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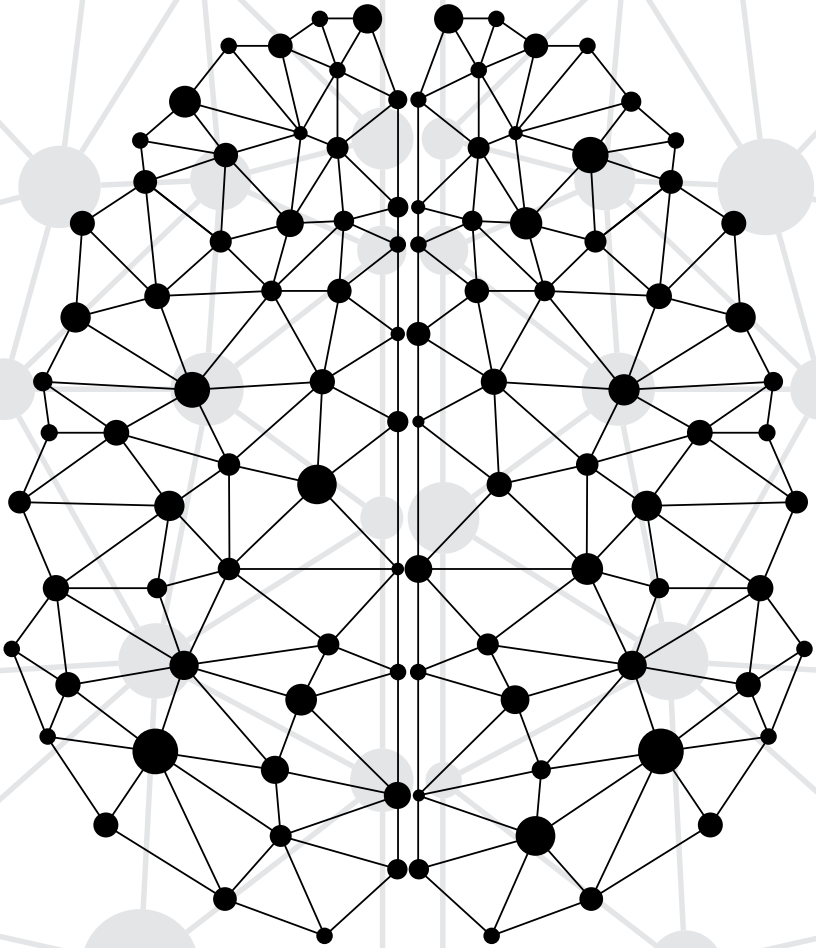

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EFFECT OF 17-BETA ESTRADIOL ON GLIOBLASTOMA CELLS MITOCHONDRIAL METABOLISM

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Glioblastoma multiforme (GBM) is the most malignant type of primary brain tumor in humans and it is often associated with a poor prognosis. High levels of estrogens exhibit oncogenic potential in various organs such as breast, prostate, endometrium and lung through their classic receptors ERα and ERβ. To this regard, ERβ has been described as a possible tumor suppressor, whereas ERα is involved in cancer progression. Several studies showed that glioblastoma cells express higher ERα receptor levels than the ERβ subtype. The aim of the present study was to evaluate in vitro the role of 17β-estradiol (E2) in GBM cell proliferation and metabolism. We observed that E2 was able to regulate positively mitochondrial dynamics, increased OXPHOS genes and mitochondrial biogenesis. In conclusion our data support the hypothesys that locally produced estrogens in glioblastoma cells may act as autocrine factors. In particular, E2 may affect glioblastoma cells by different possible mechanisms, including the regulation of estrogen receptor-mediated transcription of genes involved in cell survival, proliferation, tissue invasion and rewiring mitochondrial fitness in GBM cells.

SPINAL MUSCULAR ATROPHY: THE GLIAL RESPONSE AT CENTRAL AND PERIPHERAL LEVEL

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Context • Spinal muscular atrophy (SMA) is a fatal paediatric genetic disease, characterized by motor neuron (MN) death, leading to muscle weakness, respiratory failure and, in the most severe cases, to death. SMA is due to the mutation of the survival MN1 gene (SMN1). SMA is classically categorized as a MN disease: however there is an increasing consensus that its pathogenesis is more complex than expected, since several alterations have been also described at peripheral level. Moreover, unexpectedly, the role of neuroinflammation and glial response have never been thoroughly investigated in SMA, while representing a common pathological feature of nearly all CNS disorders. Objective • We investigated the presence of activated glial cells both at central (spinal cord) and peripheral (quadriceps) level, in WT and SMNΔ7 mice, an experimental model of SMA type II. Design • At P5 and P11 (respectively, early and late symptomatic phase), WT and SMA mice were sacrificed and the samples collected: by IF reactions, the expression of GFAP and IBA1 was evaluated. Results • Compared to control mice, at the spinal cord level, SMA pups showed a remarkable astrogliosis and microgliosis both at P5 and P11; moreover, in the SMA quadriceps sections, near the endplates, we observed an increasing GFAP-positivity during the days (never detected in WT muscular samples), whereas the IBA1-positive cell number decreased from P5 to P11 in SMA mice and remained constant in controls. Conclusions • These preliminary data suggest that in SMNΔ7 pups inflammation and glial response occur not only in the spinal cord, but also in muscles: better understanding cellular responses at central and peripheral level could be important to identify suitable cellular/molecular targets for new treatments of SMA.

EXOSOMES AS INNOVATIVE THERAPEUTIC APPROACH FOR ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset progressive neurodegenerative disease. Stem cells, in particular adipose stem cells (ASC), represent a promising therapeutic approach in the treatment of ALS. Several observations lead to the hypothesis that stem cells exert their beneficial effect through the secretion of exosomes. These vesicles enhance the repair of the damaged area releasing their content, and could be used as a cell-free therapeutic approach. We have demonstrated that ASC-exosomes have a neuroprotective effect *in vitro* model of ALS.
We wanted to assess the potential neuroprotective role of ASC-exosomes *in vivo*, on SOD1(G93A) murine model. We used two different routs of exosomes administration: intravenously and intranasal. In both cases, the ASC-exosomes administration, from clinical onset until terminal stage, point out that exosomes delay symptoms progression of treated animals. Moreover, a higher number of motoneurons in the lumbar tract of spinal cord was detected in the ASC-exosomes treated animals compared to untreated group, demonstrating that ASC-exosomes have a neuroprotective effect in *in vivo* model of ALS.
In order to identify the molecules responsible for this neuroprotection, we performed the protein content characterization of ASC-exosomes, that allow us to identify some of the molecular pathways by which exosomes explain their function.
Finally, to monitor the tracking and the homing of ASC-exosomes after *in vivo* administration, we set up a protocol to label exosomes with superparamagnetic iron oxide nanoparticles, which allow their detection with the magnetic resonance imaging (MRI). The labelled ASC-exosomes were then administered intranasal and were detected, by MRI, in the injured region of the brain of SOD1(G93A) animals.

EFFECTS OF PERINATAL EXPOSURE TO BISPHENOL A (BPA) IN EAE MODEL OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a multifactorial disease, overlapping genetic, epigenetic and environmental factors. A better definition of environmental risks is fundamental to understand its etiology and the observed increase of female-to-male ratio. The exposure to Endocrine Disruptor Compounds (EDCs, i.e. exogenous substance or mixture that alters functions of the endocrine system) fully represents one of these risks. Bisphenol A (BPA) is an EDC present in some plastics and epoxy resins, which has been controversially implicated in the etiology of MS and its role in the disease onset and progression has not been univocally defined yet.
This study starts investigating the effects of perinatal exposure (from mating until weaning) to European TDI for BPA (4µg/kg BW/day) in one of the most widely used murine model of MS, the Experimental Autoimmune Encephalomyelitis (EAE). We evaluated, by daily examination, the consequences of BPA exposure on the disease onset and progression (rotarod performance and clinical score) and on some physiological parameters (body weight, food intake, vaginal opening, ano-genital distance).
We have observed that BPA perinatal exposure has different effects on males compared to females, both on pathological and physiological parameters. Ongoing analysis will help us define the actual impact on BPA exposure both on peculiar tracts of the pathology (demyelination and inflammation) and on physiological parameters.
The analysis of the consequences of the exposure to BPA will help better decipher not only the role of these environmental factors on the pathology but also the role of exogenous and endogenous estrogens on the characteristic of the MS.

