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GIUSEPPE STERZI (1876-1919) : NEUROANATOMIST AND HISTORIAN OF ANATOMY.

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Giuseppe Nazzareno Sterzi was born on March 19, 1876 in Cittadella (Padua). He took his M.D. in Pisa on July 12, 1899, defending a thesis on the morphology of meninges, and, a few months later, because of his exceptional ability, he was appointed 'Aiuto' (reader) in the institute of Anatomy of Padua. In 1910 he was appointed Professor and Chairman of the Anatomy institute of the University of Cagliari. In five years of hard work, he reorganized and modernized the institute attracting many students to his lab. In the Summer of 1915 Sterzi volunteered into the Italian army where he served as medical officer. At the end of World War 1, Sterzi, valiantly, chose to remain in his office of director of the military hospital of Arezzo during the postwar Spanish epidemic flu. In the winter of 1919 he contracted the fever and died on February 17, at the age of 43, leaving a loving wife a four sons. Although his research activity encompassed no more than 15 years, his scientific production is impressive considering that it consists even of 3 large textbooks of which he was the sole author. Apart from the article on the endolymphatic sac, and the one on the subcutaneous tissue, the themes treated by Sterzi are relevant to neuroanatomy and history of anatomy. Sterzi approach to morphology has been mainly based on comparative embryology and anatomy; the following topics may be recognized in his publications: 1) The meninges (1899-1902). 2) The vasculature of the spinal medulla (1900-1904) and of the brainstem (1913). 3) The hypophysis (1904). 4) The regio parietalis (of diencephalon) in lower craniates (1905). 5-6). The studies on the development of the longitudinal cerebral fissure (1912) and that on the significance of the human encephalon and telencephalon (1914). In 1907 and 1909 he published two pioneering textbooks, greatly appreciated by his contemporaries, on the central nervous system of cyclostomes and selachians and, in 1914, a tract in 2 volumes on human neuroanatomy. On his intention these books should had been the extremes of a series, never accomplished because of the untimely death. Sterzi's historical studies were mainly focused on Casserius's and Fabricius, two leading anatomists of Padua's golden age. Just one century ago, he discovered, in the Marciana library of Venice, Fabricius's tabulae pictae: a body of almost 200 painted tables that represents the first coloured atlas of human and comparative anatomy, and houses, as Sterzi briefly mentioned, many important discoveries.

NEUROSCIENCES AND INNOVATION: THE DEPARTMENT OF NEUROSCIENCE AND NEUROTECHNOLOGIES OF THE ITALIAN INSTITUTE OF TECHNOLOGY

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The Department of Neuroscience and Neurotechnologies is the research platform of the IIT Foundation devoted to the study of "neural plasticity", the ability of the nervous system to adapt and remodel in response to environmental stimuli. The research infrastructure of the Department consists of electrophysiology, neurobiology, neurochemistry and neuroengineering laboratories which allow a multidisciplinary approach to the neuroscience problems. The main objective of the research program is the study of the cellular mechanisms underlying synaptic transmission and plasticity in live neurons, in order to understand the changes in information transfer and processing involved in the generation of higher brain functions, such as learning and memory, as well as in the pathogenesis of neuropsychiatric disorders. The study of the basic properties of neurons is fundamental for the generation of hybrid neuro-electronic interfaces (biochips) capable to function as high sensitivity biosensors or as devices for bidirectional interactions between brain and robotic bodies. Such approach represents a link between molecular/cellular neuroscience and the generation of new neurorobotic paradigms with the objective to create experimental models mimicking the behavior and the computing capabilities of natural neuronal networks.

THE NEURAL STEM CELLS: INVISIBLE CELLS?

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Stem cells are cells capable of self-renewal, yet in parallel, they can generate a differentiated progeny. Adult stem cells are present in many organs allowing the physiological renewal of cells, as well as tissue repair/regeneration. Their activity is regulated within stem cell 'niches', and their morphology/identity can vary in different functional states, what makes them rather 'invisible'.

The mammalian brain is made up of a perennial tissue, unable to renew its cellular elements. This is the reason why neurons present at birth last throughout life. And, if many of them will die, no new cells will be generated. At least, this is what was thought until some years ago. In the last decade, such static vision did change, thus allowing neurons to enter the ever expanding universe of stem cells, etching a strict dogma of neurobiology and opening new perspectives in neuroscience.

Nevertheless, many open questions still remain, leaving the issue of neural stem cells (NSCs) somehow unresolved. First of all, the ultimate in vivo identification of these cells. Then, the remarkable discrepances observed between in vitro and in vivo data, corresponding to a really different behavior of NSCs in different environmental conditions. The remarkable differences observed in different mammalian and non mammalian species. Finally, last but not least, the poor knowledge of what really happens in the human brain.

On the other hand, a wide and detailed chapter has been written during the last fifteen years concerning adult neurogenesis, namely the fate of the NSC progeny in vivo ultimately giving rise to new neurons within specific brain areas. In such studies, many other important questions have been addressed and solved: the anatomical localization of NSCs in specific neurogenic niches of the forebrain subventricular zone (SVZ) and hippocampal subgranular zone (SGZ), their fine cellular organization and, more recently, the origin of adult NSCs from the primitive germinative layers. Here, another important breakthrough consisted of the discovery that radial glia cells do act as stem cells during development, then persisting throughout life in neurogenic niches.

Yet, after the huge progresses reported above, the mammalian central nervous system (CNS) mainly remains consisting of a perennial tissue, uncapable of self-renewing and self-repairing. This reality is well represented by the fate of radial glia, that preserve stem cell properties within the restricted neurogenic niches but widely transform into mature astrocytes in the rest of the CNS. Starting from this point, present and future research will focus on the existence of local, parenchymal progenitors endowed with different degrees of progenitor/stem cell potential. This latter feature seems to be remarkably variable in different mammalian and non mammalian species, thus leaving open the hope for some regenerative processes that could derive from exploitation of the invisible cells.

