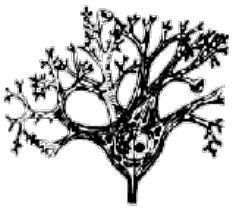
Gruppo Italiano per
lo Studio della Neuromorfologia



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EFFECTS OF COX-1 INHIBITION IN AN *IN VIVO* MODEL OF NEUROINFLAMMATION

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Neuroinflammation is closely related to the pathogenesis of neurological disorders, including neurodegenerative diseases. The hallmark of neuroinflammation is considered to be the microglial activation. Activated microglia secret an array of pro-inflammatory factors, such as prostaglandins, whose accumulation contributes to neuronal damages. Prostaglandin endoperoxide synthases or cyclooxygenases (COX-1 and COX-2), are the pharmacological targets of nonsteroidal anti-inflammatory drugs (NSAIDs), commonly used to treat pain and inflammation. A number of epidemiological studies have shown that early use of NSAIDs significantly reduces the risk of developing neurodegenerative diseases, such as Alzheimer's disease. Since it was reported that COX-1, owing to its predominant localization in microglia, is a major player in mediating the inflammatory response, the goal of this study was to evaluate in an *in vivo* model of neuroinflammation the effects of COX-1 inhibition using the selective inhibitor Mofezolac.
We adopted a model of neuroinflammation represented by 129SV mice intracerebroventricular-injected with LPS. A group of mice intraperitoneal received the selective COX-1 inhibitor, Mofezolac, or vehicle once a day for 10 days. On the 7th day, mice were anesthetized and positioned in a stereotactic apparatus and vehicle or LPS (5 μg) was administered; mice were killed 3 days later.
We observed a sustained astrocyte and microglial activation in the brain of LPS treated mice. In addition COX-1 expression resulted significantly increased after LPS treatment. Conversely, Mofezolac treatment, prior LPS challenge, determined a reduced expression in terms of COX-1 as well as a significantly decrease of both astrocyte and microglial activation. Interestingly, mofezolac resulted also able to regulate the NF-κB signaling pathway preventing IκB phosphorylation.
Overall these results demonstrated that Mofezolac was able to attenuate pro-inflammatory responses through the NF-kB signaling pathway modulation, thus suggesting a potential therapeutic use of this inhibitor in the treatment of neurodegenerative diseases with marked inflammatory component.

THE ALPHA9ALPHA10 NICOTINIC RECEPTOR ANTAGONIST ALPHA-CONOTOXIN RGIA PREVENTS NEUROPATHIC PAIN INDUCED BY OXALIPLATIN TREATMENT

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Oxaliplatin, a third-generation of platinum drug, is widely used alone or in combination with 5-fluorouracil and leucovorin to treat metastatic colorectal, ovarian, and pancreatic cancers. However, oxaliplatin is associated with common and severe side effects. Chronic neuropathy develops after long-term treatment with this drug, leading to pain and loss of sensory and motor function. The numerous analgesic compounds currently available are largely ineffective.
Cholinergic modulation is one of the pharmacological methods to the treatment of pain. Our previous study showed that the antagonism of the $\alpha9\alpha10$ nicotinic acetylcholine receptor (nAChR) subtype by the peptide α-conotoxin RgIA (RgIA) produces dose-related pain relief in rat models of nerve injury-evoked neuropathy and persistent inflammatory pain, and neurotoxicity induced by the antineoplastic drug vincristine.
Based on these findings, we hypothesized that in a rat model of oxaliplatin-dependent neuropathy (2.4 mg kg^{-1} oxaliplatin intraperitoneally daily for 21 days) RgIA could be able to counteract the neurotoxic phenomena elicited by chemotherapic treatment.
Here we demonstrate that RgIA (2 and 10 nmol injected intramuscularly once a day concomitantly with oxaliplatin treatment), reduces hypersensitivity and significantly prevents the oxaliplatin-dependent alterations of L4-L5 DRGs (dorsal root ganglia). Consistent with our previous results, in the spinal cord the numerical increase of microglia and astrocyte cell density evidenced in oxaliplatin-treated rats is partially prevented by RgIA treatment. Nevertheless, the administration of the α-conotoxin is able *per se* to elicit a numerical increase and a morphological activation of microglia and astrocytes in specific brain areas.

CEREBROVASCULAR NEURO-INFLAMMATION IN SPONTANEOUSLY HYPERTENSIVE RATS BRAIN: FACT OR FICTION?

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Spontaneously hypertensive rats (SHR) represent a model of hypertension and of vascular injury. In the past decade, SHR were also considered as a model of vascular dementia (VaD). Several studies have shown that cerebrovascular changes in SHR may mimic brain vascular disease diseases of hypertensive individuals.

Vascular and cerebrovascular changes during hypertension are often linked to inflammatory processes. Inflammation frequently affects vascular endothelium, perivascular astrocytes and other glial cells forming the blood brain barrier (BBB). This inflammatory reaction may lead to neuro-inflammation with consequent damage of brain tissue.

A significant brain atrophy and a reduction of white matter volumes, with areas of white matter demyelination and of circumscribed BBB dysfunction were found in SHR. Micro- and macrogliosis in deep cortical regions were also observed.

Based on these findings we confirm the inflammatory components of hypertension. We can also define neuroinflammation entity in SHR, using appropriate methodological approaches and inflammation markers. These included endothelial adhesion molecules [intercellular adhesion molecules-1 (ICAM-1); platelet endothelial cell adhesion molecule-1 (PECAM-1) and vascular cell adhesion molecule-1 (VCAM-1)], pro-inflammatory interleukins (IL-1□, IL-6, TNF□ and IL-18) and markers of BBB integrity (AQP4, S-100□, and claudin-5).

