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**Proceedings of the
30th National Conference
of the Italian Group for the Study
of Neuromorphology
“Gruppo Italiano per lo Studio
della Neuromorfologia” G.I.S.N.**

PROCEEDINGS

November 12-14, 2020

*University of Torino
Torino (Virtual Event) - Italy*

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The *European Journal of Histochemistry* was founded in 1954 by Maffo Vialli and published till 1979 under the title of *Rivista di Istochimica Normale e Patologica*, from 1980 to 1990 as *Basic and Applied Histochemistry* and in 1991 as *European Journal of Basic and Applied Histochemistry*. It is now published under the auspices of the University of Pavia, Italy.

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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INTRODUCTION

A BRIDGE AMONG HISTORY AND NEW MULTIDISCIPLINARY APPROACHES: THE ROLE OF G.I.S.N. IN THE FIELD OF NEUROMORPHOLOGY

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This special issue collects the abstracts of the lectures and communications presented at the 30th National Conference of the Italian Group for the Study of Neuromorphology (G.I.S.N.), a scientific association founded to promote and develop neuroscience education and research preferentially related to the field of morphology of nervous system. The community of Italian scientists is particularly involved in the field of Neurobiology and Neuroanatomy, due to a long historical tradition starting with Gabriele Falloppia (1523-1562) and Bartolomeo Eustachio (1500-1574) that described the organization of the ear, Marcello Malpighi (1628-1694), who applied the recently dis-

covered microscope to the study of the nervous system, Giovanni Battista Morgagni (1682- 1771) who linked brain alterations to neurological diseases, and Luigi Rolando (1773-1831) who described human cerebral convolutions. Neuroanatomical studies had a great boost with the development of fixation and staining techniques, and the contribution of Camillo Golgi (1843-1926) in this field was a milestone recognized by the award of the Nobel Prize (1906). This long tradition of neuroanatomical studies has been perpetuated by several disciples of these giants of the early period of medical studies and has come down to our days, as evidenced by the Nobel Prize awarded in 1986 to Rita Levi Montalcini (1909-2012). In almost all Italian universities, anatomists are engaged in the study of the nervous system from different points of view, with experimental studies involving animal models of neurological diseases, the link between neural structures and behavior, the effects of the environment or drugs on neural circuits and structures. Several researches are now devoted to fMRI studies that seek to elucidate the connectivity or modifications of the human brain under normal and pathological conditions. These different approaches converge towards the actually consolidated awareness of the unique peculiarity of the nervous system, where the structure is intimately joined to the function, laying the foundations of the growing field of neuroscience nowadays embracing central, peripheral and enteric nervous systems. All these lines of research, and many others, are represented in our group and have been discussed in our meetings. We hope that reading these abstracts can give a picture of the state of the art of Neuromorphology in Italy.

G.I.S.N. Board of Directors

MAIN LECTURES

PRION AND PRION-LIKE DISEASES: EVIDENCE OF PROTEIN MISFOLDING PROPAGATION IN HUMANS

Giorgio Giaccone

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Many common neurodegenerative diseases such as Alzheimer's disease (AD) are characterized by the accumulation of misfolded proteins in the central nervous system. For instance, AD and cerebral amyloid angiopathy are characterized by extracellular deposition of A β -amyloid (in AD in association with intraneuronal accumulation of the microtubule associated protein tau), Parkinson disease by intraneuronal accumulation of α -synuclein, atypical parkinsonisms such as progressive supranuclear palsy and corticobasal degeneration and a subset of cases of frontotemporal dementia by tau formation. Hence, these misfolded proteins are considered disease-specific biomarkers and their identification and localization in the CNS is required for a definite diagnosis. A group of disorders named transmissible spongiform encephalopathies or prion diseases, are caused by a misfolded form of the prion protein, named prions, or PrP^{Sc}. Prion diseases are transmissible as the protein with abnormal conformation is able to convert the normal protein to the pathologic form. A puzzling aspect of prion diseases lies in the fact that the same protein (PrP^{Sc}) can cause a variety of diseases which are phenotypically heterogeneous. For instance, the sporadic forms of Creutzfeldt-Jakob disease, the most common human prion disease, are currently classified in six subgroups and every form has its own peculiar features, in terms of clinical manifestations and neuropathological features. In recent years, several lines of evidence indicate that the ability to transmit misfolding to their native counterparts is not limited to PrP but is shared, although with different efficiency, by the proteins that accumulate in AD, Parkinson disease, frontotemporal dementia. This implies that two innovative diagnostic techniques, named Protein Misfolding Cyclic Amplification (PMCA) and Real Time Quaking Induced Conversion (RT-QuIC), that are able to detect traces of the misfolded proteins in CSF and in other peripheral tissues mimicking *in vitro* in a very rapid manner the pathological processes of protein misfolding which occur *in vivo* may be extended from prion diseases to more common aging-related neurodegenerations. On the other hand, the potential risk of transmission is also extended and was recently confirmed for A β by the recognition of iatrogenic forms of cerebral amyloid angiopathy linked to neurosurgical procedures performed decades before the onset of the pathology.

NEUROANATOMICAL BASIS OF BRAIN ENERGY METABOLISM IN THE MAMMALIAN BRAIN

Corrado Calì

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Understanding the mammalian brain's computational efficiency represent, to date, an ambitious challenge. Growing evidence suggests that the key to unveil such mystery relies in its complex energy management system. Brain energetic demand in particular, seemed to rely on the metabolism of glucose. In the early 90s, the discover that lactate, an intermediate product of the glucose metabolic pathway, plays a central role in neuronal energy supply, brought a paradigm shift into the field. More than 20 years of research highlighted the importance of the astrocyte-neuron lactate shuttle (ANLS), a novel hypothesis describing how glia acts a metabolic bridge between vasculature and neurons (the so called neuro-glia-vasculature unit, or NGV). Increasing evidence highlighted the central role of lactate in the physiology of the brain, as well as its benefits in a number of pathological conditions, including stroke, epilepsy and drug addiction. Nevertheless, cellular and molecular mechanisms through which lactate is exerting its function remains still unclear. Although lactate is a well-known byproduct of glycolysis, a series of recent studies have highlighted how lactate derived from glycogen, a storage mechanism of glucose expressed specifically in astrocytes, is pivotal for learning and memory. Following this alternative pathway, we have decided to investigate the localization of glycogen and analyze its distribution in several brain areas and under different physiological and pathological conditions, in order to infer the possible sites of lactate utilization in the brain. The complex structural arrangements between neurites and glial processes can be hardly resolved with conventional microscopy methods, therefore we decided to adapt microconnectomics techniques based on 3D electron microscopy (3DEM) to extract high-resolution three-dimensional models of brain parenchyma to conduct these assessments. We took extensively advantage of the most recent visualization techniques, and developed virtual reality (VR) tools to perform complex analysis in 3D. This approach is similar to what early observers like Golgi and Ramon y Cayal used to do, by hypothesizing the functional role of brain components by their morphology but revised using much higher resolution imaging techniques and VR visualization.

SESSION I - BRAIN PLASTICITY: FROM NORMAL BRAIN FUNCTION TO PATHOLOGY

IMMATURE NEURONS IN THE AMYGDALA OF CAT AND MARMOSET

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Brain plasticity is important for preventive and therapeutic approaches in neurological diseases. In mammals, the genesis of new neurons (adult neurogenesis) is spatially restricted to small stem cell niches and appears to be reduced from mice to humans. A population of undifferentiated, "immature" neurons (INs) is known to be present in the mouse paleocortex (piriform cortex); these INs are generated pre-natally, do not divide in adulthood, yet, retain expression of markers for immaturity. Recently, we showed that cortical INs may represent a reservoir of "young" cells in the neocortex of large-brained mammals, also extending in subcortical regions. Here, we focused on amygdala of two mammalian species endowed with different density of cortical INs: the gyrencephalic cat, showing high IN density, and the lissencephalic marmoset, a non-human primate with very low amount of INs. Three young-adult cats and three adult marmosets were considered. Whole brain hemisphere and amygdala volumes were evaluated in both species, by using histologically-stained coronal sections scanned with Axioscan. To study INs, both quantitative and qualitative analysis were carried out: doublecortin was employed as a marker for immaturity and Ki-67 antigen to check for cell proliferation. Then, stereological cell countings were performed using optical Fractionator on StereoInvestigator software. Populations of INs were found in the amygdala of both species (in contrast with very low amount reported in mouse), with a significant higher presence in marmoset. Hence, a mammal with low amount of cortical INs (marmoset) shows higher density of INs in the most prominent subcortical region, and vice versa. This study confirms that gyrencephalic mammals, generally characterized by reduction in stem cell-driven adult neurogenesis, rely on populations of young neurons within brain regions underlying important cognitive functions.

MORPHO-FUNCTIONAL AND CLINICAL CORRELATES OF THE SUPERIOR COLLICULUS

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Higher brain functions represent an emerging property of the complex organization of the central nervous system (CNS) and its cortico-subcortical networks. The intrinsic circuitry of the superior colliculus (SC), a laminated structure of the dorsal mid-brain, integrates visual stimuli from retina (sensory cells) with conjugate ocular movements, saccades and head turning (premotor neurons). Tecto-spinal projections, that terminate on the

motor neurons of the upper cervical spinal cord, activate neck and arm muscles, orienting responses of the head and eyes. However, the activity of the SC goes well beyond the visuomotor functions: it is central for learning a novel movement but also to detect unpredictable, biologically salient events that can trigger interruption of ongoing behavior and contributing to higher-order decision making. Recently, dysfunction of the nigro-tectal pathway, one of the main afference of SC, has been involved in the pathogenesis of cervical dystonia (CD), the most common form of adult-onset idiopathic isolated focal dystonia. Experimental reduction of the inhibitory GABAergic input from the SNpr to the SC results in increased excitability and abnormal burst firing of the visual sensory neurons, a subsequent increased excitability of the premotor cells that finally causes a movement disorder resembling CD. In conclusion, despite its low hierarchical importance, SC represents a crucial structure for human brain functions, a new actor of neurological disease pathogenesis and a novel putative target for treatment of dystonic patients.

