pathophysiologic target to treat dementia and further study of leriglitazone's ability to reduce the amyloid accumulation at the very beginning of the disease.

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## THE STRESS IMPACT ON AMYOTROPHIC LATERAL SCLEROSIS

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Stressors can induce several cellular changes that are similarly associated with neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS). ALS is a motor neuron (MN) disease characterized by progressive degeneration of both upper and lower MNs, with consequent muscle atrophy, weakness and premature death. The aim of the study was to investigate how stress influences ALS pathogenesis by using two *in vitro* experimental models. First, NSC-34 cells (naïve, $hSOD1^{WT}$ and $hSOD1^{G93A}$) have been exploited as *in vitro* model of murine MN-like cells. hSOD1 cells express human SOD1 gene (WT or mutated), through a doxycycline-inducible promoter. The cells have been differentiated in MN-like cells with retinoic acid for 4 days: on one hand, the MN differentiation was validated by Incucyte® neurotrack analysis software, able to automatically detect and measure neuron length and cell body clusters. On the other hand, a cholinergic marker, ChAT was detected by immunofluorescence and the signal was quantified through corrected total cell fluorescence. To mimic stress condition, WT and mutated-hSOD1 cells underwent oxygen and glucose deprivation (OGD) using 100µM of CoCl2 and low glucose medium. Cell damage was assessed studying mitochondria metabolism through MitoTracker™ Red CMXRos, and evaluating the protein levels of HIF1α and caspase3: interestingly, we observed a reduced ability of mutated cells to endure stress conditions. Moreover, 39 ALS-related genes were analyzed both in stressed and no-stressed conditions by RT-qPCR. Then, PPI, GO and pathway enrichment analyses highlighted the key role of some genes in mutated cells under stress: *Ang, Col1a1, Col1a2, Col4a1, Col4a2, Gsk3b, Tgfb1*. As second *in vitro* model, human MNs (hMNs) differentiated from hiPSCs derived from a TPD-43 patient were similarly stressed by OGD, and compared to healthy controls. Neurite length, branch points and cell bodies clusters have been studied through Incucyte® neurotrack analysis software: the morphological results highlighted a reduced ability of the mutated hMNs to endure stress conditions, similarly to NSC-34 cells. Moreover, *Ang, Col1a1, Col4a2, Gsk3b* and *Tgfb1* were significantly deregulated in the mutated human cells compared to controls. Our analyses demonstrated that stress can indeed affect the ALS pathogenesis and revealed the key role of collagen (*Col*) genes in all the stressed experimental models representing a novel therapeutic target.

## CHOLINERGIC TREATMENT ON BRAIN VOLUME IN MCI

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Biomarkers can improve the accuracy of diagnosis in the assessment of cognitive dysfunctions, and their use is becoming a standard in the diagnostics of neurodegenerative disorders. Cognitive dysfunctions are characterized by a decrease in the weight and volume of the brain, due to cortical atrophy, with widening of the grooves and flattening of the convolutions. Brain atrophy mainly involving the hippocampus is related to the progression of cognitive impairment and the conversion from mild cognitive dysfunction to overt dementia. The CARL (Choline Alphoscerate in miLd cognitive dysfunction) study evaluates the efficacy of choline alphoscerate in mild cognitive impairment (MCI) patients with associated vascular damage. The trial has investigated the ability of treatment to stabilize and/or slowing hippocampal, entorhinal, neocortical atrophy and ventricular dilation. This randomized controlled trial has recruited 60 patients receiving the cholinergic precursor choline alphoscerate (1,200 mg/day) or placebo (in a 1:1 ratio) and were evaluated at the beginning of the study and after 12 months. Volume ($mm^3$) of hippocampus, entorhinal cortex, neocortex and dilation of lateral ventricles were assessed from T1-weighted MRI at the baseline and after 12-month of treatment by the FreeSurfer v7.4.1 software. The evaluation of the patients that have reached the final endpoint of one year of treatment, has confirmed that the study of mesial temporal and hippocampal atrophy represents a sensitive and suitable approach for dementia diagnosis. The hippocampal region shows signs of degeneration before the onset of cognitive symptoms and therefore represents the first region to degenerate. The atrophy rate is higher in the group treated with placebo compared to those receiving choline alphoscerate. The above findings suggest a possible activity of choline alphoscerate in the treatment of MCI, deserving larger and more extensive studies.

