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ENTERIC NERVOUS SYSTEM DEVELOPMENT AND ORGANIZATION

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The enteric nervous system (ENS) is the intrinsic nervous system of the gut which controls the motility, secretion and blood flow of the gastrointestinal tract. The ENS is a collection of interconnected ganglia which are embedded within the gut wall and are composed of a vast number of neurons (subdivided into many different subtypes) and many more glial cells. As most other parts of the peripheral nervous system in vertebrates, the ENS is derived from neural crest cells which originate primarily from the vagal region of the neural tube during embryogenesis. Upon delamination from the dorsal neural tube, prospective enteric neural crest cells migrate ventrally, invade the foregut and migrate rostrocaudally to colonize the entire organ. Failure of complete colonization of the gut leads to dysmotility of the aganglionic segment and obstruction, a hallmark of Hirschsprung's disease. During the last two decades many of the signalling pathways and transcription factors that control the migration and differentiation of enteric neural crest cells have been identified. Nevertheless, the genetic and molecular mechanisms that regulate the assembly of diverse enteric neuron subtypes into functional neural circuits within the gut wall remain unknown. This paucity of understanding is the complexity of the ENS in adult animals and the lack of analytical tools to dissect the arrangement of its constitutive axonal tracts relative to the main axes of the gut.

To address these limitations we have been developing genetic and molecular tools in order to increase the resolution of analysis of the mammalian ENS. In particular, by combining a *Sox10CreER*^{T2} transgene with a Cre-dependent reporter allele of the *Rosa26* locus (*Rosa26EYFP*) and administering limiting amounts of tamoxifen, we have been able to label subsets of enteric neurons born at specific time points during embryogenesis. Analysis of the trajectory of the projections of enteric neurons born at E11.5-12.5 demonstrated that the vast majority of them was arranged parallel to the long axis of the gut and directed anally. These findings suggest that genetically controlled cell intrinsic mechanisms specify the organization of axons of specific subsets of enteric neurons. This idea is further supported by analysis of mouse embryos in which core components of the planar cell polarity pathway (such as Celsr3 and Fzd3) have been deleted specifically in neural crest derivatives. Interestingly, in contrast to the wild-type embryos, axons of Celsr3-deficient enteric neurons are randomized relative to the gut axes. To explore the physiological consequences of the altered pattern of organization of enteric neuron axons in these mutants, we video recorded the peristaltic activity of colonic preparations from wild-type and Celsr3-deficient gut and generated spatiotemporal maps of major peristaltic waves, such as the colonic migrating motor complex (CMMC). This analysis has revealed reproducible changes in the frequency, distance travelled and orientation of CMMCs in Celsr3 mutants relative to their wild-type counterparts.

Our studies provide proof of principle to the idea that the complex enteric networks in mammals are built around relatively simple and genetically controlled modules of axonal organization that obey spatial rules. Understanding such rules will enhance our understanding of the pathogenesis of several poorly defined gastrointestinal diseases of neurogenic origin

SIMULTANEOUS GOLGI-COX AND IMMUNOFLUORESCENCE USING CONFOCAL MICROSCOPY AND APPLICATIONS

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Visualization of neuronal elements is of fundamental importance in modern neuroscience. Golgi-Cox impregnation is a widely employed method that provides detailed information about morphological characteristics of neurons, but none regarding their neurochemical features. Immunocytochemical procedures, on the other hand, can provide a high degree of biochemical specificity but poorer morphological details, in particular if compared to Golgi-Cox impregnation. Hence, the combined use of these two approaches is highly desirable especially for confocal microscopists that can exploit the advantages of both methods simultaneously.

We have developed an innovative procedure that allows, by applying Golgi-Cox impregnation and immunofluorescence in the same histological section, to obtain high-quality histological material, with a very simple and inexpensive method. This procedure is based on three simple fixation steps: 1) a paraformaldehyde perfusion followed by a standard post-fixation to stabilize the subsequent immunofluorescence reaction; 2) the classical Golgi-Cox impregnation and 3) an immunofluorescence reaction in previously impregnated material. This combination allows simultaneous visualization of **i)** the structural details (Golgi-Cox impregnated neurons), **ii)** the antigens' characterization, **iii)** the anatomical interactions between discrete neuronal elements and **iv)** the 3D reconstruction and modeling. The method is easy to perform and can be reproducibly applied by small laboratories and expanded through the use of different antibodies. Overall, the method presented in this study offers an innovative and powerful approach to study the nervous system especially by using confocal microscopy.

Very recently, this method was used to study some brain areas along the dopaminergic pathway. In particular, in the striatal medium spiny neurons, spines morphology as well as Tyrosine Hydroxylase and Post synaptic density 95 changes were studied in ethanol dependent rats. The relation between spine shape, synaptic function and morphological rearrangements of spines, appear to be associated with behavioral plasticity ethanol induced.

MARCELLO MALPIGHI E I SUOI AMICI: NEUROSCIENZIATI ANTE-LITTERAM

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The term 'Malpighi's friends' does not simply express the usual solidarity among scientist working with the same intents, but a sort of climate appearing in Italy in a very difficult moment. Curiously enough, usually people forget that the thirty years war ended when Malpighi was 20 years old, and Italian scientists, after Galileo's trial, had a very difficult time. Very often, scientist have very hypertrophic egos. Therefore, Malpighi's friends appear to be a very unusual feature in the history of science.

Marcello Malpighi (1628-1694) was the founding father of microscopic anatomy (3). He achieved a series of sensational breaktroughs during his scientific career. He discovered the capillary circulation of the lung, the structure of the renal glomerulus and of other parts of the nephron, the structure of the spleen, of the lymph nodes, of many glands and of the skin.

Malpighi also discovered the microscopic structure of plants, being thus considered the founder of modern plant morphology. Moreover, although Malpighi was not able to conceive the existence of cells, he was probably the first to identify the red blood cells.

There is something more, however. Malpighi's perception of tightly packed glands in the cerebral cortex is usually considered to be a major mistake by him, and caused the widespread criticism of microscopy and its replacement by injection technique. The eclipse of the microscope ensued until it was rediuscovered and triumphed in the 19th century.

One cannot simply disparage Malpighi's studies in the central nervous system, however. This is Adelmann's translation of Malpighi's de cerebro (1):

'the cortex is a crop of very small glands. In the gyri, where the white roots of the nerves come to an end or, if you prefer, arise, these glands are fitted together so as to form the outer surface of the brain. They are oval, but pressure makes them angular, and they are almost equidistant from one another. Their outer portions are covered by the pia mater and penetrated deeply by its blood vessels. The inner part of each gives off a white nerve fiber as its excretory vessel, and the white medullary substance of the brain is formed by the gathering of many such fibers into bundles." Of course, there are flaws in this description, especially in considering the cortex of the spinal cord identical to that of the brain. On the other hand, there is something alluding to the connection between neuron and axon, just as in Golgi 200 years later.

The exploration of human brain remained a very difficult task until the 19th century, because of its extremely difficult preservation at autopsy. For instance, the temporal lobe was described only in 1584, when Giulio Cesare Aranzi (1530-1589), from the Bologna University, produced the first description of the hippocampus (4).

Therefore, Malpighi appears to be the most audacious scholar in delving in the human brain in depth. He was not alone, however. René Descartes' weird theories about brain's function had evoked profound skepticism in a young scholar from Copenhagen: Niels Stensen (1638-1686), better known as Nicolaus Steno. Therefore, he decided to migrate from Paris to Tuscany. There, he started working in touch with Malpighi and other very interesting Italian scientists: Stefano Lorenzini (after 1652- after 1721) and Lorenzo Bellini (1643-1704). The four men cooperated very strictly together and were bound by a profound friendship often appearing with moving expressions in their relatively abundant correspondence collected by Howard B. Adelmann. Like Malpighi, his friends made a large use of animal dissection, because of their easier availability and preservation. They were especially concerned with the central nervous system of sharks, because of its minor complexity. An interesting result consisted in the first demonstration of the electrical organs of torpedo. Steno was the first to observe them, probably because he was profoundly interested in correlating structure and function of skeletal muscle. The peculiarity of torpedo's organs was stressed very accurately, and anticipated Galvani's observations (Osservazioni Intorno Alle Torpedini Fatte Da Stefano Lorenzini Fiorentino).

However, Lorenzini's ampullae appear the most interesting and enduring discovery by the group of Malpighi's friends. They appeared as a sort of complex cutaneus glands (in accord with Malpighi's definition of glands) concentrated in the head of sharks. Modern neurophysiologists established their relevance in perceiving the variation of electric fields generated by swimming animals. Very recently a study re-evalueted the experiences by Malpighi's friends in showing the very effective sensory coordination in sharks (2).

