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Proceedings of the 32nd National Conference of the Italian Group for the Study of Neuromorphology "Gruppo Italiano per lo Studio della Neuromorfologia" G.I.S.N.

November 25-26, 2022

University of Campania, Luigi Vanvitelli
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The *European Journal of Histochemistry* is the official organ of the Italian Society of Histochemistry and a member of the journal subcommittee of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC), and has been an influential cytology journal for over 60 years, publishing research articles on functional cytology and histology in animals and plants.

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- nerve and muscle cell biology;
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table of contents

Proceedings of the 32nd National Conference of the Italian Group for the Study of Neuromorphology "Gruppo Italiano per lo Studio della Neuromorfologia" G.I.S.N.

MAIN LECTURES

"WHAT A FEELING IMAGING THE BRAIN": THE GENESIS OF ALGORITHMS FOR COMPUTATIONAL NEUROIMAGING

F. Esposito

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Advancements in brain imaging methodology have largely expanded the scope of modern human brain *in vivo* studies in both physiology and pathology. Particularly, a large amount of high field brain MRI studies has tremendously improved the possibilities of clinical neuroscientists for finding out and establishing, as well as monitoring over time, the links between brain morphology and function by proper combination of empowered acquisition hardware and image reconstruction software. However, in the last decade, the development of brain imaging techniques has been also increasingly affected by the progress in computational models and algorithms from computer science research. Thus, while advanced image acquisition processing techniques have been classically adapted to solve typical brain imaging problems, such as multi-modal image registration, normalization and segmentation, the focus of a larger body of brain imaging research has moved towards the search for novel computational solutions, consisting of mathematical model and computer algorithms, with enhanced capabilities at extracting more in-depth information from rapidly growing multi-center image databases. In this presentation, we will present some recent applications where the most significant contribution to the application comes from the novel application of advanced computational models and algorithms, suggesting how the multi-disciplinary field of brain imaging is nowadays perceived as the ideal place of meeting and development of new ideas and tools among a plethora of biomedical researchers from different disciplines.

NEW INSIGHTS INTO SYNAPSE-MITOCHONDRIA INTERACTIONS

E. Korkotian

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While neuronal mitochondria have been studied extensively in their role in health and disease, the rules that govern calcium regulation in mitochondria remain somewhat vague. In the present study using cultured rat hippocampal neurons transfected with the mtRCaMP mitochondrial calcium sensor, we investigated the effects of cytosolic calcium surges on the dynamics of mitochondrial calcium ($[Ca^{2+}]m$). Cytosolic calcium ($[Ca^{2+}]c$) was measured using the high affinity sensor Fluo-2. We recorded two types of calcium events: local and global ones. Local events were limited to a small, 2–5 μm section of the dendrite, presumably caused by local synaptic activity, while global events were associated with network bursts and extended throughout the imaged dendrite. In both cases, cytosolic surges were followed by a delayed rise in $[Ca^{2+}]m$. In global events, the rise lasted longer and was observed in all mitochondrial clusters. At the end of the descending part of the global event, $[Ca^{2+}]m$ was still high. Global events were accompanied by short and rather high $[Ca^{2+}]m$ surges which we called spikelets and were present until the complete decay of the cytosolic event. In the case of local events, selective short-term responses were limited to the part of the mitochondrial cluster that was located directly in the centre of $[Ca^{2+}]c$ activity, and faded quickly, while responses in the neighbouring regions were rarely observed. Caffeine (which recruits ryanodine receptors to supply calcium to the mitochondria), and carbonyl cyanide m-chlorophenyl hydrazine (CCCP, a mitochondrial uncoupler) could affect $[Ca^{2+}]m$ in both global and local events. A computational model to simulate the fundamental role of mitochondria in restricting calcium signals within a narrow range under synapses, preventing diffusion into adjacent regions of the dendrite, was created. Results indicate that local cytoplasmic and mitochondrial calcium concentrations are highly correlated. This reflects a key role of signalling pathways that connect the postsynaptic membrane to local mitochondrial clusters.

REMODELING SYNAPSES: FROM MICE TO HUMANS

A. Vlachos

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A defining feature of the brain is its ability to adapt the structural, functional, and molecular properties of synaptic contacts in a stimulus-dependent manner. In the human cortex, direct experimental evidence for activity-dependent synaptic plasticity is scarce. As a consequence, also the role of plasticity in health and disease remains not well understood, and effective therapeutic interventions based on the modulation of synaptic plasticity are limited. My presentation focuses on our recent work, which demonstrated coordinated structural and functional changes of excitatory synapses in cortical tissue obtained from mice and humans. I will discuss the role of Ca^{2+}-dependent signalling pathways and intracellular Ca^{2+} stores and present a translational framework that will eventually enable us to study the role of synaptic remodelling in the intact human brain using transcranial magnetic stimulation.

SESSION I
NEURODEGENERATION

GLYCINERGIC SYSTEM ALTERATIONS IN SMA

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Spinal muscular atrophy (SMA) is a genetic neurodegenerative disease caused by *SMN1* mutation and consequent SMN protein lack. It affects both the CNS [causing motor neuron (MN) degeneration in brainstem and spinal cord] and peripheral districts (*e.g.*, heart and skeletal muscles). The available therapies are effective, but unfortunately show several limitations including high costs, still unknown long-term effects, some side effects, and disregarding of SMN-independent targets. Therefore, the identification of new targets and therapeutic strategies is necessary. Recently it has been observed that SMA shares mitochondria alterations with other neurodegenerative diseases (*e.g.*, Parkinson's Disease, Alzheimer's Disease and Amyotrophic Lateral Sclerosis) and therefore these organelles can be considered new promising therapeutic targets. Searching for mitochondrial *SMN1*-anticorrelated genes by a bioinformatic approach, we identified *Gcsh* as a possible candidate gene: to confirm this prediction, we then performed rt-PCR and Western Blot to assess its expression *in vivo*, in SMA mice (murine model: SMNdelta7 mice, representative of a severe form of the disease). We observed the upregulation of the *Gcsh* expression in SMA lumbar spinal cord compared to WT, at early pathological stage (postnatal day 5, P5). Moreover, immunofluorescence analysis revealed a significant GCSH upregulation in MN soma, suggesting possible cell-specific alterations. Since *Gcsh* codifies for a subunit of the glycinergic cleavage system, we decided to investigate other possible alterations in glycinergic system. In fact, we assume that a reduction in MN inhibition, together with the lack of SMN, could contribute to the loss of MN due to their hyperexcitability. To verify our hypothesis, we stereologically counted (by Steroinvestigator software) and morphologically analysed (by Neurolucida software) a class of glycinergic inhibitory interneurons that are involved in MN recurrent inhibition in the ventral horns of spinal cord: the Renshaw Cells. Compared to age-matched WT mice, calbindin D-28k staining revealed a significantly reduced cell density in the late stage of pathology (P12), together with a remarkable reduction of the soma area and dendrite length. Some of these alterations, although not significant, were already present at P5. We intend now to deepen these preliminary results, in order to find out new early-altered pathways in the pathology and new drug targets for SMA therapy.

INDUCTION OF AMYLOID-β DEPOSITION IN ENTORHINAL-HIPPOCAMPAL SLICE CULTURES

R. Cirillo[1], P. d'Errico[2], A. Vlachos[2], M. Papa[1]

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Amyloidosis is a common shared feature of many neurodegenerative disorders. One of the main hallmarks of Alzheimer's disease (AD) is the aggregation and deposition of amyloid-β peptide (Aβ). To date, little is known regarding the mechanisms by which Aβ plaques form. With the final goal to generate an *ex-vivo* model of amyloidosis and to better understand both the β-amyloidosis process and the role of microglia, we induced amyloid-β deposition in entorhinal hippocampal slice culture model, which offers the advantages of an easier investigation approach. Hippocampal slice cultures (HSCs) were obtained from P6 5xFAD mice pups. HSCs were treated once with brain extract from aged FAD5x mice and the culture medium was continuously supplemented with synthetic Aβ1-40 and Aβ1-42. In order to evaluate the putative Aβ accumulation, the HSCs were treated at different time points (3,5,9 weeks) and analyzed by immunostaining. While bared or no Aβ deposition was observed in hippocampal slices following 9 weeks of treatment, Aβ accumulation emerged in HSCs at earlier time points. Larger Aβ plaques (6E10+) were detected in the slices treated with Aβ 1-40 compared to Aβ 1-42. In our experimental model, microglia (Iba1+ cells) clustering was observed around the plaques at the earliest time point which disappears later, suggesting a time-dependent microglial response to Aβ accumulation. These preliminary results underscore the importance of validating this *ex vivo* amyloidosis model with a farther aim to better understand how microglia respond to Aβ plaques and whether this process can be altered. Thus, our study could provide crucial information regarding the mechanisms underlying amyloidosis and microglial response, with the future prospect of treating amyloidogenic diseases.