In the SHR cerebrovascular tree, the expression of endothelial adhesion molecules was significantly increased in comparison to normotensive Wistar-Kyoto rats. Moreover in SHR BBB was impaired with an increase of immunoreactivity for AQP-4 and a decreased expression of claudin-5. In SHR brain, an obvious glial reaction was found for both glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes and for microglia.

The above data suggest that SHR represent a reliable model of brain cerebrovascular damage and neuroinflammation related to hypertension.

IMMUNOCHEMICAL DETECTION OF BDNF IN THE BRAIN OF A RAT MODEL OF DEPRESSION

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Several lines of evidence show a relationship between alterations in the mechanisms that control the expression of neurotrophic factors and mood disorders. In particular, support for the role of brain-derived neurotrophic factor (BDNF) in the pathogenesis of depression and related deficits in neuronal plasticity comes from evidence that a reduction of BDNF expression has been found in postmortem brains and serum of depressed subjects and that the BDNF gene is required for the response to antidepressant drugs. With the aim to contribute to the characterization of the molecular and neuronal systems involved in the pathogenesis of depression and in the mechanism of action of the antidepressant treatments, here we use the outbread Roman High- (RHA) and Roman Low-Avoidance (RLA) rat lines, psychogenetically selected for rapid versus poor acquisition of active avoidance, respectively, and bearing several behavioral characteristics closely resembling the cardinal symptoms of depression, to investigate on the immunochemical occurrence of BDNF in selected areas of the RHA and RLA rat brain by means of western blot (WB) and immunohistochemistry. WB analysis indicates that the relative levels of BDNF patently and markedly differed in the hippocampus, where they were significantly lower in RLA vs RHA rats. In the remaining examined areas, namely the prefrontal cortex, the caudate-putamen complex proper, the core and shell regions of the nucleus accumbens and the ventral tegmental area, the relative BDNF levels did not show statistically significant differences. In tissue sections, BDNF-like immunoreactive (LI) material labelled neuronal cell bodies, proximal processes and varicose nerve fibers, with an uneven distribution in telencephalic cerebral cortex, hippocampus, amygdala, nucleus accumbens, caudate-putamen complex proper, thalamus and ventral tegmentum of the midbrain. Densitometric analysis of immunostained brain sections were used to quantify differences among the two rat lines. The results obtained provide a morphological evidence for a differential expression of BDNF in specific areas of RLA vs RHA rat brains and may form the morphological basis to understand the regulation of the trophic machinery in depression.

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IMMUNOCHEMICAL DETECTION OF TRKB RECEPTOR IN THE BRAIN OF A RAT MODEL OF DEPRESSION

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The outbread Roman High- (RHA) and Roman Low-Avoidance (RLA) rat lines were psychogenetically selected for rapid versus poor acquisition of active avoidance, respectively, and differ in many behavioural traits that closely resemble the cardinal symptoms of depression. Beyond the monoamine hypothesis of depression, compelling evidence suggests that mood disorders are characterized by reduced neuronal plasticity. Consistently, it has been shown that exposure to stress and antidepressant treatment modulate the expression of neurotrophic molecules and their relevant receptors, and that these changes show an anatomical specificity. With the aim to characterize the molecular and neuronal systems involved in the pathogenesis of depression and in the mechanism of action of the antidepressant treatments, here we investigate on the immunochemical occurrence of trkB, the high affinity tyrosine-kinase receptor for brain-derived neurotrophic factor (BDNF), in selected areas of the RHA and RLA rat brain by means of western blot (WB) and immunohistochemistry. WB analysis indicates that the relative levels of trkB patently and markedly differed in the prefrontal cortex and the hippocampus, where they were lower in RLA vs RHA rats, and in the caudate-putamen complex proper where, by contrast, they were higher in RLA vs RHA rats. No statistically significant differences were seen in nucleus accumbens and ventral tegmental area. In tissue sections, trkB-like immunoreactive (LI) labelling was mainly localized to neuronal cell bodies and proximal processes, unevenly distributed in the telencephalic cerebral cortex, the hippocampus, and the ventral tegmentum of the midbrain. Densitometric analysis of immunostained brain sections revealed that differences among the two groups are consistent to a good extent with WB data. As a whole, the finding of a different expression of trkB receptor in the RLA vs RHA rat brains implies the occurrence of an altered neuronal responsiveness to BDNF in specific brain regions and may contribute to outline the molecular and morphological basis for the distinct vulnerability to depression in the two rat lines.

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SYNAPTIC PLASTICITY IN ANIMAL MODELS OF ALCOHOLISM.

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Dendritic spines are tiny protrusions that actively participate in integrative functions of nerve cells. These structures are heterogeneous in size and shape and quickly modifiable by experience in a continuous process of synaptic plasticity. The study of dendritic spines is simply method to evaluate physiological and pathological changes of neuronal activity.

In animal models of drug addiction, especially in neurons of mesolimbic way, the dendritic spines are profoundly affected by treatments. In Particular, the medium sized spiny neurons (MSN) of the Shell of the nucleus accumbens seem to be particularly sensitive to different stages of an addiction process. Based on anatomical findings we reported changes in number of dendritic spines of MSN upon withdrawal from opiates, cannabinoids and ethanol. The reduction was found 'strictly' to second order dendritic branches where, dopamine-containing terminals impinging upon spines, make synaptic contacts. More recently we described that the long-thin spines seems preferentially affected from ethanol withdrawal raising the possibility that cellular learning of these neurons may be selectively hampered. We also found that the decrease of this particular type of spines was strictly accompanied by a reduction of tyrosine hydroxylase and postsynaptic density 95 immunostaining.

Here we present the results of the restoration on the density of long thin spines of accumbal MSN in ethanol withdrawal rat after treatment with L-dopa. These results suggest a potential relationship between spine shape, synaptic function, and morphological rearrangements of the spines as forms of developmental or experience-dependent plasticity.