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THE BEHAVIORAL NEUROENDOCRINOLOGY LABORATORY AT THE NEUROSCIENCE INSTITUTE CAVALIERI OTTOLENGHI (NICO)

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The Behavioral Neuroendocrinology Laboratory at NICO is a research unit developed to continue the tradition of neuroendocrine researches at the University of Torino. The long-term topic of our laboratory is the study of the interactions among gonadal steroids, neural circuits and behavior. We are particularly interested in the description of sexually dimorphic circuits and of their variations in physiological or experimental conditions. In particular, we studied peptidergic (vasopressin, NPY) and nitrergic pathways.
In recent years, we developed a new research line on the effects of endocrine disrupting chemicals (EDCs). EDCs are environmental substances (synthetic or natural) that may impact endocrine function and, therefore, they may have long-term consequences, especially if exposure occurs during embryonic development. Most of EDCs are agonists or antagonists of androgen or estrogen receptors, therefore they may interfere with brain and behavior sexual differentiation.
We used two animal models: the mouse and the Japanese quail. In the quail, we investigated the effect of DES, genistein or DDE, administered in eggs, on the differentiation of male sexual behavior and of the parvocellular sexually dimorphic vasotocin system. In the mouse we investigated the effects of perinatal exposure to bisphenol A (BPA) or genistein on the sexual differentiation of NO producing system and of the kisspeptin system. We investigated also the organizational effects of these EDCs on sexual, social, and explorative behaviors. Our data suggest that precocious exposure to EDCs through maternal administration (in mice) or in egg deposition (in quail) may permanently alter some sexually dimorphic circuits and influence in a gender-oriented way some behaviors. In particular, the timing of exposure to EDCs is a critical factor, such that the effects of a particular EDC will vary over the lifecycle of the animal as well as across species and phyla. Therefore, exposure to EDCs during embryonic development has consequences beyond impaired function of the reproductive axis. These compounds are therefore, in addition to gonadal steroids and neurosteroids, a third player within the nervous system for its development and differentiation. The evolutionary implications of having them in the normal food supply for certain human populations (i.e. phytoestrogen derivatives from soy), as well as for wild and farm animals should stimulate a wide discussion about their beneficial or adverse role.

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I SESSION
SYSTEMATIC, CHEMICAL AND DEVELOPMENTAL NEUROMORPHOLOGY

DISTRIBUTION OF IMMUNOREACTIVITY FOR ALR (AUGMENTER OF LIVER REGENERATION) IN THE NORMAL ADULT MOUSE PROSENCEPHALON

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The augmenter of liver regeneration (ALR) plays a role as a growth factor, supports cell proliferation acting as an anti-apoptotic factor, regulates heavy metals homeostasis, and is a protective factor against oxidative stress. Firstly detected in liver, ALR is expressed in all organs and particularly in liver, muscle and brain. In the present study, we investigated the distribution of ALR in the normal adult mouse prosencephalon, using biochemical and morphological methods.

Methods. Brains were removed from skull and fixed by immersion in a fixative solution containing a mixture of aldehydes and picric acid. By a sopracollicular section, we subdivided each brain in a cranial part, corresponding to the whole prosencephalon, and a caudal part, corresponding to mesencephalon and rombencephalon. (Only the cranial part was used in this study.)

For the biochemical procedures, the prosencephalon was subdivided in 3 fragments of approximately corresponding sizes (named fragment 1, 2 and 3), which were subjected to western blot analyses. For morphological analyses, paraffin coronal sections were obtained from the whole prosencephalon, and sections coming from fragment 1 (anterior), fragment 2 (middle) and fragment 3 (posterior) were subjected to light microscopy immunohistochemistry for ALR.

Results. As shown by western blot, ALR is highly expressed throughout the prosencephalon, without significant difference in the 3 fragments examined. As shown by immunohistochemistry, ALR immunoreactivity was found in many neurons belonging to specific neuronal populations of the cerebral cortex (allocortex, isocortex), basal nuclei (striatum, amygdala) and diencephalon (thalamus, hypothalamus).

ALR is highly expressed in the normal mouse prosencephalon, but displays specific patterns of distribution within its neurons, suggesting the existence of specific roles of neuronal ALR, which might be of importance as a growth factor and/or as a protective factor. The availability of a detailed atlas on the distribution of ALR in the brain of mice is the first step to evaluate changes in the expression of this protein in experimental or pathological conditions.

NOVEL GABA SYNAPSES BETWEEN GOLGI CELLS IN THE CEREBELLAR CORTEX

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In the cerebellar cortex, Golgi cells contribute to information processing by providing feed-back and feed-forward inhibition to granule cells. Because Golgi cells are the only elements that control the activity of granule cells, they are crucial for filtering glutamatergic inputs to Purkinje cells through the mossy fiber-parallel fiber pathway. The excitability of Golgi cells is modulated by GABAergic and glycinergic synapses that they receive from distinct types of cerebellar neurons. However, the organization and function of these inhibitory circuits remain elusive. Here we show that the basolateral dendrites of Golgi cells located in the granule cell layer are targeted by at least two types of GABAergic axons. Using transgenic mice characterized by strong GFP labeling in Golgi cells (Pax-2-GFP mice) as well as immunohistochemistry with Golgi cell markers, we show that $GABA_A$ receptor clusters are distributed along the basolateral dendrites at sites contacted by VGAT-positive axon terminals. These contacts differ from synapses located in the glomeruli in that they contain the alpha2 and alpha3 subunits of $GABA_A$ receptors, but not the alpha1 subunit. Triple labeling for neurogranin (a marker of a subpopulation of Golgi cells), VGAT and calbindin shows that only a small part of VGAT-positive boutons contacting the basolateral dendrites are positive for calbindin and therefore belong to the axon collaterals of Purkinje cells. Conversely, neurogranin-positive cells are contacted by numerous Golgi cell axons immunolabeled for GlyT2 and mGluR2/3. These data provide the first anatomical evidence for the existence of Golgi-to-Golgi cell contacts in the cerebellar granular layer. The identification of Golgi-to-Golgi cell synapses calls for a revision of the cerebellar circuit diagram and changes our view of how synaptic inhibition sculpts information transfer through the mossy fiber pathway.

FURTHER STUDIES ON THE HISTOLOGY AND CHEMICAL NEUROANATOMY OF THE HUMAN CUNEATE NUCLEUS

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Previous data from this group showed that, at variance with that of laboratory mammals, including primates, the human cuneate nucleus (CN) contains one or more clear-cut subregions with neurochemical features similar to those of the protopathic relay nuclei. There, a distinctive localization of areas immunoreactive to substance P (SP) is paralleled by that to several other neuropeptides, trophic factors and cognate receptors, and neuroplasticity marker proteins, with distribution patterns strictly alike those present in the spinal dorsal horn and spinal trigeminal nucleus.
In this work we present further data showing that immunoreactivity to the transient receptor potential cation channel subfamily V member 1 (TRPV1) also occurs in those CN subregions with a distribution comparable to that detectable in the superficial layers of the spinal trigeminal nucleus. Moreover, using Nissl and myelin stainings we show a strict histological similarity between the immunohistochemically identified CN subregions and the spinal trigeminal nucleus. As observed previously for other markers, those subregions are clearly identifiable throughout ontogeny from fetal to adult life. Finally, using serial sections stained by ABC immunoperoxidase for SP, we provide a tridimensional reconstruction of the CN subregions as detectable with this labelling.
The present data reinforce our previous findings and support the concept of the existence, in the human CN, of discrete regions with histological and neurochemical features typical of the protopathic sensory nuclei superficial layers, including the substantia gelatinosa. The origin and functional involvement of such innervation remain to be elucidated.

STUDY OF THE CHOLINERGIC SYSTEM IN THE HUMAN CEREBELLAR CORTEX BY IMMUNOHISTOCHEMISTRY FOR VESICULAR ACETYLCHOLINE TRANSPORTER (VAChT)

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INTRODUCTION. The cholinergic system is widely distributed in the nervous system. Acetylcholine (Ach) acts as a neurotransmitter in somatic, visceral and psychic nervous circuits, and dysfunction of the cholinergic system is involved in neurodegenerative and psychiatric disorders (1). The cholinergic neuron phenotype is characterized by a common locus (cholinergic locus), where the gene of choline acetyltransferase (ChAT), the enzyme that biosynthesizes Ach, and the gene of vesicular acetylcholine transporter (VAChT), that transports Ach into synaptic vesicles, coexist (2). Numerous immunohistochemical studies on the cholinergic system in the mammalian cerebellum, mainly based on immunohistochemistry for ChAT, evidenced ChAT immunoreactivity in fibers and neuronal bodies of the cerebellar cortex. The fibers were observed in all the layers of the cortex, in a subpopulation of mossy fibers that originate from cholinergic neurons localized in brain stem nuclei (3); the neuronal bodies were identified as subpopulations of Golgi and granule neurons (4,5,6). Immunohistochemical studies evidenced VAChT immunoreactivity in: axon terminals localized in all the layers of the cortex; subpopulations of stellate and basket neurons; a subpopulation of Purkinje neurons; and in a subpopulation of mossy fibre terminals (7,8,9). No immunohistochemical demonstration of VAChT exists in the human cerebellar cortex. The aim of this study is to evaluate the distribution of VAChT immunoreactivity and to characterize morphologically VaChT- immunoreactive elements in the human cerebellar cortex.

MATERIALS AND METHODS. Fragments of human cerebellar cortex were taken at autopsy 24-36h after death, fixed in a picric acid and aldehyde solution, embedded in paraffin, sectioned into 5μm sections, and subjected to light microscopic immunohistochemical techniques using a rabbit anti-VAChT polyclonal antibody.

RESULTS. VAChT immunoreactivity was observed within neuronal bodies distributed in all layers of human cerebellar cortex. It was detected in: subpopulations of stellate and basket neurons, in the molecular layer; a subpopulation of Purkinje neurons, in the homonymous layer; in subpopulations of granules and large neurons, in the granular layer. Moreover, VAChT immunoreactivity was also observed in neuronal processes distributed throughout the cortex and in putative terminals in the spaces of Held in the granular layer.

CONCLUSION. This study supplies the first immunohistochemical demonstration of VAChT, marker of the cholinergic neurotransmission in the human cerebellar cortex. The motor and cognitive functions of the cerebellum (10) are possibly mediated by its cholinergic system.