AGE-RELATED MORPHOLOGICAL CHANGES IN NERVE CELLS

Ennio Pannese

During normal aging, widespread loss of nerve cells does not occur; what loss there is limited in extent (probably no more than 10%) and restricted to specific regions of the nervous system.
The commonest age-related structural changes undergone by nerve cells are as follows: dendrites decrease in the number and length and many dendritic spines are lost; axons decrease in number and their myelin sheaths may become less compact and undergo segmental demyelination and remyelination; and significant loss of synapses occurs. These changes probably make a significant contribution to the behavioural impairment and cognitive decline that may accompany normal aging.

THE G.I.S.N. RESEARCH GROUPS: DEPARTMENT OF HUMAN ANATOMY AND HISTOLOGY "RODOLFO AMPRINO" - BARI UNIVERSITY

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The main interest of the research group is the analysis of the neurovascular unit (NVU) in the developing human brain and during neurological diseases and tumours. The NVU is a complex morphofunctional entity composed of interacting cellular and acellular elements, endothelial cells, pericytes and astrocyte endfeet, together with basal lamina molecules, associated growth factors, regulatory molecules, and enzymatic complexes. One of the main objectives of the study is the analysis of the brain-specific and blood-brain barrier (BBB)-specific differentiation of the NVU's components paralleled by the analysis of their mutual interactions and involvement in brain vascularization. The results demonstrate that an early BBB-endothelial phenotype is present during human brain development and that the whole NVU seems to be precociously involved in the parallel and apparently opposite processes of barriergenesis and angiogenesis. The response of the NVU to these events is revealed by the complex phenotype of its cells, characterized by the presence of BBB-specific molecules together with angiogenic markers. Activated pericytes and perivascular astrocytes show a tight interplay with endothelial cells in the growing microvessels, owing to their position, these cells mediate endothelial cross-talk within the NVU, contribute to barrier differentiation and maintenance, and interacting with matrix molecules play a key role in controlling and guiding the endothelial sprouting. Considering that in brain diseases and malignant tumours vascularization could result from different mechanisms, an increased understanding of NVU cell relations and phenotypes during normal brain development may improve knowledge on pathological angiogenesis and contribute to the identification of new diagnostic and prognostic criteria and therapeutic strategies.

TRADITIONAL MASKS AND NEUROLOGICAL DISEASE

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The history of the representation of the human face is as old as graphic and artistic representation of Man. Human faces may, of course, show signs of disease, the most common (and striking) deformity being facial paralysis. The first medical study of peripheral facial paralysis is attributed to Avicenna, and Charles Bell described it in 1821. Independently from "official" medical studies, artistic representations of facial paralysis are documented since the Antiquity (for example, in Roman statues). Especially, facial paralysis (central or peripheral) has been depicted in traditional masks of many world regions: in the Iroquois (Canada's first people) masks, in the masks of Eskimos, the Japanese Kyogen masks, the Sri Lanka *sanni* ("illness") masks. Most of these masks have a ritual significance. For example, the Iroquois "False Face Society" was a collection of healers who used special masks with spiritual properties they carved themselves. Africa is probably the major center of world's masking traditions; interest on African masks was spurred at colonial times and masks have become emblematic of the culture of sub-Saharan Africa. Although African masks depicting disease are rather rare, facial deformity, such as facial paralysis and changes attributed to epilepsy, is found in masks deriving from different countries. Especially striking are the Congolese *Mbangu* masks (sickness masks) of Pende and the Ibo sickness mask of Nigeria. Facial paralysis was also depicted on African traditional utilitarian objects, such as figurative calabashes to keep liquid medicines. In African culture, images of deformation are considered to represent malevolent forces activated upon transgression of moral values, or diseases attributed to sorcery or punishment for wrongdoing, or to provide a lesson not to laugh at sickness, or a wish for healing. All these pieces of classical or traditional art offer vivid historical representations of neurological disease.

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I SESSION
SYSTEMATIC, CHEMICAL AND DEVELOPMENTAL NEUROMORPHOLOGY

OLFACTORY ENSHEATHING CELLS: MORPHO-FUNCTIONAL FEATURES

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Olfactory Ensheathing Cells (OECs) constitute an unusual population of glial cells, present in the olfactory system, showing exceptional plasticity and supporting olfactory neurogenesis. OECs share properties with both Schwann cells (SCs) and astrocytes of CNS. Several studies reveal that OECs produce both various growth factors and adhesion molecules. Immunocytochemical techniques have demonstrated that OECs express a high number of markers, such as S-100, GFAP, p75NTR, 04, vimentin, nestin, neuropeptide Y and calponin. Moreover, during the past decade, OECs have attracted attention for their ability to stimulate the directional regrowth of injured axons and for their functional plasticity. These properties might render them potential clinical agents and of trophic support to CNS injury. Since OECs represent a source of trophic factors, in our studies we have evaluated: 1) the effect of rat OECs on the CNS neurons in culture; 2) the expression of some markers in OECs grown in different conditions.

1) We have focused our researches on hypothalamic and hippocampal neuronal survival and neurite outgrowth in the presence of OECs, compared to the action of some trophic factors, such as bFGF, NGF and GDNF in a co-culture model. Immunohistochemical technique, with poly and monoclonal antibodies (PGP 9.5 and S-100), were used. Our results have demonstrated that both in hypothalamic neurons/OECs co-cultures and in hippocampal neurons/OECs co-cultures, the number of neurons was significantly increased in comparison with control cultures and these neurons exhibited a dense axonal outgrowth. Moreover, we show that in both co-culture models, bFGF, NGF and GDNF support them differently. In conclusion, OECs exert a positive influence on the survival and axonal outgrowth of hypothalamic and hippocampal neurons "in vitro.