OREXINERGIC INNERVATION OF THE OLFACTORY BULB: A LINK BETWEEN THE NOSE AND SLEEP-WAKE-REGULATORY HYPOTHALAMIC NEURONS

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A wealth of experimental and human data has implicated damage of neurons which contain orexin (OX)/hypocretin peptides in the sleep disorder narcolepsy. OX neuronal cell bodies, which play a key role in the regulation of sleep-wake stability, are located in the lateral hypothalamus. Previous work has shown that experimental influenza A virus intranasal infection can lead to narcoleptic-like sleep-wake changes in mice. This set of findings recalls attention on the neural links between neurons of the olfactory mucosa, located in the nasal cavity and therefore directly exposed to the environment, and hypothalamic sleep-wake-regulatory systems. In particular, neurons of the olfactory epithelium reach directly the olfactory bulb (OB) and, through retrograde axonal transport from OB neurons, they can reach neural stations that innervate the OB. Orexinergic innervation of the OB has been previously described. However, OX neuronal cell bodies which project to the OB have not been hitherto investigated. To unravel the organization of this pathway, we here injected the fluorescent tracer Fluoro Gold (FG) in the OB of adult wild-type mice. We then used double immunofluorescence with anti-FG antibodies to reveal retrogradely labeled neurons, and anti-OX-A antibodies to simultaneous reveal orexinergic cell bodies. The experimental findings have revealed that neurons distributed in the lateral hypothalamus are retrogradely labeled from the OB, though in lower numbers with respect to neurons retrogradely labeled in the same area from spread of FG injection into the prefrontal cortex. The identification of the orexinergic phenotype of hypothalamic neurons retrogradely labeled from the OB is currently in progress.

THE THALAMIC PARAVENTRICULAR NUCLEUS AS A NODE IN DIENCEPHALIC SLEEP-WAKE REGULATORY SYSTEMS

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The paraventricular thalamic nucleus (PVT), the dorsal component of the thalamic midline involved in the regulation of state-dependent, emotional and affective behaviour, is innervated by multiple cortical and subcortical sources and projects to the prefrontal cortex and limbic targets. Diencephalic afferents to PVT include intrathalamic input deriving from the reticular thalamic nucleus (Rt), the sheet of GABAergic neurons (in which parvalbumin is colocalized with GABA) implicated in sleep regulation. Diencephalic afferents to PVT also include fibers of the neurons which contain the orexin (OX)/hypocretin peptides, implicated in wake regulation, and reside in the lateral hypothalamus. Sites of possible interaction of thalamic and hypothalamic sleep-wake-regulatory systems have not been hitherto identified in the brain, and we hypothesized that PVT could represent a candidate for such interaction. We thus here verified whether the above two sets of input reach the same or different PVT neurons. In adult mice, by multiple fluorescent immunolabeling, we visualized simultaneously PVT neuronal cell bodies (revealed by calretinin immunostaining), OX-A synaptic endings (revealed by the coexpression of OX-A + synaptophysin), Rt synaptic endings (revealed by the coexpression of parvalbumin + synaptophysin). We thus obtained striking evidence of an almost 100% convergence of OX-A innervation and Rt innervation on the same neuronal cell bodies in PVT. In addition, Rt synaptic puncta were apposed to PVT somata which did not receive orexinergic innervation. The data highlight PVT as a unique node in sleep-wake-regulatory systems within the diencephalon. Thus, the balance between excitatory orexinergic input and inhibitory Rt input could directly regulate the activity of PVT neurons projecting to the prefrontal cortex, hippocampus and amygdala affecting day/night behavioural oscillation, which represents a key adaptive feature for the animal's survival.

CELL-CELL INTERACTION IN THE NEUROVASCULAR UNIT AND BBB MODULATION AS A THERAPEUTICAL APPROACH FOR DRUG TRANSPORT/TARGETING TO BRAIN

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The blood-brain barrier, a dynamic interface separating the brain from systemic circulation, is the major entry route for therapeutic compounds to the central nervous system. The blood-brain barrier phenotype of the endothelial cells of brain microvessels includes tight interendothelial junctions, the lack of pinocytosis and fenestrae, transendothelial transport pathways, and a metabolic barrier. The primary role of the blood-brain barrier is to create ionic homeostasis for neuronal functions, but it also provides the central nervous system with nutrients, protects it from toxic insults and enables communication with the periphery. The formation and maintenance of these organ-specific characteristics are based on a cross-talk between the main cell types of the neurovascular unit: brain endothelial cells, pericytes, astroglia, microglia and neurons. Co-culture of these cell types is a valuable tool to reveal cell-cell interactions and also represents a new and better model to study drug delivery across the blood-brain barrier. The problem of drug transport at the blood-brain barrier is two-fold: the great majority of neuropharmaceutical candidates, hydrophilic molecules, biopharmaceuticals, and efflux transporter ligands do not penetrate the blood-brain barrier, while unwanted side effects develop, if a drug with main peripheral action crosses the blood-brain barrier. Overcoming the major mechanisms restricting drug transport at the level of blood-brain barrier, tight interendothelial junctions, efflux transporters and the enzymatic barrier can lead to better drug penetration to brain. In addition, there are physiological transport pathways, the carrier systems and the adsorptive and receptor-mediated transports, which can be exploited for drug targeting. Strategies of drug delivery and targeting to brain include modification of the molecules, modification of the blood-brain barrier functions, and circumvention of the blood-brain barrier. Some of the techniques based on these strategies are already in clinical use, while others are promising new possibilities to improve the therapy of central nervous system diseases.