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THE CELL CYCLE AND LINEAGE PROGRESSION OF NEURAL PROGENITORS IN THE VENTRICULAR-SUBVENTRICULAR ZONE OF ADULT MICE.

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Three types of proliferating progenitor cells are involved in the generation of new neurons in the ventricular-subventricular zone (V-SVZ) of the adult rodent brain. The neural stem cells or primary progenitors (B1 cells) generate intermediate progenitors (C cells), which in turn give rise to neuroblasts (A cells). We have determined the cell cycle dynamics for each cell type and estimated the number of times they divide in vivo. We used thymidine analogues according to three different paradigms to evaluate the total length of the cell cycle (T_C) and lengths of S (T_S), G2, M and G1 phases for GFAP-GFP+ B1 cells, Ascl1+C cells, and doublecortin (DCX)+ A cells in whole mounts of the lateral and medial walls of the lateral ventricles. The most common dividing cell type in the V-SVZ were C cells, with a heterogeneous T_C 18-25h and a surprisingly long T_S of 14-17h. A cells, had a T_C of 18 hrs and T_S of 9h. B1 cells accounted for 8,6% of the proliferating cells in the V-SVZ and had a surprisingly short cell cycle of 17-18h and a T_S of 4h. Long-term proliferation dynamics suggest that, following the initial division of B1 cells, C cells divide three times and A cells one, possibly two. These data provide essential information on how adult neural stem cells maintain neurogenesis throughout life in the V-SVZ.

II SESSION/II SESSIONE
ANIMAL MODELS OF NEUROPATHOLOGIES

THE NIGROSTRIATAL SYSTEM OF PARKIN KNOCK OUT MICE: TIME-COURSE EVALUATION OF CALCIUM BINDING PROTEINS

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Parkin is the most common causative gene of juvenile and early-onset familial Parkinson's disease. It codes for an E3 ubiquitin ligase involved in the ubiquitin proteasome system. To investigate the pathogenic mechanism by which loss of parkin function causes Parkinson's disease, recent studies focused on a mouse model bearing a germline disruption in parkin. Parkin knock out (KO) mice have subtle abnormalities of behaviour, dopamine neurotransmission and calcium homeostasis, but no massive loss of dopaminergic neurons. The importance of calcium in neuronal function has been amply demonstrated in neuropathological models, sometimes by means of calcium-binding proteins (CaBPs) that, interestingly, are selectively expressed by subpopulations of dopaminergic neurons. Moreover, a relative sparing of CaBPs-expressing dopaminergic neurons has been reported both in human and experimentally induced Parkinson's disease. We investigated the expression of two CaBPs, calbindin (CB) and calretinin (CR), in the substantia nigra pars compacta (SNc) and striatum of two and seven months old parkin KO mice. Western immunoblotting showed that parkin KO mice display normal levels of CB in SNc and striatum at both the examined ages, whereas it is pointed out a significant decrease of CB immunoreactive dopaminergic neurons in SNc only at 2 months. Interestingly, a physiological age-dependent reduction of immunoreactive neurons (approximately 50% for both CaBPs) was observed in both wild type and parkin KO mice. Normal levels of CR in SNc and striatum were detected in parkin KO mice at both the examined ages. However, a significant reduction of CR immunoreactive nigral dopaminergic cells was detected at both ages. Moreover, a transient decrease of CR signal in dopaminergic fibers was detected in striatum of two months old KO mice. CR immunoreactivity decrease may be related to a nigral dopaminergic subpopulation and striatal projection that is more vulnerable in Parkinson's disease. Our results provide a preliminary insight into calcium-related and presymptomatic alterations in Parkinson's disease.

INTRACISTERNAL DELIVERY OF hMSCs REGULATES THE EXPRESSION OF IMMUNOMODULATORY MOLECULES AND ENHANCES MOTONEURON SURVIVAL IN SOD1 MICE

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We studied the effects and the mechanisms of action of intracisternal delivery of human mesenchymal stem cells (hMSCs) on the progression of disease in the mutant SOD1 G93A mouse, an experimental model of human familial ALS. In order to treat the animals at the disease onset, mice underwent a battery of behavioral tests starting from the pre-symptomatic phase.

When early symptomatic, male mice received 300,000 hMSCs into the lumbar cistern; after two weeks, the animals were sacrificed and their lumbar spinal cords collected. Sham-operated mice received saline. Surviving hMSCs (previously labeled with bisbenzimide) were found both in the meninges and into the spinal parenchyma.

The decrease in motor performance was significantly delayed in the transplanted mice compared to the sham-operated ones. In addition stereological counts showed a higher number of lumbar motoneurons in transplanted animals than in controls, thus indicating a delayed neuronal death. In another set of experiments, we investigated the expression of several cytokines in the lumbar spinal cord by microarray strategy: in particular, we observed a significant increase of the anti-inflammatory murine IL10, IL13a and vascular endothelial growth factor mRNAs in the lumbar spinal cord of transplanted mice.

Our study confirms the role of stem cell therapy as a promising tool in the treatment of ALS, by providing evidence that the minimally-invasive injection into the lumbar cistern can be as effective as intraparenchymal injection. Moreover, it identifies one of their mechanisms of action, indicating that hMSCs can exert a paracrine role in the diseased spinal cord, modulating the endogenous secretion of immunomodulatory molecules and trophic mediators.

AUTOPHAGIC PROCESS IS INVOLVED IN MOTONEURON DEGENERATION IN A MOUSE MODEL OF SMA.

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Spinal Muscular Atrophy (SMA) is a recessive autosomal neuromuscular disease, caused by the deletion of the telomeric survival motoneuron gene (SMN1), characterized by motor impairment, muscle atrophy, and premature death following motoneuron (MN) degeneration. Emerging evidence supports that regulation of autophagy, a physiological process that leads to the degradation of long-lived and misfolded proteins, contribute to pathogenesis of MN degeneration.
We investigated the relationship between autophagy and MN degeneration in the SMNdelta7 mouse model of SMA II (the intermediate form of the disease) which leads to motor impairment by postnatal day 5 (P5) and to death by P13. Motor behavior was assessed daily with a battery of tests: tail suspension, righting reflex and hindlimb suspension tests.
Lumbar spinal cords from P9 wild type (wt) and SMA pups were collected for MN counts, histological, immunohistochemical and Western Blot (WB) analyses. Beclin1 and LC3-II, autophagic markers, were increased in the lumbar spinal cord of SMAII compared to wt pups, both at immunohistochemical and WB analyses.
To clarify the role of autophagy, we performed intracerebroventricular administration of an autophagy inhibitor, 3-methyladenine (3-MA) (2 μL: 33 mM), that potently inhibits maturation of autophagosomes, in SMA pups at P3. 3-MA significantly improved motor performance in SMA pups compared to controls injected with saline. In addition, stereological counts (using the StereoInvestigator software and a microscope with a motorized stage interfaced to a computer) on Nissl-stained sections showed a delayed MN death in treated pups ($11.25*10^3$ ± 1,59) compared to sham-operated ($5.75*10^3$ ± 0.15) ($p<0.01$). Moreover, inhibition of autophagy by 3-MA suppressed autophagosome formation, caspase-3 activation and the appearance of TUNEL-positive neurons, thus reducing cell death, underlining that apoptosis and autophagy pathways are intricately intertwined.
Therefore, the expression of autophagic markers is induced, and autophagy is likely involved in MN death in SMAII, suggesting that it might represent a promising target for delaying the progression of SMA in humans as well.

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ROLE OF JNK IN AUTOPHAGIC CELL DEATH IN THE CINGULATE CORTEX IN A KA-INDUCED RAT MODEL OF EPILEPSY.

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The c-Jun N-terminal kinase (JNK) is involved in apoptotic cell death in the rat hippocampus in kainic acid (KA)-induced epileptic seizures and JNK blockade by a cell penetrating specific inhibitor (D-JNKI-1) dramatically prevents neuronal cell death. Here we studied the role of autophagy and apoptosis in the cingulate cortex and the effects of JNK inhibition.
Epileptic seizures were induced by intraperitoneal (i.p.) injection of 15 mg⁄kg KA. One hour after the injection, rats showed the first symptoms, and only animals that reached the 4th and 5th stages of the Racine scale were included. To minimize suffering and prevent mortality, 2 h following the onset a single i.p. injection of 4 mg⁄kg diazepam blocked epileptic seizures. D-JNKI1 (DJ 0.3 mg ⁄ kg,) was injected i.p. 2 h following KA injection in the KA-DJ ⁄ 1d and KA-DJ ⁄ 5d groups. Rats were killed 1/5 days following KA injection, and perfused. A series of cryostat sections for each animal was Nissl-stained and neuronal counts performed with StereoInvestigator software (MBF). Other sections were immunoreacted: i) for P-c-Jun as a marker for JNK activity as its elective target associated with microtubule-associated protein-2 (MAP2) (to confirm that JNK was involved in neuronal death); ii) for glial fibrillary acidic protein (GFAP) (to evaluate astrogliosis); iii) for Beclin-1 and LAMP-1 (as markersofautophagic processes); iv) for activated caspase 3 (as marker of apoptosis).
In Nissl-stained sections, an increase in necrotic cell death was found in KA-treated rats, both at 1 and 5 days, and neuronal density decreased by 40% at 1 day and by 70% at 5 days. Following D-JNKI-1 treatment, necrotic profiles almost disappeared at 1 day. Neuronal density was fully prevented by JNK inhibition at 1 day, and showed a 15% decrease at 5 days. C-Jun immunoreactivitycolocalized with MAP2-immunoreactivity, showing that JNK was activated in neurons. Markers of autophagy were present in KA-treated rats and were almost absent following its inhibition. On the contrary, neither in Nissl-stained sections nor in activated caspase 3 immunoreacted sections apoptotic markers were found. In parallel, there was a striking astrogliosis in KA-treated rats, partially prevented by JNK inhibition.
Therefore, in the cingulate cortex of rats KA-induced epileptic seizures induce a marked neuronal death, mostly due to necrotic and autophagic phenomena. This death is correlated with JNK activation: in fact, JNK inhibition almost completely prevents neuronal death and appearance of autophagic markers.
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BRAIN ACTIVITY CHANGES IN AN ANIMAL MODEL FOR MULTIPLE SCLEROSIS CAN BE HIGHLIGHTED BY FUNCTIONAL MAGNETIC RESONANCE IMAGING.