2) In other studies, we have examined the expression of different markers in rat OECs grown both in serum containing medium and in serum-free medium added with different growth factors (bFGF and GDNF). OECs cultures were immunostained for calponin, nestin, PGP 9.5 and MAP-2. Our results have shown a different expression of nestin and calponin, depending on the presence of bFGF or GDNF. Moreover, the expression of neuronal markers (PGP 9.5 and MAP-2) increased in OECs grown in serum-free medium both treated with growth factors and without them. These results suggest that the highest expression of neuronal markers in serum-free medium might be explained by serum containing molecules that inhibit the effect of growth factors on the PGP 9.5 and MAP-2 expression.

CHARACTERIZATION OF THE NEONATAL OLFACTORY BULB ENSHEATHING CELL LINE (NOBEC)

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Among the various cell populations that can be transplanted in the nervous system, olfactory ensheathing glial cells have raised great interest over recent years. Here, we provide an *in vitro* characterization of the NOBEC (Neonatal Olfactory Bulb Ensheathing Cell) line that was obtained from primary cells dissociated from rat neonatal olfactory bulb (OB) and immortalized by retroviral transduction of SV40 large T antigen. Light and electron microscopy investigation showed that NOBECs are a homogeneous cell population both at structural and ultrastructural level. RT-PCR, Western blotting and immunocytochemistry showed that NOBECs express the glial markers S100, GFAP (Glial Fibrillar Acid Protein) and p75NGFR as well as NRG1 (neuregulin-1) and ErbB1-2-3 receptors while they are negative for ErbB4. In addition, NOBECs are immunopositive for calponin, nestin, vimentin and CD133. In particular, vimentin (its expression is modulated during cellular maturation) showed a higher labelling expression compared to the other markers. Moreover, NOBEC present a strong immunoreaction to the neuronal markers, such as Protein Gene Product (PGP 9.5) and Microtubule Associate Protein-2 (MAP-2).

Yet, NOBECs exhibit a high proliferation and migration basal activity and can be transducted with vectors carrying GFP (Green Fluorescent Protein) and NRG1 cDNA. Functional stimulation by means of NRG1-III-β3 overexpression through viral transduction induced a significant increase in cell proliferation rate while it had no effect on cell migration.

It can be concluded that NOBEC cell line retain glial features both morphologically and functionally and can thus be used for both *in vitro* assays of glial cell manipulation and *in vivo* experimental studies of glial cell transplantation in the nervous system.

TYROSINE HYDROXYLASE IN CHICK EMBRYO BRAIN DEVELOPMENT

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Tyrosine hydroxylase (TH) catalyses the first, rate limiting step in catecholamine biosynthesis. In vertebrates the catecholamines dopamine, noradrenaline and adrenaline play important roles in many physiological functions in the central and peripheral nervous systems as well as in the endocrine system. Thus the regulation of TH expression and activity is crucial for neuronal and hormonal functions that involve catecholamines. TH is regulated by many ways: among them, gene expression modulation, alternative RNA processing, allosteric modulation by polianions (mainly RNA), site-specific phosphorilation, feedback inhibition, ubiquitination. The importance of TH expression during embryonic development have been demonstrated by the targeted inactivation of the TH gene that determines, in mice, midgestional lethality. In most species is present a single TH mRNA, that following translation produces a single form of the protein. In some species, however, alternative splicing of the primary transcript can result in different forms of TH mRNA that encode for different protein isoforms with specific regulative properties.

Considering the importance of the TH expression during embryonic development and the presence in many species of multiple TH protein/mRNA forms led us to study the enzyme expression during chick embryo brain development.

By means of western blotting we found that in the chick embryo brain, together with the expected form of ~65 kDa, is present a TH protein showing a molecular weight of ~75 kDa from E (embryonic day) 8 to E14. The analysis of the cDNA sequence of TH obtained from chick embryo brains at E8-E14 revealed the possible presence of a mRNA splice variant that presents a nucleotide insertion which encodes for an additional aminoacidic sequence in the regulative N-terminal domain of the protein. We also found that TH immunoreactive bands showing an higher molecular weight were immunoreactive for ubiquitin, too.

These results suggest that during chick embryo brain development is transiently present a TH protein of ~75 kDa; the possible mechanisms involved in this process are alternative splicing and/or ubiquitination.

THE BDNF/TRKB SYSTEM IN THE HAIR CELLS OF ZEBRAFISH

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The lateral line system (LLS) is a complex mechanoreceptor system present in fishes and larval amphibian, formed by a morpho-functional unit called neuromast. The neuromast can be localized at the body surface and within canals, and consist of sensory hair cells, basal/support cells and mantle cells. The sensory hair cells are regarded to be analogous to the mammalian inner ear sensory hair cell, on the basis of their morphology and genetic profiles. In zebrafish the LLS undergoes a continuous sensory cells renewal from quiescent elements, and the molecular mechanisms regulating this process are now rather well known. For this reason the zebrafish has become an excellent model for genetics and developmental biology studies. Particularly the LLS of zebrafish has been used as a model for cell polarity and collective cell migration, as well as hair cell loss and regeneration. The brain-derived neurotrophic factor (BDNF) is a neurotrophin that acts on the responsible cells surface binding a specific receptor called TrkB, a protein with an estimated molecular weight of 145 kDa. BDNF and TrkB have been previously detected in LLS cells, and could play an important role in the development and maintenance of hair cells of fishes and mammals. Here, we used qRt-PCR, western blot, immunohistochemistry as well as morpholino injections to analyze the involvement of the BDNF/TrkB system in the biology of hair cells of wild type and transgenic zebrafishes strains ET4, (Et (krt4:GFP)sqet4) from 4 days postfertilization (dpf) to the 120dpf adult stage. The qRT-PCR results demonstrated that the levels of BDNF and TrKB mRNAs are maximal at 20dpf, and progressively decrease up to the adult stage. These results are supported by western-blot and immunohistochemical findings. At 4 dpf BDNF and TrkB were detected exclusively in the hair cells of the LLS neuromast. During development and, particularly in the adult stage, TrkB expression remains constant in all hair cells, while the BDNF is restricted to a small subpopulation of sensory cells. Moreover, TrkB was found in a subpopulation of neurons in the anterior and posterior lateral line ganglia. The morpholino injections, designed to knockdown translation of BDNF in transgenic zebrafish, demonstrated that the block of BDNF gene during the first phases of development leads to dramatically morphological changes in the hair cells of LLS neuromast. These results, taken together, demonstrated that BDNF/TrkB system is necessary for normal development of hair cells in the LLS.