A NOVEL MITOCHONDRIAL DISORDER CHARACTERIZED BY CHRONIC INTESTINAL PSEUDO-OBSTRUCTION AND LEUKOENCEPHALOPATHY CAUSED BY MUTATIONS IN THE *LIG3* GENE

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Context: Chronic intestinal pseudo-obstruction (CIPO) is a severe gut dysmotility mimicking an intestinal sub-occlusion without demonstrable mechanical causes. Objective: We aimed to identify the mutations in a family with three siblings, affected by CIPO and brain abnormalities, born from healthy unrelated parents. All affected members shared several clinical features including malnutrition, constipation/diarrhea and severe dysmotility involving the entire gut, with at least one well-established intestinal sub-occlusive episode, muscle wasting, leukoencephalopathy, signs of cerebellar atrophy in the oldest brother and thinning of the corpus callosum in the youngest one. Design: The study was a genetic and functional analysis of a novel gene involved in this familial, syndromic form of CIPO. Results: Exome sequencing in the affected sibs revealed two novel heterozygous variants, p.K537N (paternal) and p.G964E (maternal) in LIG3, DNA ligase involved in nuclear and mitochondrial DNA repair. In the zebrafish model, LIG3 ablation reproduced brain alterations and impaired gut transit reminiscent of the human condition. Rescue of the phenotype was obtained with wild-type LIG3, but not with the two variants, indicating that they are loss-of-function mutations. The p.K537N variant, mapping to the last base-pair before the donor splice-site, induced the skipping of the corresponding exon, in patient's skin fibroblasts. The p.G964E variant, residing in a C-terminal binding site for XRCC1, impaired LIG3/XRCC1 binding. Compared to controls, patients' skin fibroblasts revealed marked mitochondrial morpho-functional impairments, i.e. decreased ATP synthesis, fragmented mitochondria and decreased membrane potential. Conclusion: Our data indicate a novel mitochondrial gastrointestinal encephalopathy due to mutated *LIG3* gene.

QUANTITATIVE CHANGES OF ENTERIC NEURONS AND AN INCREASED INTER-GANGLIONIC DISTANCE CORRELATE WITH CLINICAL FEATURES IN PATIENTS WITH SEVERE DYSMOTILITY

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Context • Current methods used to demonstrate the enteric neuropathies are mainly based on classic qualitative histopathological evaluation and criteria for interpretation may be limited by inter-observer variability. Objective: Evaluate the feasibility to quantify enteric neurons and inter-ganglionic distance in patients with severe dysmotility (SD) using paraffin sections and correlate data with clinical features. Methods: Jejunal full-thickness biopsies were collected from 32 well characterized SD patients (16-77 years; 22 F); and from n=8 controls (47-73 years 4F). A thorough symptom questionnaire was obtained prior to surgery. Patients were categorized according to a previous qualitative histopathological evaluation: n=10 with an apparently normal (AN) neuro-muscular layer; n=14 with inflammatory (INF) changes throughout the neuromuscular layer; and n=8 with degenerative neuro-muscular alterations (DEG). Neurons were stained using neuron specific enolase antibody and neuronal cell bodies/ganglion along with inter-ganglionic distance were calculated by three skilled pathologists. Statistical differences were assessed using student's t-test and the correlation with symptoms/signs was carried out using the Spearman correlation test. Results: The final concordance among the three operators was 80%. MP and SP neurons were decreased in SD ($P<0.001$) and in the tree subgroups ($P<0.0001$ in MP and $P<0.05$ in SP) *vs.* controls. Specimens with INF and DEG showed less MP (but not SP) neurons compared to AN ($P=0.0224$ and $P=0.0044$). Inter-ganglionic distance increased in SD and in the tree subgroups *vs.* controls ($P<0.01$). The number of sub-occlusive episodes increased along with an augmented distance between ganglia ($P=0.001$) and the reduction in MP and SP neurons and the increased inter-ganglionic distance correlated with abdominal distension and pain ($P<0.01$). Conclusions: Quantitative analysis showed a low discordance rate (20%) and identified an overall 50% reduction of MP and SP neuronal cell bodies; the distance between two proximal MP was increased vs. controls, corroborating further the concept of a critical neuronal mass loss. This neuronal reduction was indeed correlated with severe clinical manifestations. Finally, quantitative neuronal abnormalities can be also demonstrated in patients with AN histopathology.

THE CORTICOTOPIC ORGANIZATION OF THE HUMAN GLOBUS PALLIDUS

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The basal ganglia system is topographically organized in parallel, functionally segregated circuits within the striatum and subthalamic nucleus. Our group has recently challenged the classical view on the basal ganglia network by showing tractographic evidences of a cortico-pallidal pathway in humans, confirming previous findings in cats and non-human primates. This additional pathway superimposes a more direct cortical control on basal ganglia output that bypasses the major relay in the striatum and parallels the hyperdirect pathway. Herein, we will provide a comprehensive characterization of the topographical organization of the direct cortico-pallidal pathway within the internal (GPi) and external globus pallidus (GPe) by applying probabilistic tractography on high-resolution, multi-shell diffusion-weighted magnetic resonance images. The GPi resulted topographically organized in three connectivity clusters, namely frontal, dentate and parietal. The anterior portion of the GPi was mainly covered by the clusters of the dentate nucleus, whereas moving in anterior-posterior ventro-dorsal direction the GPi was mainly covered by the clusters connected to the frontal and parietal lobes. On the other hand, all four lobes but the dentate nucleus clustered in the GPe following a frontal, parietal, temporal, occipital topographical organization. In addition, the local connectivity of each sub-region supported the hypothesis of bilateral associative and sensorimotor functional regions occupying the anterior and mid-posterior portions of the nuclei respectively. This is the first evidence that the cortico-pallidal pathway is topographically organized within the GPi and GPe into distinct anatomical and functional zones, thus opening new perspectives in deep brain stimulation of the GP in movement disorders.

α-SYNUCLEIN INTERACTION WITH MICROTUBULES IN NEURONS: A RELEVANT EVENT IN HEALTH AND DISEASE?

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Context • Looking for the physiological and pathological role of the presynaptic protein α-synuclein, multiple partners and a plethora of neuronal functions have been intensively investigated. Among them, the interaction with tubulin remains controversial. Objective • Here, we deeply investigated the interplay between α-synuclein and tubulin using a set of complimentary approaches ranging from *in vitro* assays with pure proteins to analyses in human brain. Results • First, by microscale thermophoresis we found that the A53T and A30P variants of α-synuclein, which are linked to the pathogenesis of Parkinson's disease, significantly loose affinity for tubulin if compared with the wild-type protein. Next, by TEM and CryoEM we demonstrated that wild type and mutated forms of α-synuclein differently impact on microtubule ultrastructure causing changes in the diameter and the protofilament number of the *in vitro* assembled microtubules. Finally, we analysed the interplay of α-synuclein with post-translationally modified tubulins in *post-mortem* brains from patients affected by Parkinson's disease focusing on those brain regions that are involved in the disease. We found that a region-specific redistribution of acetylated α-tubulin occurs in patient samples with respect to controls. This seems to be correlated with the pathological oligomerization and aggregation of α-synuclein. Conclusions • Collectively, our data support the concept that the interaction with tubulin/microtubules might actually be crucial for α-synuclein in both physiological and pathological contexts.
This work was supported by Fondazione Grigioni per il Morbo di Parkinson, Milan (Italy).