ROLE OF NG2 PROTEOGLYCAN IN NEUROINFLAMMATION AND BLOOD-BRAIN BARRIER DYSFUNCTION DURING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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NG2 is a membrane associated proteoglycan whose expression is developmentally regulated in different cell types, and also appears modulated in adult normal brain and in pathological conditions. NG2-expressing cell types in mouse CNS comprise oligodendrocyte precursor cells (OPCs), activated angiogenic pericytes and non-CNS resident macrophages. All these NG2+ cell types appear to react to the acute phase of autoimmune encephalomyelitis (EAE), the purported animal model for multiple sclerosis, characterized by inflammatory infiltrates, areas of demyelination, and blood-brain barrier (BBB) alteration. The observations of accumulation of shed extracellular domain of NG2 at the glial scar, increased proliferation of NG2+ OPCs, activation of NG2+ pericytes for immunosurveillance, and migration of NG2+ macrophages through the BBB at inflammatory demyelinated lesions, altogether suggest a role for NG2 in EAE. In this study, we investigated EAE in NG2KO mice and found a significantly mild EAE disease both at clinical and neuropathological level. In NG2KO EAE, demyelination and neuroinflammation were reduced, NG2+ OPC number did not change during the disease course, and tight junction protein distribution and BBB function appeared preserved. In addition, NG2 expression was observed for the first time in encephalitogenic T cells and bone-marrow-derived dendritic cells (DC), allowing the upgrade of the list of NG2-expressing immune cell types possibly involved in demyelinating diseases. In NG2KO mice, the encephalitogenic T cell response shifted toward a less inflammatory profile and DC expressed reduced level of IL-12, a cytokine involved in the differentiation of naïve T cells into Th1 cells. On the whole, these findings suggest that milder immune response might be of prime relevance in the attenuated EAE disease displayed by NG2KO mice together with reduced loss of OPCs at the remyelination stage and reduced BBB leakage.

METABOLIC SYNDROME, OBESITY AND CEREBROVASCULAR INJURY RELATIOSHIPS

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Metabolic syndrome (MetS), defined by a constellation of an interconnected physiological, biochemical, and metabolic factors is directly related to obesity. Obesity is a relevant medical challenge being associated with the development of chronic diseases affecting also nervous system. Obesity and MetS, increase the risk of atherosclerotic vascular disease, type-2 diabetes mellitus (T2DM) and all cause mortality.
In the central nervous system vasculature, the blood brain barrier (BBB) is the site of exchange between blood and neurons, and a damage of these barriers may disturb physiological balance between these two compartments.
The obese Zucker rats (OZRs), with a mutation in leptin receptor, represent a model of obesity characterized by the simultaneous occurrence of hyperglycaemia, hyperinsulinaemia and hyperlipidaemia.
The aim of this work is to characterize BBB alterations of OZRs compared to their cohort lean Zucker rats (LZRs) to assess possible cerebrovascular injury related to obesity. In male OZRs and LZRs, different BBB markers were assessed by immunochemical and immunohistochemical techniques. Aquaporin-4 (AQP4) as a water channel protein expressed by astrocyte and glucose transporter protein-1 (GLUT1) involved in the glucose passage across the endothelial cells were studied. On the other hand changes in the expression of adhesion molecules Claudin-5 and E-cadherin were evaluated.
In older OZRs intracerebral arteries, revealed a decrease of lumen with an increase of wall area. BBB of older OZRs revealed an increased expression of AQP-4 possibly related to an oedema formation. A downregulation of GLUT1 was found in OZRs of 12 weeks of age. This might be the adaptive reaction to prevent excessive glucose entering in neuron. On the contrary, in older OZRs an increase expression of GLUT1 was assessed. No changes were noticeable in the expression of adhesion molecules in the model investigated.
These findings suggest that OZRs developed specific vascular and BBB changes. This could contribute to clarify the influence of MetS and obesity in the brain and the correlation with cerebrovascular dysfunctions.

EFFECTS OF TREATMENT WITH ENVIRONMENTAL ENDOCRINE DISRUPTORS ON HYPOTHALAMIC POMC EXPRESSION

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Endocrine disrupting chemicals (EDCs) are environmental contaminants that alter functions of the endocrine system causing adverse health effects in an intact organism or its progeny. Exposure to EDCs may cause tumors, malformations, and behavioural alterations. Some EDCs are now recognized as 'metabolic disruptors: exposure to them may alter energy metabolism and/or increase body weight and abdominal fat.
We investigated the effects of some xenoestrogens identified as putative metabolic disruptors (bisphenol A, BPA; diethylstilbestrol, DES; tributyltin, TBT) on hypothalamic POMC system of adult male mice. POMC cells are found in the hypothalamic arcuate nucleus (ARC), while fibers innervate mainly the paraventricular (PVN) and the dorsomedial (DMH) nuclei. This system is activated by peripheral signals like leptin and reduces food intake while increasing energy expenditure.
Mice were fed for 4 months with a base phytoestrogen-free diet (to avoid estrogenic interferences) adding two different concentrations of TBT, DES, BPA and E_2. At the end of the experiment, the animals were weighted, killed and dissected brains were cut and sections were processed for POMC immunohistochemistry.
We found no significant differences in body weight of exposed adult mice. Our data showed a significant decrease of POMC expression in the ARC at the highest dose of DES, the lowest dose of E_2 and both doses of BPA. POMC expression in the PVN was not affected by any treatment, whereas in the DMH we observed always a decrease of the immunoreactivity (except for the lowest dose of TBT).
These results indicate that some EDCs may alter the hypothalamic circuits that control food intake and energy metabolism. The differences among E_2 and the other molecules suggest that POMC expression may be under regulation of metabolic pathways that are not directly influenced by estrogens. Further studies are required to elucidate all potential interactions between environmental substances and POMC system in different conditions, as for example in case of perinatal exposure.