BDNF AND TRKB IN THE MESOCORTICOLIMBIC SYSTEM OF ROMAN RATS AFTER ACUTE STRESS

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The brain-derived neurotrophic factor (BDNF) has a role in the pathogenesis of depression and related deficits in neuronal plasticity as shown by evidence that a reduction of BDNF expression occurs in postmortem 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, with the aim of assessing the effect of a 15 min session of FS, by means of Western blot and immunohistochemistry, we use the Roman rats to investigate on the immunohistochemical occurrence of BDNF and its receptor trkB in the ventral tegmental area (VTA), nucleus accumbens (Acb) (core and shell) and prefrontal cortex (PFC). WB analysis indicates that levels of BDNF patently and markedly changed after FS as compared to controls and between the examined areas; thus, the VTA and Acb core showed lower BDNF expression level, and the PFC higher BDNF expression level (in both the anterior cingulate and infralimbic/prelimbic areas) in RLA vs RHA rats. As for the trkB, after FS its expression paralleled that of BDNF, with the exception of the PFC where changes were observed only in the infralimbic area. In tissue sections, BDNF- and trkB-like immunoreactive (LI) material labelled neuronal cell bodies, proximal processes and varicose nerve fibers, with an uneven distribution in the VTA, Acb and PFC. We have recently reported that acute stress, i.e. forced swimming (FS), has effects on the expression of BDNF and trkB in the hippocampus of Roman rats. The results obtained provide evidence that a differential expression of BDNF also occurs in the mesocorticolimbic system

of RLA vs RHA rat brains, and are consistent with the hypothesis that the differences in the BDNF/trkB signaling and neuroplasticity are involved in the susceptibility of RLA rats vs resilience of RHA rats to stress-induced depression.

PURE SPREADING OF FOCALLY INDUCED LONG-LASTING SEIZURES WITHIN LIMBIC SYSTEM DESTROYS BASAL FOREBRAIN CHOLINERGIC NUCLEI

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Status epilepticus (SE) of limbic onset might cause degenerative phenomena in different brain structures, and may be associated with chronic cognitive and EEG effects. In the present study SE was evoked focally by microinfusing picomolar doses of cyclothiazide+bicuculline into the anterior extent of the piriform cortex (APC) in rats, the so-called area tempestas, an approach which allows to evaluate selectively the effects of seizure spreading through the natural anatomical circuitries up to secondary generalization. In the brain of rats submitted to SE we analyzed neuronal density, occurrence of degenerative phenomena (by Fluoro-Jade B-FJB- staining) and expression of heat shock protein-70 (HSP-70) in the piriform cortex, the hippocampus and ventromedial thalamus. We further analyzed in detail, the loss of cholinergic neurons, and the presence of FJB- and HSP-70 positive neurons in basal forebrain cholinergic areas, *i.e.* the medial septal nucleus (MSN, Ch1), the diagonal band of Broca (DBB, Ch2 and Ch3) and the Nucleus basalis of Meynert (NBM, Ch4). In fact, these nuclei are strictly connected with limbic structures, and play a key pivotal role in different cognitive functions and vigilance. Although recent studies begun to investigate these nuclei in experimental epilepsy and in persons with epilepsy, conflicting results were obtained so far. We showed that after severe and long-lasting, focally induced limbic SE there is a significant cell loss within all of the abovementioned cholinergic nuclei ipsi- and contra-laterally to the infusion site. In parallel, these nuclei show also FJB and heat shock protein-70 expression. Those effects vary depending on the single nucleus assessed and on the severity of the SE seizure score. We also showed the occurrence of cell loss and degenerative phenomena in limbic cortex, hippocampus and limbic thalamic areas. These novel findings show direct evidence of SE-induced neuronal damage which is solely due to seizure activity ruling out potential confounding effects produced by systemic pro-convulsant neurotoxins. A damage to basal forebrain cholinergic nuclei, which may underlie cognitive alterations, is documented for the first time in a model of SE triggered focally.

α-SYNUCLEIN IMBALANCE AS A POTENTIAL MARKER OF EPILEPSY

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α-Synuclein (syn) is a protein widely expressed in brain tissue, mainly in presynaptic terminals. Its physiological role and its contribution to pathologies, such as Parkinson's disease (PD) and dementia with Lewy bodies (DLB), are unclear. Both PD and DLB, besides α-syn accumulation, show sleep dysfunction and EEG alterations, which in DLB frequently become epileptic seizures. Interestingly, recent studies unraveled the alteration of α-syn expression both in animal model and in human epileptic brains, suggesting the involvement of α-syn imbalance in the pathogenesis of epilepsy. Therefore, our aim was to analyze α-syn expression in a murine model of a genetic sleep-related epilepsy and in human epileptic brain. In particular, in wild type (WT) and transgenic (TG) mice we analyzed α-syn immunolocalization in three different types of synaptic terminals identified by means of the relative vesicular neurotransmitter transporters (VGLUT1, VGAT, VACHT) in sample cortical and striatal areas, such as Prefrontal (PFC) and Somatosensory cortex (SS) and Corpus Striatum (CS). Our results revealed an imbalance of α-syn expression in PFC and CS of TG mice. Moreover, CS displayed an increasing number of both VGAT and VACHT immunopositive synaptic terminals expressing α-syn in TG mice, suggesting an alteration of GABAergic and cholinergic circuits. Subsequently, post-mortem human brain sections were used to set the experimental protocols for the detection of α-syn both in control subjects and in patients affected by PD as positive controls for synucleinopathy, revealing the synaptic localization of α-syn in controls and the presence of Lewy bodies and neurites, as expected, in PD patients. On these bases, we set up experiments to investigate its expression in post-surgical human tissues from patients with Temporal Lobe Epilepsy due to Hippocampal Sclerosis (TLE-HS), the most prevalent form of chronic focal epilepsy, and with Focal Cortical Dysplasia (FCD), a developmental cortical malformation. Preliminary results showed: i) a severe loss of α-syn staining in sclerotic hippocampi, due to the synaptic density reduction; ii) the pathological white matter in FCD displayed α-syn immunopositive baskets surrounding dysmorphic neurons not correctly migrated. The present study provides new insight to understand with a completely new approach the pathogenesis and/or the histopathological consequences of different types of epilepsy.

SESSION II - NEURODEGENERATION AND NEUROPROTECTION

α -SYNUCLEIN OLIGOMERS IN SKIN BIOPSY AS BIOMARKER FOR PARKINSON'S DISEASE

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*The authors equally contributed to the study

The pathological hallmark of Parkinson's disease (PD) is α -Synuclein inclusion formation in the brain areas affected by neurodegeneration. PD is now considered as a multisystemic disorder and α -Synuclein-related pathology is also present in the peripheral nervous system, that could be exploited to unravel novel disease-related mechanisms. α -Synuclein oligomers have recently been indicated as 'a new hope' in the search of a reliable biomarker for synucleinopathies, including PD and multiple system atrophy. The oligomeric species of α -Synuclein consist in small aggregates of the protein, which occur in the early stage of the pathology, preceding and probably triggering the formation of the fibrillar conformation present in Lewy bodies. In the present study we explored α -Synuclein oligomers using the proximity ligation assay (PLA), an innovative approach to detect *in situ* protein interactions, in the peripheral nervous system by focusing on skin biopsies. We conducted a comparative analysis in a cohort of PD patients (n=38) and healthy subjects (n=29), including a subgroup of monozygotic twins discordant for the disease (n=19). In this case-control study, we observed previously undetected α -Synuclein oligomers within synaptic terminals of autonomic fibers in skin biopsies and proposed a method for their quantification, namely the PLA score. This score was found to have good sensitivity (82%), specificity (86%) and positive predictive value (89%). Intriguingly, although no difference in median values was detected between consecutive healthy controls and healthy twins, the prevalence of healthy subjects positive for PLA score was significantly greater in twins than in the consecutive cohort (47% vs 14%). This suggests that genetic predisposition is important, but not sufficient, in the aetiology of the disease and strengthens the contribution of environmental factors. All these findings endorse the hypothesis that α -Synuclein oligomers could be used as a reliable diagnostic biomarker for PD. Furthermore, this important starting point opened the way to investigate the molecular mechanisms involved in triggering α -Synuclein oligomerization and aggregation, including cytoskeletal remodeling in the peripheral and central nervous system.

PROTECTIVE EFFECTS OF PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE IN AN *IN VITRO* MODEL OF ALS

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the loss of upper and lower motor neurons (MNs). Not all MNs are susceptible to degeneration in ALS: in fact neurons of the oculomotor nucleus, controlling eye movements, are more resistant as compared to hypoglossal nucleus MNs. The analysis of *post mortem* samples from ALS patients has shown a differential genomic pattern between the two nuclei. Among identified genes, adenylyl cyclase activating polypeptide 1 (ADCYAP1) gene, encoding for pituitary adenylyl cyclase-activating polypeptide (PACAP), was found over-expressed in the oculomotor vs hypoglossal nucleus, suggesting that the peptide could exert a role on MNs in ALS. In the present study, we investigated the potential ability of PACAP to counteract MNs degeneration, by using a motor neuron like hybrid cell line (NSC 34) expressing human superoxide dismutase (SOD1) G93A mutation, as an *in vitro* model of ALS. Our results showed that PACAP promotes cell viability following serum deprivation, *via* EGFR transactivation mediated by protein kinase A stimulation. Furthermore, PACAP significantly decreased hypoxia-induced mutant SOD1 accumulation by modulating the autophagy process through the activation of the MAPK/ERK survival signaling pathway. Overall, our data demonstrated that PACAP exerts a protective role in MNs during ALS progression, suggesting that the different vulnerability of some cranial nerve motor nuclei could be due to differential expression of PACAP and its receptors in MNs.