ASSESSMENT OF THE EFFECTS CAUSED BY MECHANICAL STIMULATION ON PERIPHERAL NERVOUS SYSTEM

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Context • Since 1990 low back pain and neck pain are the leading causes of disability worldwide, the increase in interventional procedures and opioid prescriptions has not led to a lessening of the burden of chronic pain, so new pathways needs to be followed and testing novel approach in a reliable and feasible way is of paramount importance.
In the last decades it has been shown that the selective repeated tension of the Peripheral Nervous System (PNS), also known as neurodynamic treatment (NDT) can be successful in pain modulation of patients affected by chronic and acute back and neck pain. Although it's relevant clinical effects on pain and disability, nowadays the biological effects involved in the NDT still unknown and no standard protocols are available. Objective • The present study aims to assess the effects of NDT on the cells of PNS in order to develop a protocol of treatment for animal models of acute and chronic pain. Design • A repeated 4 arms randomized controlled trial was performed for each immortalized cell line. Setting • The study was conducted in the laboratories of the Department of Clinical and Biological Sciences, University of Turin Orbassano (To). Participants • Based on the principle of the 3R (Refinement, Replacement, Reduction) the research was started using in vitro models, in particular immortalized cell lines of motor and sensitive neurons (NSC34 and 50B11). Intervention • To assess the behaviour of the PNS cell populations to repeated mechanical stimuli a bioreactor was developed and used ad hoc. Protocols were tested starting from those reported in literature and refined by trials results. Experiments were performed using pre-coated silicone membranes and cells were seeded on them and repeated tension protocols were administered using the bioreactor. Morphological, genetic and protein expression analysis were performed. Results • A standardized protocol of NDT was possible to be defined. Preliminary results have shown that NDT seems to have no side effects and can affect neurites orientation, cell differentiation and avoid apoptosis. Interestingly, a protocol of NDT downregulates TLR2, a gene linked to mechanical allodynia and also up regulates genes involved in neurites growth and in cell defense mechanism against oxidative stress. Conclusions • Results from our preliminary experiments are promising and they suggest that NDT can be standardized being translatable in clinical practice and promote the regeneration processes in motor and sensory neurons.

THE TIMING OF MICROGLIA

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Microglia constantly scan the microenvironment by highly motile processes providing for defence against tissue damage and restoration of tissue homeostasis. In basal conditions, transient microglia-synapse interactions seem to be involved in synaptic activity regulation. We recently reported the occurrence of a daily reorganization in the input to orexin-containing neurons, which reside in the lateral hypothalamus (LH) and play a key role in wakefulness stability. The investigation of microglia morphology in the LH was also carried out in transgenic CX3CR1^{GFP} mice, in which microglial cells are tagged with green fluorescent protein (GFP). Diurnal changes in microglial morphology and microglia interaction with orexin cell bodies has been investigated in the LH of homozygous CX3CR1$^{GFP/GFP}$ mice, in which, however, fractalkine receptor may not be functional. The present study was therefore aimed at the investigation of microglia plasticity in the LH of heterozygous CX3CR1$^{+/GFP}$ mice, in which only one copy of the gene encoding fractalkine receptor is replaced by GFP. Video-recordings of freely moving mice was performed to assess the predominant vigilance state before sacrifice at two time points (night and day) in antiphase. Triple immunofluorescence in confocal microscopy was used to visualize orexin neurons and synaptic excitatory and inhibitory contacts, using presynaptic and postsynaptic markers). Similarly to the homozygous mice, 3-D reconstructions of GFP-labelled microglia in the LH of the heterozygous mice indicated a trend towards plastic diurnal changes in microglial cell morphology, which at night (when nocturnal rodents are predominantly awake) was endowed with more ramified processes than at daytime (the period of sleep predominance).

BLOOD-BRAIN BARRIER REPAIR BY ANNEXIN A1 DURING METABOLIC IMBALANCE

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The blood-brain barrier (BBB), a complex structure that controls the central nervous system homeostasis, is composed of endothelial cells, sealed by tight junctions (TJs), pericytes, astrocytes endfeet, and non-cellular components. In homeostatic conditions, the cells, by contact-dependent interactions and a wide variety of secreted factors, induce and maintain the barrier properties. Annexin A1 (AnxA1), an anti-inflammatory mediator expressed in brain microvascular endothelial cells, regulates the BBB integrity. A relation between unbalance diet and cognitive impairment has been demonstrated to be associated with BBB dysfunctions together with moderate neuroinflammation. With the aim of understanding the impact of metabolic imbalance and the possible role of AnxA1 on BBB function, we studied the expression and distribution of TJ proteins, claudin-5 and occludin, vascular basal lamina molecules, laminin isoforms, and AnxA1, in brain microvessels of wild type (WT) mice fed with chow diet (CHOW) and with a high fat diet (HFD), by immunofluorescence confocal microscopy. The BBB structural components have also been studied after administration of human recombinant AnxA1. The results showed an altered distribution of TJ proteins and a modified expression of laminin isoforms in HFD mice compared with WT mice. After AnxA1 administration, the brain microvessels seemed to recover the BBB features, suggesting a reparative effect of AnxA1 molecule during metabolic imbalance.

INVOLVEMENT OF PACAP-ADNP AXIS IN DIABETIC RETINOPATHY

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Diabetic retinopathy (DR) is a microvascular complication of diabetes leading to vision loss. Hyperglycemia causes hypoxic microenvironment and activation of inflammatory pathway involving some cytokines resulting in blood retinal barrier (BRB) impairment. In particular, triggering of hypoxia-inducible factors (HIFs) conduces to aberrant expression of some target genes such as vascular endothelial growth factor (VEGF).
In previous papers, we have demonstrated a protective role of pituitary adenylate cyclase-activating peptide (PACAP) in DR. It is well known that its effect in central nervous system is mediated through the activation of activity-dependent neuroprotective protein (ADNP). To date the retinal effect of PACAP-ADNP axis has not been investigated, yet. To determine its involvement in early phase of diabetes, we have tested modulatory activity of ANDP smallest active element, known as NAP, on hypoxic and inflammatory event characterizing early stage of DR.
The immunolocalization analysis, conducted by using confocal microscopy, have revealed that a single intraocular administration of NAP reduced retinal expression of HIF-1α and HIF-2α that were more intense in the inner nuclear layer (INL), in the outer plexiform layer (OPL) and in the photoreceptor layer (RCL) of STZ-injected rat. Moreover, intravitreal injection of NAP reduced the signal intensity of VEGF in RCL and in ganglion cell layer (GCL) as compared to STZ group. This peptide is also able to reduce expression of inflammatory cytokine IL-1β and its related receptors IL1-RI and IL1-RII in OPL, inner plexiform layer (IPL) and RCL of diabetic retinas.
A further characterization of PACAP-ADNP axis involvement in DR could lead to identification of new therapeutic targets counteracting efficiently retinal damages.

ANALYSIS OF THE THROMBIN PATHWAY IN SCHWANN CELLS AND OF ITS ROLE IN PERIPHERAL NERVE REPAIR AND REGENERATION

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Complex nervous systems require rapid nerve impulse transmission. Myelination by Schwann cells (SCs) in the peripheral nervous system (PNS) is essential for efficient saltatory conduction of action potentials.
At the center of the function of myelinated axons are the nodes of Ranvier. In the PNS, nodes are contacted by interdigitating microvilli that project from the end of the SCs to closely appose the nodal axolemma.
On the SC plasma membrane we and others have reported the presence of a protease activated-receptor, PAR1 which is concentrated at the level of the nodes. This receptor is a sensor for proteases (in particular for thrombin).
Thrombin generation is generally believed to have long-term beneficial effects for tissue repair. In this connection, our previous data indicate that PAR1 activation on SCs favors their ability to promote axonal regrowth after lesion. On the other hand, it has also been reported that an excessive generation of thrombin can be detrimental for nerve functions. In peripheral nerves after injury a rapid rise in thrombin activity can be measured and when thrombin is applied to peripheral nerve fibers it determines a conduction block.
Data presented here analyze the morphological alterations in the architecture of the nerve fibers associated with PAR1 overstimulation by thrombin or agonist peptides. A complete rearrangement of myelin is also detected following PAR1 activation. These effects of thrombin on nerve fibers can be overcame by a specific PAR1 inhibitor or controlling calcium entry through BAPTA-AM administration.
PAR1 on SCs may represent a novel possible target for pharmacologic therapies in order to preserve neuronal health during nerve injury.