KISSPEPTIN AND PARAVENTRICULAR NUCLEUS: EFFECTS OF BISPHENOL A ON CD1 FEMALE MICE

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The kisspeptin neurons express estrogen receptor α (ERα); thus, they are direct targets for estradiol action. Kisspeptin fibres from anterior ventral periventricular region, AvPV, and arcuate nucleus, Arc, project to the GnRH neurons and in a few other locations, including the hypothalamic paraventricular nucleus (PVN), the most important center for relationship among neural circuits controlling food intake/energy metabolism. The innervation of the PVN by the kisspeptin is dimorphic and heterogeneous, with changes during the estrous cycle and the development. The presence of a higher amount of fibres within the medial PVN suggest that kisspeptin could directly innervate parvocellular elements related to the control of energy metabolism and food intake. The kisspeptin system is an important target for the action of Bisphenol A (BPA), a endocrine disrupting chemical present in the environment and that can interfere with the synthesis, metabolism and action of endogenous hormones.
In this study we analyzed the effect of treatment with BPA (5-10-40 μg/kg bw/day) from gestational day 11 (G11) to postnatal day 8 (PND8) on kisspeptin fibres in PVN of CD1 female mice during the postnatal development (PND12 to PND30). Kisspeptin immunoreactivity was evidenced by using a rabbit antibody (AC#566, gift from I. Franceschini and M. Keller, Tours, France) and we quantified the extention of immunoreactivity by calculating the Fractional Area covered by the signal using Image J software.
Our results indicate that the perinatal treatment with BPA decreases the percentage of kisspeptin-ir fibers in PVN during the postnatal development and the higher dose has more effect with a strong decrease at PND21. Moreover, there is the presence of significant difference of BPA's effect among the medial and the lateral portion of the PVN, the treatment has more effect on the lateral portion, in particular on the dorsal lateral part.

CTIP2 AND SATB2 TRANSCRIPTION FACTORS ARE NOT TYPE AND TARGET SPECIFIC IN CORTICAL PROJECTION NEURONS

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Cortical neurons are characterized by a great heterogeneity in terms of morphology, laminar position, functional properties and connectivity. In particular, pyramidal cortical projection neurons can be ascribed to two major classes: the intra-telencephalic (IT) neurons projecting to ipsilateral and/or, through the corpus callosum, to contralateral cortex (CPN); and subcerebral projection neurons (SCPN), that project to different targets below the cortex. In order to identify these different classes through immunohistochemical studies, many markers with characteristic expression patterns have been selected. Among these, Satb2 and Ctip2 are transcriptional factors involved in the differentiation respectively of callosally-projecting neurons (CPN) and subcortically-projecting neurons (SCPN). They are co-expressed in a small population of neurons during embryonic development, but they were thought to become mutually exclusive around birth. In our study we observed that a population of double labeled cells (C+/S+) can be found not only in postnatal stages, but becomes even more consistent in adulthood. Through retrograde labeling we showed that Ctip2/Satb2 neurons project either to the contralateral cortex or to subcortical targets. Most of the sub-cortical projecting C+/S+ neurons, but not all, do not reach the spinal cord and project to brainstem nuclei. Therefore these results show that Satb2 and Ctip2 are not mutually exclusive and cannot be used alone as markers to indentify and distinguish CPNs from SCPNs.
Our data, and others in recent literature, suggest that a variety of transcription factors orchestrate the fate of cortical neurons and that the interplay among transcription factors, and even their quantitative expression, may influence their final destination. Therefore, manipulation of cortical neuron fate may be more complex than expected and reorienting cell fate in disease a challenging issue.

ROLE OF JNK1 IN OLIGODENDROCYTE DIFFERENTIATION

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The C-Jun N-terminal kinase (JNK) pathway participates in several physiological and pathological mechanisms by phosphorylation of downstream molecules. JNK is expressed in three isoforms, which seem to play, at least in part, different roles. JNK1 KO mice show several morphological alterations in the central nervous system, such as reduced development of the corpus callosum. In fact, JNK1 is involved in regional apoptosis during early brain development, in microtubule dynamics, in the regulation of dendritic architecture and in interneuron migration during cortical development.
The aim of this project was to investigate the role of JKN1 in the development of myelin tracts. IN particular, we focused on oligodendrocyte development. The somatosensory cortex of JNK1 ko mice was reacted with PDGFRalpha antibodies to label oligodendrocyte precursors cells (OPCs) and with myelin binding protein (MBP) ab to label the mature, myelinating ones. The immunohistochemical and cell counting analysis revealed a significant increase of OPCs at P7 and P15 in KO mice compared to WT, in parallel with a lower grade of myelination at P15 and P90.
Therefore our findings suggest that JNK1 plays a consistent role on oligodendocyte differentiation. Further experiments will investigate the molecular mechanisms of these differences. The myelin JNK1 KO mice defect could be due to an OPC maturation deficit or to an increased mature oligodendrocyte apoptosis. Moreover, it would be interesting to analyze how the oligodendrocyte myelination defect is connected to dendritic and axon architecture of the developing cortex. In conclusion, our purpose is to clarify the JNK1 role in the cellular and molecular mechanisms of myelination, and the development of great cortical tracts.

NEUROTROPHIC FACTORS CONJUGATED TO IRON OXIDE NANOPARTICLES: A PROMISING TOOL FOR PROMOTING PERIPHERAL NERVE REGENERATION.