## THE SUBMUCOSAL PLEXUS IN THE PIG *VS* HUMAN COLON

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The pig is a valuable model to investigate gastrointestinal (GI) pathophysiology for its homologies with humans. These include: i) structural similarities; ii) both are omnivores, colon fermenters, with similar microbiota; iii) equivalent structures of the enteric nervous system (ENS). In the GI, neurons are organized in ganglionated plexuses: the myenteric plexus (MP), between the longitudinal and circular muscle; and the submucosal plexus (SMP), in the submucosa. Both ganglionated plexuses exhibit morphological features differing among species. In large mammals, such as pigs and humans, the SMP is multilayered and subdivided into inner (ISP, close to the mucosa), and outer (OSP, near the circular muscle) plexuses. We demonstrated that the density of enteric neurons in pigs is higher in ISP than OSP in ascending (AC) and descending colon (DC) and that neurons in both SMPs expressed immunoreactivity (IR) for choline acetyltransferase (ChAT), neuronal nitric oxide synthase (nNOS) and substance P (SP). Here, we compared the distribution and neurochemical profiles of ISP and OSP neurons in the AC and DC specimens from pigs (18 Yucatan, 3 F, 25-30 kg) and humans (14 patients; 6 F, age: 48-86; clean margin from resected colonic adenocarcinoma). In whole-mount submucosal specimens, neurochemical profile was assessed with HuC/D, ChAT, nNOS and SP antibodies using confocal imaging and Imaris software for quantification. In both pig and human colon, the highest density of neurons was observed in the ISP of AC. The overall HuC/D-IR/$mm^2$ was nearly 4 times greater in pig *vs* human. The density of HuC/D-IR neurons in ISP of the AC was higher than in the DC ($P<0.01$), whereas it was comparable in OSP of AC and DC in both pigs and humans. The ChAT-IR neurons in human (36-43%) and pig (31-44%) colon was comparable in ISP and OSP in AC and DC. Conversely, the percentage of nNOS-IR neurons was similar in ISP and OSP in the human AC and DC (16-22%) and differed in the pig, where the nNOS-IR neurons in the ISP of AC was significantly lower than OSP (15% *vs* 45%; $P<0.001$) and both ISP and OSP of DC (38-42%, $P<0.001$). The percentage of SP-IR neurons was smaller in human (15-20%) *vs* pig (23-26%) in both AC and DC. SP-IR varicosities were detected in the ganglia of pig and human colon. Our data show quantitative and neurochemical similarities in pig *vs* human submucosal neurons, supporting the use of this animal model to explore neuromodulation mechanisms in human colonic disorders.

## INVOLVEMENT OF PKCε IN ALS MOTOR NEURON DEGENERATION

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Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset neurodegenerative disorder, characterized by progressive degeneration of motor neurons in the brain and spinal cord. Multiple studies have reported the impairment of the protein kinase C (PKC)-mediated signal transduction mechanisms in ALS, describing the aberrant expression or activity of single PKC isozymes. In the present investigation, we analyzed the PKCε cellular distribution in human motor cortex specimens and described a significant reduction of PKCε mRNA and protein activation state in a subset of sporadic ALS patients at terminal stages. Furthermore, we investigated the steady-state levels of PKCε in the doxycycline-activated NSC-34 motor neuron cell carrying human wild-type (WT) or mutant G93A SOD1, and the biological long-term effect of its transient agonism by Bryostatin-1. Results demonstrated that the phosphoPKCε/PKCε ratio decreased in the mutant cells where the

brief pulse-activation of PKCε by Bryostatin-1 induced G93A-SOD1 long-term survival.
Overall, these data support a role for PKCε in ALS pathogenesis and sustain its pharmacological modulation as a candidate therapeutic strategy at least for a subgroup of sporadic ALS patients.