EPIGENETIC MODULATION IN SOD1(G93A) ALS MICE

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ALS is a neurodegenerative disease that affects motor neurons (MNs). Transcriptional dysfunction which involves a defect in histone homeostasis has recently been implicated in MN degeneration. Histone homeostasis strongly depends on the activity of histone deacetylases (HDACs). These enzymes, which includes an important group known as sirtuins (SIRT) have been implicated in cellular processes such as cell death. Recent studies from our lab have demonstrated that the combination of two epigenetic drugs, MS-275 (which inhibits HDACs) and Resveratrol (an activator of the AMP-activated kinase (AMPK)-sirtuin 1 pathway) provided neuroprotective effects and improved motor performance in ALS mice. However, MS-275 is currently not approved for clinical trials. Several studies have indicated that Valproate, another pharmacological inhibitor of HDACs, improves cell survival by promoting histone acetylation, gene transcription and protein synthesis in cancer and ischemic stroke, and is currently being used in clinical trials. To improve the translational power of this approach, the overall aim of this study was to investigate the efficacy of MS-275 replacement with

Valproate, and explore for the first time in ALS mice, the effect of a combination of these two epigenetic drugs, Valproate and Resveratrol, to modulate histone homeostasis and directly protect MNs from neurodegeneration. Experiments were performed using SOD1(G93A) mice separated into treated and control groups. Animals in the treated group were administered Valproate (40 µg/kg) and Resveratrol (136 µg/kg) in combination every day from post-natal day 50 until the end stage of the disease. Behavioural tests were carried out to test motor function. Stereological count of MNs in the lumbar tract was performed to determine MNs survival and the acetylation state of histone 3 (H3) was examined by immunofluorescence staining. Western blot was carried out to detect the acetylation of RelA protein in the lumbar tract. Overall results showed that the drugs improved motor performance of treated animals and significantly delayed the loss of motor function. Stereological count showed drugs protected the MNs from death and a significant increase in the MNs number was observed in the treated group. Immunofluorescence revealed a decrease in acetylation of H3 in the control group and a restoration of H3 acetylation after drug treatment, while Western blot analysis also showed a restoration of RelA protein acetylation state.

EFFICACY EVALUATION OF THE GHRH AGONIST MR409 IN A SMA MURINE MODEL

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Spinal Muscular Atrophy (SMA) is a pediatric neurodegenerative disease caused by the deletion or mutation of the telomeric gene "survival motor neuron 1" (*SMN1*), resulting in the loss of MNs in the brainstem and in the spinal cord. Patients also show a progressive skeletal muscular atrophy and neuromuscular junction (NMJ) defects. Nowadays, despite their effectiveness, *SMN*-dependent available therapies have different limitations (difficult administration, several adverse effects, high costs and poor efficacy in milder patients or in late-treated people): investigating *SMN*-independent treatments and targeting other (peripheral) districts could be a turning point to bypass some of these crucial aspects. Here we focused on skeletal muscles, evaluating the role of MR409, a growth hormone-releasing hormone (GHRH) agonist that has already shown a remarkable activity in preventing apoptosis and proteolysis in an *in vitro* model of muscle atrophy. To this aim, from postnatal day 2 (P2) to P12, we daily administered vehicle or MR409 (1 mg/Kg and 2 mg/Kg) to *SMN*delta7 mice (a well-known murine model of SMA). We observed a progressive weight gain, especially with the highest dose, as well as a significant improvement in motor behavior. Proportionally to the administered dose, these promising results positively correlated with histological and molecular analyses on quadriceps and gastrocnemius, respectively a proximal and a distal hindlimb skeletal muscle, sequentially affected in the pathology. Indeed, H&E staining showed a significant increase in the size of the muscular fibers; moreover, immunofluorescence analyses on NMJs revealed their increased maturation (*i.e.*, a higher

monoinnervation) and a reduced denervation of the endplates. Finally, molecular analyses exhibited an enhanced expression of different isoforms of myosin heavy chains (MYH1, MYH2, MYH7 and MYH8) and of markers of myogenesis and muscular damage repairing (respectively, Myogenin and MyoD1), as well as a remarkable downregulation of MuRF1 and Atrogin-1 (whose increased expression seems correlated with muscular atrophy). Thus, our results suggest MR409 as a new promising therapeutic approach for the treatment of SMA, possibly in combination with *SMN*-dependent therapies.

ASC-EXOSOMES ADMINISTRATION: A THERAPEUTIC APPROACH FOR ALS AND SMA

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Therapeutic strategies for fatal neurodegenerative diseases as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) have currently provided little or no satisfactory results. Interest in stem cells for the treatment of neurodegenerative disorders is increasing and could represent a promising approach thanks to their beneficial action that seems to be due to the paracrine release of exosomes, main mediators of intercellular communication. Given their ability to stimulate the nerve regeneration, synaptic plasticity and neuronal protection, our group also demonstrated that exosomes isolated from adipose mesenchymal stem cells (ASC-exosomes) were involved in cell adhesion and negative regulation of the cells apoptotic process in an *in vitro* model of ALS, thanks to the release of their content, especially proteins, miRNA and mRNA. In addition, we tested and demonstrated that repeated administrations of ASC-exosomes delivered by intravenous injections exert a neuroprotective effect in the SOD1(G93A) murine model of ALS. To further investigate the beneficial effects of ASC-exosomes, in our recent study the administration has been tested *via* intranasal, as a different and non-invasive administration route, in the SOD1(G93A) mice and *via* intracerebroventricular delivery in the *SMN* 7 murine model, the most widely used model of SMA. Indeed, despite these two neurodegenerative diseases are caused by different pathogenetic mechanisms and affect different targets, they share some pathological dysfunctions, they are both characterized by the progressive loss of motor neurons (MNs) in the spinal cord and brainstem and they both lead to a progressive and highly disabling motor decline. The results showed that ASC-exosomes could improve the motor performance of animals, evaluated by specific motor tests both in treated SOD1(G93A) and *SMN* 7 mice; they could also decrease the astrocytes activation and protect lumbar spinal cord MNs from neurodegeneration, validated by a significant reduction in cleaved caspase-3 activation observed in SMA spinal cord after treatment. Moreover, in the peripheral tissues the outcomes showed a higher innervated neuromuscular junctions' number and an attenuated skeletal muscle atrophy in the treated SOD1(G93A) group. These data could allow to better understand the mechanisms underlying ALS and SMA and to evaluate the promising use of ASC-exosomes as a therapy in neurodegenerative diseases.

MITOCHONDRIAL ALTERATIONS IN SPINAL MUSCULAR ATROPHY

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Spinal Muscular Atrophy (SMA) is due to a mutation/deletion of the Survival Motor Neuron 1 (*SMN1*) gene which affects motor neurons (MNs) in children and young adults following a decrease in the levels of the functional SMN protein; this results in motor impairment, muscle atrophy and premature death. The current experimental therapies for SMA aim at restoring SMN protein levels: Spinraza-Biogen, Zolgensma-AveXis/Novartis and Risdiplam-Genentech/Roche have been approved by the Food and Drug Administration. However, their long term effects are still under evaluation, especially in adults. Although the genetic cause of SMA has been identified, many aspects of its pathogenesis remain elusive and novel biological targets are investigated to develop new therapeutics and to monitor the efficacy of the existing treatment. We focus on mitochondria since already at early stages in SMA their function, number, area and transport are significantly altered in axons of spinal MNs. We characterized subcellular and mitochondrial alterations (such as size, amount, area and cristae length and density) in MNs from TEM images of the SMA mouse model SMNdelta7 compared to age-matched control mice. By fractionation, we isolated mitochondria from the spinal cord of mice at postnatal day 7 and after 2D gel and MALDI-TOF mass spectrometry we identified differentially expressed proteins and, after enzymatic assays, dysfunctional proteins in SMA. Moreover, in order to better study the mechanisms underpinning mitochondria dysfunctions, we cultured primary fibroblasts (MEFs) and we stained mitochondria with the MitoTracker before performing a time-lapse live imaging by confocal microscopy. From the multidimensional quantitative image analysis we evaluated the differences in mitochondria number, distribution and trafficking. Briefly, mitochondrial morphology and dynamics, analyzed from TEM images of SMNdelta7 pups and by the toolset MiNA from ImageJ in MEFs, revealed severe alterations of both mitochondrial networks and anatomical structures. The defects that we described may contribute to SMA disease pathogenesis. Interestingly, since mitochondria take part in a plethora of processes in order to preserve cellular homeostasis and genomic integrity and their dysfunctions are reported in other neurodegenerative diseases, they could represent a potential therapeutic target to implement *SMN*-dependent therapies.