CEREBRAL CORTEX ALTERATIONS IN A SMA MOUSE MODEL

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Spinal Muscular Atrophy (SMA) is a severe neurodegenerative disease of the early childhood, caused by the mutation/deletion of the survival motor neuron (SMN1) gene. The lack of functional SMN protein induces the selective degeneration of spinal motor neurons (MNs), resulting in progressive skeletal muscle denervation and atrophy. However, little is known about the effects of SMN deficiency in the brain and only recent studies in both animal models and human patients highlighted, interestingly, that also the brain is affected by SMN loss. For instance, functional alterations in cerebral neural networks have been reported in SMA models, and imaging studies on patients suggested the occurrence of a reorganization of cortical grey matter, due to the progressive neurodegeneration in the spinal cord. Thus, we focused on the sensorimotor cortex of SMA 7 mice (a severe SMA model), to investigate the effect of SMN deficiency on the cortical cytoarchitecture. We analysed early (postnatal day 5) and late (P11) symptomatic animals, comparing SMA mice with their wild-type (WT) littermates. We investigated both projection neuron and interneuron distribution by immunofluorescence analysis, using different markers to identify specific neuronal subtypes. We confirmed a lower cell density in layer V of SMA cortex. Indeed, looking at different projection neuron subtypes, we found that both corticospinal (Ctip2-positive) and callosal (Satb2-positive) neurons are reduced at P11 in SMA cortex by about 40%, suggesting that SMN reduction could affect the upper MNs as well. These neuronal cell types in SMA also show alteration in some morphological traits, as observed by morphological analysis using retrograde tracers. Furthermore, we observed a differential distribution of interneuron populations (parvalbumin and somatostatin-positive cells), in both supragranular and infragranular layers, in SMA sensorimotor cortex compared to WT. Overall, we found a remodelling in cortical cytoarchitecture in SMA which could contribute to the etiopathology of the disease. Knowing the involvement of cerebral cortex in SMA will contribute to unravelling the dynamics of progressive degeneration occurring in the disease and will be also useful in designing new comprehensive treatments strategies, for better clinical outcomes

SESSION II
NEURO-METABOLIC COUPLING AND BRAIN-GUT AXIS

NEUROPLASTICITY OF ENTERIC NERVOUS SYSTEM IN ANIMAL MODEL OF OBESITY

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An altered functional innervation of the intestine by the enteric nervous system has been demonstrated in various obese animal models. Pathological changes in histological features confirm neuronal plasticity in obesity conditions. Since we have already assessed central nervous system alterations in obese animals, the current study will aim at providing the long-term peripheric effects of obesity on the enteric nervous system in obese rats. Here, we investigated the potential obesity-related alterations in the small and large intestines. We studied rats with obesity induced by diet after long-term exposure to a high-fat diet compared with animals fed a standard diet. We examined whether consuming this diet induced a temporal progression of changes in glial cells and neurons along the innervation of the gut using different markers through immunohistochemical and immunochemical techniques to visualize the morphological and functional modulations of the heterogeneous neuronal population of the gut wall. Obesity developed after five weeks of a high-fat diet. Concerning the results in obese conditions, myenteric neurons showed a reduction in neuronal marker staining, suggesting a degeneration associated with obesity. An increased glial reactivity was found in the jejunum of obese rats, which pointed out a suffering condition of nervous tissue that leads astrocytes to protect the neuronal microenvironment. On the contrary, a reduced expression of the glial fibrillary acidic protein was found in the ileum and the distal colon of treated rats suggesting a neuronal degeneration related to lipotoxicity. Moreover, the network of vesicular acetylcholine transporter was more evident in the intestine of obese rats than in lean controls to counteract cholinergic hypofunction of motoneurons in dysmetabolic conditions. Finally, we found fewer nitrergic neurons in the duodenum and the jejunum of obese animals, and we speculate that a hypercaloric diet reduced inhibitory neuromuscular transmission. In conclusion, consuming a high-fat hypercaloric diet makes a temporal progression of changes in glial cells and neurons along the innervation of the gut of obese animal models, causing several gastrointestinal alterations. Damages to the enteric glia could also affect the integrity of enteric neurons, increase the network of cholinergic neurons, and decrease nitrergic neurons. These findings suggest that obesity induces myenteric neurodegeneration.

GLUTAMINE RESCUES LIG3-INDUCED DEFECTS IN CIPO

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We identified a new recessive syndrome due to mutations in LIG3 in patients with chronic intestinal pseudo-obstruction (CIPO), leukoencephalopathy, epilepsy, migraine, stroke-like episodes and neurogenic bladder. LIG3 mutations affected mitochondrial DNA (mtDNA) maintenance, leading to mtDNA depletion and severe bioenergetic defects. In this study, we aimed to recover the mitochondrial impairment and develop a targeted treatment. Skin-derived fibroblasts from patients (1-1; 3-2) and controls were grown with 2 or 6 mM L-Gln. Methods included growth analysis in Incucyte; Western blot; live-cell fluorescent staining; RNAseq. Patients were followed up for L-Gln treatment. Gastrointestinal Symptom Rating Scale (GSRS) questionnaire was administered at t=0 and t=8 months after treatment. The mitochondrial network in mutant cells was fragmented and the respiration rate highly impaired. In mutant vs control cells, we observed a reduced expression of the Krebs' cycle enzyme Oxoglutarate Dehydrogenase (P=0.0005, Student's t-test). In control cells lysosomal and mitochondrial probes co-localized, whereas in mutant cells the fluorescent signals were distinct, indicating that dysfunctional mitochondria were not removed. Although autophagy was activated in mutant fibroblasts, shown by LC3B cleavage, a severe reduction of mitofusin-2 or PTEN-induced kinase 1 (PINK1) blocked mitophagy. As the addition of L-Gln 6 mM increased the survival of cells with mtDNA defects, we studied cell growth in 2 vs 6 mM L-Gln. In control fibroblasts 6 mM L-Gln resulted in a decreased growth. Conversely, mutant fibroblasts grew more efficiently in 6 mM L-Gln. RNAseq analysis of treated vs. untreated cells revealed that the most significantly upregulated gene was the mitochondrial tRNAPro (*MTTP* gene, 7.485-fold-change, adjusted p=0.0018). These data suggested to offer as compassionate use a daily enteral supplementation with a dipeptide containing L-Gln to three sibs affected by LIG3 mutations (0.3-0.5 g L-Ala-L-Gln/kg body weight). This compound has long been in use safely for other uses. After 8-month treatment, abdominal pain decreased and bowel function improved as indicated by the GSRS evaluation. Our study identified specific positive effects in LIG3-mutant cells grown with high L-Gln. This translational option in available patients showed encouraging results in reducing symptom severity without noticeable side-effects.

IMPACT OF EARLY-LIFE STRESS AND GONADAL HORMONES ON REWARD SYSTEMS OF ABA RATS

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**These authors contributed equally to this work*

Anorexia nervosa (AN) is a psychiatric illness in which the main symptoms are a disrupted perception of the body and its shape and disturbed eating that leads to self-starvation. A disturbance of the brain serotonergic network predates the onset of AN and should contribute to premorbid symptoms of anxiety, inhibition, and vulnerability to dietary restrictions. Moreover, puberty-related steroids and stress may enhance serotonin (5-HT) and dopamine (DA) dysregulation and aggravate the disease. In fact, these two systems are of greater interest in AN because their central pathophysiological role in patients is still limited. Based on our previous study, here we investigate the effects of early-life stress and of the absence of gonadal hormones in Activity Based Anorexia (ABA) affected rats. Four groups of gonadectomized (GDX) rats (GDX, MS-GDX, ABAGDX, MS-ABA-GDX) of both sexes were used, applying maternal separation (MS) to mild stress induction (3 hours/day, from PND1 to PND15) and the ABA protocols (2 hours of running wheel followed by 1 hour of food access, from PND37 to PND42) to provide symptoms like hyperactivity and weight loss, resembling anorectic patients' conditions. The exploratory and anxiety-like behaviours were evaluated using the open field (OF) and elevated plus maze (EPM) tests. Last, to investigate the impact on serotonergic and dopaminergic systems, immunohistochemical analysis was performed to quantify the presence of 5-HT in the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN) and of the DA in the ventral tegmental area (VTA) and substantia nigra (SN). The ABA protocol induced hyperactivity and a reduction in anxiety-like behaviours in rats of both sexes compared to controls (GDX). In addition, the behavioural phenotype alteration appeared to be sex-dependent by combining MS and ABA in GDX rats. Of particular interest were the results of 5-HT+ cells in GDX rats that changed from MS and ABA, or the combination of the two, showing distinct effects in the two sexes, especially in the dorsal part of DRN. Although serotonergic and dopaminergic systems in anorexic rats develop in the presence of gonadal hormones, their suppression may have altered the activation of those systems, whereas MS may have affected their organization, influencing the behaviour observed in ABA rats.

S100B PROTEIN AND MICROBIOTA

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S100B is a small EF-hand calcium-binding protein expressed by definite neural and extraneural cell types, being also present in enteroglial cells and in the enteric nervous system-ENS. Notably, it has been detected in biological fluids as a biomarker. It belongs to the S100 family that is located as a cluster on chromosome 1q21, while *S100B* gene is located at 21q22.3 and codifies for a multifunctional protein involved in several physiological processes. Modulations of *S100B* have been implicated in several disorders and pathogenetic pathways, including inflammatory processes and extracellular activity interacting with the Receptor for Advanced Glycation Endproducts. S100B was detected in the intestinal lumen and in feces of healthy individuals (Di Liddo et al 2020), but also associated to amplification of the Clostridium difficile toxin-induced colonic damage in Inflammatory Bowel Disease (IBD). In previous *in silico* studies, we showed the differential capability of S100B to interact with the proteome of a healthy microbiota (Orsini et al 2020), suggesting a possible role at the mucosa-microbiota barrier, in the ENS and in the gut microbiota axis. Our *in vivo* experiments in mouse models disclosed a relationship between S100B levels and microbiota biodiversity, further supporting the hypothesis of a possible interaction with the mechanisms involved in gut microflora equilibrium. Consistently, S100B-dependent effects on microbiota could not be observed in presence of drugs interfering with S100B functional pathways, such as Pentamidine. The relative occurrence of bacteria at risk for IBD such as Clostridium was also influenced by the presence of the protein, suggesting a putative protective role of S100B in the gut lumen. The whole of the observed results supports a role for S100B in the microflora equilibrium and opens new perspectives proposing S100B as a therapeutic target for the modulation of gut microbiota axes and disease pathways, including IBD and several chronic conditions.