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## MODERATE AEROBIC EXERCISE UP-REGULATES ADNP IN SPECIFIC RAT BRAIN AREAS

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Moderate aerobic exercise largely improves cognitive function and overall brain health. Exercise enhances adult neurogenesis and synaptic plasticity, particularly in regions associated with learning and memory, such as the hippocampus, and motor learning and coordination, such as the cerebellum. Activity-dependent neuroprotective protein (ADNP) is a neuroprotective protein essential for embryonic development, proper brain development, and neuronal plasticity. To date, no study has investigated the effect of moderate exercise on ADNP expression and distribution in the rat brain. The current investigation aimed to analyze moderate exercise's effects on the ADNP expression and neuronal activation measured by the microtubule protein β-Tubulin III. To this end, twenty-four rats were selected and evenly distributed into two categories: sedentary control rats and rats exposed to moderate physical activity on a treadmill for 12 weeks. Our results showed that moderate aerobic exercise increases the expression of ADNP and β-Tubulin III in the dentate gyrus hippocampal region and cerebellum. Moreover, we found a co-localization of ADNP and β-Tubulin III in both dentate gyrus and cerebellum, suggesting a direct association of ADNP with adult neuronal activation induced by physical exercise. In conclusion, the results suggest that the positive role of moderate PA could be partly mediated by up-regulation and interaction between ADNP and β-Tubulin III in specific brain areas.

## ACTIVITY OF THIOCTIC ACID AND CHOLINERGIC PRECURSORS ON NEUROINFLAMMATION

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An essential part of neuroinflammation in hypertension regards microglia activation and astrogliosis. Studies have demonstrated that chronically elevated blood pressure leads to adverse glial activation, amplified brain inflammatory mediators, and increased risk of neurodegeneration. In our study, we investigated the potential anti-inflammatory effects of (+)-thioctic acid (TIO) and cholinergic precursors, α-glyceryl-phosphorylcholine (α-GPC) and cytidine-5'-diphosphocholine (CDP-choline), alone or in association, in the hippocampus of 24-week-old spontaneously hypertensive rats (SHR). The same compounds were applied *in vitro* lipopolysaccharide (LPS)-induced BV2 microglial activation. After four weeks of treatments with cholinergic precursors and TIO alone or in association, systolic blood pressure values were slightly reduced. Morphological and western blot results in the hippocampus of SHR rodents showed that treatments with TIO and CDP-choline, decreased gliosis and microglial activation, accompanied by reduced expressions of nuclear factor-kappa B and interleukin-1 beta. Similarly, α-GPC alone and in association with TIO countered astrogliosis and decreased the level of tumor necrosis factor-alpha and nuclear factor-kappa B. For *in vitro* results, the cell line was incubated for 24 h with LPS and different compounds. MTT assay results showed no significant difference in cell viability between different groups compared to the untreated cells. LPS increased the expression of ionized calcium-binding adapter molecule 1, nuclear factor-kappa B, and interleukin-1 beta. The protein expressions of the previous markers were downregulated with both TIO and CDP-choline, but not with TIO and α-GPC. Collectively, our results showed that the treatments with TIO and cholinergic precursor compounds act in different modes on neuroinflammation, and it is achieved by the downregulation of certain inflammatory markers. This proposes that they may have therapeutic potential for the treatment of neurodegenerative diseases accompanied by microglial activation. However, further studies are also needed to clarify the molecular mechanisms involving the cholinergic pathway.