IDENTIFICATION OF SUBPOPULATIONS OF CEREBELLAR GRANULES BY IMMUNOHISTOCHEMISTRY

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Although numerous data have indicated that the cerebellum is involved in a progressively increasing number of functions, including cognitive and affective functions, simplified views on the morphofunctional organization of the cerebellum are still predominant. These views take into account only 5 neuronal types in the cerebellar cortex, the so-called 'traditional' neurons, and a basic nervous circuitry consisting of 2 types of afferences, which are excitatory, incoming by means of the climbing and mossy fibres, and only one type of efferences, which are inhibitory, provided by the Purkinje neurons. Moreover, no substantial changes would be present in the different regions of the cerebellar cortex. Accordingly, the granules, which represent the most common neuronal type in the whole CNS, have usually been considered a homogeneous population of neurons. They receive excitatory, glutamatergic, inputs from mossy fibres and inhibitory, GABAergic, inputs from Golgi neurons axons at synaptic glomerular complexes, and send their axon to the molecular layer, where it gives rise to the parallel fibres, which mainly synapse on dendritic processes of the Purkinje neurons. All granules are considered as excitatory, glutamatergic neurons.
In the present study, we aimed to evaluate the existence of granule subpopulations by specific neuronal markers. The study was conducted on fragment of postmortem human cerebellar cortex by light microscopy immunohistochemistry. The markers examined were: the glutamate; the vesicular transporters of glutamate, V-GLUT-1 and V-GLUT-2; the neuropeptides, VIP and motilin; the calcium binding proteins, calbindin D28K and D9K.
The results of this study confirmed the glutamatergic nature of granules (positivity of their bodies to glutamate; positivity of their axon terminals to VGLUT-1), but also revealed granule subpopulations displaying positivity to motilin, to vasoactive intestinal polypeptide and to the calbindin D28K and D9K. In conclusion, this study demonstrates that the granules are a non-homogeneous neuronal population of the cerebellar cortex.

II SESSION
TROPHIC FACTORS, NEUROMEDIATORS AND RECEPTORS

A TROPHIC FACTOR EXTRACTED FROM RAT PERIPHERAL NERVOUS SYSTEM.

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Evidence is presented for the trophic role on muscle of some substances extracted from rat sciatic nerve. These substances were obtained by elution of soluble proteins of rat sciatic nerve on a DEAE Sephadex A-50 column. The acidic proteins were eluted in twelve peaks. The peak six was further separated and purified on acrylamide gel electrophoresis.

The results show that peak "six", with the highest protein content, consists of a homogeneous group of proteins. The proteins of peak six were injected daily in rat gastrocnemius muscle previously enervated by cutting of the sciatic nerve in the second day after birth. The macroscopic features demonstrate that the injected proteins restore the muscular atrophy induced by enervation.

Neurotrophic factors are small proteins that exert survival-promoting and trophic actions on neuronal and/or non neuronal cells . Neurotrophic factors regulate growth of neurons, associated metabolic functions such as protein synthesis, and the ability of the neuron to make the neurotransmitters that carry chemical signals which allow the neuron to communicate with other neurons or with other targets (muscles, glands, etc.). In addition to their physiological role, neurotrophic factors can be administered exogenously (i.e., pharmacologically) as pure proteins. Administered pharmaco-logically, neurotrophic factors can prevent cell death caused by various insults including nerve injury, brain trauma, local enervation and exposure to toxins. They can also prevent the cell death, metabolism, and functions of organs and/or tissue by its innervated.

ACTION OF NEUROTROPHIC FACTORS ON SOME ENZYMATIC ACTIVITIES OF DENERVATED MUSCLES

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The purpose of this study is to show the effects of some peripheral nerve sub-fractions on skeletal muscles after cutting of the afferent motor nerve. The nerve sub-fractions were obtained by elution of homogenates of vagal and hypoglossal rabbit nerves. Some fractions of these nerves were injected every day in rat gastrocnemius muscle previously denervated by the cutting of the sciatic nerve on the second day after birth.

Observations were made after 28 days of treatment. The gastrocnemius muscle was observed, weighted and photographed. Frozen cryostat sections of the muscle were stained with hematoxylin-eosin (HE) Lactate dehydrogenase (LDH) Succinate dehydrogenase (SDH) Adenosintriphosphatase (ATP) and Acetylcholinesterase (Ache).

The morphological results show that the cytoplasm is normally represented and the nuclear structures well preserved. The activities of LDH, SDH ATPase decrease in the atrophic muscles in comparison to normal muscles, while in treated muscles all these same enzymatic activities are about well preserved. The analysis of the ACHE activity in the treated muscles demonstrates numerous and well-conserved Ache positive elbow-shaped formations. The sub-neural apparatus also appears intact. Our results demonstrate that the nerve sub-fractions notably reduce the atrophy induced by the cutting of the motor nerve. Such factors carry out their own action thus contributing towards maintaining the structure of the motor end-plate complete, which otherwise degenerates in atrophic muscles.

ANDROGENS AND DEVELOPMENT OF THE BST PARVOCELLULAR VASOPRESSIN SYSTEM

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In rodents, the limbic arginine vasopressin (AVP) system is sexually dimorphic and sensitive to gonadal steroids. In the bed nucleus of the stria terminalis (BST), the medial amygdala (AMY) and the lateral septum (LS) the AVP immunoreactivity (AVP-ir), fibers and cells, is higher in male that in female. Androgen (AR) and estrogen receptors (ER) are localized in these nuclei. AVP expression is regulated by testosterone (T) in these nuclei: it decreases after gonadectomy and T treatment of castrated males restores the expression of AVP to a level comparable to intact ones.