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For peripheral nerve regeneration nerve conduit are a valid alternative to nerve autotrasplant. Chitosan tube has been shown to be successful for short nerve gap, indeed for extended injury (gap longer than 10mm) innovative nerve conduits are needed. One approach is the delivery of neurotrophic factors (NTFs) within the conduit to promote cells invasion of the tube and to facilitate the axon elongation in it.
Here we investigated, *in vitro*, the bioactivity of three selected neurotrophic factors conjugated to iron oxide nanoparticles (IONP-NTFs), analysing the neurite outgrowth inductive activity in adult or neonatal DRG culture, where factors were administrated in the medium or mixed in a matrix (NVR gel). We compared IONP-NTFs and bone marrow-derived stem cells genetically engineered to overexpress those neurotrophic factors (NTF-BMSCs). Using the same system we analyzed the stability of IONP-NTFs compared to the non conjugated NTFs. Next we investigated *in vivo* how the enrichment of chitosan tube with NVR gel and IONP-glial derived neurotrophic factor (IONP-GDNF) could improve nerve regeneration in rat sciatic injury model. The hollow tube or the tube enriched with NVR gel alone or with GDNF were compared with the tube filled with NVR and IONP-GDNF. Functional and morphometrical analysis were performed.
We demonstrated that the conjugation with IONP preserves the bioactivity of the factor and increases its stability. From the *in vitro* results the IONP-NTFs have the same ability to induce neurite outgrowth as the non conjugated molecules but with a greater stability. Our *in vivo* study confirms that chitosan tube enriched with a matrix with GDNF give a more successful result in term of regeneration and functional recovery respect to the empty tube, moreover there are no detectable differences between free versus conjugated NTFs at the time point evaluated.

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PROTECTIVE EFFECT OF GROWTH FACTORS ON TISSUE TRASNGLUTAMINASE OVEREXPRESSION INDUCED BY β-AMYLOID IN OLFACTORY ENSHEATHING CELLS. A STUDY *IN VITRO*

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Tissue transglutaminase (TG2) is a calcium dependent protein implicated in numerous physiological and pathological cellular processes, including signal transduction, cellular survival, differentiation, apoptosis, cancer and neurodegenerative diseases, such as Parkinson and Alzheimer diseases (AD). TG2 activity has been detected in normal and AD brains and it has been proposed that the protein is involved in development of abnormal insoluble neurofilaments. Furthermore, it has been demonstrated that Amyloid-beta (Aβ) is a substrate for TG2, which is a reactive acceptor and donor sites responsible for the TG-catalysed formation of polymers.
In previous studies, we demonstrated a relationship between TG2 and Growth Factors (GFs) in astrocytes and Olfactory Ensheathing Cells (OECs), a cell type capable of continous neurogenesis throughout lifetime. Specifically, we showed that TG2 overexpression induced by some stressors was down-regulated by GFs exposure in OECs.
Herein, we assessed the effect of some GFs (bFGF and GDNF) on TG2 overespression induced by native full-length peptide (Aβ 1-42) or by fragments, such as Aβ (25-35) or Aβ (35-25, as control). To monitor cell viability, MTT test was used, whereas cell morphological features was examined by immunocytochemical procedures, using anti-Vimentin and anti-Caspase-3 cleavage. TG2 expression levels were evaluated immunocytochemically.
Our results demonstrate that when OECs were exposed to Aβ (1-42) or Aβ (25-35) fragments for 24 h, TG2 expression was increased. The pre-treatment of cells with GFs significatively decreased the TG2 positive cells, when compared with the untreated controls. In addition, Vimentin expression in treated and untreated-OECs remained unchanged, while Caspase-3 cleavage was modulated by GFs in stressed cells with Aβ (1-42) or Aβ (25-35), when compared with the controls.
Our data suggest that OECs exposed to GFs, which are able to modulate TG2 levels, could be an innovative mechanism to contrast TG2 expression, which plays a key role in AD.

EXOSOMES: A NOVEL THERAPEUTIC APPROACH FOR ALS?

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Amyotrophic lateral sclerosis (ALS) is a fatal progressive neurodegenerative disease and mutations in superoxide dismutase gene (SOD1) are the major genetic contributor to ALS. Therapeutic strategies for ALS are actually minimally effective on patients' survival and quality of life. Stem cells represent a promising therapeutic approach in the treatment of neurodegenerative diseases and their beneficial effect seem to be due through a paracrine effect via the release of exosomes.
In the present study we wanted to assess the neuroprotective effect of exosomes derived from adipose stem cells (ASC) on *in vitro* and *in vivo* models of ALS, and to monitor the homing of exosomes after their *in vivo* administration.
In *in vitro* experiments, the administration of ASC-exosomes after oxidative insult (H_2O_2) on motoneuron-like cell line (NSC-34) naïve and transfected with different human mutant SOD1 gene (G93A, G37R, A4V), protected cells from oxidative damage, with a significantly increase of cell viability. In *in vivo* experiments, the intravenously injection of ASC-exosomes in SOD1(G93A) mice at clinical onset until terminal stage point out that exosomes delay symptoms progression and postponed lifespan of treated animals.
Our results demonstrate that ASC-exosomes have a neuroprotective effect in *in vitro* and *in vivo* models of ALS, indicating a possible new strategies as therapy in this neurodegenerative disease.
Moreover, we set up a new protocol to label exosomes with superparamagnetic iron oxide nanoparticles, which allow to evaluate the tracking and the homing of exosomes *in vivo* with a non-invasive technique, as magnetic resonance imaging.

EPIGENETIC MODULATION IN THE AMYOTROPHIC LATERAL SCLEROSIS MURINE MODEL

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that cause degeneration of motoneurons of central nervous system. Currently there is no effective therapy for ALS.
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 non-histone proteins, notably 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 NFkB complex. The NFkB 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 modulate the acetylation of RelA in the spinal cord and motor cortex of SOD1(G93A) mice and slow down the disease progression of ALS. In order to promote a proper acetylation of NFkB, a combination of the HDAC 1-3 (HDAC class I) inhibitor Entinostat MS-275 and the sirtuin 1 (HDAC class III) 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 show a significant improvement of motor performance ($p<0.05$) of treated group versus control group in Paw grip endurance and Rotarod tests. Furthermore we observed statistically differences in a delay of onset of disease ($p<0.05$) in the treated group compare to the untreated once.
Our study reveals that the combined epigenetic drugs delay the degeneration process that occurs in ALS, representing a future promising therapy for this disease.