## EFFECT OF DEXAMETHASONE OR ESTRADIOL ON THE EXPRESSION OF NESTIN, NEUROFILAMENT, BETA-TUBULIN AND MAP KINASE IN BONE MARROW MESENCHIMAL STEM CELLS CULTURES

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The aim of the present investigation is to study the effects of DEX or E2 treatment during differentiation towards neural cell line of rat BM-MSCs in culture. In order to better characterize biochemically our *in vitro* model, we evaluate by western blotting and immunocytochemical analysis some neural lineage markers (nestin, neurofilament, β-tubulin) and MAP-Kinases. An enhanced expression of the neural markers and MAP-Kinase in DEX- treat-

ed BM-MSCs cultures is found. In addition, E2-treatment increases MAP-Kinase and b-tubulin expression, but it decreases nestin and neurofilament expression. In conclusion, our findings highlight a significant up and down modulation of nestin, neurofilament, β-tubulin and MAP- Kinases expression in neurosteroids-treated BM-MSCs. In particular, our results clarify the molecular mechanism involved during eventual differentiation of these stem cells treated with DEX and E2, addressed towards a neural cell line, that may express neurotrophic receptors and release neurotrophines particularly implicated during neurogenesis processes. In addition, immunocytochemical data obtained in E2 treated-cells support evidences concerning an up and down modulation of nestin, neurofilament, beta-tubulin and MAP- Kinase expression, as observed by western blot analysis. Furthermore, α-Lipoic Acid (ALA) reduces Iron-induced Toxicity and Oxidative Stress in a Model of Iron Overload. Human mesenchymal stem cells (HS-5) and animals (zebrafish, a very little fish having a nuclear genome very similar about 75% to human nuclear genome) were treated for 24h with ferric ammonium citrate (FAC) 120 μg/mL in the presence or absence of ALA 20 μg/mL. In conclusion, ALA may represent a valuable tool to be used in iron overload conditions because of its pleiotropic mechanisms of action, impacting on various, important pathophysiological mechanisms involved in cellular dysfunction and organ injury, as well as in astroglial cell cultures of nervous system.

## MULTIMODAL GRADIENTS OF THE HUMAN PULVINAR

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The pulvinar, the largest nucleus in the human thalamus, is a complex, highly interconnected structure. Through a dense, organized network of cortical and subcortical areas, it provides adequate cooperation between neural systems, which is crucial for multiple high-order functions such as perception, visuospatial attention, and emotional processing. Such a central role is made possible by a precise internal topographical organization, which is mirrored by anatomical connections as well as by the expression of neurochemical markers. While being traditionally subdivided into sub-nuclei, each characterized by distinct connectional and morphological features, recent studies in both primate and human brains have highlighted that this topographical organization only marginally aligns with the conventional histological subdivision. Instead, it has been delineated in the context of continuous gradients of cortical connections along the dorsoventral and mediolateral axes. While this multi-gradient organization has been extensively documented in primate models, it remains relatively underexplored in the human brain. The present work leverages high-quality, multi-modal structural and functional imaging data of 210 healthy subjects from the Human Connectome Project (HCP). Additionally, we incorporate a recently published whole-brain, large-scale, positron emission tomography (PET) atlas detailing 19 neurotransmitters and receptors distributed across the human brain. By applying diffusion embedding analysis to tractography, functional connectivity, and receptor co-expression data, we identify and characterize multiple topographically organized gradients of structural connections, functional coactivation, and molecular binding patterns. We demonstrate that such gradients converge on a shared representation along dorso-ventral and medio-lateral axes of the human pulvinar. This representation aligns with transitions in both structural and functional connectivity, spanning from lower-level to higher-order cortical regions. Moreover, it is paralleled by gradual changes in the expression of molecular markers associated with key neuromodulatory systems, including serotoninergic, noradrenergic, dopaminergic, cholinergic, and opioid systems. We contend that our findings mark a significant stride towards a more comprehensive understanding of pulvinar anatomy and function, providing a nuanced characterization of its role in health and disease.