To investigate the possible effects of the chronic lack of estrogens or androgens on this system, we used knock-out mice lacking a functional aromatase gene (ArKO) [determining a chronic lack of estradiol (E2) during the whole life], and *Tfm* rats, with a spontaneous mutation of the AR gene (they are insensible to androgens).

Comparing ArKO and wild type (WT) mice we demonstrated a significant decrease of the AVP positivity in LS, and AMY. This reduction could be due to a lack of both organizational or activational effects of estrogens. Therefore, we treated male ArKO and WT mice with E2 in adulthood. This treatment restored AVP-ir in the LS of ArKO males to levels of intact WT males, suggesting that the mouse AVP limbic system is influenced by activational effects of the estrogenic metabolite of T and that the differentiation of the AVP system may depend on androgens or sex chromosomes rather than estrogens. To better clarify the role of T aromatization during the development, we compared ArKO and WT mice after a treatment with E2 or only with oil, during the first ten days of postnatal life (critical period). The treatment with E2 of ArKO male does not stimulate a further differentiation of the AVP system in any of considered regions; whereas, in ArKO female it increases the AVP-ir in LS and BST. These results suggest that the development of AVP system in ArKO males is not sensitive to estrogens during the early postnatal period. On the contrary, in ArKO females the absence of E2 in the perinatal period reduces the differentiation of AVP system. In conclusion, these results suggest a crucial role for androgens in males and for estrogens in females for the differentiation of the limbic AVP system.

In Tfm rats, that are not exposed to T during the whole life, we detected a significant decrease of the immunoreactivity in BST and AMY of Tfm males in comparison to Wild-type males, hence confirming the important role played by androgens in the differentiation of AVP system.

The results obtained in these different models indicate that (at least in male rodents) androgens may have an important role to determine the differentiation of the limbic AVP system.

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BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) IN THE HUMAN BRAINSTEM FROM PRENATAL AGE TO ADULTHOOD

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Initially characterized for its ability to promote survival of peripheral sensory neuronal subpopulations during programmed cell death, the brain-derived neurotrophic factor (BDNF) is now known to affect the viability and maintenance of numerous peripheral and central neuronal groups in various animal species throughout life. However, data regarding the expression of BDNF in the human nervous system, and all the more those describing its changes with age, are limited. With the aim of gaining information relevant to the involvement of the trophin in health and plasticity of human brain neurons, we examined by immunohistochemistry the normal distribution and temporal profile of BDNF expression in the human brainstem nuclei from prenatal to adult life.
Western blot tests of antibody specificity and trophin immunodetectability in autoptic tissue, performed on human and rat brain homogenates, showed a single 28 kDa protein band, compatible with both the precursor and mature BDNF, up to 72 hours postmortem. In tissue sections, BDNF-like immunoreactive (LI) neuronal perikarya and nerve fibers and terminals occurred at all examined ages with uneven distribution. The density of immunoreactve elements and their staining intensity showed variable changes with age in different territories. BDNF labelled cell bodies were observed within sensory and motor nuclei of cranial nerves, dorsal column nuclei, olivary nuclear complex, peryhypoglossal nuclei, reticular formation nuclei, pontine nuclei, locus caeruleus, raphe nuclei, substantia nigra, red nucleus, and quadrigeminal plate. Codistribution of BDNF with the polysialylated-neural cell adhesion molecule (PSA-NCAM), a neuroplasticity marker also known to modulate the BDNF neuronal responsiveness, was observed. Neurochemical characterization of BDNF-LI neurons in the substantia nigra pars compacta revealed that about 70% of them were also labelled for tyrosine hydroxylase. BDNF-LI nerve fibres occurred within the gracile and cuneate fasciculi, trigeminal spinal tract, oculomotor nerve, solitary tract. The results obtained support the involvement of BDNF and/or its precursor in processes subserving cell development, connectivity, maintenance and plasticity of different neuronal systems in the human brainstem, and recommend the analysis of the relevant high and low affinity receptors to advance in the definition of the possible functional role and interplay of the different forms of the molecule.

NEURAL ANGIOGENESIS IS MEDIATED BY A CROSS-TALK BETWEEN NERVE GROWTH FACTOR AND VASCULAR ENDOTHELIAL GROWTH FACTOR IN THE BRAIN OF MDX DISTROPHYC MICE

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Increased angiogenesis and an altered blood-brain barrier have been reported in the brain of dystrophin-deficient mdx mouse, an experimental model of Duchenne muscular dystrophy. To further elucidate the mechanisms underlying angiogenesis in Duchenne muscular dystrophy, in this study we evaluated whether nerve growth factor (NGF) and NGF receptors (NGFRs) are involved, then correlated NGF-NGFRs expression with vascular endothelial growth factor (VEGF) and its receptor -2 (VEGFR-2) content and matrix metalloproteinases-2 and –9 (MMP-2 and-9) activity, by confocal laser microscopy and immunohistochemistry. Results showed that neurons, astrocytes and ependymal cells were strongly labelled by NGF in mdx brain, expressing NGFRs on glial and endothelial cells. In controls, NGF faintly labeled neurons and astrocytes, whereas endothelial cells were negative for NGFRs. Immunogold electron microscopy demonstrated NGFRs gold particles on endothelial cells in mdx brain, while in controls few particles were recognizable only on glial endfeet. Western blotting and real time polymerase chain reaction (RT-PCR) demonstrated a higher expression of NGF and NGFRs mRNA and protein in mdx brain as compared to controls, and increase of VEGF-VEGFR-2 and active MMP-2 and -9 content. Overall, these data suggest that in the brain of mdx mice, an up-regulation of the NGF-NGFRs system might be involved directly, or indirectly through the activation of VEGF-VEGFR-2 and MMP-2 and -9, in the angiogenic response taking place in this pathological condition.