AUTONOMIC INNERVATION IN MODELS OF RAT LIVER HYPERPLASIA

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The autonomic neural network represents one of the key players in maintaining liver homeostasis and regeneration after hepatic damage. However, the mechanisms governing alterations in hepatic innervation in different injury contexts are largely unknown. To contribute to the knowledge of the autonomic innervation in liver regeneration under different conditions of injury and repair, here we investigate the distribution of tyrosine hydroxylase (TH)- and choline acetyltransferase (ChAT)-containing nerve fibers, to indicate adrenergic and cholinergic nerves, respectively, in rats under different conditions of liver damage and repair. By immunohistochemistry and assessment of the density of nerve fibers labeled for tyrosine-hydroxylase (TH) and choline acetyltransferase (ChAT), three models of induced hepatic regeneration were examined: the carbon tetrachloride (CCl4) intoxication, with two treatment periods of 14 weeks (wk) and 18 wk; the partial hepatectomy (PH); the thyroid hormone (T3) treatment, for a total of 33 rats. For a semiquantitative evaluation of TH and ChAT immunohistochemical labelling, representative microscopic fields, taken from each experimental group, were blindly analyzed by quantitation of the density of TH- and ChAT-like immunoreactivity/cm2 and by the percentage of portal tracts harboring labeled nerve fibers. TH- and ChAT-like immunoreactive (LI) nerve fibers were detectable mostly in the portal spaces. The TH-LI ones occur only around blood vessels while ChAT-LI nerve fibers are localized around the neoductal clusters in the CCl4 rats. The quantitation of the TH-LI nerve fiber density in the portal areas showed a decrease in the CCl4 14wk treatment and after PH, and an increase after T3. The measurement of ChAT-LI nerve fiber density within the portal areas revealed an increase in the CCl4-treated rats while showing no change in the PH and T3-treated rats.
The density of perivascular TH- and ChAT-containing nerve fibers depends upon the type of subacute/chronic hepatic hyperplasia. The observed changes suggest a finely tuned autonomic modulation of hepatic blood flow depending on the type of subacute/chronic induced hyperplasia, while the characteristic occurrence of the periductal cholinergic innervation under the CCl4-induced post-necrotic regeneration implies a selective parasympathetic role in regulating the regenerative potential of the rat cholangiocytes.

SESSION III
BRAIN TUMORS

PITUITARY ADENYLYL CYCLASE-ACTIVATING PEPTIDE (PACAP) COUNTERACTS GLIOBLASTOMA PROGRESSION BY INTERFERING WITH HYPOXIC SIGNALLING PATHWAY

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Glioblastoma multiforme (GBM) represents the deadliest brain cancer, with poor prognosis since therapeutic resistance and relapse characterize it after surgery. GBM is a heterogeneous mass, containing infiltrating cells, blood vessels, secreted molecules, and surrounding matrix. Uncontrolled cell proliferation creates hypoxic niches inside the tumour core favouring cancer development. The hypoxic microenvironment induces intracellular transcription of hypoxiainducible factors (HIFs) that translocating into the nucleus activating transcription of many downstream target genes including epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) gene. Intracellular hypoxia is also responsible of epithelial to mesenchymal phenotype transition (EMT) of the cells, an event also responsible for malignant progression. Since recent data have demonstrated that pituitary adenylyl cyclase-activating peptide (PACAP) is involved GBM progression, we aimed to characterize the molecular mechanisms underlying this effect. Results showed that PACAP act in GBM by interfering with hypoxic signaling pathway. In particular, PACAP is able to modulate HIFs expression resulting in downregulation of VEGF expression and its release in extracellular compartment, reduction of EGFR transactivation and it is able to counteract EMT process. These findings contributed to elucidate the modulatory role exerted by PACAP in GBM malignancy.

CHANGES OF THE BRAIN-TUMOR INTERFACE AND NEURO-IMMUNE MODULATION DURING GLIOBLASTOMA PROGRESSION

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The Glioblastoma multiforme (GBM) represents the most prevalent phenotype and the most malignant of all primary brain tumours. The cellular heterogeneity, the intimate interaction with the environment and its scarcely predictable progression rely on

the different anatomical and biological signatures of GBM, but many of the features ensuring the immune-escape of malignant cells remain unresolved. This interplay induces molecular interaction and eventually genetic and epigenetic modifications that could play a pivotal role. Here we try to consider the dynamic interactions between resident glia, innate immune system and extracellular matrix (ECM). The time-related changes in the GBM environment will be characterized through a syngeneic mouse model of primary GBM. The GL261 glioma cells have been injected in the right striatum of C57Bl/6 mice, without immunodeficiency, and animals were sacrificed after 7, 14, and 21 days (7D, 14D, 21D). The tumour development was verified with an innovative 3D tomographic imaging before *ex vivo* immunostaining, and Western blotting. Moreover, to validate our analysis in the human pathophysiology we used a transcriptomic database considering the changes in transcription of homologous genes. The dynamic of GBM progression is granted by the silencing of microglia/macrophage activity, within the growing bulk. The astrocytic reaction surrounded as a dense scar the growing GBM. The immune function mostly impaired were recruitment and antigen presentation, while the invasion was prompted by the ECM modification. The present study shed new light on the role of brain-tumour interaction, guiding the urge for time-dependent models that take into account all neurovascular and parenchymal components of the central nervous system.

SESSION IV
NEUROINFLAMMATION AND NEURODEGENERATION

THE ROLE OF GLIA IN THE BLOOD-BRAIN BARRIER INTEGRITY

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The blood-brain barrier (BBB) is an extremely selective structure sharing dynamic interactions both with neurons and glial cells, thus directly contributing to the formation of the neuro-vascular unit (NVU).The NVU term was leap out more than 20 years ago highlighting the close relationship between brain cells and cerebral vessels.[1]The complexity of the NVU is mainly due to the interconnection and the mutual influence of the single cellular compartment[2] and, among the plethora of different kind of cells, glial cells play a pivotal role in maintain the physiological brain homeostasis, together with the contribution for the BBB formation.[3] On the other hand, the glial compartment alteration, has been demonstrated to indirectly affect the brain cells.[4] The aim of this study is to better understand the mechanisms underlying the alterations of the BBB, the first structure involved against toxic insult, as well as the role of glial cells, the *primum movens* in neurodegenerative disorders.Cell viability, oxidative stress marker as well as glial activation marker was evaluated both during physiological and toxic condition, such as the presence of cadmium acetate, at different concentration (1-10 μM), on astrocyte and microglial cell lines alone (DITNC1 and BV-2, respectively), or in co-culture with rat brain endothelial cells (RBE4). Our preliminary data indicate that, during physiological condition, astrocytes seems to reinforce the BBB integrity increasing the brain endothelial cells viability and better localizing the claudin 5 tight junction. On the other hand, microglial cells do not alter the brain endothelial cells viability during co-culture condition, in comparison to monoculture (RBE4 alone). Moreover, during cadmium acetate toxic insult, the presence of astrocytes in co-culture lend a better protection to RBE4 cells, in comparison to microglial cells. The microglia-dependent lack of protection might be explained by a more sensitivity to oxidative stress during toxic stimulation, especially at lower times (4 hours). In conclusion, these preliminary data on glial cells behavior on BBB preservation and alteration underline the main role of astrocytes in reinforcing and preventing the brain endothelial cells dysregulation. However, once microglial cells are damaged, this deleterious effect dramatically reflects on BBB integrity.

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SPATIOTEMPORAL DYNAMICS IN THE NEUROVASCULAR UNIT FOLLOWING PERIPHERAL NERVE INJURY

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The central nervous system (CNS) is significantly perturbed following peripheral nerve injuries and leads to maladaptive plasticity under chronic conditions. However, the molecular sequence for the system dysfunction is unknown and depends on the regionality of the neurovascular unit and its anatomic connections. In the present study, we investigated the spatiotemporal dynamics of the spinal neurovascular unit using the spared nerve injury (SNI) model. Rats were transcardially perfused at 24 h, 48 h, and 7 days after SNI or SHAM surgery. Immunohistochemistry and immunofluorescence techniques were performed to analyse the morphology and the molecular profile of the neurovascular unit including neurons, blood-brain barrier, microglia, macrophages, and extracellular matrix. Our results revealed a time-dependent and region-specific response in the dorsal and ventral horn of the lumbar spinal cord following peripheral nerve injury. Double-wave reactive gliosis (Iba1, GFAP) triggers neurovascular remodelling with an early increase in vascular density (laminin), thrombin signalling (PAR-1), and polarization of astrocytic endfeet (AQP-4).
Our findings shed new light on the CNS early modifications after nerve injury, which may be crucial for preventing the consequent maladaptive rewiring of the neurovascular unit, and prompt further studies to develop novel multi-targeted and time-dependent therapies.