## A MORPHOLOGICAL DETECTION OF PIGMENT AND NEUROTRASMITTERS IN THE HUMAN DENTATE NUCLEUS

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Neuromelanin (NM), is a black brown pigment localized in the cytoplasm of catecholaminergic neurons of the substantia nigra pars compacta (SNpc) and of the locus coeruleus (LC). Although, NM is a bioactive compound in these brain regions, its functions and properties are not fully known. Several studies have outlined to NM a neuroprotective role in the SNpc and in the LC as a potent antioxidant. Furthermore, NM is also involved mitochondrial oxidative dysfunction especially in Parkinson's disease, Alzheimer's disease, and in some psychiatric disorders. This is due to mainly a dysregulation of NM precursors, and NM accumulation. Moreover, although studies have demonstrated the existence of a cerebellar dopaminergic system, the presence of NM in the cerebellum is essentially denied. NM in the human dentate nucleus in a condition known as neuromelanosis has rarely been demonstrated. Therefore, the aim of this study was to evaluate in the neurons of human dentate nucleus the presence of NM and to identify their neurotransmitter phenotype. The study was carried out on fragments of postmortem human dentate nucleus 36-48h after death. Each fragment was fixed in an aldehyde and picric acid solution or in neutral buffered formalin, embedded in paraffin, cut into 5μm sections, and subjected to light microscopy depigmentation histological protocol and to immunohistochemical procedures with polyclonal antisera to serotonin (5-HT), dopamine transporter (DAT), dopamine receptor type 2 ($DRD_2$), neurotensin (NT). Immunoreaction were revealed by streptavidin-biotin technique and 3, 3'-diaminobenzidine (DAB) or DAB-nickel. For positive control of the depigmentation procedure, section of midbrain containing SNpc has been used. Depigmentation protocol demonstrate the existence of NM in large neuron types of the human dentate nucleus. Immunohistochemical results demonstrate a positivity for all the antigens in neuronal cell bodies and processes of small and large neuron types; in a subpopulation of large neuron types the

co-presence of NM and of positivity to 5-HT, DAT, $DRD_2$ NT has been also detected. These results demonstrate in the human dentate nucleus the presence of NM in monoaminergic and peptidergic large neurons types. Finally, we suggest a possible role of this large neuron types in neurodegenerative disorders such as ataxias and Parkinson's disease.

## SKIN BIOPSIES' POTENTIAL FOR THE STUDY OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide. Besides affecting the central nervous system (CNS), where the loss of dopaminergic neurons of the *substantia nigra* and the presence of insoluble aggregates called Lewy Bodies are the two main pathological hallmarks, PD involves also the peripheral nervous system (PNS). Indeed, increasing evidence highlighted the presence of altered α-synuclein, the main component of the Lewy Bodies, in peripheral samples from PD patients. Focusing on the fibers innervating the sweat glands of the volar forearm skin biopsies, we have previously shown that oligomeric α-synuclein, a toxic and aggregation-prone species, can distinguish idiopathic PD (iPD) patients from healthy controls. This initial finding led us to consider the periphery as a easy accessible window to comprehend the pathology and possibly stratify different parkinsonism. First, we observed that peripheral oligomeric pathology is a feature of both iPD and genetic *GBA1*-PD patients compared to controls. Moreover, investigating their synaptic density, idiopathic patients can be distinguished from genetic ones. Then, we moved on the study of microtubule cytoskeleton system. Indeed, numerous evidence indicate that alteration in microtubule regulation, caused by post translational modification (*e.g*. acetylation) or by microtubule binding proteins (*e.g*. TAU) are involved in PD pathology. Here we found that acetylated α-tubulin is decreased in fibers innervating the sweat glands in patients' skin biopsies. To note, we have very recently shown that alterations of acetylated α-tubulin are present in the CNS in PD. Although our results need further investigation, they indicate that skin biopsies are a powerful site to investigate PD pathology. In conclusion, this work again highlights the involvement of the PNS in the pathology of PD and lays the groundwork to reliable assays to distinguish and stratify patients.