MATERNAL SEPARATION IN ANOREXIC RATS: SEXUALLY DIMORPHIC EFFECTS IN THE REWARD SYSTEM

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Context • Anorexia nervosa (AN) is a complex, potentially life-threatening eating disorder characterized by self-starvation and excessive weight loss. Moreover, there are some genetic, biological, psychological and sociocultural factors that regulate the onset of this disease. The attachment during the postnatal period with the mother can have an important role in the pathogenesis of AN. Our study showed that the Maternal Separation (MS) induces alterations in the behavior of anorexic rats, obtained with the Activity-Based Anorexia (ABA) model: in females, the MS induces an increase of the hyperactivity during the ABA procedure and a less anxious phenotype. In males, instead, the anxiety behavior increase in MS anorexic rat in comparison with not handling ABA rats. Objective • These data suggested us to investigate the effect of the MS on the reward system. Setting • We performed a quantitative analysis of the dopamine neurons in the ventral tegmental area (VTA) and in the pars compacta of the substantia nigra (SNpc) and of the serotonin neurons in the dorsal raphe nucleus (DRN). Results • The results showed that in females the ABA protocol induces an increase of dopamine neurons in comparison with the control group and, only in females, the MS anorexic rats show a further increase in VTA. Regarding the serotoninergic system, only female rats showed a strong increase in MS anorexic groups in comparison with the ABA animals. Conclusions • Data obtained with the morphological analysis seems to explain some of the behavioral changes in anorexic animals after the maternal separation, including the increase of hyperactivity in MS anorexic females rats. However, future studies will be necessary to expand the study to the whole dopaminergic and serotoninergic pathways.

A LIGHT MICROSCOPY STUDY ON THE NEUROTENSIERGIC SYSTEM IN THE HUMAN CEREBELLUM

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Neurotensin (NT) is widely distributed in the central nervous system of mammals. NT play a considerable role in neuromodulation mechanisms, in Parkinson's disease and in schizophrenia. Data on the presence of neurotensiergic neurons in the cerebellum of mammals are not available. In the cerebellum of non-primates were detected the presence of few neurotensiergic extrinsic fibers, and of the neurotensin receptor subtypes NTR_2 and NTR_3. The aim of this study of chemical neuroanatomy, was to ascertain whether in the human cerebellum an intrinsic neuronal neurotensiergic system exist, taking fragments of postmortem human cerebellar cortex and dentate nucleus 36-48h after death, and subjecting them to light microscopy immunohistochemical procedures using rabbit and goat polyclonal antibodies respectively against NT and NTR_1. For positive controls were used fragments of rat intestine subjected to the same procedure.
In the cerebellar cortex, NT and NTR_1 were detected in the molecular layer, in subpopulations of stellate and basket neurons; in the Purkinje neuron layer, in a subpopulation of Purkinje neurons; in the granular layer, in subpopulations of granules, Golgi neurons and non-traditional large neurons (candelabrum, synarmotic, perivascular neuron). NTR_1 positivity was also observed in form of fine 'puncta' (putative terminals) in the neuropil and in close relationship to the wall of microvessels. In the dentate nucleus, NT and NTR_1 were observed in small associative neuron types and in several large projective neuron types and in the perivascular neuron. The present study reports the existence of an cerebellar neurotensiergic neuronal system may involved in neuromodulation mechanisms of intrinsic circuits, in the microvascular innervation, and in projective circuits to the midbrain areas such as the connections between the dentate nucleus and the substantia nigra and the ventro tegmental area, recently demonstrated, where the neuromodulatory action of NT is well known.

NOVEL INSIGHT ON THE FINE MECHANISMS OF THE ACTION OF METHAMPHETAMINE WITHIN CATECHOLAMINERGIC NEURONS

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Methamphetamine (METH) is abused worldwide and it represents a threaten for public health. METH exposure induces a variety of detrimental effects. In fact, METH produces a number of oxidative species, which lead to lipid peroxidation, protein misfolding and nuclear damage. Cell clearing pathways such as proteasome (UP) and autophagy (ATG) are involved in METH-induced oxidative damage. Although these pathways were traditionally considered to operate as separate metabolic systems, recent studies demonstrate their interconnection at functional and biochemical level. Very recently, the convergence between UP and ATG was evidenced within a single organelle named autophagoproteasome (APP), which is suppressed by mTOR activation. In the present research study, the occurrence of APP during METH toxicity was analyzed. In fact, co-immune-precipitation indicates a binding between LC3 and P20S particles, which also recruit p62 and alpha-synuclein. The amount of METH-induced toxicity correlates with APPs levels. Specific markers for ATG and UP, such as LC3 and P20S in the cytosol, and within METH-induced vacuoles, were measured at different doses and time intervals following METH administered either alone, or combined with mTOR modulators. Different approaches were used to document the effects of mTOR modulation on METH toxicity and the merging of UP with ATG markers within APPs. METH-induced cell death is prevented by mTOR inhibition while it is worsened by mTOR activation, which correlates with the amount of autophagoproteasomes. The present data, which apply to METH toxicity, are also relevant to provide a novel insight into cell clearing pathways to counteract several kind of oxidative damage.

NEUROIMMUNE MECHANISMS IN SYMPTOM GENERATION IN PATIENTS WITH NON-CELIAC GLUTEN / WHEAT SENSITIVITY

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Context• Gastrointestinal (GI) symptoms, e.g. abdominal pain and bloating, along with extra-GI manifestations, represent dominant clinical features of patients with non-celiac gluten/wheat sensitivity (NCG/WS). Typically, these patients develop GI and extra-GI symptoms few hours or days after gluten/ wheat protein ingestion. The mechanisms underlying symptom generation in NCG/WS remain poorly understood. Objective• This study was designed to define the role of neuro-immune interactions focusing on submucosal plexus (SMP) neurons and mast cells (MCs). Participant • Thirty-four patients with self-reported NCG/WS, 28 with celiac disease (CD), 13 with functional dyspepsia (FD) and 24 controls (HC) were enrolled. Intervention • SMP samples isolated from duodenal biopsies were analyzed by immunohistochemistry to obtain quantitative data on neuronal and MCs density and the percentage of MCs in close vicinity to nerve endings. Appropriate statistical tests were applied to compare the four groups and correlate the results to the clinical features and appropriate symptom questionnaire. Results• The number of SMP neurons was not different among groups. In NCGS/WS, the number of MCs was not different form HC, being slightly increased *vs.*CD ($P=0.07$), and decreased *vs.* FD ($P<0.05$). MCs in proximity to nerves were a common feature in all three groups of patients *vs.* HC ($P<0.001$). In NCGS/WS, MCs infiltration was correlated to bloating ($P=0.001$) and abdominal pain severity ($P=0.03$), and the percentage of MCs in proximity to neurons correlated with symptom severity ($P=0.05$), bloating and abdominal pain ($P=0.01$). Conclusions• Submucosal MCs infiltration and the close proximity to nerves can be a histopathological feature underlying abdominal pain and bloating severity in NCG/WS patients.