THE BLOOD VESSELS NETWORK PROVIDES A PATHWAY TO MIGRATING SCHWANN CELLS TO COLONIZE NERVE CONDUITS

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The peripheral nervous system has the ability to regenerate itself when damage, but this property is not sufficient to guarantee a functional recovery after a damage with the loss of nervous substance. Indeed, the use of autograft or conduit to repair severe nerve injury is required to bridge the gap and avoid axon dispersion. Although the autograft technique is a surgical gold standard, the conduits are widely used to repair small gaps because of their comparable efficacy. A deeper comprehension of the cellular interactions inside a hollow tube could improve the design of nerve conduits in order to be able to repair even longer gaps. Until now, the migration of Schwann cells on endothelial cells has only been studied on the nerve bridge model, a spontaneous formation of nervous tissue to connect nerve stumps 2-3 mm apart. In this study, we investigate if endothelial cells provide a pathway to migrating Schwann cells also inside the nerve conduits used in the surgical practice to repair nerve gaps. Median nerves of adult female rats were injured and repaired with 10 mm chitosan conduits. The regenerated nerves, collected at different time points (7-, 14-, 21- and 28-days after the repair), were cut into slices 50 μm thick to be immunolabeled and analysed by confocal imaging. Our results show endothelial cells to form a dense capillary network, while only polarized vessels are used by clusters of Schwann cells to migrate from the two nerve stumps within the conduit. In conclusion, angiogenesis plays a key role to restore the functional architecture of the nerve by forming a framework for the migration of newly formed Schwann cells within conduit. The use of methods to boost vascularization might be an interesting strategy to support and enhance nerve regeneration when angiogenesis is impaired, as in long gap.

SESSION V
NEURODEGENERATION - SYNUCLEINOPATHIES

LINKING STRUCTURAL DEFECTS TO METABOLISM IN PARKINSON'S DISEASE

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Neurons are cells that strictly rely on a functional and highly specialized microtubular cytoskeleton, which is crucial to ensure brain health and physiology. Indeed, defective microtubules represent a common feature of several neurodegenerative disorders, including Parkinson's disease (PD). Post translational modifications (PTMs) of Tubulin and microtubules are key regulators of microtubule dynamics, and an unbalanced homeostasis of PTMs is reported to precede and likely trigger neurodegeneration by affecting microtubule stability in neurons. Using an immunohistochemistry approach on autoptic brain tissues and skin biopsies we demonstrated that tubulin acetylation, the PTM associated with more stable, flexible and resilient microtubules, is either reduced or redistributed in both central and peripheral nervous systems of PD patients. On this ground, we moved to an *in vitro* disease-model represented by neuron-like cells overexpressing either wild-type alpha-synuclein or its disease-related mutation A53T. In both conditions we observed lower levels of acetylated-tubulin and a more complex neurite branching, suggesting a change in microtubules stability and cell architecture. Since the effects of an unbalanced microtubule homeostasis is well known to be reflected on axonal transport and mitochondria trafficking, we searched for modifications of the metabolic state in cells overexpressing wild-type or A53T alpha-synuclein. In particular, we disclosed an uncoupled mitochondrial metabolism in transfected cells, reporting a switch towards a more glycolytic dependent metabolism, such as happens under hypoxia. Intriguingly, the administration of microtubules targeting agents (MTAs) was able to revert the phenotype by increasing tubulin acetylation in our model. On the other hand, MTAs treated cells were featured by an unbalanced glutamine metabolism, suggesting a close relationship between microtubules dynamics and cell metabolism. These data altogether sustain a key role for microtubules in PD's ethiopathogenesis, opening novel insights for research and therapy.

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ACETYLATED TUBULIN AFFECTS α-SYNUCLEIN PATHOLOGY

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Parkinson's disease (PD) belongs to a group of neurodegenerative diseases called "Synucleinopathies", characterized by α-Synuclein's alterations. This little protein is one of the most abundant of the human brain and, despite its physiological role is only partially elucidated, its involvement in pathology is a matter of fact. α-Synuclein is the main component of Lewy bodies (LBs) that, together with dopaminergic neurons' loss, are the pathological hallmarks of PD. The causes and the mechanisms that trigger its aggregation and accumulation in neurons remain unknown. The hypothesis is that α-Synuclein starts to form small aggregates, called oligomers, that grow to form fibrils and finally LBs, where α-Synuclein is stacked together with other intracellular elements like vesicles, organelles, and proteins. Among them, the presence of cytoskeletal proteins drawn our attention for different reasons. First, defects in microtubule regulation and dynamics are known to be involved in PD. Second, α-Synuclein is emerging as regulator of microtubule cytoskeleton. Third, very recently we highlighted a link between microtubule alterations and LB morphogenesis. Indeed, by investigating *post-mortem* human brain affected by PD, we observed a redistribution of acetylated tubulin in neurons that is linked to α-Synuclein aggregation. Starting from these considerations, we aimed to get mechanistic insights into α-Synuclein and acetylated tubulin interplay. Taking advantage of murine primary mesencephalic neurons, we first revealed that endogenous α-Synuclein colocalizes with acetylated tubulin along microtubule cytoskeleton in physiological condition. Then, we treated cells with tubacin, a selective inhibitor of HDAC6 that leads to an increase of acetylated tubulin, and we found that this interplay is altered. Thus, we suggest that α-Synuclein interacts physiologically with acetylated microtubules in neurons and that changes in acetylation regulate this interplay. Then, to investigate what could happen in pathological conditions, we moved to a neuroblastoma cell line overexpressing human α-Synuclein. Our preliminary results indicate that over-acetylation of microtubules promotes α-Synuclein aggregation into oligomers. All together our data support the hypothesis that alteration of the physiological interplay between α-Synuclein and tubulin can be a key actor in triggering α-Synuclein pathology.

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α-SYNUCLEIN GLIAL CYTOPLASMIC INCLUSIONS AS PRUNED LEWY NEURITES

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Synucleinopathies are neurodegenerative disorders characterized

by the accumulation of insoluble fibrillar α-synuclein (α-Syn). Parkinson's disease (PD) and dementia with Lewy bodies are characterized by intraneuronal α-Syn aggregates called Lewy bodies in the somas and Lewy Neurites in the neuronal processes, in multiple system atrophy (MSA) α-Syn aggregates are also found within oligodendrocytes (OLs) and named Glial Cytoplasmic Inclusions (GCIs). The origin of GCIs remains however a mistery because OLs barely express α-Syn, even in the MSA context. Mass spectrometry of α-Syn aggregates from PD and MSA suggests that GCIs are preassembled in neurons and secondarily incorporated into OLs. This possibility is compatible with the observation that intracerebral injections of α-Syn preformed fibrils (PFFs) in mice can induce a delayed pathology in OLs (after 18 to 24 months), long after the neuronal α-Syn pathology has peaked. We identified a new fibril strain of α-Syn (i) sharing biophysical characteristics with fibrils amplified from MSA patients, and (ii) uniquely capable of seeding neuronal nuclear inclusions reminiscent of the ones observed early in MSA. Our goal was to evaluate whether this particular strain of PFFs could also cause the buildup of GCIs in Ols *in vivo*. 129SV mice received unilateral intrastriatal injections of PFFs and were sacrificed after 6 or 28 weeks. α-Syn pathology was evidenced using the phosphoS129 α-Syn immunoreactivity. We found that even though PFFs were injected unilaterally, after 6 weeks phosphoSyn-positive neurons were present bilaterally in the layer V of the cortex, suggesting a fast retrograde progression of the pathology in both pyramidal and intra-telencephalic neurons. We thus scrutinized the anterior commissure, a myelinated intra-telencephalic fiber tract connecting both hemispheres: we observed many pSyn-positive Lewy Neurites oriented parallel to the main axis of the commissure, traveling at distance from OLs perikaria, and with no major sign of OL α-Syn pathology at this stage. After 6 months however, the Lewy Neurites were instead contorted, forming tangles in-between as well as inside the perikarya of the mature interfascicular OLs (Olig2 and Tau positive), indicating the pruning of the Lewy neurites by OLs. Our PFFs strain can thus induce a progressive model of neuronal α-synucleinopathy that can quickly transform into an OLs synucleinopathy typical of MSA, with GCIs acquired from neuronal processes.

POLY(ADP-RIBOSYL)ATION IN LEWY BODY MORPHOGENESIS

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Parkinson's disease (PD) etiology is still not clear and therapies currently pursued are not resolutive. Understanding the mechanism that leads to α-Synuclein aggregation and neuronal death is crucial for designing mechanism-based treatment strategies. In this field, Poly(ADP-ribosyl)ation (PARylation) has emerged as an important post-translational modification (PTM) catalyzed by PARP1 enzyme, that could be implicated in neurodegenerative diseases. In the context of PD, *in vivo* and *in vitro* studies have proposed PARylation to be able to induce α-Synuclein aggregation, and to activate a mitochondrial-mediated cell death pathway known as Parthanatos pathway. In addition, we previously demonstrated that PARP1 enzyme translocates from neuronal nucleus to cytoplasm in post-mortem human brain of PD patients. Interestingly, an increase of PAR polymers has been detected in the cerebrospinal fluid of PD patients. Based on that, the aim of the present study was to investigate the still unexplored PARylation state in post-mortem human brain obtained from patients in the late stage of PD (Braak stage 6), linking it with α-synuclein aggregation. Our results indicate that PAR polymers translocate from nucleus to cytoplasm of neurons, where they co-localize with different form of α–synuclein aggregates, including Lewy bodies. Moreover, using Proximity ligation assay (PLA) and 3D reconstruction, we focused on α-synuclein oligomers, which are the early stage and considered the main toxic species of aggregation, and demonstrated, for the first time, that they are PARylated. Furthermore, once translocated into the soma of substantia nigra dopaminergic neurons, PAR polymers also co-localize with mitochondria in PD patients. All together, these data point out an important involvement of PARylation in triggering α-synuclein aggregation and Parthanatos cell death pathway, helping to get novel insights into the pathological mechanisms leading to neurodegeneration and to find a target with the potential to halt or mitigate PD pathology.