THE ENZYME A20 IN A MURINE MODEL OF DEMYELINATION

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The ubiquitin-editing enzyme A20, codified by TNFAIP3 gene, is a central gatekeeper in inflammation through the inhibition of the pro-inflammatory factor NF- κ B. TNFAIP3 has been identified as a susceptible gene for several inflammatory and autoimmune disorders such as Multiple Sclerosis (MS). Data demonstrated the A20 down-regulation in blood of MS patients. Close to the well-known role of A20 in the systemic immune system, evidence in human tissues and murine models suggesting a function of A20 also in glial cells are emerging. Based on this evidence, we aim to unveil a possible involvement of A20 in a de- and re-myelination process accompanied with gliosis without the recruitment of peripheral inflammatory infiltrates. To this purpose, we took advantages of a toxin-induced focal reversible demyelination model, based on the injection of lysolecithin in the mouse corpus callosum, where peripheral immune cells do not infiltrate the CNS. We analyzed the corpus callosum of lysolecithin- and vehicle-injected mice sacrificed at different time points to evaluate microglia activation, oligodendrocyte precursor recruitment, oligodendrocyte precursor cells differentiation, and completed remyelination. In particular, mice were sacrificed at 1, 4, 14 and 31 days post injection (dpi). We highlighted that in physiological conditions A20 is expressed at very low levels in the CNS, whereas its expression is upregulated upon lesion in microglia cells. In particular, A20 expressing cells reached a peak of expression in all resident ramified microglial cells during the late phases, when the re-myelination process is ongoing and microglia exert an anti-inflammatory role supporting re-myelination. The results are also supported by RT Real Time PCR analysis. These results suggest that A20, in parallel with its recognized role in the peripheral inflammation, exerts a function also in microglia activation cooperating with the remyelinating processes.

QUANTITATIVE CONFOCAL MICROSCOPY OF REACTIVE ASTROCYTES IN EAE AND RECOVERED ASTROCYTES AFTER MSC TRANSPLANTATION

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Astroglia is a prominent feature of multiple sclerosis (MS) and experimental allergic encephalomyelitis (EAE), the murine model of MS. It is well known that astrocytes rapidly change their morphology upon inflammatory activation, overexpressing glial filaments and becoming hypertrophic, with increased thickness and number of processes. The morphology of reactive astrocytes has been described and qualitatively categorized but, alternatively, can be quantified as a continuous variable for parameters such as cell ramification, complexity, and shape. Confocal fluorescence microscopy is an excellent tool for making quantitative measurements in cells and tissues, but very few techniques apply to multiple astrocytes in entire low magnification 3D pho-

tomicrographs. The purpose of this study is to quantify the reactive morphology of astrocytes in 20- μ m-thick telencephalic sections from EAE-affected, EAE-affected mesenchymal stem cell (MSC)-transplanted, and naïve mice. Cell size and shape descriptors of glial fibrillary acidic protein-positive astrocytes were evaluated by means of AnalyzeSkeleton (2D/3D) plugin and the obtained individual binary astrocyte outlines were morphometrically analyzed by FracLac plugin for ImageJ, both applied for the first time in the neuropathological evaluation of EAE. The chosen descriptors included the number of astrocyte process endpoints and their cumulative length, the index of increasing complexity of cell shape (fractal dimension), the index of cell shape heterogeneity in filling the extracellular space (lacunarity), the major/minor axes (span ratio) and the maximum radius of Hull circle. Our study shows that MSCs differentially affect the response of astrocytes to neuroinflammatory noxae. In the subcortical white matter (WM), one of the brain regions primarily affected by EAE, significant differences of astrocyte ramification, fractal dimension and lacunarity were observed among the three experimental groups, whereas the same differences appeared only sketched in the cerebral cortex. In conclusion, this sensitive assessment of astrocyte morphology has indicated that MSCs attenuate the hypertrophic features of reactive EAE WM astroglia and suggest to deeply study their molecular crosstalk to empower their reciprocal regenerative activity.

PACAP AND VIP COUNTERACTS GLIOBLASTOMA AND NEUROBLASTOMA PROGRESSION

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Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP) are two neuropeptides largely distributed in the body. They are also involved in some human cancers, including glioblastoma multiforme (GBM) and neuroblastoma (NB). These are solid tumors characterized by extensive hypoxic areas. The hypoxic microenvironment induces transcription of hypoxia-inducible factors (HIFs) which in turn trigger the activation of signaling cascades responsible of cells proliferation and metastasis formation. In particular, HIFs activation is linked to epidermal growth factor receptor (EGFR) overexpression and induction of vascular endothelial growth factor (VEGF) release. Previous studies have demonstrated that PACAP and VIP promote neuroblastoma differentiation and are also implicated in counteract the invasive nature of gliomas. In the present work, we have investigated the molecular mechanisms underlying the anti-invasive effect of PACAP or VIP in GBM and NB cells. Peptides effect have been tested in U87MG glioblastoma cells and in malignant undifferentiated and all-trans retinoic acid (RA) differentiated SH-SY5Y cells, representing the benign form of NB, exposed to deferroxamine (DFX), an hypoxic mimicking agent. Our data have shown that PACAP and VIP counteract GBM cell invasiveness under hypoxia by modulating HIFs and EGFR expression through the inhibition of PI3K/Akt and MAPK/ERK signaling pathways. Furthermore, they also induce NB cell differentiation into benign form by regulating HIFs, VEGF and VEGFRs expression and distribution. Overall, our finding demonstrated the efficacious role played by PACAP and VIP in counteract GBM and NB malignancy.

SESSION III - PERIPHERAL NEUROPATHIES AND NERVE REGENERATION

A NOVEL BIOLOGICAL (ACTR-FC-NLG3) TO SUSTAIN NEUROMUSCULAR JUNCTION INNERVATION IN SARCOPENIA

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Sarcopenia is a type of muscle atrophy that occurs with aging and/or immobility. In the elderly, it is the primary cause of impaired motor performance, also responsible for increased morbidity and mortality. The prevailing approach to counteract such condition is the increase in muscle mass through inhibition of the myostatin system: however, this strategy only moderately improves muscular strength because it is not able to sustain per se innervation of the hypertrophic muscle, causing a progressive worsening of the motor performances. In this scenario, a novel protein has been created by combining the soluble activin receptor, a strong myostatin inhibitor, to the C-terminal agrin nLG3 domain (ActR-Fc-nLG3); both domains are connected via the constant region of an IgG1 monoclonal antibody. This compound has the potential of providing additional innervation to the hypertrophic muscle. We have previously demonstrated that, after ActR-Fc-nLG3 administration, young mice are capable of remarkably enhancing the endurance in rotarod motor tasks, with only a modest gain of muscle mass, compared to common myostatin inhibitors (ActR-Fc). In this work, we extended these observation by demonstrating that also in aged (2 years-old) mice, long-term administration of ActR-Fc-nLG3 increases in a sustained way the motor endurance and muscle strength, compared with the administration of the ActR-Fc alone and the control vehicle (PBS). Histological data demonstrate that this new compound administration leads to changes in neuromuscular junctions (NMJs) and to the preservation of fiber innervation, without affecting muscular fiber size. Moreover, an increase in membrane folds is observed in the postsynaptic site, offering a possible explanation to the increased endurance as a result of improved efficiency of the neurotransmission at the NMJ level. Thus, our novel biological may represent a valid option for treating disorders of the striatal muscle tissue, together with muscle dysfunction caused by altered neuronal input to the muscle, raising the hope that a therapy may be developed not only for sarcopenia but also for other neuromuscular disorders.

A NOVEL GENETIC VARIANT OF CCT5 RELATED TO MOTOR NEUROPATHY

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Identification of diseases associated with acquired or genetic defects in members of the chaperoning system (CS) is increasing in frequency as the knowledge of the system expands. The CS is composed of molecular chaperones, co chaperones, co-factors, receptors, and interactors, and their diseases are the chaperonopathies. Illustrative instances of genetic chaperonopathies are mutations in the chaperonins of Groups I (e.g., Hsp60) and II (e.g., CCT). The diseases MitCHAP60 and SPG13 are examples of the former, while a distal sensory mutilating neuropathy is caused by a mutation (His147Arg) in the CCT5 subunit equatorial domain. Recently we identify a novel homozygous CCT5 c.670C>G p.(Leu224Val) variant in the CCT5 gene causing devastating disease in a young Italian girl. The phenotype associated is mostly characterized by early-onset, demyelinating neuropathy, severe motor disability and it looks extremely different from that observed in subjects affected by p.(His147Arg) mutation. Here we show the genetic analysis and compare clinical data of our patient with those from patients carrying p.(His147Arg) variant. Furthermore, through *in silico* 3D-structure analysis and bioinformatics, we demonstrate that the novel p.(Leu224Val) mutation occurring within the intermediate domain of the CCT5 leads to an abnormal conformation of its apical domain, as a result of a "mutation resonance-effect" on the molecular anatomy of the subunit. Finally, in the present study, we also observed histopathological impairment of myofibers and an incorrect organization of sarcomeric proteins in skeletal muscle tissue from affected p.(Leu224Val) patient. This is the first time that the effects of a CCT-complex mutated subunit are evaluated on diseased skeletal muscle tissue. These preliminary data could open a new gateway leading into the field of chaperonopathies to study, for example, the impact of the mutations on the properties and functions of the subunit and its teams (functional oligomers) and networks, and the molecular mechanisms of the tissue and organ abnormalities seen in patients. The molecular processes underlying the mutation are yet to be clarified, but now we show

that impaired CCT-complex does not guarantee the function and structure of musculoskeletal tissue. Data from these investigations should be instrumental for developing screening procedures for early diagnosis, even prenatal, for genetic counseling, and for developing specific therapies centered on the chaperonin.