CHOLINERGIC ENHANCING DRUGS AND CHOLINERGIC TRANSPORTERS IN AN ANIMAL MODEL OF VASCULAR BRAIN DISORDER

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Hypertension has been related to the development of brain damage and is the main risk factor for the occurrence of cerebrovascular disease. Abnormal regulation of cholinergic neurotransmission might contribute to the cognitive impairment associated with adult-onset dementia disorders including Alzheimer's disease (AD) and vascular dementia (VaD). Cholinergic transporters control cellular mechanisms of acetylcholine (ACh) synthesis and release at presynaptic terminals. The high-affinity choline uptake transporter (CHT) recaptures choline deriving from ACh hydrolysis by acetylcholinesterase (AChE). Choline is resynthesized into ACh by choline acetyltransferase. The neurotransmitter is loaded into synaptic vesicles by its vesicular transporter (VAChT). Enhancement of deficient cholinergic neurotransmission is an important therapeutic strategy to counter adult-onset dementia. Cholinesterase inhibitors (ChE-Is) are the first class of drugs licensed for symptomatic treatment of cognitive impairment of mild-moderate stages of AD.
This study has assessed the influence of a 4 week treatment with the AChE/ChE-I galantamine, with the cholinergic precursor choline alphoscerate (alpha-glyceryl-phosphorylcholine, GPC) or with galantamine plus GFC on brain and peripheral blood lymphocyte (PBL) cholinergic transporters in spontaneously hypertensive rats (SHR). SHR were used as a model of hypertensive brain damage. In these rats an obvious cholinergic hypofunction is noticeable and therefore they could represent a model for investigating the effect of drugs on cholinergic system. Wistar Kyoto (WKY) rats which belong from the normotensive phenotype opposed to SHR were investigated as well.
Analysis performed by immunochemistry and ELISA included frontal cortex, striatum, hippocampus and cerebellum. An increased expression of CHT and VAChT was observed in brain areas investigated and in PBL of SHR compared to WKY rats . This increase probably represents an up-regulation to counter cholinergic deficits of SHR. Treatment with galantamine countered the increase of CHT and VAChT. Treatment with GPC further increased CHT and to a greater extent VAChT. Treatment of SHR with galantamine plus GPC elicited effects greater than those noticeable with single drugs . Similar results were obtained in PBL.
The effect of GFC is consistent with an increased synthesis of ACh it induces. This suggest that association between GPC and AChE/ChE inhibitors is a strategy worthwhile of being investigated. The similar behaviour observed in brain and PBL suggests that these circulating elements may be considered as a marker of the expression of brain cholinergic transporters.

III SESSION
NEURODEGENERATION, NEUROREGENERATION AND PHARMACOLOGICAL THERAPY

α-LIPOIC ACID INDUCES UP AND DOWN MODULATION OF GFAP, VIMENTIN, NESTIN, CYCLIN D1 AND MAP-KINASE ESPRESSION IN ASTROGLIAL CELL CULTURES

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α-Lipoic acid (ALA) plays a pivotal role as antioxidant and metabolic component of some enzymatic complexes involved in glucose metabolism of different cell types.
In the present investigation we studied the effect of dextrorotatory (+)-enantiomer or raceme ALA on the expression of some proliferation and differentiation markers of astroglial cells in primary cultures.
The expression of GFAP, vimentin, nestin, cyclin D1 as well as of MAP-kinase, a signalling transduction pathway biomarker, was assessed by Western blot or ELISA analysis in 15 DIV astrocyte cultures treated chronically or acutely with 100 μM ALA after pre-treatment with 0.5 mM glutamate for 24h.
GFAP expression significantly increased after (R+)-ALA acute-treatment in glutamate-pre-treated cultures.(+)-ALA acute-treatment increased vimentin expression, but decreased it after raceme acute treatment. Nestin expression drastically increased after acute raceme-treatment in glutamate-pre-treated or non pre-treated cultures, but significantly decreased after (+)ALA acute and chronic-treatments. Cyclin D1 expression augmented in raceme and (+)ALA acutely-treated astrocyte cultures pre-treated or non pre-treated with glutamate. MAP-kinase expression increased after (+)ALA acute treatment in glutamate-pre-treated or non pre-treated ones. GFAP immunostaining analysis is consistent with Western blot data.
Our results indicate a significant increased of GFAP expression as well as an "up and down" modulation of nestin and vimentin expression in 15 DIV astrocyte cultures after chronic or acute treatment with raceme or (+)ALA. These preliminary findings may represent a first step to better clarify the antioxidant and metabolic role played by ALA in proliferating and differentiating astroglial cell cultures during an interactive cross-talk between glial and neuronal cells, after brain lesions or damages.

THE TOTAL NUMBER OF DORSAL ROOT GANGLION NEURONS INCREASES OVER 40% AFTER A CRUSH LESION OF PERIPHERAL NERVES IN ADULT RATS

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Many experimental works have suggested that new-generated neurons could be added to the DRG (dorsal root ganglia) postnatally. Some authors have pointed out the possible existence of neurogenesis in adult DRGs, others have suggested that these new neurons could instead originate from the maturation or growth of preexisting immature cells. However adult DRG neurogenesis has not been confirmed *in vivo*.

In the present study, we investigated, using different experimental approaches, the effect of a nerve crush lesion on DRGs sensory neurons in adult rat. The crush injury was applied to the median, ulnar and radial nerves at their point of origin from the brachial plexus using a non-serrated clamp that could guarantee standardized and reproducible method. Animals were then sacrificed at 1, 5, 10 and 30 days after the injury and DRG corresponding only to the level C5-T1, that give rise to the fibers of the radial, ulnar and median nerves, were extracted.

Our morphological analysis in optical and electron microscopy reveals an unusual number of small size cells morphologically different from the glial satellite cells. FACS analysis performed at 1, 5, 30 days post-damage confirmed that there is no significant loss of sensory neurons.