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A NOVEL RIT2-LRRK2 INTERACTION IN LYSOSOME PROCESSING

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Lysosome dysfunction is recognized as a critical factor in Parkinson's disease (PD) pathogenesis. LRRK2 has a kinase-dependent role in autophagy and lysosomal function, and its pharmacological inhibition is effective against α-synuclein (αSyn) pathology. But the precise signaling pathways involving LRRK2 and how alterations lead to pathology haven't been clarified yet. In this work we unravel a novel functional interaction between LRRK2 and the small GTPase Rit2, previously reported to participate in MAPK-signaling, neurite outgrowth and dopamine transporter trafficking. Polymorphisms in *RIT2* locus are associated to increased risk of PD and are predicted to alter gene expression. We found that *RIT2* mRNA levels are reduced in *substantia nigra pars compacta* (SNpc) dopaminergic (DA) neurons of idiopathic PD patients and in different *in vitro* and *in vivo* PD models. Rit2 overexpression in G2019S-LRRK2 neuroblastoma cells restores lysosomal defects and diminishes αSyn accumulation, phenocopying pharmacological LRRK2 kinase inhibition. Moreover, Rit2 selective overexpression in SNpc DA neurons in A53T-αSyn virally injected mice reduces neurodegeneration and αSyn pathology. Notably, we uncovered that Rit2 interacts with LRRK2, and its overexpression inhibits LRRK2 kinase activity both *in vitro* and *in vivo*. In addition, Rit2 is required for lysosomal function since its knock-down in cell lines

and DA neurons leads to lysosomal defects. We have promising preliminary data indicating that the mechanism of Rit2 action in autophagy might involve lysosomal calcium release, which represent a convergent point in LRRK2-mediated regulation of autophagy. We propose Rit2 and its interplay with LRRK2 as novel targets for future therapeutic approaches.

SESSION VI
NEUROPLASTICITY AND BEHAVIOR

WHITE MATTER ACTIVITY PATTERNS PREDICT PERSONALITY TRAITS

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Personality is a higher-order psychological construct that refers to the unique patterns of thought, feelings, and behavior that distinguish people from each other. Personality traits lie at the core of the concept of human individuality and correlate with social and clinical features such as emotion expression, academic or job performance, or individual vulnerability to psychopathology. Among the various attempts performed to evaluate personality in a qualitative and quantitative manner, the five-factor model[1] has been affirmed as the leading psychometric paradigm in the field of personality neurosciences. With the advent of large-scale neuroimaging and connectomics, inter-individual differences in the five major personality traits featured in this model have been correlated to different aspects of brain structure and function. On the other hand, many of these findings were challenging to be reproduced reliably in larger samples or be fitted into robust predictive models. As any unimodal brain imaging modality captures only peculiar facets of the whole brain morphology and physiology, multi-modal imaging measures are likely more suitable to predict complex, higher-level behavioral measures such as personality traits. In this context, we applied track-weighted dynamic functional connectivity (tw-dFC), which is a multi-modal analysis technique combining structural, functional, and dynamic connectivity, on a relatively large sample of 205 healthy subjects from the LEMON repository. Functional white matter units derived by independent component analysis (ICA) of tw-dFC data have been recently employed to retrieve track-weighted dynamic functional connectomes and to build models predictive of individual cognitive performance.[2] Herein, we identified tw-dFC networks positively or negatively correlated to each personality trait; higher degree nodes included white matter connecting medial frontal and temporal cortices, the corpus callosum, the cingulum and subcortical structures such as the hippocampus and basal ganglia, and the cerebellum. Using a regression approach with leave-one-out cross-validation, we show that these networks can predict at least three personality traits, outperforming simple, static functional connectivity networks obtained from the same dataset.

We suggest that tw-dFC may represent an improvement over traditional connectivity methods for predicting complex behavioural measures, thus opening a new framework of analysis for a better understanding of the biological basis of human individuality.

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NANOPLATFORMS CONCEIVED FOR NOSE-TO-BRAIN DRUG DELIVERY

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Many approaches to brain delivery of bioactive compounds have emerged in recent years. Intranasal (IN) route is one of the newly focused delivery options for central nervous system (CNS) targeting, since this is the only way through which the brain is in connection with the outside environment. Nose-to-brain (N2B) drug delivery can be achieved by different mechanisms that are not yet fully understood. Drugs can be transported directly from the nasal cavity to the CNS by the trigeminal and the olfactory nerves pathway bypassing the blood brain barrier. In order to overcome some of the limitations associated with the administration of free drugs (*i.e.* rapid elimination, poor water solubility, stability etc.), the use of nanomedicine could be considered a promising approach and is under intense investigation. Nanoplatforms of various nature [polymeric nanoparticles (PNP), lipid nanoparticles (SLN, NLC) nanocrystals (NCs)], characterized by different physico-chemical and technological properties have been designed to improve drug transfer after N2B delivery. Fluorescent dye (Rhodamine B) loaded PNP have been detected after IN administration in healthy rats to evaluate their distribution in brain areas. Once confirmed PNP transport to hippocampus, assessment of drug efficacy was performed after IN administration of oxcarbazepine loaded PNP (OXC_PNP) in an *in vivo* model of epilepsy. Our results demonstrated significant protective effects against seizures with a reduced dose and number administrations of OXC_PNP compared to the neat drug. Recently, nanocrystalline drug technology approach was also investigated to overcome one of the main limitations of colloidal systems, namely the difficulty to obtain a highly concentrated drug formulation in the small volume necessary for IN administration. NCs were formulated starting from natural (curcumin, resveratrol) and synthetic molecules (carbamazepine) obtaining concentrated formulations with high drug loading, particularly useful for for the treatment of neurodegenerative diseases by the N2B strategy. NCs have been evaluated *in vitro* on Olfactory Ensheating Cells, a unique class of glia that envelopes bundles of olfactory axons, that project to the olfactory bulb which we consider to play a key role in the direct pathway of drug transport through the N2B route. Overall, nanomedicine combined with IN administration is a relevant research topic that can rise as a hope for a new era of brain diseases treatment._

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BDNF AND TRKB IN THE HIPPOCAMPUS OF ROMAN RATS: COMPARATIVE EFFECTS OF FORCED SWIM AND TAIL PINCH

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The brain-derived neurotrophic factor (BDNF) has a role in the pathogenesis of depression as shown by evidence that a reduction of BDNF expression occurs in *post-mortem* brains and serum of depressed subjects and that the BDNF gene is required for the response to antidepressant drugs. The outbred Roman High-Avoidance (RHA) and the Roman Low-Avoidance (RLA) rats are a model designed to investigate the impact of genetic and environmental factors on the neural substrate of depression. They were selected for rapid (RHA) vs. extremely poor (RLA) acquisition of active avoidance, in a shuttle-box. It has been shown that emotional reactivity is the most prominent behavioral difference between the two lines, with the RLA rats being more fearful/anxious than their RHA counterparts. Here, we use the Roman rats, submitted either to 40 min of tail pinch (TP) or to a 15 min session of forced swim (FS), to compare the impact of different aversive situations on the coping ability of the two lines and on the immunochemical occurrence of BDNF and its receptor trkB in the dorsal (dHC) and ventral hippocampus (vHC). Results obtained show that RLA rats display a depression-like behavior in FS and in TP, while their RHA counterparts in face of both stressors exhibit a proactive coping style aimed at gaining control over the stressor. According to previous data, the WB analysis showed that the basal BDNF levels are lower in the dHC and vHC of RLA than RHA rats. Moreover, in RLA rats, FS elicited opposite changes on the BDNF levels in the hippocampal subregions examined, with no alterations in trkB-LI in either subregion, suggesting that FS may hinder plastic events in RLA rats, but not in their resilient RHA counterparts. In RHA rats, TP increased the basal level of BDNF-LI and trkB in the dHC while in the vHC TP decreased trkB-LI but failed to modify the basal BDNF-LI. The immunohistochemistry showed that both the FS and TP induced in both Roman rats line-related changes that were appreciable as differential regulation of BDNF-LI neuronal structures in the CA subfields and dentate gyrus (DG) in the dHC *versus* the vHC; line-related changes were also observed in the expression of trkB. Collectively, these results demonstrate that FS and TP stress have a differential impact on the baseline BDNF signalling, eliciting different changes in the dHC *versus* the vHC, and between the two rat lines.