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Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). Magnetic Resonance Imaging (MRI) is used for MS first diagnosis and the evaluation of CNS damage extension during disease course while information about cortical area functional reorganization can be obtained by means of functional MRI (fMRI). The clinical patterns of disease evolution are highly variable and scarcely correlate with structural MRI-detected CNS damage. This phenomenon has been referred to as clinical/MRI paradox.

Experimental Autoimmune Encephalomyelitis (EAE) is the animal model for MS and can be induced in Dark Agouti (DA) rat reproducing the condition experienced by the most MS patients, i.e. the remitting-relapsing form.

fMRI observations in MS are already available in humans, but deeper knowledge on its usefulness might be gained using reliable animal models.

EAE was induced by syngenic spinal cord intrafootpad administration with clinical disease onset around 10 days post EAE induction (dpi) and the worst clinical condition reached on 14dpi without a complete symptom resolution in main animals up to 45dpi.

The brain plasticity was investigated by means of serial fMRI acquisitions performed before, 30 and 60 days after EAE induction. A train of squared pulses electrical stimulation (frequency=3Hz, current=2mA, duration=0.5ms) was delivered to the left forepaw during acquisition of MRI sensitive to Blood-Volume. A single stimulation protocol was composed of 30 images under rest condition and 10 images acquired during stimulation. After appropriate image analysis, performed using the FSL software package, the brain region activated by the applied stimulus was determined.

The week before EAE induction, electrical stimulation resulted in a localized response only in the contralateral sensory motor cortex according to previously reported results. Thirty and 60dpi, the activated area was greatly increased covering large regions of both contra and ipsilateral somatosensory cortex and extending also to extra-cortical regions.

Our results show that the DA rat EAE model is a good model in reproducing the functional reorganization of cortex observed in MS patients. It remains to be investigated whether this effect could represent an innovative platform for testing new therapeutic approaches for MS.

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ROLE OF RESVERATROL IN NEUROINFLAMMATION MODULATION IN A MPTP MOUSE MODEL OF PARKINSON'S-LIKE DISEASE

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Parkinson's disease (PD) is a neurological disorder characterized by a significant loss of dopaminergic neurons in the substantia nigra (SN) resulting in reduced striatal dopamine (DA). Recent evidence demonstrates that chronic neuroinflammation plays a key role in disease progression: the analysis of brains from PD patients has revealed increased levels of proinflammatory mediators such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-1beta, IL-6, and nitric oxide (NO). The loss of dopaminergic neurons in the SN is associated with a massive astrogliosis and excessive microglial activation.

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes the selective degeneration of mesencephalic dopaminergic neurons. MPTP-treated animals are widely used as models of PD. The robust glia activation found in MPTP-treated mice, together with proinflammatory mediators and their respective receptors expression [1], make this model particularly suitable for the study of neuroinflammation in PD.

In the present study we have used this parkinsonism model to analyze resveratrol neuroprotective effects. We observed that resveratrol treatment significantly reduced glia activation, decreasing the levels of IL-1, IL-6 and and TNF-α as well as their respective receptors in the SN of MPTP treated mice, as demonstrating by western blotting and RT-PCR analysis. This reduction is related to a possible neuroprotection since we observed that resveratrol administration limited the decline of tyrosine hydroxylase immunoreactivity in the in striatum and SN induced by MPTP injection.

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III SESSION
ENTERIC NERVOUS SYSTEM

FASTING AND DIETARY NUTRIENTS AFFECT EXPRESSION AND REGULATION OF A-TRANSDUCIN IN THE PIG GASTROINTESTINAL TRACT

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Background: The discovery that taste receptors (TRs) and signaling molecules are expressed in the gastrointestinal (GI) mucosa suggests a chemosensing role for these molecules in the gut. TRs are G protein-coupled receptors (GPCRs) sensing bitter (T2Rs), or sweet and umami (T1Rs). TRs mediate gustatory signaling by interacting with specific Gα subunits, including α-gustducin ($G_{\alpha gust}$) and α-transducin ($G_{\alpha tran}$) through the activation of different effector systems leading to intracellular $Ca2^+$ increase and transmitter release. To this end while it has been demonstrated the presence of Gα gustducin ($G_{\alpha gust}$) in several mammalian GI tract, how and to what extent nutrients affect the Gα proteins expression has not been extensively studied.

Aim: Based on a pig model, closely resembling the human gut, this study was designed to establish whether fasting and re-feeding were able to affect: 1) the expression of $G_{\alpha tran}$ throughout the GI tract; 2) the characterization of the type of cells expressing $G_{\alpha tran}$; and 3) the relationship between $G_{\alpha tran}$ expressing cells and nerves supplying the gut.

Methods: GI specimens (stomach to rectum) of 12 pigs (45 days of age), subdivided in 3 groups, i.e. control, fasted for 24h and re-fed, were fixed in 4%-paraformaldehyde, embedded either in paraffin or OCT medium and processed for single, double labelling and quantitative immunofluorescence with antibodies to $G_{\alpha tran}$, chromogranin-A (CgA), ghrelin, gastrin/cholecystokinin (Gas/CCK), somatostatin (SOM) and PGP9.5. Western Blot analysis was used to confirm the specificity of antibodies to $G_{\alpha tran}$ and $G_{\alpha gust}$ and the presence of these messengers in pig GI tract.

Results: Western blot analysis did show two major bands at the expected molecular weights (45 and 40 kDa, respectively) for $G_{\alpha tran}$ and $G_{\alpha gust}$ throughout the pig GI tract. Most $G_{\alpha tran}$-IR cells contained CgA. In the stomach, many $G_{\alpha tran}$-IR cells contained ghrelin, whereas in the upper small intestine many were IR for Gas/CCK and only few for SOM. $G_{\alpha tran}$-IR and $G_{\alpha gust}$-IR colocalized in some cells. Fasting (24h) resulted in a significant decrease in $G_{\alpha tran}$-IR cells in the cardias (29.3 ± 0.8 *vs*. 64.8 ± 1.3, $P < 0.05$), pylorus (98.8 ± 1.7 *vs*. 190.8 ± 1.9, $P < 0.0$l), cecum (8 ± 0.01 *vs*. 15.5 ± 0.5, $P < 0.01$), descending colon (17.8 ± 0.3 *vs*. 23 ± 0.6, $P < 0.05$) and rectum (15.3 ± 0.3 *vs*. 27.5 ± 0.7, $P < 0.05$). Re-feeding restored the control level of $G_{\alpha tran}$-IR cells in the cardias. By contrast, in the duodenum and jejunum, $G_{\alpha tran}$-IR cells were significantly reduced after re-feeding, whereas $G_{\alpha tran}$-IR cells density in the ileum was not changed by fasting/re-feeding. Most PGP9.5 varicose nerve fibers, running either singly or in small fascicles, throughout the lamina propria of the small bowel mucosa were seen in close spatial relationship with $G_{\alpha tran}$-IR cells.

Conclusions: Our findings provide further support to the concept that taste receptors contribute to luminal chemosensing in the GI tract and suggest they are involved in modulation of food intake and GI function induced by feeding and fasting.

EXPRESSION OF B_2 ADRENOCEPTORS WITHIN ENTERIC NEURONS OF THE HORSE ILEUM

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Objective - The activity of the gastrointestinal tract is strongly regulated through the catecholaminergic system, which acts through the activation of α and β adrenergic receptors (ARs). Since data concerning the distribution of ARs in horse intestine is virtually absent, we investigated the distribution of β2-AR in the horse ileum.
Animals - Four Italian trotter horses, two young females age 2.5 and 3.5 years, and two males age 8 months and 5 years.
Procedures - Double-immunofluorescence staining was performed on cryostat sections with primary antibodies against β2-AR, Hu, neuron-specific enolase (NSE), S100 protein and tyrosine hydroxylase (TH). Percentage of double-immunolabeled neurons was determine in submucosal plexus (SMP) and myenteric plexus (MP). In addition, the relationship between the β2-AR-immunoreactive (IR) cells and the cathecolaminergic fibers showing immunoreactivity for the enzyme tyrosine hydroxylase (TH) was also studied.
Results - The β_2-AR-immunoreactivity (IR) was observed in the following elements: the majority (95%) of the neurons located mainly in SMP; few (8%) neurons of theMP; non-neuronal elements (interstitial cells of Cajal, mast cells and immunocytes). In addition, sympathetic TH-IR fibers were observed close to neurons, ICCs and mast cells expressing β2-AR-IR. Although enteric glial cells were not β_2-AR-IR, in the SMP and MP the TH-IR fibers were observed also near glial cells.
Conclusion and Clinical Relevance - Since β_2-AR is virtually expressed in the majority of neurons located in the horse SMP and in a lower percentage of neurons in the MP, it is reasonable to retain that this adrenergic receptor could regulate the activity of both secretomotor neurons and motor neurons innervating muscle layers and blood vessels. The high density of TH-IR fibers near SMP and MP β_2-AR-IR neurons indicates that the excitability of these cells could be directly modulated by the sympathetic system. Consequently, the sympathetic system may regulate the motility, secretion and blood flow of the ileum acting also on β_2-ARs. The presence of β_2-AR in ICCs, mast cells and immunocytes indicates that catecholamines can regulate the activity of these non-neuronal cells distributed inside the gut wall. Pathogenesis of intestinal dysmotility included also imbalance of the sympathetic activity. Thus, the present work, provides a useful data concerning the cellular populations potentially involved in these pathological processes.