LOSS OF FOREBRAIN CHOLINERGIC NEURONS PURELY INDUCED BY SEIZURE SPREADING IN FOCALLY INDUCED LIMBIC STATUS EPILEPTICUS

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We induced limbic (SE) in rats by microinfusing pmolar doses of cyclothiazide+bicuculline into the anterior extent of the piriform cortex (APC), which is the brain site with the lowest threshold to seizures. This approach allows to evaluate selectively the effects of seizure spreading through the natural anatomical circuitries, differently from SE induced by systemic administration of kainate or pilocarpine.
Acutely, we assessed seizure behavior, EEG and seizure spreading [(by c-Fos and F-desoxyglucose (FDG) uptake]. Chronically, we assessed hippocampal mossy fiber sprouting, and the occurrence of neuronal loss, degenerative phenomena (by Fluoro-Jade B-FJB- staining) and expression of heat shock protein-70 (HSP-70) in the hippocampus, piriform cortex and ventromedial thalamus. We further analyzed in detail SE chronic effects in basal forebrain cholinergic areas, the medial septal nucleus (MSN), the diagonal band of Broca (DBB) and the Nucleus basalis of Meynert (NBM), as these nuclei are strictly connected with limbic structures, and play a key role in cognitive functions and vigilance.
We showed that during SE, epileptic activity spread to brain regions downstream to APC as shown by seizure features, EEG, c-Fos and FDG. Chronically, SE induces effects resembling human hippocampal sclerosis, together with cell loss and degeneration in limbic cortical and thalamic areas.
Finally, we showed a significant cell loss, FJB-staining and HSP-70 expression within MSN, DBB and NBM ipsi- and contra-laterally to the infusion site.
We provide direct evidence of SE-induced neuronal damage which is solely due to seizure activity. The damage in basal forebrain cholinergic nuclei might be particularly relevant in terms of chronic cognitive effects of limbic SE.

NEUROVASCULAR UNIT MODIFICATIONS OF GLIOBLASTOMA MULTIFORME

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Glioblastoma (GB) is the most common malignant primary brain tumor with the highest degree of vascularization among the CNS neoplasms. The GB vascular network shows an extreme variety of tumoral vessel morphology, ranging from normal-looking microvessels to glomeruloid structures. In parallel with the complex vascular architecture is the variety of its cellular constituents, growth mechanisms, and molecular triggers.
The purpose of this study is to investigate neurovascular unit (NVU) of GB vessels, analyzing their contribute to vessel growth and mutual relations in the newly formed tumoral vessels. Confocal microscopy analysis of GB tissues after multiple immunolabellings with endothelial-, pericyte-, and astroglial-specific markers was carried out.
Heterogeneity in tumour microvascular architecture reflects the different mechanisms of vessel growth and includes: a modified expression of P-glycoprotein (P-gp) from endothelial cells to tumoral cells and a diversified involvement of pericyte subsets, the latter identified by the expression of specific NG2 isoforms and by endosialin.
In GB, alternative pericyte-driven mechanisms of vessel growth significantly participate to tumoral vessel increase, possibly modifying P-gp expression. The limited impact of treatments targeting the abnormal GB vasculature reveals the need for additional targets that could be valuable to further improve therapeutic strategies.

A NEW *IN VITRO* MODEL TO STUDY THE DEVELOPMENTAL AND FUNCTIONAL FEATURES OF HUMAN BASAL FOREBRAIN CHOLINERGIC NEURONS

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Within the basal forebrain, the nucleus basalis of Meynert (NBM) provides the major source of cholinergic innervation to cortex and its degeneration has been associated to cognitive decline in neurodegenerative disorders (ND), such as Alzheimer's and Parkinson's diseases (AD, PD). Indeed, restoration of cholinergic function is targeted by the current treatments able to temporarily delay the AD progression. Moreover, recent findings by stereotactic mapping of the forebrain evidenced that NBM volume can predict early cognitive decline in PD. Since studies on the human NBM neuron biology are limited, we first decided to establish a primary culture of cholinergic neurons from the human fetal NBM (hfNBMs). An extensive characterization demonstrated the cholinergic identity of hfNBMs, mainly expressing specific markers (ChAT, VACHT, ACHE) and functional cholinergic receptors. Moreover, treating cells with the nerve growth factor (NGF), the main neurotrophin for NBM neurons, activated the specific TrkA signalling and promoted hfNBMs differentiation. When intravenously injected in NBM-lesioned rats, hfNBMs led to a significant improvement in cognitive and memory functions. In order to test whether hfNBMs could be useful to analyse the effects of known factors implicated in ND progression, such as neuroinflammation, we exposed cells to tumor necrosis factor α (TNF-α). TNF-α significantly increased the expression of the mature neuronal marker MAP2 and promoted neurite elongation, while significantly reduced immature neuronal markers (nestin, β-tubulin III) and TrkA expression. Overall, our results suggest that hfNBMs are a valid tool for investigating the biology of human NBM cholinergic neurons and their response to pathological factors.

ULTRASTRUCTURAL DISSECTION OF MITOCHONDRIA IN NEURODEGENERATION AND CEREBRAL TUMOR

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Mitochondria are highly dynamic organelles surrounded double membrane. They are key for energy production and cellular homeostasis in eukaryotic cells. Mitochondria have vital roles in calcium signalling and storage, metabolite synthesis and apoptosis. Therefore a regulation of mitochondrial number, distribution and activity is a key aspect for the maintenance of cell integrity.
A proper mitochondrial activity is pivotal to ensure cell survival, since mitochondrial dysfunction is a major hallmark of several pathologic conditions, ranging from neurodegeneration to tumors. Mitochondrial biogenesis and mitochondria-selective autophagy (mitophagy) regulate mitochondrial adaptation in response to cell malfunction. Thus, mitochondrial biogenesis and elimination of damaged mitochondria are highly regulated processes and influence both mitochondrial and cellular homeostasis.
Recent evidence indicates that an uncoupling between these processes induces altered mitochondrial function as a common denominator of several pathological conditions.
Here by using mitochondrial ultrastructure we describe the mitochondrial biogenesis, mitochondrial fission and mitophagy using in various cells as model for neurodegeneration and cerebral tumor.

EFFECTS OF RESVERATROL ADMINISTRATION ON FPR1 AND IL1Ra EXPRESSION IN A MOUSE MODEL OF NEUROINFLAMMATION

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Neuroinflammation is involved in both pathogenesis and progression of neurological disorders, including neurodegenerative diseases. In particular, recent evidences have shown that neuroinflammation may play an important role in Parkinson's disease insurgence and progression, and its modulation elicits neuroprotection. Also, a number of epidemiological studies have shown that early use drugs able to modulate inflammation, such as nonsteroidal anti-inflammatory drugs, significantly reduces the risk of developing Alzheimer's disease. Among the innovative therapeutic approaches that have been explored there is the targeting of receptors implicated in regulating and resolving the inflammation. One such family of receptors is represented by the formyl peptide receptors (FPRs), that bind different ligands, such as fMLP and lipoxin A4 (LXA4). However, the receptors are expressed in different cells and tissues, raising the possibility that FPRs have far more diverse and complex roles in biology. Interestingly, resolution of inflammation involves the formation of endogenous anti-inflammatory mediators, which signals the termination of recruitment and removal of inflammatory cells from the inflammatory locus. Among these, IL1 receptor agonist (Ra) has an important role in preventing the development or progression of inflammation. In this work we aimed to investigate in mice intracerebroventricular-injected with LPS the expression of FPR1 and IL1Ra and their possible modulation by resveratrol, a polyphenol associated with neuroprotective properties, to propose a novel anti-inflammatory and pro-resolving therapeutics in order to reduce the detrimental effects associated with neuroinflammation. We also investigated in both brain slices and lysates GFAP and Iba1 expression, markers of astrogliosis or microgliosis, respectively. Preliminary results of this study showed that resveratrol was able to modulate FPR1 and IL1Ra expression together with the reduction of both astroglia and microglia activation, suggesting its administration for the treatment of inflammatory based neurodegenerative diseases.