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A NEW ANIMAL MODEL FOR THE STUDY OF GAMING DISORDER: SEX DIFFERENCE IN BRAIN CIRCUITS

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Although game is an important part of human behavioral development, recent studies show that children and adolescents who use electronic media, for more time, may experience intra- and interpersonal risk factors. Because of its strong similarity to addictive disorders and along with social anxiety and attention deficit, loss of control over gaming has been termed as "Gaming Disorder" (GD). However, the various studies conducted in recent years show several limitations, such as exposure period, duration, and gender. The purpose of this work is to validate an animal model of GD in rat, which exhibits sex differences in susceptibility, addictive behaviour, and brain activity of areas implicated in GD. We developed, for the first time, using a new apparatus provided with a touchscreen platform, a GD rat model that resembles the fundamental features of the disorder (*e.g.*, addiction, hyperactivity). After five weeks of training, male and female Wistar Kyoto (WKY) rats were assessed for: a) their attachment to the game under different condition, b) their compulsiveness during gaming, and c) the maintenance of these conditions after a period of game pause and a reward interruption. According to the multicriteria described in the literature, it was possible to identify GD-rats in 16/18 males and in 21/21 females, which obtained scores between 66 and 99%. GD-rats showed a significant increase in frequency and duration of play, and time spent in front of the screen compared to both controls and rats which have been trained but did not develop an addiction, with a greater accentuation of these behaviours in the group of GD females. Moreover, through immunohistochemical techniques, we performed several morphological investigations in the brain of these animals: we analysed whether there was a different activation in the areas of circuits involved in addiction through the immunohistochemical detection of c-Fos positivity (a marker of neuronal activity); activation of anxiety- and stress-related regions, the mesocorticolimbic reward system, and decision-making and motor learning circuits are found to be impaired in GD groups compared with CON groups. In addition, analysing the reward system, both dopamine and serotonin immunoreactivity was found modified in the VTA and DRN of GD groups.
In conclusion, we propose a rat animal model of GD that exhibit features also found in GD patients such as the development of addiction-like behaviours, sex difference in susceptibility, and changes in brain activity. The use of our animal model of GD will allow us to further study the neurological basis of the disorder while also accounting for sex differences.

SESSION VII
GLIAL CELLS AND NEUROINFLAMMATION

ACM-GFS MODULATE DNA RNA ERK SYNTHESIS IN ASTROCYTES

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Astroglial conditioned media (ACM) and growth factors (GFs) influence the development and maturation of cultured Astrocytes. The present work has assessed the effect of ACM collected from 15, 30, 60 or 90 days *in vitro* (DIV) on developing (15 or 30 DIV) cultured astrocytes pre-treated with growth factors (EGF, bFGF, IGF-I or INS). The study was specifically designed to assess up and down modulation by exogenous growth factors during interactive crosstalk with endogenous growth factors, released in ACM harvested from different stages of maturation of astrocyte cultures.
Addition for 24 h of ACM obtained at 30, 60 or 90 DIV significantly reduced DNA labeling in 15 or 30 DIV astrocytes pretreated for 12 h with EGF. A slight but significant increase of DNA labelling was found in EGF- pretreated cultures at 15 or 30 DIV compared to control cultures. Addition of ACM obtained at 15 DIV induced a marked stimulation of DNA labeling in 12 h epidermal growth factor-pretreated 30 DIV cultures. This effect was more pronounced after EGF treatment. Addition of ACM to 15 DIV cultures from 30 or 60 or 90 DIV after 12 h pretreatment with EGF markedly inhibited DNA labelling. Addition for 24 h of ACM obtained at 30, 60 or 90 DIV significantly reduced DNA labeling in 15 or 30 DIV astrocytes pretreated for 12 h with bFGF. Reduction in DNA labelling was found in 15 DIV bFGF-pretreated cultures. Addition of ACM to 15 DIV cultures from 30 or 60 or 90 DIV after 12 h pretreatment with bFGF, markedly inhibited DNA labelling. Addition for 24 h of ACM obtained at 30, 60 or 90 DIV significantly reduced DNA labelling in 15 or 30 DIV astrocytes pre-treated for 12 h with INS. At 12 h INS pretreatment remarkably increased DNA labelling.
Addition of ACM to 15 DIV cultures from 30 or 60 or 90 DIV after 12 h pre-treatment with INS markedly inhibited DNA labelling. Addition for 24 h of ACM obtained at 30, 60 or 90 DIV significantly reduced DNA labelling in 15 or 30 DIV astrocytes pre-treated for 12 h with IGF- I. ACM Addition to 15 DIV cultures from 30, 60, 90 DIV after 12 h IGF-I pretreatment inhibited DNA. ACM collected from 15 or 60 or 90 DIV increased RNA labelling of 15 and 30 DIV astrocyte cultures, being the highest value that of 30 DIV cultures added with ACM from 90 DIV. The findings of increased DNA labelling after EGF or INS pre-treatment in 30 DIV cultures, followed by addition of ACM from 15 DIV cultures, suggest that these phenomena may depend on extra cellular signal-regulated kinase 1 (ERK1) activation.

BISPHENOLS AS NEW ENVIRONMENTAL RISK FACTORS IN MULTIPLE SCLEROSIS

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Epidemiological studies support the idea that multiple sclerosis (MS) is a multifactorial disease overlapping genetic, epigenetic, and environmental factors. A better definition of environmental risks is critical to understand both the aetiology and the sex-related differences of MS. Exposure to Endocrine Disrupting Compounds (EDCs) fully represents one of these risks. EDCs are natural or synthetic exogenous substances (or mixtures) that alter the functions of the endocrine system. Among synthetic EDCs, exposure to bisphenol A (BPA) has been implicated in the aetiology of MS, but controversial data has emerged to date. Furthermore, nothing is known about bisphenol S (BPS), one of the most widely used substitutes for BPA. As exposure to bisphenols will not disappear soon, it is necessary to clarify their role in this pathological condition, defining their impact on disease onset and progression. In this study, we examined, in both sexes, the effects of perinatal exposure to BPA and BPS in one of the most widely used mouse models of MS, experimental autoimmune encephalomyelitis (EAE). Exposure to bisphenols seemed to be particularly deleterious in males. In fact, both BPA- and BPS-treated males showed anticipation of the disease onset and an increased motoneuron loss in the spinal cord. Overall, BPA-treated males also displayed an exacerbation of the EAE course and an increase in inflammation markers in the spinal cord. Among females, the treatments did not significantly affect the analysed disease-related parameters, confirming the sex-specific effects of perinatal exposure to bisphenols also in this pathological condition. Analysing the consequences of bisphenols exposure on EAE will help better understand the role of both xenoestrogens and endogenous estrogens on the sexually dimorphic features of MS.

BENEFICIAL EFFECTS OF MSC TREATMENT ON CCL2-MICROGLIA-MEDIATED CEREBRAL CORTEX NEUROINFLAMMATION: EMERGING ROLES FOR MSC-DERIVED EXTRACELLULAR VESICLES

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Experimental autoimmune encephalomyelitis (EAE) in mouse neocortex is characterized by inflammation, demyelination and blood-brain barrier (BBB) dysfunction. Treatment with mesenchymal stem cells (MSCs) ameliorates the clinical course of EAE, significantly reducing neuroinflammation, demyelination and astrogliosis, while improving BBB function. In the present study, using the same experimental model of EAE-affected MSC-treated mice, we have investigated the cellular sources of CCL2, a chemokine claimed to be primarily expressed by astrocytes and endothelial cells, involved in leukocytes recruitment and BBB impairment during neuroinflammation. The analysis was carried out by immunohistochemistry (IHC) and dual RNAscope IHC/*in situ* hybridization, using novel microglia-specific markers, *i.e.* TMEM119 and SALL1, combined with astrocytic- and endothelial-specific markers and with antibody against CCL2. The results show the presence of activated hypertrophic microglia cells, which express high levels of CCL2 in the EAE mouse neocortex. Microglia activation and CCL2 expression in MSC-treated and untreated EAE mice, have been evaluated and compared by morphometric parameters. According to these results, the neocortex of EAE-affected MSC-treated mice is characterized by a reduced microglia reactivity and a lower CCL2 expression, together with restored BBB structure and function. The role of MSCs and the possible mechanism by which these cells counteract neuroinflammation was furtherly investigated, transfecting these cells with GFP lentivirus and analyzing their viability at the level of the mouse lungs, a site were MSCs are known to be trapped. The results show that, after venous administration, MSCs localize, in both control and EAE mice, within the alveolar septa. MSCs detected in EAE-affected mouse lungs express the multivesicular body marker CD9 and seem to release extracellular vesicles (EVs). The idea that MSC-derived EVs may be involved in the effectiveness of MSC treatment, through the interaction with endothelial cells, was supported by the immunodetection of the MSC marker endoglin (CD105). The observed clustering of endoglin at the endothelium-MSC interface indicates the glycoprotein as a potential partner in endothelium-MSC interaction and suggests a possible mechanism of MSC-derived EV release at the alveolar interstitium-capillary barrier.