MECHANICAL STIMULI INDUCE PHENOTYPIC CHANGES IN PERIPHERAL NERVES RELATED WITH PAIN RELIEF

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The selective repeated tension of the Peripheral Nervous System (PNS) also known as neurodynamic treatment (NDT) is successful in pain modulation of patients affected by nerve-related chronic and acute back and neck pain, the main cause of disability worldwide. Even if NDT reduces pain and disability the biological effects involved are still unknown and no standard protocol is available. The study aims to assess the effects of NDT on PNS cells in order to develop a standardized protocol, to define any dose response changes in PNS cells and even any side effects of NDT. We adopted *in vitro* models of motor and nociceptive neurons (NSC34 and 50B11) and later an *ex vivo* model of rat Dorsal Root Ganglia (DRG). Protocols of repeated mechanical stimuli were tested starting from those reported in literature and refined by previous trial results. Experiments were performed in triplicates seeding cells on pre-coated silicone membranes and repeated tension protocols were administered using a bioreactor. Morphological, Gene and Protein expression analysis were performed. A standardized protocol of NDT was possible to be defined. NDT protocols are able to induce dose response changes in motor, sensitive neurons and DRGs. No side effects were possible to be detected. In particular NDT is able to promote cell differentiation and to avoid apoptosis. Interestingly, the NDT do significantly affect the expression of PIEZO1 and TACAN, that are genes linked to receptors transducing respectively mechanical non painful stimuli and mechanical painful stimuli. Those results suggest that NDT promotes the regeneration processes in motor and sensory neurons with anti-allodynic effect. Even if cell lines and DRG rodent models looks distant from clinical practice the cell subpopulations and their behavior are similar to human PNS cells. Also, all variables on which the NDT protocol was defined (amplitude of elongation, number of repetition, speed etc.) still be very suitable to be translated in clinical settings.

ENRICHED CHITOSAN CONDUITS FOR PERIPHERAL NERVE REPAIR

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Repairing severe peripheral nerve injuries remains a great challenge for surgeons, as the regeneration outcomes are not usually satisfactory. In injuries accompanied by substance loss, nerve autografts are used to fill the nerve gap and to rejoin the two transected nerve stumps. Tubulization technique is an alternative that has been developed to repair nerves and to overcome the limitations accompanied by the use of autografts. It is highly efficient in repairing small gaps (up to 3 cm), while to improve the conduit efficiency in repairing long gaps intraluminal enrichment might be a good strategy. Extracellular matrix (ECM) fillers, stem cells, growth factor releasing particles or internal topographical cues are different strategies to bio-mimic the native neural tissue. In this study we combined two ECM components that are known to have a role during peripheral nerve regeneration and are endogenously released in the injured environment: fibrin and collagen. Fibrin cables are secreted by fibroblasts and help in guiding cellular migration; collagen is required for normal ECM assembly and plays an important role in the regulation of Schwann cell function. Hollow chitosan conduits were enriched with a mix of fibrin and collagen hydrogel with or without the addition of Adipose Derived Mesenchymal Stem Cells (ADMSC) and these combinations were tested in regenerating a sciatic long gap injury (15 mm). Macroscopic analysis showed that the ulcer diameter was statistically less in ADMSC fibrin-collagen enriched conduits. Muscle atrophy did not show significant differences between tested groups. Molecular analysis during the first month following injury and repair showed that soluble Neuregulin1 (a growth factor playing an important role in nerve repair) was highly upregulated in enriched conduits both at RNA and protein level. Morphometric analysis will be also performed to evaluate the final regeneration outcome following 15 weeks of repair. Based on the obtained preliminary results we can speculate that the addition of ADMSC, together with fibrin-collagen hydrogel, to hollow conduits, could enhance the regeneration outcome.

DEVELOPMENT OF NOVEL PERIPHERAL NERVE WRAPS: AN *IN VIVO* STUDY

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The aim of the study was to assess and compare the efficacy of two novel biodegradable wraps, in a rat model of peripheral nerve injury without substance loss. In particular, the wraps were made of synthetic oxidized polyvinyl alcohol (OxPVA) and natural

leukocyte-fibrin-platelet membrane (LFPm), respectively. During surgery, after sciatic nerve sharp transection, neurorrhaphy was performed. Sprague-Dawley rats (n=30) were randomly implanted with a) NeuraWrapTM (control group); b) OxPVA; c) LFPm wraps. Thus, 12 weeks later, after functional recovery tests, the animals were euthanized, and samples removal occurred. Explanted nerves underwent to morphological/morphometric studies including histological evaluations (hematoxylin and eosin staining - H&E; Toluidine-Blue staining) and immunohistochemical analyses (anti-CD3, -F4/80, -S100 - β -tubulin staining). Thus, ultrastructural investigations were also performed by Transmission Electron Microscopy (TEM) and collagen distribution was observed by Second Harmonic Generation (SHG) microscopy. According to the study results, all the implanted wraps allowed for nerve function recovery; at dissection, no dislocation of the wraps was observed, and no scar-tissue/neuromas were recognizable at the surgery site. As regards wraps biodegradation, only OxPVA and NeuraWrapTM residues were still identifiable, suggesting a higher re-absorption rate for the LFPm wraps. Histological and immunohistochemical analyses (CD3 and F4/80) both proved the absence of significant inflammatory infiltrate in all experimental groups, suggesting the biocompatibility of the implanted wraps. Then, the specific nervous origin of the repaired tissue was also verified by both immunohistochemistry (S-100 and β -tubulin) and TEM analysis. In the fascicular area, no significant collagen infiltration was observed by SHG microscopy in OxPVA samples compared to NeuraWrapTM and LFPm wraps. According to the morphometric study, OxPVA and LFPm wraps were effective in promoting nerve regeneration especially in the distal portion. Bioengineered OxPVA and LFPm wraps promoted lesion recovery and may be considered an interesting alternative to the commercial NeuraWrapTM.

SESSION IV - THE CNS AS A TARGET FOR ENDOCRINE DISRUPTORS AND OTHER POLLUTANTS

BENZO[a]PYRENE AFFECTS DEVELOPMENT AND FUNCTION OF HUMAN GnRH NEUROBLASTS

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The increasing environmental pollution represents a major concern not only for the global ecosystem, but also for human health. Endocrine disrupting chemicals (EDCs), such as benzo[a]pyrene (BaP), are widespread pollutants that can interfere with the endocrine system, altering reproductive function and embryo development. However, little is known about BaP effects on human reproductive axis at central level. The central regulatory network of the reproductive system is mediated by gonadotropin-releasing hormone (GnRH) neurons, which originate in the olfactory placode and, during fetal development, migrate into the hypothalamus. We investigated the direct effects of BaP on development of GnRH-secreting neurons taking advantage of a primary culture isolated from the human fetal hypothalamus (hfHypo). hfHypo cells express the enzymes cytochrome P450 (CYP1A1 and 1B1), required for metabolic activation of BaP and that expression was strongly induced by BaP exposure (0.2 and 10 μ M for 24 h). Moreover, treating hfHypo with BaP (10 μ M, 24 h) increased reactive oxygen species (ROS) production and influenced the total antioxidant capacity of the cells. From a functional point of view, BaP exposure (10 μ M, 24 h) significantly reduced both mRNA and protein expression of GnRH and decreased the mRNA level of the receptor for kisspeptin (KISS1R), the main physiological regulator of GnRH neuron function. In addition, since the migratory process is a crucial event for the correct maturation and functionality of GnRH neurons, we investigated the effect of BaP on pre-migratory GnRH neuroblasts isolated from the human fetal olfactory epithelium (FNC-B4). Preliminary results, using a transwell assay, indicated that BaP pre-incubation (10 μ M for 24 h) significantly reduced FNC-B4 migratory properties. In conclusion, our findings demonstrate that BaP may directly affect GnRH neuron maturation and function by altering migration process and interfering with GnRH and KISS1R expression, suggesting a possible mechanism underlying EDCs-related alterations of reproductive function.

EFFECTS OF CHRONIC EXPOSURE TO BISPHENOL-A IN PREGNANT FEMALE MICE

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Bisphenol A (BPA), an organic synthetic compound found in some plastics and epoxy resins, is one of the best known and most studied EDCs (Endocrine Disrupting Chemicals, *i.e.* an exogenous chemical, or mixture of chemicals, that can interfere with any aspect of hormone action). Exposure to BPA is especially dangerous if it occurs during specific "critical periods" of life, such as intrauterine, perinatal, juvenile or puberty periods, when organisms are more sensitive to hormonal changes. This exposure can originate, in adulthood, both physiological and behavioural alterations. In particular, we focused on the effects of exposure to BPA during pregnancy, which represents a particularly sensitive period not only for the fetus but also for the mother. In this study we treated C57BL/6 dams orally with a dose of 4 μ g/kg body weight/day (*i.e.* EFSA Tolerable Daily Intake dose) of BPA dissolved in corn oil (N=11) or with vehicle (N=8), starting with mating and continuing for 20 weeks. We monitored the dams, evaluating their body weight (daily) and food intake (once a week). During the last two weeks of treatment we followed up the estrous cycle and we performed the Three-Chamber Test to assess sociability. We did not notice differences in body weight, food intake, number of pups and female-to-male ratio in the litters, but we found that BPA-treated dams tend to have higher pup mortality and to develop an aggressive behavior towards males during mating. In addition, BPA-treated dams showed an altered estrous cycle, spending more time in estrus compared to the controls. The Three-Chamber Test revealed that the male-preference of the control mice, measured as time spent within the chamber of the male non-tester mouse, was lost in BPA-treated females. Therefore, we decided to analyze vasopressin and oxytocin systems, measuring both fractional area and number of cells, in paraventricular, supraoptic and suprachiasmatic nuclei of these animals. Although we did not find any alteration in the oxytocin system, we did observe some alterations in the vasopressin system, which could be partially linked to the behavioral alterations. These results suggest that exposure to BPA may pose a risk even in adulthood (given the long-term exposure period, the persistence of these compounds in the environment and the ability of bisphenols to accumulate in certain compartments of the body), particularly when it occurs during delicate periods such as pregnancy.