POST-INFLAMMATORY VISCERAL PAIN: GUT – SPINAL CORD RELATIONSHIP

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The management of visceral pain is a major clinical problem in patients affected by Inflammatory Bowel Diseases (IBDs) or by Irritable Bowel Syndrome (IBS), since it is experienced also in the absence of a relevant intestinal damage. The therapy for chronic visceral pain is still unsatisfactory because of the lack of knowledge about the underling pathological mechanisms. Aims of the present study was to characterize an animal model of chronic visceral pain in order to study the functional and morphological alterations in the gut and in the central nervous system. Visceral pain was induced in animals by the intrarectal injection of DNBS (30 mg in 0.25 ml EtOH 50%). Visceral sensitivity was assessed by measuring the magnitude of the abdominal response to colo-rectal distension and the behavioural alterations related to pain perception. DNBS induced a local inflammatory response peaking 3 days after its intra-rectal injection, a progressive remission started from day 7. As a consequence of the intestinal damage, the animals developed a visceral hypersensitivity which persisted also after colitis remission as confirmed by the intestine histological analysis on days 14 and 21. Fourteen days after DNBS injection, a significant morphological activation of both microglia and astrocytes (Iba1- and GFAP-positive cells), as well as an increased number of astrocytes, was observed in the dorsal horn of spinal cord. The same cellular framework was found in the ventral horn. In conclusion, the persistence of visceral pain induced by DNBS is not directly related to the intestinal damage, rather it is the result of alterations in the peripheral and central nervous system signalling and it is likely sustained by the activation of microglia and astrocytes in the spinal cord.

BRAIN ORGANOIDS FROM AN ENGINEERING POINT OF VIEW

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Cellular neuroscience focuses on 2-dimensional (2D) *in vitro* cultures (i.e. neural populations grown on a dish), *ex vivo* brain slices or on the study of animal models. While cell cultures are often over simplistic, brain slices show artefacts due to the cessation of brain activity at the moment of death, and animals cannot be used to infer how uniquely human cognitive abilities are managed at the cellular level. As a result, to date no models are capable to directly answering how perceptual experience, complex problem solving and planning arise from and relate to cellular processes.
Recent tissue engineering advances have provided 3D self-organizing models of the brain from stem cells, the so-called brain organoids, which are able to recapitulate the main functional and neuroanatomical features of the organ *in vivo*. To reveal organoid anatomy demonstrating that they faithfully reflect their *in vivo* counterparts, we designed a rigorous work-flow integrating imaging tools and image processing algorithms, applying it to mid-brain organoids.
We firstly customized the CLARITY protocol for unperfusable biological samples, such as the organoids. Since clarification parameters are dependent on sample, source and dimension, a quantitative metrics to identify the moment at which the clearing process should be terminated to avoid useless protein loss was used. The samples were then acquired using a confocal microscope for the visualization of cells deep within the construct. The datasets will be then processed using both automatic and manual segmentation tools to classify the cell types and identify specific brain regions, assessing the organoid ability to recapitulate both the regional complexity and cellular diversity.

GPER NEURONAL AND GLIAL CELLS EXPRESSION IN THE HYPOTHALAMUS OF ADULT RATS: SEXUALLY DIMORPHIC DISTRIBUTION AND DIFFERENCES DURING THE ESTROUS CYCLE

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The estrous cycle, in female mammals, is regulated by estradiol, which production and release from the ovaries fluctuate during the cycle with higher circulating levels in estrus than in diestrus phase. The physiological consequences of estradiol fluctuations may affect several hypothalamic nuclei as the arcuate (ARC), the paraventricular (PVN), the ventromedial (VMH), the lateral (LH), and the supraoptic (SON) nucleus, where classic and membrane receptors have been identified. Moreover, in vitro studies demonstrated that glial cells respond to hormones, in particular hypothalamic astrocytes in a sexually dimorphic way. Although it is known the involvement of the canonical estrogen receptors during the estrous cycle, very poor informations are available regarding G protein-coupled estrogen receptor 1 (GPER1).

We studied the expression of GPER1 within ARC, PVN, VMH, LH and SON nuclei in male and female rats during estrus and diestrus. GPER1-immunostaining experiments demonstrated that, except for VMH, the expression of GPER1 was sexually dimorphic in all ARC, LH and PVN neuroanatomical subdivisions with higher number of GPER1-ir cells in female animals. During the estrous cycle, ARC, SON and LH showed higher amount of GPER1-ir cells in estrus, and only in the anterior subdivision, while higher amount of GPER1-ir cells was achieved in all the VMH subdivisions and in the magnocellular PVN subnuclei during diestrus. Regarding hypothalamic glia, the percentage of glial cells expressing GPER1 resulted dimorphic, higher in female than male, with increased in estrus phase in VMH, ARC and LH. In addition, the analysis of coexpression of glial fibrillary acidic protein (GFAP) and GPER1 during the estrus cycle showed an increased number of astrocytes expressing GPER1 during estrus in VMH, ARC and LH, while no differences were detected in PVN and SON.

In conclusion, our data demonstrate that GPER1 expression is sexually dimorphic and fluctuates in hypothalamic neuronal and glial cells of female rats during estrus and diestrus suggesting new pathways in the regulation of hypothalamic circuits during the estrous cycle.

CHOLINERGIC TRANSMISSION IN THE BRAIN OF RATS WITH DIET INDUCED OBESITY (DIO)

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The association between obesity and cognitive health is receiving increasing recognition. Obesity and its comorbidities are linked with impaired cognitive performance and neurodegenerative pathologies such as vascular dementia and Alzheimer's disease (AD) in later life. High-fat diets (HFD) contribute to several of the key facets of AD: accumulation of β-amyloid, hyperphosphorylation of tau, and inflammation in the brain (including astrocyte and microglial activation). These neurodegenerative diseases affect the cholinergic system. This study evaluates the possible alterations of vesicular acetylcholine transporter (VAChT), of biosynthetic enzyme choline acetyltransferase (ChAT) and of acetylcholinesterase (AChE) in rats with diet-induced obesity (DIO) after 17 weeks of HFD (24 weeks of age), compared to the control rats with standard diet (CHOW). The obesity developed after 5 weeks of HFD (12 weeks of age). Body weight, systolic blood pressure, glycaemia and insulin levels were higher in DIO rats than in CHOW. No difference in total cholesterol and triglycerides was observed. In frontal cortex, hippocampus and brainstem, immunochemical and immunohistochemical techniques were performed to identify cholinergic markers. Our results showed a higher presence of VAChT and ChAT especially in frontal cortex and brainstem of obese rats. A reduced expression of VAChT was found in the frontal cortex of 24 weeks old DIO rats, while at the same age an increase of ChAT was evident in different brain areas. Based on our previous studies, in which were demonstrated astrogliosis and neurodegeneration in older DIO rats, the up-regulation in the synthesis of acetylcholine could be considered as a cholinergic recovery mechanism to overcome the brain injury.

A SILK FIBROIN CONDUIT TO IMPROVE PERIPHERAL NERVE REGENERATION: IN VITRO AND IN VIVO STUDY

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Peripheral nerve lesion are common injuries often caused by trauma or accident at work and can lead to loss of motor and sensory function. Despite the ability of the peripheral nerve to regenerate and re-innervate denervated target organs has been recognized, clinical and experimental evidences show that the regeneration is usually far from satisfactory, especially after severe injuries. In this study a Silk fibroin scaffold is used to test the regenerative potential of this biomaterial.
In vitro experiments were performed using glial RT4-D6P2T and neuronal NSC34 cell lines seeded on Silk fibroin. At this purpose, different parameters were evaluated: for RT4-D6P2T, proliferation and adhesion were evaluated 2-4-6 days after culture, for NSC34, differentiation and neurites elongation were investigated 5 days after seeding.
For *in vivo* experiments, the rat median nerve was repaired by Silk fibroin hollow tube. Samples were harvested at early (14, 24 days after nerve repair) time points, and different morphological analyses were carried out. Morphological analysis show the presence of cell types and first fibers appearance, typical of the first regenerative phase. Results obtained on RT4-D6P2T and NSC34 cell lines show that Silk fibroin represents a permissive substrate in term of proliferation and adhesion of glial cells, and differentiation and axonal elongation of NSC34 neuronal cell.