ENTERIC NEUROPLASTICITY OF SEAWATER-ADAPTED EUROPEAN EEL "(*ANGUILLA ANGUILLA*) EXPERIMENTALLY INDUCED TO SEXUAL MATURATION

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Objective of the study. European eel lives most of its life/mainly in fresh water until its spawning migration to the Sargasso Sea. During seawater adaptation the eel modifies its physiology and the digestive system adapts the body homeostasis to the new environment, drinking salt water to compensate for continuous water loss. In that period, eels interrupt food intake until spawning: the prolonged starving is associated with morphological changes of their body up to death because of the extreme effort. Thus, the eel represents a unique model to establish the adaptive changes of its digestive innervation, i.e. the enteric nervous system (ENS), to modified salinity first and starving thereafter.

Materials and Methods. The experiments were organized in two steps. First, we investigated the neuronal density of the eel ENS during prolonged starvation in seawater after hormonal treatment with standardized carp pituitary extract (CPE). Intestinal specimens from control fresh-water (of both sexes) and starved seawater-adapted female eels were obtained and assessed. Animals were classified as controls, T0, and T4. Control eels, ageing about 5-7 years, were raised in fresh-water and were normally fed. T0 eels were captured in north-Italian salty-water estuaries when they were ready for reproductive migration. T4 eels were adapted in captivity to sea-water and were treated for 22 weeks with CPE to induce sexual maturity. Secondly, we analyzed also the modification of the ENS of non hormonally-treated male eels during prolonged starvation (10 weeks) in seawater and freshwater.

The density of myenteric plexus (MP) and submucosal plexus (SMP) HuC/D-immunoreactive (Hu-IR) neurons was assessed by counting the number of Hu-IR neuronal cell bodies in 1 cm-long longitudinal cryosections; the density of myenteric plexus (MP) Hu-IR neurons was assessed also by counting ten randomly chosen fields (magnification 40x) of wholemount preparations.

Results: In cryosections of control eels, the number of MP and SMP neurons was 138± 25 and 17±8 respectively, with no statistically significant differences between sex. In T0 the number of MP was 183±15 whereas the number of SMP neurons varied between 4 and 32 (average 18). In T4 eels the number of MP and SMP neurons was 225± 30 and 61±8 respectively.

In MP wholemount preparations of control, T0 and T4 eels, the number of neurons was 140±23, 296±41, and 439±38, respectively.

In cryosections of starved untreated freshwater male eels, the number of MP and SMP neurons was 74±2 and 14±3, respectively, whereas the number of MP and SMP neurons of sea-water eels was 126±15 and 21±4, respectively.

Discussion: The progressive increasing number of Hu-IR MP and SMP neurons observed in eels adapted to salinity indicates that high salty water evokes enteric neuroplasticity necessary to maintain body homeostasis in this species.

DOPAMINERGIC MARKERS AND DOPAMINE RECEPTORS IN THE RAT GASTROINTESTINAL TRACT

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Dopamine (DA) besides of being a central nervous system neurotransmitter exerts several peripheral effects including cardiovascular, renal and gastrointestinal ones. The gastrointestinal tract is a relevant target of DA actions including stimulation of exocrine secretions, inhibition of gut motility, modulation of sodium absorption and control of mesenterial and mucosal blood flow. Moreover, several evidence suggests a protective role of DA against gastroduodenal ulcer disease. It is debated if gastrointestinal actions of DA are mediated by the catecholamine or via other catecholamines of which DA is precursors such as noradrenaline and adrenaline.

The present study was designed to assess the distribution and localization of DA stores, of DA transporter (DAT), of vesicular monoamine transporter (VMAT) type-1 and 2 and of DA D1-like and D2-like receptor subtypes in the rat gastrointestinal tract by immunohistochemical, immunochemical techniques and by RT-PCR.

DA, DAT, VMAT-1 and different subtypes of DA receptors, with predominance of D5 sites for the D1-like superfamily and D2 sites for the D2-like superfamily displayed a widespread localization in different portions of the gastrointestinal tract. Dopaminergic markers are expressed primarily by mucosal tissue, whereas DA receptors are more abundant within gastrointestinal tract vasculature and muscular layers. Dopaminergic markers and DA receptors were also found in the islands of immune tissue located throughout the gastrointestinal tract.

Localization of dopaminergic markers and different DA receptor subtypes may account for the different activities of dopamine throughout the gastrointestinal tract. Moreover it could contribute to understand the pathophysiology of gastrointestinal symptomatology of systemic diseases characterized by impairment of central dopaminergic system (e.g. Parkinson's disease).

FIBROTIC REMODELING AND DAMAGE OF THE NEUROMUSCULAR COMPARTMENT IN THE PRESENCE OF INTESTINAL INFLAMMATION

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Objective. The aim of this study has been to assess tissue remodelling of the colonic wall in a rat model of colitis, with a particular focus on the neuromuscular compartment. The rationale of the study is based on the notion that there is currently a scarcity of animal models of intestinal fibrosis, and that new methods to induce fibrosis as well as reliable parameters to evaluate the progress of fibrotic remodelling in the gut wall are highly expected. In particular, there is limited information on the pathophysiology of fibrotic processes secondary to chronic intestinal inflammation, which might account for abdominal pain and dysfunctions of intestinal transit, at least in part through alterations of the enteric nervous system.

Methods. Colitis was induced in rats by intrarectal administration of 2,4-dinitrobenzenesulfonic acid (DNBS, 30 mg/rat in 0.25 ml ethanol 50%). After 6, 12 and 21 days, the following parameters were assessed on paraffin sections from distal colonic samples: gross morphologic damage and tissue inflammatory infiltration by current histology; collagen and elastic fibers by histochemistry; HuC/D antigen for neurons, glial fibrillar acid protein (GFAP) for glial cells, nestin for myenteric plexus, and c-Kit antigen and ANO-1 for interstitial cells of Cajal (ICCs) by immunohistochemistry.

Results. The presence and severity of colitis were assessed by macroscopic and microscopic scoring. At day-6, inflammatory and ulcerative alterations were observed in all DNBS-treated rats, and they progressed towards fibrotic lesions at day-21. Inflammatory infiltrates were abundant within and around the ulcerated lesions (neutrophils and eosinophils). The presence of eosinophils persisted within the colonic wall up to day-21. Colitis was associated with a significant increase in collagen fibers, which occurred in parallel with a dramatic decrease in elastic fibers throughout the whole wall thickness. Besides collagen deposition, the colonic neuromuscular compartment of inflamed animals displayed: significant decrease in myenteric HuC/D^+ neuron density in concomitance with increased immunoreactivity for GFAP and nestin; marked neovessel formation; reduced density of intramuscular and myenteric ICCs.

Conclusions. The DNBS model of colitis shows a significant degree of bowel remodeling characterized mainly by: a) enhanced collagen deposition in concomitance with elastic fiber reduction; b) altered myenteric neuron/glia proportion; c) increased vasculogenesis; d) impairment of the ICC apparatus. Based on the present findings, the patterns of bowel tissue alterations in this model of colitis are suitable to investigate the pathophysiology of colonic inflammation and evaluate the impact of new therapeutic strategies on inflammatory bowel diseases.

MITOCHONDRIAL NEUROGASTROINTESTINAL ENCEPHALOMYOPATHY (MNGIE): THE LIVER AS A NEW SOURCE OF THYMIDINE PHOSPHORYLASE

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Background: Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is a rare autosomal recessive disease associated with the nuclear *TYMP* gene mutation. As a result, the thymidine phosphorylase (TP) enzyme activity is markedly reduced (or completely deficient) and this leads to mitochondrial DNA mutation and multiple deletions. On a clinical standpoint, MNGIE is characterized by severe gastrointestinal dysmotility (gastroparesis and chronic intestinal pseudo-obstruction) along with neurological impairment, i.e. progressive external ophtalmoplegia, cachexia, peripheral neuropathy, and diffuse leukoencephalopathy. The onset is usually between the second and fifth decades and patients' life expectancy is limited. So far, there are no established therapeutic options for patients with MNGIE. Recently, allogenic hematopoietic stem cell transplantation (AHSC) has been performed as a cellular source of TP. Current data obtained on about 20 MNGIE patients undergone AHSC transplantation showed gastrointestinal and neurological improvement, although 5-year survival rate is roughly 50%. Objective: The aim of this research project has been to test the hypothesis that the liver may serve as an alternative cellular source of TP. Methods: A number of n= 10 patients (7 males; age range: 35-55 years) undergone liver resection for focal disorders (i.e., non complicated tumor disease) was included. Margins of liver tissue unsuitable for histopathology were processed for a variety of methodological approaches aimed to identify TP protein using ELISA, Western Blotting, immunohistochemistry (IHC) and mRNA via qPCR. Results: ELISA test showed the presence of TP protein in liver tissue with a mean of 0.5 ± 0.07 ng/μg total proteins; data were confirmed by Western Blotting in which the densitometric ratio TP/GAPDH had a mean of 0.9 ± 0.5 A.U. The localization of TP with IHC was identified in hepatocyte nuclei and in reticular cells (intestinal mucosa and skeletal muscle were used as positive and negative control, respectively). TYMP mRNA specific liver expression has been found using qPCR. Conclusions: The results of this study provided evidence that the liver is an important source of TP. Thus, likewise AHSC, also orthotopic liver transplantation might be a therapeutic alternative for patients with MNGIE possibly with a better outcome in terms of survival rate.