Neurogenesis was then investigated by injecting rats with bromodeoxyuridine (BrDU). Immunohistochemical analysis in laser confocal revealed a relevant number of cells BrDU-positive. Most of the BrDU positive cells belong to the glial family although, some BrDU colocalized with neuronal markers suggesting that neurogenesis occurs in adult DRGs neurons that undergo peripheral nerve injury. These data are supported by evidence of neuronal progenitor's markers as nestin, Sox-2 expressed within the crushed DRGs 1 and 5 days after damage.

Finally, a stereological analysis, using the physical dissector method, showed a significant increase in number (42%) of DRGs sensory neurons 1 month after nerve-crush injury compared to control. All together our data support the idea that the population of DRG's neurons increased as a consequence of the nerve damage. Evidence of morphological changes in the population of cells surrounding neurons and the immunopositivity for neuronal progenitor markers, suggested the hypothesis that the increased number of neurons is due to undifferentiated precursors localized within the adult DRG.

GENE TRANSFER FOR PROMOTING NERVE REGENERATION

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Tissue engineering of peripheral nerves has seen an increasing interest over the last years and, similarly to many other fields of regenerative medicine, great expectations have risen within the general public to its potential clinical application in the treatment of damaged nerves. The biotechnological progress that makes it possible to induce therapeutic changes through gene transfer represents one of the pillars of tissue engineering and has engendered much excitement opening great perspective in many disciplines of biomedicine including improvement nerve regeneration. Gene therapy may contribute to stimulate regeneration of the peripheral nerve by locally supplying several neurotrophic factors the efficacy of which in case of exogenous application, is limited because of their fast degradation. We have recently focused our attention on viral vectors based on the adeno-associated virus (AAV), a nonpathogenic and widespread parvovirus, incapable of autonomous replication and able to transduce both dividing and nondividing cells and show a specific tropism for postmitotic cells. Because these vectors do not contain any viral genes—which are transiently transfected in trans for the packaging process—they elicit virtually no inflammatory or immune response. As a consequence, transgene expression from these vectors persists for several months in a variety of animal tissues in vivo. Vectors based on adeno-associated virus (AAV) have recently been used in phase-I clinical trials for the treatment of neurological disorders. Indeed, AAV-mediated gene transfer is a promising tool for the delivery of therapeutic gene into the central and peripheral nervous systems. In this presentation, we will address the potentiality of gene transfer for promoting nerve regeneration describing, in particular, the possibility to take advantage of the high effectiveness of skeletal muscle infection by AAVs for transferring genes along regenerating nerves by creating muscle-vein-combined scaffolds previously potentiated by AAV gene transfer.

MORPHOLOGICAL EVIDENCE THAT MPTP-INDUCED PARKINSONISM AFFECTS DOPAMINERGIC NEURONS IN MOUSE ENTERIC NERVOUS SYSTEM

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Parkinson's disease (PD) is a neurodegenerative pathology which affects dopaminergic neurons of the substantia nigra, leading to a movement disorder. Non motor symptoms commonly involve the gut. In the present study we tried to reproduce digestive dysfunctions by using the parkinsonism-inducing neurotoxin 1-methyl, 4-phenyl, 1,2,3,6,-tetrahydropyridine (MPTP) in 9-week old C57BL mice. One week after treatment with MPTP (i.p. 20 mg/kg x3, 2 h apart) we analyzed morphological changes on the nervous network of the gut: immunostaining for tyrosine hydroxylase (TH), dopamine transporter (DAT) and noradrenaline transporter (NET); fluorescent immunostaining for alpha-synuclein; while monoamine level were measured by HPLC-ED. Furthermore, behavioural and functional tests, including food consumption, stool collection and colonic transit assay, have been performed. In controls, TH immunopositivity was well evident in both myenteric and submucous plexuses, appearing as continuous markedly stained rings. From the submucous plexus nervous fibres and neurons extended to the mucosa up to the axes of the villi. DAT and NET immunopositivity was also well evident as stained rings. In MPTP-treated mice, both TH and DAT, but not NET, immunopositive neurons were reduced in both plexuses and the continuous ring-like staining was no longer evident. Moreover, alpha-synuclein fluorescence was revealed in the submucous plexus. Consistently, while noradrenaline levels were unchanged, there was a severe dopamine depletion. Finally, these morphological and biochemical features were accompanied by a functional impairment with delayed colonic transit and reduced stool weight which was reminiscent of constipation occurring in PD. Our data provide a novel and reliable model to study the altered digestive function in PD, while offer the basis to interpret the digestive dysfunction in PD as a consequence of a selective dopaminergic impairment of the gut.

MONITORING AUTOPHAGY BY ELECTRON MICROSCOPY IN A MODEL OF NEURODEGENERATION: TOWARDS THE MORPHOLOGICAL DEFINITION OF THE PHAGOPHORE

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Autophagy degrades intracellular components within eukaryotic cells by clearing long-lived proteins and organelles (mitochondria and endoplasmic reticulum). Autophagy is active under normal cell functioning, and it is believed to increases the neuron's ability to cope with a variety of stressors. The downregulation or partial inhibition of autophagy sometimes provokes or aggravates neurodegeneration. Despite autophagy is composed of several steps which are well established, the earliest step, consisting of the phagophore formation, remains non-defined. This is mainly due to the paucity of morphological studies using electron microscopy. In fact, a variety of methods including proteomics and confocal microscopy were used to study autophagy but electron microscopy remains the most accurate methods for the detection of autophagy. Electron microscopy allows the identification of the fine structure of autophagic compartments leading eventually to solve the early events as phagophore formation.
By profiting of a strong autophagy inducer (Methamphetamine) in the present study we enhanced the early step of autophagy to blow up the phagophore formation.
The data obtained suggests that the nucleation of the membranes originating from the endoplasmic reticulum is pivotal for the formation of the 'phagophore'.