MPTP-INDUCED PARKINSONISM REVEALS THE NATURE OF CATECHOLAMINE-CONTAINING NEURONS IN THE MOUSE ENTERIC NERVOUS SYSTEM

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Parkinson's disease (PD) is a degenerative condition which affects dopaminergic neurons of the substantia nigra, leading to movement impairment, although visceral activities, especially at gastrointestinal level, are also affected. In this study, an attempt was made to reproduce these digestive dysfunctions by using the parkinsonism-inducing neurotoxin 1-methyl, 4-phenyl, 1,2,3,6,-tetrahydropyridine (MPTP) in 9-week old C57BL mice. One week after treatment with MPTP (i.p. 20 mg/kg x3, 2 h apart) morphological and biochemical changes on the nervous network of the gut were examined: tyrosine hydroxylase (TH), dopamine (DA) transporter (DAT) and norepinephrine (NE) transporter (NET) by immunostaining; catecholamine levels by HPLC-ED. In control mice, TH immunopositivity was well evident in both myenteric and submucous plexuses, as continuous markedly stained rings. From the submucous plexus, nervous fibres and neurons extended to the mucosa up to the axes of the villi. DAT and NET immunopositivity also appeared as stained rings. In MPTP-treated mice, both TH and DAT, but not NET, immunopositive neurons decreased in both plexuses and the continuous ring-like staining was no longer evident. Consistently, while NE levels were unchanged, there was a severe DA depletion. These morphological and biochemical changes were accompanied by a functional impairment which was reminiscent of constipation occurring in PD. These data provide a reliable model to investigate the altered gastrointestinal function in PD, and offer the basis to interpret the digestive dysfunction in PD as a consequence of a selective dopaminergic loss, thus confirming that DA neurons would be the sole catecholamine cells within intrinsic circuitries affecting gut motility and secretions.

MULTIPLE CELLULAR INTERACTIONS IN THE AGED HIPPOCAMPUS

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Aging and neurodegenerative diseases share a condition of neuroinflammation in the central nervous system (CNS), whose cells share a thick network of interactions tightly connecting them to each other from several points of view: either morphologic, functional and pathogenetic. Such network is pivotal in the maintenance of nervous tissue functionality, and might be greatly altered by neuroinflammation. This study assessed a possible correlation between neurons, astrocytes and microglia morphology in rat models of chronic neuroinflammation and aging, by FLIM/Phasor two photon and 3D confocal analysis implemented with particle analysis. Our data suggest that, in aged rats, morphofunctional alterations of one cell population reverberate to the others affecting their functional activities and amplifying detrimental effects of inflammation.

PERIPHERAL CHEMORECEPTORS IN SWINE CAROTIDES: AN EXCITING HUNT

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The carotid bodies (CBs) are small organs localized bilaterally at the bifurcation of the common carotid artery. They work as peripheral chemoreceptors that sense changes in arterial blood O2, CO2 and pH levels and activate sympathetic-mediated cardiorespiratory reflexes to restore the altered parameters. CBs are mainly formed by chemoreceptor cells (glomus cells) surrounded by glia-like sustentacular cells in small highly vascularized cell clusters called "glomoids". A variable quantity of connective tissue surrounds glomoids and defines the capsule of the CB, giving to the organ a discrete and compact aspect in some species whilst in others is rather diffuse. CBs have been recently addressed as peripheral metabolic sensors involved in glucose homeostasis in metabolic diseases like type II diabetes. Despite the pig represents one of the best pre-clinical model of type II diabetes available, a proper anatomical characterization of pig CBs is lacking in this species. In the present work we provide a detailed and updated anatomical characterization of pig CBs as a fundamental step for further studies in this species. We identified the CB bilaterally (n=7) as a small compact corpuscle attached to internal carotid wall in the proximity of the carotid bifurcation. We observed a lobular structure where it was possible to recognize glomus and sustentacular cells in clusters resembling the glomoids as described in other species. Type I cells were identified using antibodies against neural markers such as NSE and TH, while type II cells showed marked positivity to glial markers like GFAP and S100. Neurofilament immunolabeling revealed a rich amount of dispersed neural fibers arising from the CBs without forming a well identifiable nerve structure.

SEXUAL BEHAVIOR DIFFERENCES BETWEEN ROMAN HIGH AND LOW AVOIDANCE RAT LINES ARE ASSOCIATED WITH DIFFERENTIAL EXPRESSION OF THE IMMEDIATE EARLY GENES C-FOS, ΔFOSB AND ARC IN THE LIMBIC SYSTEM

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Context • Male Roman High- (RHA) and Low-Avoidance (RLA) rats display significant differences in sexual behaviour, since RHA rats exhibit higher motivation and better copulatory performance than RLA rats. Such differences are very evident in sexually naïve rats (which copulate with a receptive female rat for the first time), and persist, after five copulatory tests, when sexual experience has been acquired. Objective • Since sexual activity is a natural reward that involves activation of limbic brain areas, we studied whether the differences in sexual activity between the two rat lines are associated to changes in the expression of the immediate early genes (IEG) c-Fos, ΔFosB and Activity regulated cytoskeleton-associated (Arc) protein as indicators of neural activation and synaptic plasticity. Setting • By means of Western blot and/or immunohistochemistry, we investigated their expression in ventral tegmental area (VTA), nucleus accumbens (Acb) and medial prefrontal cortex (mPFC) of control (no sexual behaviour), sexually naïve and experienced (exp) RHA and RLA rats. Expression levels of selected IEG in the Roman lines was compared with concomitant changes in sexual motivation and copulatory performance in relation to the level of sexual experience. Results • c-Fos, ΔFosB and Arc expression increased differentially in the VTA, Acb (core and shell) and mPFC of RHA and RLA rats. In both rat lines, the increases were very evident in naïve rats, tended to disappear in exp rats, with the exception of ΔFosB which tended to accumulate with sexual experience, and were usually higher in RHA than RLA rats. Conclusions • These findings confirm that sexual activity induces neural activation in limbic brain areas involved in motivation and reward, thereby leading to changes in the mechanisms controlling neural plasticity with the acquisition of sexual experience, and imply that changes in these mechanisms may also depend on specific biobehavioural traits.
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EFFECT OF GHRELIN ON DIFFERENT MARKER EXPRESSION IN OLFACTORY ENSHEATHING CELLS. AN *IN VITRO* STUDY

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Ghrelin (Ghre), a gut-brain peptide hormone, and its receptor (GHS-R 1A) are expressed in different part of the central nervous system. Recently, several studies describe this peptide as a neuroprotective factor showing antioxidant, anti-inflammatory and anti-apoptotic properties.
Olfactory Ensheathing Cells (OECs) are particular glial cells responsible for the continuous neurogenesis in the mammalian olfactory system. Studies on axonal regeneration in animals with a spinal cord damage have showed that OEC transplantations may restore the injury. Furthermore, the OECs are able to secrete a variety of neurotrophic factors, promote axonal growth and support the remyelination of damaged axons. The aim of this *in vitro* study was to investigate on the effect of Ghrelin on the OECs. Primary OECs were isolated from neonatal rodent olfactory bulbs. Some cells were processed immunocytochemically to the detection of Ghre and its receptor. Other cells were grown with Ghrelin peptide at different concentrations (1μM and 2μM) and times (1-3 and 7 days). To analyse the results, 3-[4, 5-dimethylthiazol- 2-yl)-2,5-diphenyl] tetrazolium bromide test (MTT) and immunocytochemical procedures were used.
Our immunocytochemical study highlights, for the first time, that OECs express both Ghre and GHS-R1A and in addition show an OEC characterization towards a neuronal phenotype, as they are Neuregulin and MAP-2 positive. Moreover, the results obtained through the MTT test indicate that Ghre at 2μM after 7 days stimulates cell viability, in fact a significant increase ($p<0.05$) of OECs was observed. Besides the cells exhibit longer fibers than controls.
In conclusion, the presence and the action of Ghre on OECs could open a doorway that leads to investigate more deeply on the world of these cells.