THE S100B PROTEIN AS A THERAPEUTIC TARGET FOR MULTIPLE SCLEROSIS PROCESSES

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S100B is a calcium-binding protein mainly concentrated in astrocytes. Its levels in biological fluids are recognized as a reliable, even predictive, biomarker of active neural distress. Mounting evidence now points to S100B as a Damage-Associated Molecular Pattern protein which, when released at high concentration, triggers tissue reaction to damage in various disorders.[1,2]

A number of correlative evidence proposes that S100B high lev-

els may play a promoting role also in multiple sclerosis (MS).[3-5] We showed that in the relapsing-remitting experimental autoimmune encephalomyelitis (EAE) mouse MS model, the inhibitor of S100B activity pentamidine (PTM) ameliorates clinical scores and neuropathologic-biomolecular parameters.[6]
Also, arundic acid (AA), an inhibitor of astrocytic S100B synthesis, in the chronic EAE mouse model, by the evaluation of clinical scores and neuropathologic-molecular analysis, induced lower severity compared to the vehicle-treated mice, particularly in the early phase of disease onset.[7] A significant reduction of astrocytosis, demyelination, immune infiltrates, proinflammatory cytokines expression and enzymatic oxidative reactivity in the AA-treated group was observed, indicating the participation of S100B in neuroinflammatory processes, reasonably as an astrocytic activity. The active participation of astrocytes in S100B-induced MS processes is currently studied.
This scenario proposes S100B as a therapeutic target for MS, as for different neural disorders appearing to share some common pathogenic features, reasonably attributable to neuroinflammation.[1,2]

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GHRELIN AND MICROGLIA FRIENDS OR FOES IN NEUROINFLAMMATION

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Neuroinflammation induces neurometabolic alterations and increases in energy consumption. Recent research has attempted to clarify the role of Ghrelin (Ghre) signaling in microglia on the regulation of energy balance, obesity, neuroinflammation and the occurrence of neurodegenerative diseases. This orexigenic hormone not only regulates food intake, energy content and glucose homeostasis, but also modulates plasticity and cognition in the central nervous system (CNS). Additionally, microglia may constitute an important therapeutic target in neuroinflammation. In fact, microglial cells are able of producing a wide range of chemokines to promote inflammatory processes being the protective cells of the CNS. These cells have also been identified as specialized macrophages sharing many phenotypical and functional characteristics. Ghre and microglia are involved in the pathophysiology of neurodegenerative diseases characterized by neuronal damage such as Alzheimer's disease and Parkinson's disease. The reported evidence show that Ghre modulates microglia activity and thus affecting pathophysiology of neurodegenerative diseases interferes in the control of neurometabolism. The aim was to evaluate the underline mechanisms by which Ghre modulates microglia activity during obesity-induced neuroinflammation by emphasizing the effects of Ghre in inducing these cells towards an anti-inflammatory phenotype. The understanding of this peptide's functions will allow for the development and implementation of new therapeutic and neurological diagnostic strategies.

ASC-EV THROUGH NASAL EPITHELIUM: EFFECT ON INJURED NEURONAL CELLS

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Adipose mesenchymal stem cells (ASCs) represent a feasible and valid alternative to other sources of mesenchymal stem cells in the treatment of several neurological disorders. Currently, the scientific community brings a growing body of evidence indicating that ASCs exert their neuroprotective and immunomodulatory effects by a paracrine mechanism through the release of extracellular vesicles (EVs). Indeed, EVs are considered important mediators in intercellular communication as they can transfer their cargo (proteins, miRNAs and mRNAs) to nearby cells promoting nerve regeneration, neuronal protection, synaptic plasticity and remyelination in different pathophysiological contexts, recapitulating the effect of origin cells. However, an EVs-based therapy needs to identify an advantageous route of administration to ameliorate their biodistribution: in this context we explore the intranasal (i.n.) route as a non-invasive strategy to deliver therapeutic agents directly to the brain. In particular, the aim of this study was to set up an *in vitro* model to mimic the i.n. delivery of ASC-EVs. Furthermore, we wanted to evaluate their neuroprotective effect after their passage through the nasal barrier on an oxidative stress-induced model of both motor neuron (NSC-34) and neuron (SH-SY5Y) cells. Regarding the study of i.n. route, it is also crucial to identify the mechanisms by which EVs are able to cross the epithelium and reach the central nervous system. To do that we set up a fluorescent labelling protocol to isolate and characterize labelled ASC-EVs and to evaluate their uptake by injured NSC-34 cells. The results showed that ASC-EVs neuroprotective effects observed in previous studies were maintained after their passage through the nasal epithelium as well, with a rescue of the neuronal cells viability after oxidative stress. Moreover, the ASC-EVs labelling protocol allowed us to isolate fluorescent EVs that will pave the way for further studies in order to clarify their passage after i.n. administration and their capture by injured cells in the central nervous system.

POSTERS SESSION

MATRIX METALLOPROTEINASES, PURINERGIC SIGNALING, AND EPIGENETICS: HUBS IN THE SPINAL NEUROGLIAL NETWORK FOLLOWING PERIPHERAL NERVE INJURY

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Nerve injury triggers multiple mechanisms in the central nervous system that are largely unknown and lead to maladaptive plasticity and chronic disorders. However, reactive gliosis made up of glial cells' response to the trauma remains a central event. Among the features of glial inflammation, we investigated the role of the purinergic system, extracellular matrix (ECM), and epigenetic involvement in the dynamic of reactive gliosis. We used the spared nerve injury (SNI) model to induce a perturbation to the central nervous system and studied the morpho-molecular sequence and the modulation of the spinal maladaptive plasticity through the *in vivo* administration of oxidized ATP (oxATP), an antagonist of P2X receptors (P2XR), and/or GM6001, an inhibitor of MMPs, in rats for 3 or 8 days. Immunohistochemistry, immunofluorescence, and Western blot techniques were performed on lumbar spinal cord samples. Our study revealed time-dependent waves of spinal reactive gliosis and modifications in the neuro-glial-ECM crosstalk via remodelling of P2XRs expression, nerve growth factor (NGF) axis (TrkA and p75), and histone deacetylases after treatments with oxATP or GM6001. Altogether, our data suggest that MMPs and purinergic inhibition impact crucial proteins in the reactive gliosis pathways, acting at different levels, from intracellular signalling to epigenetic modifications, and prompting novel therapies for the modulation of the maladaptive spinal plasticity.

NEW IMMUNOHISTOCHEMICAL DATA ON THE CEREBELLAR SYNARMOTIC NEURON TYPE

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Previous immunohistochemical studies in the cerebellar cortex revealed a wide distribution of subpopulations of different large neuron types, called 'non-traditional large neurons', which are distributed in three different zones of the granular layer. These large neuron types are mainly involved in local circuits inside the cerebellar cortex. A subpopulation of these neuron types is represented by the synarmotic neuron, which, on the other hand may rather play a projective role within the cerebellar circuitries. The synarmotic neuron cell body map within the internal zone of the granular layer or in the subjacent white substance, and the axon cross the granular layer and run in the subcortical white substance for long distance. In fact, since its first description of the synarmotic neuron a role in projective circuits has been attributed. Therefore, in the cerebellar cortex together with the Purkinje neuron, the traditional projective neuron type, the synarmotic neuron could be to represent the second projective neuron type of the cerebellar cortex. Studies on the neurochemical features on the synarmotic neuron are scanty. Currently, mainly an inhibitory GABAergic nature of the synarmotic neuron has been evidenced. The study was carried out on fragments of *post mortem* human cerebellar cortex 36-48h after death and on recent specimens of mouse cerebellar cortex. Each fragment was fixed in an aldehyde and picric acid solution, embedded in paraffin, cut into 5 μm sections, and subjected to light microscopy immunohistochemical procedures using rabbit polyclonal antibodies respectively against glutamic acid decarboxylase isoforms 65/67 (GAD65/67), neurotensin (NT), neurotesin receptor type 1 (NTR1), serotonin (5-HT), dopamine transporter (DAT) and contactin-1 (CT-1). The immunoreactions revealed in the granular layer of the human cerebellar cortex the presence of synarmotic neuronal cell bodies and processes immunoreactive to GAD65/67, NTR1, 5-HT, DAT and in the granular layer of the mouse cerebellar cortex the presence of CT-1 immunoreactive neuronal cell bodies and processes of synarmotic neurons. Therefore, this immunohistochemical study confirm the GABAergic nature of the synarmotic neuron and in addition, it open a new scenario on the neurochemical data of the synarmotic neuron, suggesting a role of this non-traditional large neuron type in neurotransmission/neuromodulation mechanisms.

IMPROVING REGENERATIVE CAPABILITIES OF PERIPHERAL NERVOUS CELLS WITH CHITOSAN MICROSTRUCTURED AND FUNCTIONALIZED MEMBRANES

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The current treatment of localized prostate cancer is radical prostatectomy with frequent iatrogenic damages to the periprostatic neurovascular bundles (NVB) that can lead to erectile dysfunctions. Our research teams previously demonstrated that the intraoperative application of a chitosan membrane is safe and able to promote a faster potency recovery. Moreover, *ex vivo* and *in vitro* studies assessed the neuro-regenerative effect of chitosan membrane on *ex vivo* cultures of autonomic ganglia and its anti-proliferative effect on metastatic prostatic cancer cells. The aim of the present study was to test *in vitro* and to develop a functionalized microstructured chitosan membrane to support and promote the main regenerative mechanisms, underlying nerve outgrowth and glial cell survival and proliferation, for an *in vivo* use perspective, to repair a lesion affecting the cavernous nerve. The chitosan, a biomaterial of natural origin, was blended with 5-

15% glycerol and micropatterned to obtain the well-established gratings (GR) and the improved asymmetric pattern with scalene triangles (SCA), both able to induce directional stimuli to cells. Moreover, the controlled release of phosphodiesterase inhibitors (PDEI) was designed to chemically promote nerve regeneration and functional recovery. The results of *in vitro* and *ex vivo* direct cultures on microstructured chitosan membranes suggest that the substrates are useful for the oriented growth of neurons, a very important step in making regeneration more effective. The *in vitro* protocol for the administration of PDEI (sildenafil-PDE5I and rolipram-PDE4I) was developed and for both stimulations an interesting gene regulation linked to the neuroprotective brain-derived neurotrophic factor (BDNF) and the proangiogenic Vascular endothelial growth factor (VEGF) in immortalized cultures of sensory and motor neurons was observed. In glial cell cultures, the administration of PDEI resulted in up-regulation of the transcription factor Krox20, which can positively influence the expression of myelin genes and in a decrease cell migration. Furthermore, the administration of Rolipram has been shown to induce an increase in neuritic extension in neuronal populations. Further investigations are underway to deepen the study of the effect of PDEI administration on organotypic cultures (dorsal root ganglia and autonomic ganglia), where neuronal and glial cells co-exist, *ex vivo* models more similar to what happens *in vivo*.