UPDATE ON ZINC PROTECTION AGAINST CD-INDUCED BBB IMPAIRMENT

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Over the years, anthropogenic factors have led to cadmium (Cd) accumulation in the environment causing various health problems. Due to its highly soluble nature compared to other metals, Cd is easily absorbed by plants giving rise to bioaccumulation phenomena. So, the diet is the primary source of Cd exposure in humans. Other sources include smoking, occupational exposure and house dust. Once inside the bloodstream, Cd is able to impair the blood-brain barrier (BBB), a specialized system that shields the brain from toxic substances in the blood. This impairment allows a greater amount of toxicant to enter the central nervous system leading to neurodegeneration. In fact, chronic exposure to Cd has been linked to numerous neurodegenerative disorders in adulthood including Alzheimer's and Parkinson's diseases. Although studies in rodents have established a Cd-dependent BBB dysfunction, how Cd may alter the cell-cell junctions in the endothelium remains elusive. In our previous studies, we investigated the signaling pathway of Cd-induced tight junctions disassembly in a rat brain endothelial cell line (RBE4), as an *in vitro* model for the study of the BBB. This phenomenon was coincident with a significant ROS production, upregulation of GRP78 expression levels, a chaperone involved in endoplasmic reticulum stress, caspase-3 activation and BAX overexpression leading to apoptotic cell death pathways. Surprisingly, the micronutrient Zinc (Zn), one of the most important microelements necessary for normal body functioning, was able to mitigate Cd harmful effects. Moreover, morphological analysis following Zn co-treatment showed the role of Zn in preventing ZO-1 dislocation and altered cytoskeleton rearrangements induced by Cd. These results highlight the protective role of Zn against Cd-induced alteration in the BBB, suggesting Zn supplementation as an effective strategy to prevent cell oxidative stress.

EXPOSURE TO PARTICULATE MATTER HAMPERS REPAIR IN A MOUSE MODEL OF DEMYELINATION

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Epidemiological studies show a strong association between exposure to air pollution – and particularly to particulate matter (PM) - increased prevalence of Multiple Sclerosis (MS) and higher rates of hospital admissions for MS and MS relapses. Beyond having immunomodulatory effects and sustaining a systemic oxidative-inflammatory response, PM may participate in MS pathogenesis by targeting also Central Nervous System (CNS)-specific processes, such as myelin repair. Here we show that, in a mouse model of lysolecithin-induced demyelination of the subcortical white matter, post-injury exposure to fine PM hampers remyelination, disturbs oligodendroglia differentiation dynamics and promotes astroglia and microglia reactivity. These findings support the view that exposure to fine PM can contribute to demyelinating pathologies by targeting the endogenous regenerative capability of the CNS tissue.

SESSION V - BRAIN AND METABOLISM

ION CHANNEL EXPRESSION IN THE BRAIN AREAS OF HIGH-FAT DIET FED RATS

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Obesity is associated with the development of cerebrovascular diseases promoting cognitive decline. High Body Mass Index has been suggested as a risk factor for Alzheimer's disease and vascular dementia and has been associated with poorer cognitive performance in population-based studies. Evidence suggests that transient receptor potential (TRP) ion channels dysfunction significantly contributes to the physiopathology of metabolic and neurological disorders. Mutations in genes encoding TRP channels are the cause of several inherited diseases in humans (the so-called 'TRP channelopathies') that affect the cardiovascular, renal, skeletal, and nervous systems. This study aimed to evaluate the effects of a high-fat diet on ion channel expression in the brain of diet-induced obesity (DIO) rats. DIO rats were studied after 17 weeks under a hypercaloric diet. Moreover, groups of DIO rats were supplemented with tart cherries seeds powder (DS) or seeds powder plus tart cherries juice (DES) to evaluate the possible protective effects. DIO rats were compared to the control rats with a standard diet (CHOW). To determine the systemic effects of high-calorie diet exposure, we examined food consumption, fat mass content and fasting glycemia, insulin levels, cholesterol, and triglycerides. qRT-PCR, Western blot, and morphological analysis were performed in the frontal cortex and hippocampus. After 17 weeks of fat diet, rats increased significantly their body weight in comparison to the CHOW rats. No differences in body weight were observed in DS and DES rats compared to age-matched DIO rats. In DIO rats TRPC1 and TRPC6 were up-regulated in the hippocampus, while they were down regulated in the frontal cortex. In the case of TRPM2 expression instead, was increased both in the hippocampus and in the frontal cortex. All these data are confirmed by immunohistochemical and Western blot analysis. Supplemented DIO rats showing a different modulation on TRPC1, TRPC6, TRPM2, and TRPV1 ion channel expression in the hippocampus and the frontal cortex, possibly related to the positive effects of anthocyanins on reactive gliosis. The identification of neurodegenerative changes in DIO rats involving the ion channels expression may represent the first insight to better characterize the neuronal changes occurring in obesity. Further studies are needed to clarify the benefits of tart cherry supplementation on the prevention of cerebrovascular alterations.

MUSIC EFFECT ON WEIGHT, GHRELIN EXPRESSION IN RAT HYPOTHALAMIC NEURONS

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Recent studies highlighted that music stimuli play an important role in brain physiology, in some areas related to emotions, food intake and body weight, such as the amygdala, the hippocampus and the hypothalamus. Furthermore, music seems to influence the regulation of the hypothalamic-pituitary-adrenal axis, in the sympathetic nervous system and in the immune system, thus affecting metabolism and energy balance. This leads us to believe that music can have positive effects on the physiological mechanisms directed to metabolic recovery. There are different frequencies to which music can be tuned, today the most used is at 440 Hz, while in the past the 432 Hz frequency was more utilized showing particular effects on brain. Ghrelin (Ghre), a gut-brain peptide hormone, regulates food intake in the hypothalamus; in the last years, it has aroused particular interest for its antioxidant, anti-inflammatory and anti-apoptotic properties. In our previous investigation, we reported that musical stimuli at 432 Hz modified the Ghre expression in the rat, increasing beneficial effects on metabolism. In this study, we used this frequency and we focused our attention on body weight, Ghre expression and neuron morphology in hypothalamic cultures. To investigate the role of music, we utilized newborn pups from pregnant rats; they were divided in two groups: Gr1 without music stimuli, Gr2 with music stimuli at 432 Hz during both the perinatal period and the postnatal period, some for three days (P3) and others for six days (P6). Our results showed that music increased the body weight of pups; in addition, an enhanced Ghre expression in hypothalamic neurons and their axonal elongation were highlighted by immunocytochemical techniques. The expression of Ghre in the β 3-Tubulin positive neurons increased significantly in both Gr1 and Gr2 from P3 to P6. In particular, the increase of the expression of Ghre in the neurons was statistically significant at P3 between Gr1 and Gr2; this increase became highly significant at P6. In addition, the Ghre/ β 3-Tubulin positive neurons both of Gr1 and Gr2 showed a significant physiological elongation of the processes from P3 to P6. These results suggest that the musical frequency at 432 Hz could stimulate the orexigenic Ghre effects influencing the increase in body weight and affecting the number of hypothalamic neurons expressing Ghre.

SESSION VI - NOVEL APPROACHES FOR NEURO-ANATOMICAL/MORPHOLOGICAL STUDIES IN THE HUMAN NERVOUS SYSTEM

BUILDING A 7T – RADIOLOGICAL-ANATOMO-TOPOGRAPHICAL ATLAS OF THE HUMAN BRAIN

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The anatomical organization of the central nervous system represents one of the most complex fields of anatomical sciences. The purpose of this project is to build an Atlas of the *ex vivo* human brain employing ultra-high field 7T MRI with anatomico-microscopical validation and 3D reconstruction of both MRI scans and histological sections. One human brain deriving from the Body Donation Program of the Institute of Human Anatomy of the University of Padua was sampled after a 48 h post-mortem delay and fixed in 4% paraformaldehyde for 30 days. The specimen was then placed in an airtight cylinder filled with perfluoropolyether, a fluid which is hypointense in all the MRI sequences, paying attention to remove as much air as possible from the ventricles and the depth of the sulci. The specimen first underwent ultra-high field 7T MRI performed with a Discovery MR 950 scanner (GE Healthcare) equipped with a 2ch-Tx/32ch-Rx head coil for imaging the whole brain. The specimen was then anatomically sectioned in order to isolate the brainstem, which subsequently underwent MRI employing a custom-built Tx/Rx birdcage coil for the acquisition of high-resolution sequences of the brainstem. MR images were used to design and create two cutting boxes, one for each specimen. These are boxes containing a housing for the specimen designed as a negative plaster cast and equidistant fissures which act as guides for the anatomical section of the sample. The specimens were then sectioned and stained with Hematoxylin and Eosin, Klüver-Barrera and Weigert-Pal and were used as the reference for the MR images analysis. 7T MRI of the brainstem revealed structures generally evaluated through light microscopy, such as the accessory medial and dorsal olivary nuclei, the oculomotor nucleus, the solitary tract, the medial and dorsal longitudinal fasciculus, the nucleus ambiguus and the mesencephalic tract of the trigeminus. Whole brain imaging revealed several structures that are difficult to visualise through conventional MRI at high magnetic field, such as the subthalamic nucleus, the dorsal anterior nucleus of the thalamus, the continuity between the substantia incerta and the extended amygdala. MR images accurately represented the morphological organization of brainstem and sub-cortical structures, as revealed by comparison with anatomico-microscopical sections.