INVOLVEMENT OF MUSCLE-SPECIFIC MIRNA-206 IN THE SPINAL MUSCULAR ATROPHY PATHOGENESIS

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Spinal muscular atrophy (SMA) is a fatal paediatric genetic disease, characterized by motor neuron (MN) death, leading to progressive muscle weakness, respiratory failure, and, in the most severe cases, to death. SMA is due to the deletion or mutation of the telomeric survival MN gene (SMN1), on chromosome 5. Its homologous, SMN2 gene, only produces a limited amount of functional protein which can modulate SMA severity.
Specific or general changes in the activity of ribonucleoprotein containing micro RNAs (miRNAs) play a role in the development of SMA. Additionally miRNA-206 has been shown to slow amyotrophic lateral sclerosis progression by promoting a compensatory regeneration of neuromuscular synapses. Therefore, we correlated the morphology and the architecture of the neuromuscular junctions (NMJs) of the quadriceps, a muscle which is affected early in SMA, with the expression levels of miRNA-206 in a murine model of intermediate SMA (SMA II).
Our results showed a decrease in the percentage of type II fibers, an increase in atrophic muscle fibers and a remarkable accumulation of neurofilament (NF) in the pre-synaptic terminal of the NMJs in the quadriceps of SMA II mice. Furthermore, molecular analysis highlighted a direct link between miRNA-206-HDAC4-FGFBP1, and in particular, a strong up-regulation of this pathway in the late phase of the disease.
We propose that miRNA-206 is activated as survival endogenous mechanism, although not sufficient to rescue the integrity of motor neurons. We speculate that early modulation of miRNA-206 expression might delay SMA neurodegenerative pathway and that miRNA-206 could be an innovative, still relatively unexplored, therapeutic target for SMA.

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CAN A DELAYED REPAIR OF A PERIPHERAL NERVE INJURY LEAD TO A COMPLETE REGENERATION?

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Peripheral nerve regeneration and complete functional recovery after severe injuries not always occurred. Many factors can influence the complete recovery: patient age, lesion site, injury severity, size of the gap between damaged nerve stumps and time interval that elapses before performing surgical repair.
The poor outcome occurring after a long delay can be due to loss of the neuron ability to regenerate, loss of the Schwann cell ability to support regeneration and, of course, progressive muscle atrophy.
The aim of this study was to investigate the nerve regeneration after delayed repair and to study the degenerative processes of the denervated distal nerve stump.
Adults Wistar rat median nerves were transected and the distal parts were analysed after 3, 6 and 9 months of degeneration. Morphological and biomolecular analysis showed atrophic Schwann cells, a lot of collagen fibers and fibroblasts and a significant reduction of soluble NRG1already after 3 months of degeneration.
Immediately after median nerve transection or after 3 and 6 months, the median nerve was repaired with a neurotization with the ulnar nerve. The distal part of regenerating nerves were analysed after 6 months of regeneration. During regeneration, functional analysis was performed using grasping test and a functional recovery was observed only in the group repaired immediately (approximately 50% of the animals), and not in groups repaired with a delay of 3 and 6 months. Despite these functional results, morphological analysis showed regenerating fibers in all groups even if the quantitative analysis showed that in the 6-months delayed group they are fewer and smaller compared to immediate repair. Moreover biomolecular analysis on the 6-months delayed repaired nerve showed that soluble NRG1 maintain a low expression also after 6 months of regeneration.

MOVING TOWARDS THE COMPREHENSION OF THE HUMAN BRAIN CONNECTOME: PERILS, PITFALLS AND HIGHLIGHTS OF TRACTOGRAPHY

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Neurons do not work as isolated entities but interact with each other through afferent and efferent connections, so that the different sensory, motor and cognitive tasks can be realized. Brain connectivity is intended to describe the "morpho- functional strength" of such interactions. In the last twenty years, several non-invase, in-vivo brain-mapping imaging techniques have tremendously contributed to our understanding of the brain organization and function.
Understanding the organization and integrity of the underlying white matter circuitry is of of uttermost importance in assessing brain normal function and the pathophysiology of some of the brain disorders.
Until recently, the organization of white matter in the human brain has been studied post-mortem with fiber dissection, through indirect assessment of tracts' functional associations from studies of functional impairments in trauma patients, or with invasive tracer techniques in primates.
However, the limitations of these techniques have been overcome by the introduction of Diffusion Tensor Imaging (DTI) and white matter fiber tractography. To date, DTI is known as one of the most powerful tools for tracking neural connections non-invasively in-vivo in humans estimating properties of preferential anisotropic diffusion of magnetically labelled water molecules along myelinated axons. Since DTI suffers from several limitations, many multi-tensor models DTI have been recently developed and used for improving the evaluation of neural connectivity in normal and pathologic conditions. One promising approach is Constrained Spherical Deconvolution (CSD), a diffusion modelling technique that allows reliable estimation of one or more fiber orientations in presence of intravoxel orientational heterogeneity and provides data less affected by reconstruction biases and artefacts. Using this innovative approach we have recently shown.

COLONIC DYSFUNCTIONS IN EXPERIMENTAL PARKINSON'S DISEASE

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Parkinson's disease (PD) is a degenerative neurological disorder, which is often associated with gastrointestinal disturbances, mostly represented by constipation and defecatory dysfunctions, which have received considerable attention owing to their potential value as early marker of disease onset [1]. Our study aims at evaluating whether motor dysfunctions and neuropathological alterations occur in colon from rats with experimental PD.