NEUROPROTECTIVE EFFECT OF COMBINED EPIGENETIC DRUGS IN AMYOTROPHIC LATERAL SCLEROSIS MURINE MODEL

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that cause degeneration of motor neurons of central nervous system, for which no effective therapy exists.
Defects in histone homeostasis has been recently implicated in the pathogenesis of neurodegenerative diseases, including ALS. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) catalyze the acetylation and deacetylation, respectively, of histone proteins. HATs and HDACs use as substrates also transcription factors, such as nuclear factor (NF) kB. Transcriptional dysregulation occurs in human sporadic ALS and in the SOD1(G93A) mouse model. Five DNA-binding proteins can compose the NF-kB complex. The NF-kB dimer p50/RelA has a dual, neuroprotective or neurotoxic effect depending on its acetylation state.
Our aim is to test if the treatment with epigenetic drugs modulates the acetylation of RelA in the spinal cord of SOD1(G93A) mice, slowing down the disease progression of ALS. In order to promote a proper acetylation of NF-kB, a combination of the HDAC 1-3 inhibitor MS-275 and the sirtuin 1 activator Resveratrol were administered intraperitoneally every day in SOD1(G93A) mice at beginning of 50 day of life, until the death of the animals.
Behavioral tests showed a significant improvement of motor performance ($p<0.05$) of treated group versus control group. Furthermore a delay of pathological onset and an increase of survival rate ($p<0.05$) were detected in the treated group compare to the untreated once. Moreover, the epigenetic treatment elicited a neuroprotective effect on the lumbar spinal cord motoneurons of treated group compare to control group, accompanied by increased levels of protein products of NF-kB-target genes, Bcl-xL and brain-derived neurotrophic factor.
Our study reveals that the combined epigenetic drugs delay the degenerative process that occurs in ALS, representing a future promising therapy for this disease.

CHANGES IN IMMUNOCHEMICAL OCCURRENCE OF BDNF AND TRKB IN THE LIMBIC SYSTEM ARE RELATED TO DIFFERENT COPULATORY PATTERNS BETWEEN ROMAN HIGH- AND LOW-AVOIDANCE RATS

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Context • Roman High- (RHA) and Low-Avoidance (RLA) outbred rats were selected for a respectively rapid vs. poor acquisition of the active avoidance response in the shuttle-box. When exposed to stressors, RLA rats are reactive copers (i.e. show hyperemotional responses to novelty), and prone to develop depression-like behaviors, while RHA rats are proactive copers and resilient to stress-induced depression. In the presence of a sexually receptive female rat, Roman male rats display major differences in sexual activity that concur with the strongly different coping styles of the two lines. Thus, sexual motivation and copulatory performance, usually more pronounced in RHA vs. RLA rats, are evident in naïve rats during the first copulation, persist when sexual experience has been acquired, and involve activation of limbic brain areas. Objective • Mood disorders show reduced neuronal plasticity whose neurochemical and cellular underpinnings include the impaired brain-derived neurotrophic factor (BDNF)-trkB signalling as shown in the hippocampus of Roman rats. This study aims to clarifying the possible role of BDNF in mesolimbic neuronal plasticity. Setting • By means of Western blot and/or immunohistochemistry, here we report on the immunochemical presence of BDNF and trkB in ventral tegmental area (VTA), nucleus accumbens (Acb) and medial prefrontal cortex (mPFC) of control (i.e., no sexual behaviour), sexually naïve and experienced (exp) RHA and RLA rats. Results • As a general rule, BDNF and trkB expression levels showed different, often opposite, changes in the VTA, Acb and mPFC of naïve and exp vs. control rats. Thus, for example, after the first copulation BDNF increased in the Acb core and shell in RHA rats but decreased in RLA rats, while in sexually exp rats increased only in the VTA of RHA rats. TrkB changes were similar to those of BDNF in the Acb shell, while were opposite in the VTA and mPFC. Conclusions • These findings confirm that sexual activity induces neural activation in limbic brain areas involved in motivation and reward, thereby leading to changes in the mechanisms controlling neural plasticity with the acquisition of sexual experience, and imply that changes in these mechanisms may also depend on specific biobehavioural traits.
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APP-DEPENDENT GDNF GENE EXPRESSION DRIVES NEUROMUSCULAR JUNCTION FORMATION

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The physiological function of the Amyloid Precursor Protein (APP) remains poorly understood. APP has been extensively studied for its involvement in Alzheimer's disease (AD) since its amyloidogenic processing leads to the formation of beta-amyloid peptide (Aβ), the major constituent of senile plaques that are typical AD lesions. We contributed to a more comprehensive picture of APP regulatory network and physiological function by showing that APP-dependent regulation of the Glial cell line-Derived Neutrophic Factor (GDNF) drives the process of neuronal and muscular maturation involved in neuromuscular junctions (NMJs) formation. APP function is likely to rely in important part mainly to the regulation of APP target genes expression. So far, the identity of genes regulated by APP is intense matter of debate and, in addition, these genes are often difficult to relate to the phenotype observed in APP-deficient mice. APP-dependent GDNF transcription appears as critical for the muscular phenotype observed in APP null transgenic mice (APP-/-). Knowing that GDNF is a major neurotrophic factor, involved in PNS and CNS neuron survival, and that GDNF has been suggested marker of AD, it is of particular relevance to understand how APP controls GDNF expression, and how these pathways are related to AD pathological process. We show here the morphological analysis of the NMJs from the tibialis anterior of APP knock-out mice hindlimbs and the nerve-muscle co-culture system we set-up to build neuro-muscular contacts *in vitro*.

NEUROECOTOXICOLOGY: EFFECTS OF ENVIRONMENTAL HEAVY METAL EXPOSURE ON THE BRAIN OF AFRICAN GIANT RATS

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Increased exploitation of minerals has led to pollution of confined environments as documented in the Niger Delta in Nigeria. Information on the effects on the brain of such exposure is limited. Due to its exploratory activities, the African giant rat (*Cricetomys gambianus*) provides a unique model for ecotoxicological research to determine levels of animal and human exposure to different environmental pollutants. Aim of the present study is to unravel neuropathological features of this animal sampled from agro-ecological zones of Nigeria with different levels of heavy metal exposure. With ethically approved procedures, the animals have been collected in the field in three regions of Nigeria according to the previously determined data on heavy metal exposure: mangrove/fresh water swamp (high level of vanadium and selenium); woodland/tall grass savanna (lead, selenium and zinc); rain forest (low levels of heavy metals). Histochemical and immunohistochemical analyses have been conducted, focusing on different cell types. Interesting results have been up to now obtained concerning dopaminergic neurons of the substantia nigra pars compacta (SNC). Stereological cell counts of tyrosine hydroxylase (TH) cells showed a significant loss (-41.8%) of SNC dopaminergic neurons in the animals exposed to vanadium and selenium (mangrove), and (-50.7%) in those exposed to lead, selenium and zinc (woodland/tall savanna), compared to those from rain forest zone. Similarly, a significant loss (-39.9% and -40.8%, respectively) of parvalbumin-containing (PV) interneurons in the cingulate cortex has been documented in same animal groups compared to those of rain forest. These are the first "neuroecotoxicological" findings in distinct neuronal cell groups. Further analyses are in progress.