SEGREGATED AND INTEGRATED FUNCTIONAL TERRITORIES OF THE HUMAN GLOBUS PALLIDUS

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The most accepted model on basal ganglia functional anatomy, mainly derived from multimodal evidences collected from non-human primates, suggests that connections from functionally homologous regions of the cerebral cortex, striatum and subthalamic nucleus are likely to converge on overlapping, yet identifiable regions of the globus pallidus (GP). Herein, in order to provide a comprehensive, unified framework of basal ganglia connectivity and functional topography, we test the hypothesis that the striatopallidal, subthalamopallidal and pallidothalamic pathways are spatially coherent and topographically organized within the GP by using tractography-derived connectivity based parcellation (CBP) on a 3T MRI dataset of 100 healthy subjects. The two-stage hypothesis-driven CBP approach proposed herein revealed that the striatopallidal, subthalamopallidal and subthalamopallidal pathways are topographically organized in anterior limbic, intermediate associative and posterior sensorimotor territories within the internal (GPi) and external GP (GPe). Our results suggest that the general topographical organization of connectivity parcels is highly consistent across the GPi and GPe, regardless the bundle of interest, reinforcing the idea that different pathways, running parallel and in series to one other, may share the same spatial organization pattern. Indeed, we found high similarity among functionally homologous connectivity maps derived from the striatopallidal, subthalamopallidal and pallidothalamic tracts, as indicated by good-to-high Dice coefficient values. In order to investigate the possible clinical or pathophysiological relevance of our results, we evaluated the spatial relationship between the sensorimotor GPi connectivity maps obtained in the present study and optimal stimulation sites as previously identified in dystonic patients, showing that the coordinates of such optimal stimulation sites are located along the lateral border of striatopallidal and pallidothalamic sensorimotor maps, whilst covered a more central position within subthalamopallidal maps. Taken together, our findings suggest that functionally homologous afferent and efferent connections may share similar spatial localization within the GP and that the pallidal connectivity maps obtained in the present study may be employed during pre-operative targeting in order to ameliorate clinical outcomes as well as to improve our current knowledge of side effects during post-operative evaluation.

TRACTOGRAPHY-BASED VIM IDENTIFICATION: A METHODOLOGICAL PERSPECTIVE

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The ventral intermediate nucleus (Vim) of thalamus receives its afferent connections from contralateral dentate nucleus and mainly projects to primary motor area, thus representing the prototypical target for tremor suppression in functional neurosurgery settings. For this reason, structural connectivity-based

parcellation (CBP) of thalamus is emerging as a promising resource for functional neurosurgery, allowing for individualized Vim targeting. However, results of CBP rely on methodological variables which have been poorly investigated in existing literature. Herein, we present a novel thalamus parcellation protocol, by testing it on high quality data of 210 healthy subjects from the Human Connectome Project repository. Structural CBP of thalamus has been carried out employing two different signal modelling techniques: diffusion tensor model (DTI) and constrained spherical deconvolution (CSD). Each parcellation pipeline has been performed applying either hard-segmentation or a 25% threshold on connectivity maps. Summarizing, four different pipelines have been implemented for each subject. Reproducibility of each pipeline was then assessed calculating inter-subject similarity measures. Finally, the spatial relations between connectivity maps, Vim histological maps and an optimal stimulation point for essential tremor have been characterized. CSD-based pipelines resulted to be more reproducible than DTI-based ones; moreover, higher reproducibility was observed when a threshold-based approach was applied as voxel classification criterion. Among motor related connectivity maps, the precentral gyrus map resulted to be the most reproducible, whilst dentate connectivity maps exhibited the lowest reproducibility. Dentate and precentral connectivity maps exhibited higher overlap with histological Vim maps. In addition, the optimal stimulation point for essential tremor was located into the overlap area between connectivity maps of dentate nucleus and precentral gyrus. Taken together, our results suggest that a pipeline combining CSD signal modeling with a threshold-based approach is able to highlight the thalamic voxels connected both to precentral gyrus and to contralateral dentate nucleus with high reproducibility, thus potentially representing a powerful tool for identifying the ideal target to stimulate/ablate in prospective functional neurosurgery studies.

IN VIVO IDENTIFICATION OF THALAMIC NUCLEI USING TRACK-DENSITY IMAGING

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The thalamus is a core structure of the human brain and its nuclei present distinct cyto-, myelo- and recepto-architectonical features; each of these nuclei has important implications in various key aspects of brain physiology and many of them show selective alterations in a wide range of brain disorders. In addition, both surgical stimulation and ablation of specific thalamic nuclei have been proven to be useful for treating different neuropsychiatric diseases. As thalamic nuclei show very poor contrast on conventional MRI scans, the development of novel techniques for the *in vivo*, non-invasive visualization and identification of thalamic structures has represented a major challenge for human neuroimaging research in the last decades. While conventional methods based on stereotactic and histological atlases usually rely on just a few anatomical specimens and may then underestimate inter-individual variability, methods based on clustering of structural or functional connectivity are inherently limited by poor spatial resolution and often fail in the identification of smaller nuclei. Herein, we present a protocol for histologically-guided delineation of thalamic nuclei based on track-density imaging (TDI), which is an advanced imaging technique that exploits high angular resolution diffusion tractography to obtain

super-resolved white matter maps with high anatomical detail. We tested this protocol on i) six healthy individual 3T MRI scans from the Human Connectome Project database, and on ii) a group population template reconstructed by averaging 100 healthy subject's MRI scans from the same repository. We demonstrate that this protocol can identify up to 13 distinct thalamic nuclei with very high reliability (intraclass correlation coefficient: 0.995, 95% confidence interval: 0.992-0.998; total accumulated overlap: 0.43) and high similarity to the most recent histological thalamic atlas. We show that the obtained thalamic maps can be successfully used to study thalamic connectivity profiles *in vivo* using both structural and functional neuroimaging. We suggest that such protocol, by bringing together the advantages of histological and connectivity-based approaches, may have potential implications both for basic and translational research, as well as for pre-surgical planning purposes.

TRANSLATIONAL STUDY OF THE HUMAN CEREBELLAR DOPAMINERGIC SYSTEM, ITS INTERCONNECTIONS AND ROLE IN NEUROLOGIC AND PSYCHIATRIC DISORDERS

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The cerebellum is not considered a dopaminergic area, though a cerebellar role in Parkinson's disease (PD) and schizophrenia (SCZ) were suggested. In rodents cerebellum the presence of extrinsic dopaminergic fibres and of few Purkinje neurons in the cerebellar cortex were evidenced, instead in human brain data of cerebellar dopaminergic neuronal system and of interconnections between the cerebellum and the midbrain dopaminergic nuclei A₉ and A₁₀ are both lacking. The goal of this study is to make in the human cerebellum an analysis by means of an immunohistochemical and Diffusion Magnetic Resonance Tractography (DMRT) approach on the presence of an intrinsic cerebellar dopaminergic system and interconnections among the dentate nucleus to A₉ and A₁₀. Fragments of autoptic human cerebellum were fixed in an aldehyde-picric acid solution, embedded in paraffin, cut into 5 µm sections and subjected to light microscopic immunohistochemistry with rabbit polyclonal antibodies for dopamine transporter (DAT) or dopamine receptor type 2 (DRD₂). A 3T Achieva Philips scanner was used; a SENSE 8 channels head coil, acquiring T1 weighted 3D TFE, DTI sequences: data were analyzed using the contrasted spherical deconvolution technique (CSD). DAT and DRD₂ positivity were detected in the cerebellar cortex in Purkinje and synarmonic neurons. In the dentate nucleus DAT and DRD₂ positivity was observed in several neuron types. Moreover, we demonstrated with CSD, interconnections of the dentate nucleus to A₉ and A₁₀. This study demonstrates the existence of a cerebellar intrinsic neuronal dopaminergic system and the presence of direct dentate nucleus interconnections to the A₉ and A₁₀ nuclei. Finally, we suggest that the cerebellum may be involved in dopamine-related brain disorders and may be a critical element for transcranial stimulations, a new non-pharmacologic therapeutic approach for PD and SCZ.

A NEUROSCIENCE MULTIDISCIPLINARY ASSESSMENT OF CORPUS CALLOSUM AGENESIA

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The corpus callosum (CC) is the mainly brain interhemispheric commissural structure in placental mammals. In human CC reaches its maximum complexity and size relative to whole brain volume. The CC fibers originated mainly by neocortical neurons of the layers III, V, VI, and are mainly composed by myelinated fibres and by a minor number of unmyelinated fibres, which present a heterogeneous neurochemical composition. Cytologically consists of oligodendrocytes, astrocytes, and few neurons. Though the CC fibers are topographically subdivided in 7 regions the functional distribution of the CC fibers are still little known. Recently, in the CC regions a different expression profile of regulating proteins involved in oxidative stress and in Ca-regulation signalling were demonstrated. The agenesis of the corpus callosum (AgCC) is a developmental disorder characterized by a complete or partial absence of CC fibers, often the AgCC are neglected or undiagnosed. Studies evidenced the coexistence between the AgCC and other brain abnormalities, instead, the influences of AgCC on the brain abnormalities and on the neurologic and psychiatric symptoms are scanty. Therefore, the aim of the study was to investigate in a neglected AgCC clinical case by means of morphological, clinical and neuropsychological approach the influence of the AgCC on other brain abnormalities, clinical symptoms, neuropsychological value. The analysis evidenced the presence of other brain abnormalities, neurologic and psychiatric symptoms, neuropsychological impairment closely related to the AgCC. This multidisciplinary assessment can play a relevant role to evidence the presence of undiagnosed AgCC or CC abnormalities related to neurologic and psychiatric diseases such as multiple sclerosis, cognitive impairments; prognosis of stroke sequelae, Marchiafava-Bignami disease, Korsakoff's syndrome, autism spectrum disorders.