EVIDENCE OF RET/GDNF-RELATED APOLIPOPROTEIN B (APOB) ACTIVATION AND ALTERED EXPRESSION IN PATIENTS WITH CHRONIC INTESTINAL PSEUDO-OBSTRUCTION (CIPO)

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Background: CIPO is a severe dysmotility syndrome characterized by recurrent intestinal sub-occlusive episodes with no detectable mechanical causes. CIPO can be secondary to a variety of diseases or idiopathic in origin when no apparent causes can be detected. In this latter group, genetic defects may play a role in altering enteric neuro-muscular integrity thereby leading to severe gut dysfunction and symptom generation. The RET/GDNF pathway regulates neural crest-derived cells development. RET mutations play a pathogenic role in Hirschsprung's disease (HSCR), but apparently not in CIPO, where the involvement of RET signalling downstream mediators remain largely unknown. In a mouse knock-in carrying the HSCR-*ret*^{C620R} mutation, *Apob* expression was lower in homozygous mice (aganglionosis phenotype), whereas markedly increased in heterozygous mice (hypoganglionosis phenotype).[1] Objective: As an index of RET/GDNF impaired pathway, this study was designed to evaluate *ApoB* expression in a neuronal cell line (Neuro2A) and in CIPO patients. Methods: Neuro2A cells (ATCC, UK) were cultured according to previously validated protocols. *RET* and *APOB* mRNA silencing was obtained by transfecting Neuro2A cells with pRS-shRNA vector specific for mouse *ret* and *apob* respectively. RNA was extracted from blood samples from 20 consecutive CIPO patients (13 F, age range: 18-55 yrs) defined by clinical, radiologic and manometric criteria,[2] and from age-sex-matched asymptomatic controls (3 F, age range: 24-34 yrs). *Apob* expression was assessed by qPCR. Western blot analysis was performed with antibodies against GDNF family receptor alpha 1 (GFRα1) and APOB on sera of CIPO patients. Western Blot analysis was performed on *Apob*-silenced and GDNF-treated Neuro2A to test the expression of HuD protein. Results: *Apob* expression in Neuro2A was activated by the RET/GDNF pathway via MAPK P38 activation, and *ret* silencing abolished this activation. We investigated whether *Apob* activation was related to enteric neuronal development by assessing HuD expression in GDNF-treated Neuro2a. *Apob* silencing abolished the increase in GDNF-induced HuD expression. Similarly to mice heterozygous for the mutation *ret*^{C620R}, *APOB* mRNA expression was markedly abnormal in CIPO patients compared to healthy subjects. In CIPO, Western Blot analysis revealed a marked increase of APOB and GFRα1, the (GPI)-linked cell surface receptor for GDNF that mediates RET activation. Conclusions: APOB upregulation plays a role in neuronal development/differentiation as demonstrated by Neuro2A cells and *ret*^{C620R} mice. The aberrant expression of APOB and GFRα1 in CIPO suggests an altered neuronal development/differentiation playing a role in a subset of patients with this severe dysmotility syndrome.

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IV SESSION
THE PERIPHERAL NERVOUS SYSTEM

BORTEZOMIB-INDUCED PERIPHERAL NEUROPATHY: STUDY OF PROTEASOME INHIBITION AND MICROTUBULE STABILIZATION MECHANISMS IN RAT MODEL

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Bortezomib is an antineoplastic agents that is often used to treat the multiple myeloma and of some lymphomas. Although its well-known antitumor activity, its effectiveness is limited by the highly incidental development of severe peripheral neuropathy (BIPN). This neuropathy is characterized by dysesthesia, numbness and a painful sensations. In order to obtain a pre-clinical model to improve our understanding of BIPN molecular pathways, we performed an rat model in which bortezomib (0,20 mg/kg) was administered three times weekly for eight weeks, followed by a four-week follow up period.
At the end of the treatment period, we assessed nerve conduction velocity (NCV) and pathological changes in caudal nerve, dorsal root ganglia (DRGs), and sciatic nerve. Afterwards, we verified the involvement of proteasome inhibition and also we evaluated the microtubule stability in sciatic nerve by comparing the distribution of acetylated tubulin between polymerized (P) and soluble (S) fractions by western blot experiments.
The neurophysiological evaluation demonstrated a reduction in NCV both at eight weeks and after the follow up period: at the pathologic analysis, caudal nerves were the most affected structures, whereas DRGs showed a vacuolization in the cytoplasm in sensory neurons and of satellite cells, although the sciatic nerves evidenced a mild axonopathy. Proteasome activity assay was performed on peripheral blood mononuclear cells (PBMCs), sciatic nerve and brain: PBMCs and sciatic nerve recovered quickly in the acute setting, while maintaining strong inhibition until 48 hours after last injection when the drug was chronically administered. Moreover, at six and eight weeks of treatment, we observed the increase of acetylated alpha-tubulin in the polymerized fraction in sciatic nerves of BTZ-treated animals as compared with control, that returning at baseline during follow up period.
In conclusion, our results demonstrated that BTZ is able to induce a toxic effect on peripheral nervous system by the inhibition of proteasome and the stabilization of microtubule. Consequently, to deepen the study on these two possible neurotoxic mechanisms that underlying BIPN, will be important for developing neuroprotective drugs.

EXPRESSION OF TRPV1, CGRP AND SUBSTANCE P IN SPINAL PRIMARY AFFERENT NEURONS IN A RAT MODEL OF BORTEZOMIB-INDUCED PERIPHERAL NEUROPATHY

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Bortezomib (BTZ), a selective proteasome inhibitor, is an antitumor drug used to treat multiple myeloma. A side effect of BTZ therapy is a painful peripheral neuropathy (PN), often seen after the first treatment cycles and refractory to management. Our goal was to study the possible neurochemical changes involved in the development of BTZ-induced PN. With this aim, we used a well-established rat model of BTZ-induced PN, whose hallmarks are allodynia and axonopathy of peripheral nerves. Two groups of Wistar rats were used: one group received a single dose of BTZ (0.20 mg/kg), the other a 8-week-long treatment (0.20 mg/kg/day three times/week). The expression of TRPV1 and sensory neuropeptides CGRP and SP were examined in L4-L5 dorsal root ganglia (DRG), spinal cord segments, and sciatic nerves by means of western blot, reverse transcriptase-polymerase chain reaction (PCR) and immunohistochemistry.

BTZ treatment affects TRPV1 expression by inducing an increase of protein levels that is already obvious in the DRG after a single dose and occurs in DRG and spinal cord after chronic treatment. Moreover, TRPV1 mRNA and CGRP mRNA are down-regulated after the chronic treatment. Morphometric analysis of TRPV1-, CGRP- and SP-like immunoreactive (LI) DRG neurons shows that the proportion of TRPV1- and CGRP-LI neurons increased after BTZ treatment. Labeled neurons were mostly of small- and medium-size; however, after BTZ treatment, they underwent a rearrangement in size distribution, with differential changes involving the relative frequency of sized neurons. A BTZ-induced decrease of DRG neurons coexpressing TRPV1 and neuropeptides also occurs, being already evident for TRPV1/SP after acute scheduling, and involving colocalization of TRPV1 and either neuropeptide after chronic treatment. No BTZ-induced changes in immunolabeling are appreciable in the dorsal horn of the spinal cord and sciatic nerve.

These observations will be useful to deeply understand the pathophysiology of the BTZ-induced neuropathic pain and allow potential more targeted therapies.

NEUROPATHIC PAIN IS REDUCED IN ACID SPHINGOMYELINASE DEFICIENCIENT MICE

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Neuropathic pain is a disabling condition that develops after damage to the nervous system. Unfortunately, actual tools for analgesia are only partially successful. Current literature supports a role for the inflammatory cytokines in neuropathic pain pathogenesis: among them interleukin-1 (IL-1)-β is particularly interesting since it is elevated in the cerebrospinal fluid of individuals with chronic neuropathic pain. Based on recent evidence that acid sphingomyelinase (ASM) is necessary and sufficient to control the formation and release of microvesicles containing IL-1β by glial cells, this study evaluated, by a multidisciplinary approach, the onset and the maintenance of neuropathic pain in the knockout mouse for ASM (ASMKO) following the sciatic nerve crush.

Adult (1 and 5 month-old) male ASMKO and age-matched wt mice underwent sciatic crush lesion. The variables considered were: thermal (Plantar test) and mechanical sensitivity (Dynamic Plantar Aesthesiometer apparatus), digital nerve conduction velocity (NCV) and action potential amplitude, walking track analysis (SSI) followed by ultrastructural and morphometric analyses of sciatic nerves. Since ASM KO mice are a model of Niemann Pick disease Type A, the disease progression was monitored by rotarod test.