THALAMIC MORPHO-FUNCTIONAL CHANGES FOLLOWING ELECTRICAL STIMULATION OF THE AXOTOMIZED TRIGEMINAL NERVE

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To understand the morpho-functional changes of the thalamic nuclei following stimulation of the transected infraorbital branch of trigeminal nerve. Continuous electric stimulation was applied to the proximal stump of axotomized left infraorbital branch of trigeminal nerve, 12h/day for four weeks. Brain sections were immunostained for cytochrome oxidase (CyO), paravalbumin (Pv) and calbindin (Cb) and quantified in the ventral posteromedial o (VPM), posterior o (PO) and reticular (Rt) thalamic nuclei. Intragroup comparisons between left and right sides and intergroup comparisons between control, axotomized and stimulated-axotomized animals were performed. Axotomization of trigeminal nerve reduced the number of positive Pv and Cb cells in the Rt and the CyO density in all the analyzed thalamic nuclei. Electrical stimulation of the proximal nerve stump restored the cellular density in the Rt and the CyO density. Trigeminal nerve transection induces morpho-functional changes in the thalamus that might trigger chronic neuropathic trigeminal pain. These maladaptive changes are rescued by peripheral electric stimulation, that might represent a potential therapeutic strategy.

ACETYLCHOLINE PRECURSORS ATTENUATE NEUROINFLAMMATION IN LPS-STIMULATED BV2 CELLS

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Choline-containing phospholipids, choline alphoscerate (α-GPC), and cytidine 5′-diphosphocholine (CDP-choline) are both acetylcholine precursors crossing the blood-brain barrier. As procholinergic nootropic agents, studies have provided their neuroprotective effects. Currently, there is a limited number of studies concerning whether they have a similar effect in treating cognitive impairment. Indeed, contradictory results have been reported in their mechanisms of action on the neurovascular units. Since microglia play a crucial role in neuronal damage and protection, this study investigated the effects of α-GPC and CDP-choline on the inflammatory response in activated microglia using an immortalized murine microglial cell line (BV-2) stimulated with lipopolysaccharide (LPS). BV2 microglia were treated with or without LPS and were incubated with LPS and different concentrations of both acetylcholine precursors for 24 h. MTT assay, immunocytochemistry, and Western blotting methods were utilized. MTT assay did not show significant changes in cell viability after treatments at different concentrations. Here, we report no differences in untreated cells. On the contrary, morphological changes and an increase in ionized calcium-binding adapter molecule 1 (Iba1) expression were found in LPS-stimulated BV-2 cells. In addition, the nuclear translocation of nuclear factor-kappa B (NF-κB) and the up-regulation of inflammatory interleukin-1β (IL-1β) were accompanied by an increase in oxidative state proteins and lipid peroxidation in LPS-treated BV2 cells. These alterations were reversed after the treatments with both α-GPC and CDP-choline. Our data demonstrate that these compounds attenuate equally LPS-induced neuroinflammatory responses and suggest insights to explain their therapeutic role in brain disorders characterized by vascular impairment.

STRATEGIES TO IMPROVE PROSTATIC NERVE REGENERATION AFTER RADICAL PROSTATECTOMY

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Prostate cancer is the most frequent cancer among males surpassing the lung and the colorectal cancers, representing the second cause of cancer mortality in industrialized countries. The current treatment of localized prostate cancer in patients with a life-expectancy >10 years is radical prostatectomy (RP). Unfortunately, in patients who undergo RP, frequently iatrogenic

damage to the periprostatic neurovascular bundles (NVB) occurs, leading to erectile dysfunction and impairment in quality of life. Recently, new strategies to improve the regeneration of the prostatic nerves are arising to reach the functional recovery. Among these, chitosan, a derivative of chitin obtained from the exoskeleton of crustaceans with useful properties in intraoperative field such as hypoallergenicity, biocompatibility, bioavailability and lack of toxicity. At this purpose, *ex vivo* experiments performed on autonomic explant ganglia have been showed the neuro-regenerative effect of a flat chitosan membrane reporting a higher neurite outgrowth. At the same time, in *vitro* experiments on metastatic cancer cell lines displayed a lower proliferation rate when cultured with chitosan coating and dissolution products. The safety and the ability of the flat chitosan membrane to promote nerve regeneration was tested in clinical urological resulting in higher potency recovery rate in patients that have undergone radical prostatectomy. To improve the regenerative performance achieved by the flat membrane, nanostructured membranes with two different topographies, grating arrangement and a zig-zag pattern were used to repair cavernous nerve of adult male rats: a 3 mm of cavernous nerve was bilaterally transected and repaired with chitosan membranes (flat, grating, zig-zag). 60 days after the surgical procedure, samples were harvested and morphological analysis were carried out to identify the presence of nerve fibers. In order to obtain a three-dimensional visualization of the nerve fibers on the membranes, a whole-mount immunolabeling and clearing technique "iDISCO" was used allowing to detect the pathway of nerve fibers on the whole membrane. These *in vivo* results provide the first experimental evidence supporting the ability of the chitosan membrane to allow autonomic axonal regeneration demonstrating the safety of the device for clinical use and supporting its application as an effective adjunct strategy to reach the functional recovery after RP.

VITAMIN C EFFECTS ON THE GSK3β SIGNALING PATHWAY

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Neuroinflammation is a defence mechanism finalized to the preservation of the brain in response to inflammatory stimuli. However, a prolonged inflammatory status can be deleterious since neuroinflammation is implicated in the pathogenesis of neurodegenerative disorders together with oxidative stress. Vitamin C (Vit C) is known to have anti-inflammatory and antioxidant properties but, although its neuroprotective effects have been elucidated, the underlying molecular mechanisms remain unclear. Since glycogen synthase kinase 3β (GSK3β) is a serine/threonine kinase which acts as a potent driver of inflammation, this may suggest that targeting GSK3β pharmacologically could provide a therapeutic mechanism to control neuroinflammation. In this study, we have investigated the role of GSK3β inactivation in the context of Vit C neuroprotective effects by using a well-known 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced animal model of Parkinson's disease (PD) and LPS-treated BV2 cells as a cellular model for neuroinflammation. We found that Vit C promotes the p-p38 MAPK-induced inactivation of GSK3β alleviating the inflammatory responses and boosting the antioxidant responses. In addition, the anti-inflammatory M2 phenotype is favoured over the pro-inflammatory M1 phenotype, and the microglial reactivity reduced. *In silico* analysis suggests that Vit C may antagonize with the activated form of p38 MAPK, proposing a role in maintaining low levels of p-p38 in an inflammatory context by aiming the GSK3β pathway towards anti-inflammatory and anti-oxidant responses. Collectively, these results demonstrate that Vit C exhibits substantial neuroprotective effects through the modulation of GSK3β pathway, attenuating pro-inflammatory and up-regulating anti-inflammatory processes.

DYNAMICS OF REACTIVE GLIOSIS IN THE PROGRESSION OF GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBM) is the most aggressive brain tumor with a malignant prognosis and is characterized by intra-tumor and inter-tumor heterogeneity. The tumor microenvironment (TME), which includes brain resident cells, extracellular matrix (ECM), and vascular components, contributes to the GBM heterogeneity and is a key regulator of tumor progression. To untangle the relationship between the GBM and the brain microenvironment, time-dependent molecular targets and regionalization of the pathological process should be determined. GL261 glioma cells were unilaterally injected into the striatum of immuno-competent C57Bl6J mice, and the brains were extracted after 7, 14, and 21 days (7D, 14D, 21D). Immunohistochemistry and Western blotting analysis were performed. The tumor bulk was established at 14D with necrotic areas persisting at 21D. The tumor growth was paralleled by a differential response from the astrocytes and microglia, which make up the reactive gliosis. Reactive astrocytes (GFAP+) density in the peritumoral region increased in response to GBM progression. Surprisingly, microglia (Iba1+, TMEM119+ cells) were scarcely detected at the earliest stages of GBM development, while macrophages (Iba1+ cells) infiltrate the brain tissue at 21D. In addition, antigen-presenting function and microglia/macrophages-associated inflammation were compromised (MCP-1/CCL2, MHC-II) and then restored in the final stages, suggesting a differential regulation for tumor-associated microglia and macrophages during GBM growth. The intricate dynamics of the reactive gliosis also led to a time-dependent expression of the extracellular matrix components and variable tissue integrity. Our findings contribute to characterizing the brain remodeling during the GBM progression and prompt further studies for the development of novel muti-targeted therapies dependent on the disease phase.