CHARACTERIZATION AND VISUALIZATION OF THE HUMAN NEURONS (POST-MORTEM) COMBINING THE GOLGI'S METHOD AND IMMUNOFLUORESCENCE

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The Golgi's method is still one of the most used methods to study the central nervous system at the microscopic level. In fact, its ability to "stain" the neurons in a very detailed manner makes it a low-cost instrument even within the reach of the smallest research laboratories. However, the complete absence of biochemical information that accompanies this method, greatly limit its use, nevertheless this information is provided by immunocytochemical procedures. Previously these procedures were used as an alternative to Golgi's method, forcing the investigator to

renounce the morphological detail. Some time ago we successfully patented a method that combines the two procedures obtaining both the morphological detail and the biochemical information of the sample, but this procedure can only be applied to experimental animals due to recommended fixation procedures in rat. With this project we want to develop a new method (Golgi's staining + immunofluorescence) that is applicable to post-mortem human brain specimens, which are often kept in formalin, even for a long time, waiting for the possibility of being used. Our aim is providing scientific community with a tool for the neuro-anatomical study of a large amount of neurodegenerative diseases directly on humans, in order to give more precise indications with respect to animal experimentation.

EVIDENCE FOR A2A-D2 RECEPTOR-RECEPTOR INTERACTIONS IN THE CAROTID BODY

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The carotid body (CB) is an arterial chemoreceptor located at the carotid bifurcation and typically constituted by 'neuron like' chemo-sensitive type I cells and 'glial-like' supportive type II cells. There is great consensus in recognizing the CB as a multi-purpose sensor-activity organ, exerting its role through the activation of afferent sensory fibers. In fact, to counteract environmental variations like hypoxia, hyperoxia, acidosis, type I cells produce many different neurotransmitters/neuromodulators to restore balance; among them, adenosine and dopamine are included, and they can interact with adenosine receptor A_{2A} and dopamine receptor D₂, respectively. From a functional perspective, the reciprocal influences of the two receptor monomers in a A_{2A}/D₂ receptor complex would be particularly intriguing; this event has been proved in many different tissues but never in the CB. The aim of this work was to demonstrate close proximity (*i.e.* a distance lower than 10 nm) between the receptor molecules A_{2A} and D₂ as colocalization is a necessary condition to have direct receptor-receptor interactions. In turn, this will allow supporting the hypothesis of heterodimers formation. Native CB tissues were obtained from Sprague Dawley rats (n=5) and human donors (n=5) from the Body Donation Program of Padua University. The fixed tissues were paraffin embedded and processed according to routine protocols. After hematoxylin and eosin (H&E) staining, immunohistochemistry and *in situ* proximity ligation assay (PLA) were both performed to verify the presence and the localization of A_{2A} and D₂ receptors. The H&E staining showed the characteristic CB morphology for all the investigated samples and immunohistochemistry revealed positive A_{2A} and D₂ elements for all specimens. After PLA assay and confocal evaluation, red clusters surrounding DAPI-stained nuclei were detected suggesting the possible existence of A_{2A}/D₂ receptors heterodimers. These data give new insights about both CB basic organization and its ability to possibly undergo plastic changes according to environmental stimuli.

SESSION VII - ENTERIC NERVOUS SYSTEM AND GUT-BRAIN AXIS

MODELING ENTERIC NEUROPATHY: THE RAD21 KNOCK-IN MOUSE

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RAD21 is a double-strand-break repair protein and a critical component of the cohesin complex with key roles in several cellular functions including transcriptional regulation. A novel RAD21 missense mutation has been shown to cause severe gut dysmotility, specifically chronic intestinal pseudo-obstruction (CIPO), in a consanguineous family. RAD21 immunoreactivity (IR) was detected in a subset of enteric neurons of the mouse enteric nervous system. In order to investigate how the Rad21 mutation might contribute to gut sensory-motor dysfunction, this study was designed to provide a quantitative and qualitative characterization of myenteric neurons in the colon of wild type (WT) and genetically re-constructed Rad21 conditional knock-in (Rad21KI) mice carrying the novel Ala626Thr mutation. Immunohistochemical analysis was performed in whole mount myenteric plexus preparations, using a pan-neuronal marker HuC/D, choline acetyltransferase (ChAT, a cholinergic marker for excitatory motor neurons) and neuronal nitric oxide synthase (nNOS, a nitrinergic marker for inhibitory motor neurons). Compared to WT, there was about 30% reduction of HuC/D myenteric neurons/field in Rad21KI mice, reminiscent of the CIPO phenotype observed in patients. Subsets of HuC/D-IR myenteric neurons of WT mouse colon displayed either ChAT-IR (43.71±3.45) or nNOS-IR (30.84±5.40). In Rad21KI mice HuC/D/ChAT-IR neurons/field were 45.13±4.27, while HuC/D/nNOS-IR were 14.13±1.40 neurons/field. These preliminary findings of a reduction of the overall myenteric neurons with a selective reduction of inhibitory motor neurons in Rad21KI mice, suggest an involvement of this gene alteration in gut motility dysfunction. Further studies will be required to validate Rad21KI as a model to better understand enteric neuropathy in CIPO patients.

MICROBIOTA AND COGNITIVE DECLINE IN AGEING

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The gut-brain axis and the intestinal microbiota are emerging as key players in health and disease. Shifts in intestinal microbiota composition affect a variety of systems, however, evidence of their direct impact on cognitive functions is still lacking. We tested whether faecal microbiota transplant (FMT) from aged donor mice into young adult recipients affected the hippocampus, an area of the central nervous system (CNS) known to be affected by the ageing process and related functions. Young adult mice were transplanted with the microbiota from either aged or age-matched donor mice. Following transplantation, characterization of the microbiotas and metabolomics profiles along with a battery of cognitive and behavioural tests were performed. Label-free quantitative proteomics was employed to monitor protein expression in the hippocampus of the recipients. We report that FMT from aged donors led to impaired spatial learning and memory in young adult recipients, whereas anxiety, explorative behaviour and locomotor activity remained unaffected. This was paralleled by altered expression of proteins involved in synaptic plasticity and neurotransmission in the hippocampus. Also, a strong reduction of bacteria associated with short-chain fatty acids (SCFAs) production (*Lachnospiraceae*, *Faecalibaculum*, and *Ruminococcaceae*) and disorders of the CNS (*Prevotellaceae* and *Ruminococcaceae*) was observed. Finally, the detrimental effect of FMT from aged donors on the CNS was confirmed by the observation that microglia cells of the hippocampus fimbria, acquired an ageing-like phenotype; on the contrary, gut permeability and levels of systemic and local (hippocampus) cytokines were not affected. These results demonstrated that age-associated shifts of the microbiota have an impact on protein expression and key functions of the CNS. Furthermore, these results highlight the paramount importance of the gut-brain axis in ageing and provide a strong rationale to devise therapies aiming to restore a young-like microbiota to improve cognitive functions and the declining quality of life in the elderly.

SESSION VIII - BRAIN TUMORS AND CHEMOTHERAPY-INDUCED NEUROTOXICITY

MICROENVIRONMENT IN A SYNGENEIC MODEL OF GLIOBLASTOMA

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Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor with a malignant prognosis. GBM is characterized by high cellular heterogeneity and its progression relies on the interaction with the central nervous system components. This interplay induces metabolic, (epi)-genetic, and molecular rewiring in both domains. In the present study, we aim to characterize the time-related changes in the GBM landscape, using a syngeneic mouse model of GBM. GL261 glioma cells were injected in the right striatum of immuno-competent C57Bl6J mice. Animals were sacrificed at 7, 14, and 21 days (7D,14D,21D) from the inoculation of the cells. A group of animals was subject to valproic acid (VPA) treatment and sacrificed after 21 days from the tumor cells inoculation. The tumor development was assessed through 3D tomographic imaging and brains were processed for immunohistochemistry, immunofluorescence, and RNA microarray technology. Our results showed the dynamics of the tumor progression, being established as a bulk at 14D and surrounded by a dense scar of reactive astrocytes. GBM growth was paralleled by a decrease of the innate immune system response while the invasive phase was characterized by changes in the extracellular matrix, as shown by the analysis of tenascin C. The late phase of the disease correlated with molecular modifications of the non-tumor cells and impairment in the antigen-presenting functions, that may be reversed by valproic acid treatment. The present study emphasizes the role of functional changes in the microenvironment during the GBM progression and the chance of modulation, fostering the development of novel multi-targeted, time-dependent therapies in an experimental model similar to the human disease.

Mn AND Mg PROTECT AGAINST THE OXALIPLATIN-DEPENDENT NVU IMPAIRMENT

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Oxaliplatin is a well-known chemotherapeutic drug largely used for metastatic colorectal cancer. Even though oxaliplatin has showed beneficial effects in tumour reduction, it is able to induce neuropathic pain, thus leading for dose reduction and therapy discontinuation. Recently, it has been demonstrated the oxaliplatin-dependent alteration in a blood-brain barrier (BBB) *in vitro* model that may, at least in part, explain the mechanism by which oxaliplatin treatment triggers neuropathic pain. Also, glial cells, an integral part of the so-called neurovascular unit (NVU), have a pivotal role in eliciting neuropathy. However, very little is known how counteract these deleterious effects. We postulate that the antioxidant properties of manganese (MnCl₂) and magnesium chloride (MgCl₂) can protect glial compartment from oxaliplatin-induced damage. In order to validate our hypothesis, we performed molecular and morphological assays to monitor the effectiveness of MnCl₂ and MgCl₂ during oxaliplatin treatment on different cell lines. Our data show the oxaliplatin-dependent effects on ROS production, endoplasmic reticulum stress, and caspase-3 activation. On the contrary, both MnCl₂ and MgCl₂ treatment are able to reduce oxidative and endoplasmic reticulum stress, as well as to retrieve caspase-3 activation. Moreover, BBB tight junction dysfunction and glial markers were analysed by immunofluorescent analysis. In conclusion, our results showed the ability of MnCl₂ MgCl₂ to oxidative and ER stress induced by oxaliplatin, suggesting that MnCl₂ and MgCl₂ could be a new tool to counteract the chemotherapy-dependent NVU alterations.