The results obtained emphasize the possible relevance of IL-1β in the development of neuropathic pain. The absence of IL-1β release in ASMKO animals alleviates the development of peripheral neuropathy and peripheral nerve function abnormalities. The ASMKO mice never develop thermal hyperalgesia. Regarding mechanical sensitivity, by the day 5, in the 5 mos-old ASMKO the paw becomes hyposensitive. Even though the functionality of the fibers is preserved in all animals, morphological data suggest a delay in the regenerative process in the ASMKO compared to the WT mice. Moreover, our results are also consistent with recent studies that suggest an involvement of the sphingomyelin-ceramide metabolism in neuropathic pain.

In conclusion, targeting IL-1β production and secretion may represent a new therapeutic strategy for the treatment of neuropathic pain.

ERBB2 RECEPTOR: EFFECTS OF ITS CONSTITUTIVE OVEREXPRESSION ON ADULT MOUSE MEDIAN NERVE REGENERATION.

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Schwann cells are involved in many important aspects of peripheral nerve development and biology as well as in nerve repair. Neuregulin-1 (NRG1) and its receptor, the heterodimer ErbB2/ErbB3, have emerged as key mediators of axon–Schwann cell interactions and regulators of Schwann cell development, proliferation, migration and myelination.

While the effects of ErbB2 deletion in the peripheral nervous system have already been analyzed, the consequences of its overexpression have not been investigated yet. In this study we therefore studied the effect of constitutive ErbB2 receptor overexpression on adult mouse median nerve in physiological and regenerative conditions.

Physiologically, the grip function controlled by the median nerve in transgenic mice (BALB-neuT) was similar to wild-type (BALB/c). Stereological assessment of healthy nerves showed that peripheral nerves develop normally in ErbB2-overespressing animals (BALB-neuT) with no difference in number and size of myelinated fibers compared to wild-type mice (BALB/c). No differences in Schwann cells number between BALB/c and BALB-neuT were seen in electron microscopy.

By contrast, the overexpression of ErbB2 appeared to speed up the nerve regeneration after a median nerve crush injury, as shown by a faster functional motor recovery. Stereological results showed a higher number of regenerated myelinated fibers with a thinner axon and fiber diameter in BALB-neuT mice. Moreover, BALB-neuT showed more Schwann cells nuclei profiles.

Real time PCR analysis, performed two days after injury, revealed a decreased expression of all the ErbB receptors and of the transmembrane (type III) NRG1 isoforms both in BALB/c and BALB-neuT mice. By contrast, the level of the soluble NRG1 isoforms (type I/II, alpha and beta) increased and, intriguingly, the expression level in BALB-neuT mice was significantly higher than in BALB/c animals.

Altogether, these results suggest that constitutional ErbB2 receptor over-expression does not influence the physiological development of peripheral nerves, while it improves nerve regeneration following traumatic injury, possibly strengthening the up-regulation of soluble NRG1 isoforms.

NEUROPROTECTIVE ACTIVITY OF THIOCTIC ACID IN CENTRAL NERVOUS SYSTEM LESIONS CONSEQUENT TO PERIPHERAL NERVE INJURY

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Peripheral neuropathies have a heterogeneous etiology being caused by autoimmune diseases, exposure to drugs or toxic substances, infections, trauma or metabolic diseases such as diabetes. Neuropathies are in general characterized by hyperalgesia and allodynia with alterations of muscular sensivity and functions. Thioctic acid is an antioxidant existing in nature and expressed in two optical isomers designated as (+)- and (-)-thioctic acid. (+)-Thioctic acid is the naturally occurring stereoisomer, although synthetic (commercially available) thioctic acid is mainly the mixture of (+) and (-)-enantiomers. The antioxidant has been proposed as a potential therapeutic agent in the treatment or prevention of several pathologies related to an imbalance of the oxidoreductive status. The purpose of the present study was to assess if compression of sciatic nerve, induced by loose ligation of it, is accompanied by an increased oxidative stress and by central nervous system (CNS) changes. This study has also investigated the effect of treatment with enantiometers of thioctic acid on oxidative stress and on CNS damage induced by peripheral nerve injury.
Loose ligation of the right sciatic nerve was performed in spontaneously hypertensive rats (SHR), used as a model of increased oxidative stress, and in normotensive Wistar-Kyoto rats (WKY) (reference group). Animals with sciatic nerve ligation were left untreated or were treated intraperitoneally for 14 days with (+/-)-(25 and 50 mg/Kg/day), (+)-(25 mg/Kg/day), (-)-(25 mg/Kg/day) thioctic acid. Analysis was centered on astrogliosis and neuronal injury phenomena at level of the motor cortex (M1 and M2) and sensory cortex (S1HL) areas, and of hippocampus. Morphological evaluation has assessed the expression of glial fibrillary acid protein (GFAP), of the myelin basic protein (MBP), and for the neuronal component the phosphorylated 200 KDa neurofilament protein (NFP).
In the brain of SHR astrogliosis, with hypertrophy of astrocytes, and neuronal damage accompanied by decreased expression of NFP was observed. Sciatic nerve ligation raised astrogliosis and increased oxidative stress markers. Treatment with the antioxidant reduced astrogliosis and neuronal damage in the different brain areas analyzed. (+)-Thioctic acid was more active than (+/-)- or (-)-enantiomers in countering astrogliosis and neuronal damage.
The above results demonstrated a neuroprotective effect elicited by thioctic acid on CNS lesion consequent to peripheral nerve injury. The demonstration of an activity of thioctic acid, both on the levels of oxidative stress and on CNS, suggests that appropriate antioxidant strategies may represent a therapeutic approach in the treatment of compressive neuropathies.

V SESSION
TROPHIC FACTORS, NEURODEGENERATION, NEUROREGENERATION

TESTOSTERONE AND ESTRADIOL REGULATE PROLIFERATION IN SVZ OF THE ADULT MALE RAT

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Adult neurogenesis is a process occurring within different regions of the Central Nervous System: i.e. in the olfactory bulbs, in the subventricular zone (SVZ), and in the dentate gyrus (DG). Gonadal hormones may have a role in regulating this phenomenon. In a previous studiy we demonstrated an activational role of Testosterone (T) on neuroblasts' proliferation in male rat SVZ. Quantitative analysis revealed that the rate of cell proliferation in the lateral wall of the lateral ventricle is positively associated to the levels of circulating T; in fact, in castrated animals there was a significant decrease of the number of newborn cells.
However, these data do not clarify if this effect is based on a purely androgen-dependent mechanism or if estrogenic metabolites may be also involved. To this aim, in the present study, we analyzed 6 experimental groups: Sham operated male rats, castrated (CX) treated with vehicle (sesame oil), T, Estradiol (E2), dyhydrotestosterone (DHT), or with both hormones (E2 + DHT). All the animals received two intraperitoneal injections of BrdU at an interval of six hours and were sacrificed one day after the last BrdU administration. The rate of neurogenesis was evaluated through quantitative counting of immunocytochemically stained BrdU-positive cells performed by stereological methods on six sections per animal, taken in an intermediate rostrocaudal level (about from 1.60 mm to 0.70 mm anterior to Bregma).
Our data showed no significant difference between CX+T, CX+E, CX+DHT+E and sham while the number of BrdU+ cells was statistically lower in CX and CX+DHT in comparison with others groups. These results indicate that both testosterone and estradiol, the aromatic metabolite of testosterone, and not DHT, the reduced metabolite, increase cell proliferation. At present, we are trying to identify what cell population (A or C type) is affected by hormonal treatments within the SVZ. To this aim, we are performing quantitative analyses by the identification of 4 markers: BrdU (to identify cell proliferation), PHH3 (to identify cell in mitosis), DCX (for A cells) and MASH1 (for C cells).

EFFECT OF DIFFERENT GROWTH FACTORS ON OLFACTORY ENSHEATHING CELLS EXPOSED TO HYPOXIA AND/OR SERUM DEPRIVATION: IMPLICATION FOR THERAPEUTIC APPROACHES

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Olfactory Ensheathing Cells (OECs) are the glial cells derived from the olfactory placode that envelop olfactory axons through a migration process from the olfactory epithelium to the bulb. OECs, as a source of neurotrophic growth factors, promote axonal growth *in vitro,* while *in vivo* they produce myelin, supporting remyelination of damaged axons. Consequently, OECs transplantation has emerged as a proper experimental therapy to induce anatomical and functional repair of spinal cord injuries (SCI). Conversely, the functional recovery of SCI after OECs transplantation is limited. This might be ascribed to the microenvironment at the lesion site, which shows deprivation of growth factors, nutrients and oxygen. To mimic this condition and to evaluate its possible effect on OECs survival, we used an *in vitro* approach by growing primary neonatal mouse OECs under hypoxic condition and/or serum deprivation. As OECs exhibit antigenic features of both astrocytes and Schwann cells (SCs), we compared OECs survival with that of schwannoma cells (RT4-D6P2T cell line) and primary cultured astrocytes under the same experimental conditions. OECs and astrocytes were prepared from neonatal mouse olfactory bulbs and all glial cellular types were grown in different conditions: some cells were cultured both in DMEM/FBS and in DMEM/FBS with different growth factors (GFs), such as NGF, bFGF, GDNF and their combination. Other cells were grown both with serum-free DMEM/F12 medium and with DMEM/F12 added with above mentioned GFs. Cells grown in these conditions were also exposed to an anoxic insult, by inverting coverslips with cells in their wells and thus lowering the concentration of oxygen. After a week, cultures were analyzed by immunocytochemistry and cell viability was evaluated by MTT assay. Our results show that both serum and oxygen deprivation induce a reduction of the cellular survival and that the three cell types were differently sensitive to the tested stress conditions, with OECs being the most sensitive. Moreover, OECs survival was rescued by bFGF under serum deprived or hypoxic condition, but not in a condition of drastic serum deprivation and hypoxia. bFGF resulted effective also on the other cell types, whereas the effect of the other GFs was negligible.
This model, simulating secondary events during SCI, suggests that administration of bFGF might be considered useful to sustain cell survival after transplantation of OECs either alone or in combination with other glial cell types.