PD was induced in rats by injection of 6-idroxydopamine (6-OHDA) into the medial forebrain bundle. Functional and morphological studies were carried out 4 and 8 weeks after 6-OHDA injection. Colonic muscle contractions were recorded in organ baths after electrical and carbachol-induced cholinergic stimulation. Colonic tissues were also examined for myenteric HuC/D^+ neurons and $S100^+/GFAP^+$ glia; choline acethyltransferase ($ChAT^+$); muscarinic receptors; malondialdehyde (MDA); tumor necrosis factor (TNF); inflammatory cells (eosinophils, mast cells).

At both time points 6-OHDA-induced nigrostriatal denervation was associated with: altered electrically evoked neurogenic and carbachol-induced cholinergic contractions; decreased ChAT immunopositivity in the myenteric ganglia; increased expression of colonic muscarinic receptors; increased tissue levels of MDA (membrane oxidative stress) and TNF (tissue inflammation); mast cell and eosinophil infiltration associated with $GFAP^+$ myenteric glia activation.

Our findings suggest that experimental PD, elicited by nigrostriatal dopaminergic degeneration, is associated with impaired colonic excitatory cholinergic neurotransmission and neuroinflammation, which together may account for enteric dysmotility.

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INTESTINAL EPITHELIAL BARRIER DISFUNCTION IN PATIENTS WITH CHRONIC INTESTINAL PSEUDO-OBSTRUCTION

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Chronic intestinal pseudo-obstruction (CIPO) is a rare condition characterized by severe gastrointestinal impairment causing a clinical picture mimicking a mechanical obstruction lacking any occlusion. Although degenerative or inflammatory neuro-interstitial cells of Cajal-muscular abnormalities are the main pathogenetic mechanisms underlying gut dysfunction, other factors, i.e. intestinal epithelial barrier (IEB) abnormalities, may trigger the initial insult.
To assess the expression of occludin and zonula occludens-1 (ZO-1), two major components of tight junctions (TJs), as markers of IEB in CIPO.
n=26 clinically and histopathologically characterized CIPO pts (15 F; 16-75 yrs) were studied. CIPO cases were subdivided in 3 groups according to histopathology: A) apparently normal n=7; B) inflamed n=8; C) degenerative n=10. Patients (n=8; 3 F; 48-73 yrs) undergoing elective surgery for uncomplicated neoplastic diseases served as controls. Jejunal full thickness biopsies were processed to assess occludin and ZO-1 mRNA and protein expression using q-PCR and WB.
Compared to controls, total occludin protein showed a marked decrease in CIPO pts ($P<0.05$); and a tendency to a decreased mRNA expression. Occludin oligomers, an index of occludin assembly in rafts TJs, were detected only in 19% of CIPO pts while all controls showed normal oligomerization. ZO-1 protein and mRNA expression did not change in CIPO vs controls. Interestingly, the tree sub-groups of CIPO showed a selective reduction of only one of the two analyzed components. Specifically, occludin decreased in group-A and B ($P<0.05$), while ZO-1 diminish only in group-C ($P<0.01$).
IEB was altered in patients with CIPO as identified by the reduction of at least one of the TJ components. The abnormal occludin oligomerization is indicative of TJ dysfunction, which increases the possibility of noxious agents passing through the intestinal wall. A better knowledge of IEB altered molecular mechanisms can provide a basis to novel targeted therapies.

SMALL VESSELS ABNORMALITIES IN THE GI TRACT OF PATIENTS WITH MITHOCONDRIAL NEUROGASTROINTESTINAL ENCEPHALOMYOPATHY (MNGIE)

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MNGIE is a genetic disorder leading to a dysfunctional thymidine phosphorylase (TP) converting nucleosides in the savage pathway. TP, however, is also known as *endothelial cell growth factor-1* being implicated in angiogenesis. Since MNGIE patients show severe gastrointestinal (GI) dysmotility, i.e. recurrent chronic intestinal pseudo obstructions (CIPO), and malabsorption (contributing to cachexia), we tested whether gut dysfunction may rely upon an impaired microvessel formation.
To verify whether blood vessel abnormalities occur in MNGIE vs. other severe dysmotility, e.g. non-MNGIE CIPO, through morphometric assessment of the GI submucosal microvasculature.
N=3 MNGIE (3M; 22-32 yrs), n=14 non-MNGIE CIPO (8M; 16-75 yrs) and n=5 (3 M; 48-73 yrs) patients (operated for uncomplicated neoplastic diseases - i.e. controls) were examined. Jejunal full thickness biopsies were formalin fixed, paraffin embedded, and sections were stained using 1% orcein to identify the vasculature elastic fibers. Vessel density was quantified as vessels/μm^2 of submucosa and subdivided in five classes: **a**) >501; **b**) 500-301; **c**) 300-101; **d**) 100-51; **e**) <50 μm.
In MNGIE vessels increased significantly vs. controls (20±1.5 vs. 10±2.9 vessels/μm^2, respectively; $P<0.01$). Also non-MNGIE CIPO showed a trend to an increased number of vessels. However, based on size, vessels with the lowest diameter (**class-e**) drastically increased in both MNGIE (~50%; $P<0.01$) and non-MNGIE CIPO (~40%; $P<0.05$) vs. controls. Conversely, ~75% of vessels with the widest diameter (**class-a)** were lost both in MNGIE ($P<0.001$) and non-MNGIE CIPO ($P<0.05$), while **class-c** vessels decreased in MNGIE (~60%; $P<0.001$) and non-MNGIE-CIPO (~35%; $P<0.01$) vs. controls. The structure of microvasculature, mainly **class-a** and **-b**, resulted markedly abnormal in MNGIE.
Submucosal microvascularization is markedly altered in MNGIE and, to a lesser extent, in non-MNGIE CIPO. These findings provide a morphological support to understand the severe GI dysfunction, including malabsorption, in CIPO of MNGIE and non-MNGIE origin.