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## FIRST VISUALIZATION OF GATA1 IN MICE BRAIN

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GATA1 is a transcription factor involved in the development and the maturation of erythropoietic cells. At present, there are no clear evidence about the expression of GATA1 in neuronal lineage, in contrast to the widely described neuronal expression of GATA2. Studies conducted on red-blood cells have shown that GATA1 regulates the transcription of the alpha synuclein gene (SNCA). In the present study, we aimed at determining the detailed GATA1 expression in the brain and the effects of GATA1 knocking down on the expression of a-syn and on the survival of different neuronal subtypes in the brain. The study was performed using control mice with CD1 background and a mice model of GATA1 knock down (STOCK GATA1tm2Sho/J). After morphological and morphometric comparison of the brains of the two groups of animals, immunohistochemistry (IHC) and immunofluorescence (IF) were used to analyze the expression of GATA1 protein and its co-expression with several markers of neuronal populations. A stereological count of different neuronal cell types in the olfactory bulbs (OB) and in the midbrain dopaminergic (DA) nuclei was performed. RNAscope was used to detect the expression of GATA1 mRNA. Finally, the expression levels of a-syn were analysed by ELISA and the cyto-morphological features of GATA1 expressing neurons were visualized by Transmission Electron Microscopy (T.E.M.). The brains of CD1 mice are 21% larger in size than those of GATA1low mice. GATA1 protein is expressed in discrete brain-stem and telencephalic regions. OB are distinguished by its expression in different types of peri-glomerular neurons. Ultrastructural analysis revealed the expression of GATA1 in vesicles-like structures in the cytoplasm and at perinuclear level. RNA scope demonstrated the presence of GATA1 mRNA in the same neuronal populations. As expected.ù, expression of GATA1 was reduced in mice, where GATA1 expressing neurons appeared shrinker and morphologically altered. Finally, we noticed that the expression of GATA1 correlates with altered expression and aggregation of a-syn. Our results prove, for the first time, that GATA1 is expressed in the brain and particularly in the peri-glomerular layer of the OB. Regulation of SNCA gene and contribution to maturation and survival of DA neurons, represent key biological functions of GATA1, which request detailed exploration to link GATA1 to neurodegeneration in course of synucleinopathies.

## GATA1LOW MICE: A NEW MODEL FOR NEURODEGENERATION?

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GATA1 is a transcription factor predominantly involved in the development of erythropoietic cells. Expression of GATA1 has been found in the brain and more specifically in the olfactory bulb. GATA1 regulates the transcription of numerous genes, such as alpha-synuclein gene (SNCA), and Olig2 and TCF3, essential for neurodevelopment and myelination. This suggests that this transcription factor, despite being known just for its hematopoietic properties, plays also an important role in myelination, inflammatory activity, autophagy homeostasis and glial cell development. In this study, we compared CD1 control mice and a knockdown model of GATA1 (STOCK GATA1tm2Sho/J). Immunohistochemistry (IHC) and immunofluorescence (IF) were used to analyse GATA1 protein expression in all brain regions. Myelin was studied in both its lipid component, through Luxol fast blue, and its protein component, using IF for anti-myelin basic protein (MBP). Double IF was done to study colocalization between MBP and the autophagy marker MAPLC3β and between GATA1 and TGFβ. GATA1 expression was found in the substriatal region, in the pontine grey and in the dorsal nucleus of the vagus. The myelination of the brains was found altered in the GATA1low mice. The myelin fibers were analyzed and quantified, showing a significant increase in the number of small, medium and large amplitude fibers in the $GATA1_{low}$ mice. The expression of MAPLC3β showed increased levels in $GATA1_{low}$ in young mice and in CD1 in old individuals. Furthermore, increased colocalization between MBP and MAPLC3β in the $GATA1_{low}$ model indicated increased autophagic process at the myelin fibers. TGFβ was studied together with GATA1 and the two molecules have shown colocalization. Our findings reveal that GATA1 is expressed in specific brain regions, including the sub-striatal area, pontine grey, and dorsal nucleus of the vagus, extending its known distribution in the central nervous system. The observed alterations of myelin in $GATA1_{low}$ mice, coupled with the significant reduction in the density of myelinated fibres, indicate a critical role for GATA1 in maintaining myelin integrity. The increased levels of MAPLC3β and its colocalization with MBP in $GATA1_{low}$ mice suggest enhanced autophagic activity within myelin fibres, contributing to myelin degradation. The colocalization of TGFβ with GATA1 further suggests an interaction that may influence neuroinflammatory or neurodegenerative pathways.