XENOESTROGENS AND ANXIETY IN MICE: SEXUALLY DIMORPHIC EFFECT OF POSTNATAL EXPOSURE TO GENISTEIN

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Genistein is a phytoestrogen, produced by Leguminosae, largely present in human and laboratory animal diets, sharing structural features with the 17beta-estradiol, so it can bind estrogen receptors, exerting both estrogenic and antiestrogenic activity.

Genistein is present in soybased infant formulas widely used in many countries. Phytoestrogens are considered to have beneficial effects on adults, but little is known about the effects of massive neonatal exposure, that could cause long-term neurological alterations.

To test this hypothesis we orally administered to CD1 female and male mice from postnatal day 1 (PD1) to PD8: genistein (50 mg/kg in sesame oil), estradiol (50 mg/kg in sesame oil) or only vehicle. To measure the anxiety-like behavior we used the Elevated Plus Maze (EPM) and Open Field (OF): the analysis of mice activity on EPM during a five min trial revealed no differences of both gender and treatment. For the OF, we observed a significant effect of both gender and treatment: control male spent more time and cover a longer distance in the center area than female.

Genistein-exposed females show a significant increase of the time spent and the distance covered in the center area, wheareas genistein-exposed males show a significant decrease of two parameters.

Due to the involment of arginine-vasopressin (AVP) in the control of stress axis, we have also investigated the magnocellular vasopressin system of the paraventricular nucleus (PVN) in its different subdivisions. We have observed a decrease of the number of AVP neurons in PVN medial magnocellular (PaMM) and lateral magnocellular (PaLM) regions of genistein-treated females. No effects were observed in the posterior magnocellular (PaMP) region of females, as well as in the PVN of genistein-exposed males. Thus, precocious direct exposure to genistein has sexually dimorphic effects not only on anxiety behavior, but also on the magnocellular AVP system of the PVN, one of the key centers to control the stress-axis.

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TRPV1-, CGRP- AND SP-IMMUNOREACTIVE INNERVATION OF SCALP ARTERIES IN PATIENTS SUFFERING WITH CHRONIC MIGRAINE

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The vanilloid receptor TRPV1 and the neuropeptides calcitonin gene related peptide (CGRP) and substance P (SP) appear to be differently involved in migraine pain. A role of scalp arteries and a beneficial effect of their ligature in chronic migraine (CM) is suggested by several data. In this study, we show the occurrence of TRPV1-like immunoreactive (LI) nerve fibres in human scalp arteries and provide quantitative data on the TRPV1-, SP- and CGRP-LI innervation of specimens from CM patients and control subjects.

Samples were obtained at surgery from patients affected by treatment-resistant CM and other intracranial pathologies as controls. All patients gave informed consent. ABC immunoperoxidase was performed with antibodies against TRPV1, CGRP, SP and the pan-neuronal marker protein gene product 9.5 (PGP9.5). Immunoreactive nerve fibres in vessel cross sections were quantified as ratio of the total length in μm of fibre segments detectable in each section to the section area.

TRPV1-, CGRP-, SP- and PGP9.5-LI nerve fibres occur in the adventitia with varied density for each marker and among specimens. Innervation density showed statistically significant differences between CM and control group for TRPV1, CGRP and SP, and not for PGP9.5 (t-test). Analysis of the ratio of TRPV1-, CGRP- and SP-positive fibers to PGP9.5-positive ones for each artery showed a statistically significant higher amount of TRPV1-LI fibres in CM compared to control samples, while the peptide-positive fibers, though more abundant in CM tissue, did not significantly differ between the two groups.

To our knowledge, this is the first demonstration for the occurrence of TRPV1-LI innervation of human scalp arteries. Our data further indicate that, compared to controls, in subjects affected by CM, TRPV1- and, in lower measure, CGRP- and SP-LI innervation is strengthened. This supports the viewpoint of a role of scalp arteries and the involvement of TRPV1 and possibly CGRP and SP in CM.

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SYNAPTIC AND MOLECULAR DETERMINANTS OF RETT SYNDROME, AN X-LINKED FORM OF MENTAL RETARDATION.

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Rett syndrome (RTT) is a postnatal progressive disorder with no efficient treatment that manifests mainly in girls during early childhood that is caused in the majority of cases by mutations in methyl-CpG-binding protein 2 (MeCP2). Effective animal models for RTT are available and show morphofunctional abnormalities of synaptic connectivity. No treatment is currently available for this disease. This absence mostly stems from our ignorance about the mechanisms leading from the genetic defect to the alterations occurring at cellular and neural system level. To study the effects of RTT on neuronal organization, we produced Mecp2-KO/Thy1-GFP mice expressing GFP only in selected neuronal populations of the forebrain. The analysis using confocal microscopy on fixed tissue revealed neuron-specific abnormalities of dendritic spines structure and number. In addition, using two-photon microscopy to image spines in-vivo through a cranial window we found that dendritic spines short-term motility was drastically reduced in presymptomatic KO animals compared to WT littermates. Next, we found that the synaptic defects associated with RTT may be caused by abnormal protein synthesis rates. Phosphorylation levels of rpS6, a component of the 40S ribosomal subunit, is severely altered in different neuronal subpopulations across the brain of presymptomatic MeCP2-KO mice and in symptomatic heterozygous female mutants. Moreover, severe defects of protein synthesis initiation were present the brain of presymptomatic Mecp2 mutants that were not restricted to specific subset of transcripts. Finally, we discovered a general dysfunction of the Akt/mTOR signaling associated with the disease progression. Our results indicate that defects in the AKT/mTOR pathway are responsible for the altered translational control in Mecp2 mutant neurons and disclosed a novel putative biomarker of the pathological process. Importantly, this study provides a novel context of potential therapeutic interventions to restrain or ameliorate RTT.

MORPHINE WITHDRAWAL PRODUCES ERK-DEPENDENT AND ERK-INDEPENDENT EPIGENETIC MARKS IN NEURONS OF THE NUCLEUS ACCUMBENS AND LATERAL SEPTUM

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Epigenetic changes such as covalent modifications of histone proteins represent complex molecular signatures that provide a cellular memory of previously experienced stimuli without irreversible changes of the genetic code. In this study we show that new gene expression induced *in vivo* by morphine withdrawal occurs with concomitant epigenetic modifications in brain regions critically involved in drug-dependent behaviours. We found that naloxone-precipitated withdrawal, but not chronic morphine administration, caused a strong induction of phospho-histone H3 immunoreactivity in the nucleus accumbens (NAc) and in the lateral septum (LS), a change that was accompanied by augmented H3 acetylation (lys14) in neurons of the NAc. In the same regions morphine withdrawal induced the phosphorylation of the epigenetic factor methyl-CpG-binding protein 2 (MeCP2) in Ser421. These epigenetic changes were accompanied by the activation of members of the ERK pathway as well as increased expression of the immediate early genes (IEGs) *c-fos* and activity-regulated cytoskeleton-associated protein (Arc/Arg3.1). Using a pharmacological approach, we found that H3 phosphorylation and IEG expression were partially dependent on ERK activation, while MeCP2 phosphorylation was fully ERK-independent. These findings provide new important information on the role of the ERK pathway in the regulation of epigenetic marks and gene expression that may concur to regulate *in vivo* the cellular changes underlying the onset of the opioid withdrawal syndrome.

A CHEMOKINE SYSTEM COORDINATES HUMAN CEREBRAL CORTEX DEVELOPMENT AND VASCULARIZATION

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The blood-brain barrier (BBB) appears to develop as soon as cerebral cortex microvasculature begins to form during foetal development. TJ proteins are expressed together with barrier-specific transporters and enzyme systems very early during foetal life stages, and the BBB is present and reveals a significant degree of functional tightness. The tightness of the barrier continues to increase gradually during mid to late gestation and, to a lesser degree, in the newborn period. Recent studies have demonstrated that the canonical Wnt/β-catenin pathway and the Wnt7a/7b growth factors in addition to their function on brain vascularization might participate to the differentiation of a BBB phenotype in endothelial cells. Moreover, it has also been suggested that the entire process of vascularization is not only driven by demands for oxygen and nutrients by the growing neuroblasts but that an overlapping sequence of signalling molecules produced in one system (vascular) may influence development of the other system (neural) and mutually coordinate cell proliferation, migration, and differentiation. Our hypothesis is that other brain-specific signalling pathways could be implicated in brain angiogenesis and in BBB induction, in addition coordinating cerebral cortex vascularization with cortex development. Members of the CXC system of chemokines, in particular ligand CXCL12, a potent migration and differentiation factor, and receptors CXCR4 and CXCR7, represent good candidates to sustain these processes. The analysis carried out during human cerebral cortex development, by immunohistochemical and confocal microscopy methods, demonstrates CXCL12 and its receptors differentially expressed by different cell types, suggesting an important role for this chemokine ligand-receptor axis in the communication between radial glia cells, migrating neuroblasts and growing microvessels.