METHAMPHETAMINE PROMOTES THE EXPRESSION AND AGGREGATION OF THE PRION PROTEIN PRPSC *IN VIVO* IN DIFFERENT BRAIN AREAS

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The cellular prion protein (PrPc) is physiologically expressed within selected brain areas. Alterations of the secondary structure of this protein leads to the scrapie-like prion protein (PrPsc), which is a misfolded PrPc protein owing the same primary structure, which precipitates in the cell. In fact, when high amount of PrPc occurs, this protein cannot be metabolized and undergoes alteration of the secondary structure, thus leading to detectable amount of PrPsc. Prion protein metabolism is partly dependent on the activity of protein clearing systems as witnessed by the deleterious effects of proteasome and autophagy inhibitors in the clearance of PrPsc. So far, the presence of PrPsc has been detected in infectious, inherited or sporadic neurodegenerative disorders; however, the ability of METH to increase oxidative stress, altering UP and autophagy systems, and misfolding cell proteins led us to explore the potential consequence of METH administration on the expression of PrPc and PrPsc. We explored a variety of brain areas known to be the preferentially targeted by METH to detect the presence of PrPc by using SDS PAGE immunoblotting, immunohistochemistry and immunoelectromicroscopy. We found that METH administration consistently increases PRPc amd PrPsc *in vivo* and *in vitro* as detected by a variety of antibodies directed against PrP with or withouth proteinase K. The present findings, while fostering novel molecular mechanisms which can lead to prion disorders, also show that infectious, sporadic and inherited degenerative conditions can be mimicked at subcelllular level by a drug of abuse.

TRANSPLANTATION OF MESENCHYMAL STEM CELLS INTO RAT PELVIC MUSCLES

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Bladder incontinence represents a major problem for urologic patients of both sexes, due to iatrogenic sphinteric lesions in males and to pelvic floor weakness in females. Stem cell transplantation has been suggested as therapy to prevent incontinence, aimed not only to bulking effect but also to strengthen the urethral external sphincter and the other muscles of the pelvic floor. Therefore, several different types of stem cells are currently under investigation. Here, we decided to use human mesenchymal stem cells (hMSCs) due to several advantages on other cell types: first of all, they have been shown to be able to differenziate into muscle cells; then, they can be collected from the same patient needing transplantation and, in any case, they have immunomodulatory properties, i.e. they rarely induce host reaction. hMSCs were collected from human donors, following informed consent, and were expanded in vitro. They were prelabeled in culture with bisbenzimide overnight before transplantation. We transplanted hMSCs into the external sphincter and the ischiocavernosus muscles of Sprague-Dawley rats of both sexes. At different time intervals (briefly: one day, one month, four months) from transplantation rats were sacrified and perfused with fixative, and sections of the urethra and pelvic floor were cut on the cryostat. Single sections were examined at the fluorescence microscope to detect transplanted cells, and immunoreacted with a battery of antibodies to detect their differentiation in different cell types. In order to study double staining (bisbenzimide and antibodies) we used a Leica Confocal Scanning Laser Microscope. We succeeded to observe large numbers of bisbenzimide-positive cells in the muscles and surrounding the urethra, thus suggesting a very good survival of stem cell-derived elements, even at longer survival times. Moreover, we could find bisbenzimide-positive cells labeled with muscle-specific antibodies, thus suggesting a differentiation of MSCs into the muscular lineage. Therefore, MSCs can represent an efficent therapy for bladder incontinence and bone marrow a good reservoir of stem cells for the patient.

CHOLINE-CONTAINING PHOSPHOLIPIDS ACTIVITY ON BRAIN NEUROTRANSMITTER TRANSPORTERS: FURTHER INSIGHTS IN THE MECHANISM OF ACTION OF OLD, SAFE AND EFFECTIVE DRUGS

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Phospholipids are important components of all mammalian cells with a variety of biological functions: (1) they form lipid bilayers that provide structural integrity necessary
for protein function, (2) they function as an energy reservoir (eg, triglycerides), and (3) they serve as precursors for various second messengers such as arachidonic acid, docosahexaenoic acid, ceramide, 1,2-diacylglycerol, phosphatidic acid, and lyso-phosphatidic acid.

CDP-choline (cytidine-5'-diphosphocholine, CDP) and choline alphoscerate (L-alpha-glyceryl-phosphorylcholine, GFC) are phospholipids and phospholipid derivatives respectively used in clinical practice for treating sequelae of cerebrovascular accidents and cognitive disorders. They affect biosynthesis of phospholipids, brain metabolism and neurotransmitter systems.

The influence of i.p. treatment for 7 days with choline-equivalent dose (0,67 mmol/Kg/day of choline) of CDP and GFC was investigated in rat brain. Dopamine (DA), serotonin (5-HT) and acetylcholine (ACh) levels and DA plasma membrane transporter (DAT), vesicular monoamine transporters (VMAT-2), 5-HT transporter (SERT), choline transporter (CHT) and vesicular ACh transporter (VAChT) were assayed in frontal cortex, hippocampus and striatum by HPLC with electrochemical detection, Western blot and ELISA.

CDP did not affect DA levels, which were increased by GFC in frontal cortex as well as 5-HT in frontal cortex and striatum. GFC treatment increased ACh concentrations in frontal cortex and hippocampus. DAT was stimulated in frontal cortex by both CDP and GFC, whereas VMAT1, VMAT2 and 5-HT-T were unaffected. CHT was decreased by CDP and GFC in frontal cortex and hippocampus. In frontal cortex VAChT was increased after treatment with GFC while treatment of CDP increase the VAChT expression in the striatum.

These results suggest that CDP and GPC affect to a different extent DA, 5-HT and ACh levels and transporter systems. The activity of the compounds on parameters investigated may account for the more favourable cognitive and mood profile observed in clinical studies with GPC compared to CDP. Moreover, these data suggest that choline-containing phospholipids besides to their role on brain cholinergic system possess monoaminergic activities the relevance of which should be further investigated.

IV SESSION
ENTERIC AND PERIPHERAL NERVOUS SYSTEM

CYCLOOXYGENASE ISOFORMS IN NEUROMUSCULAR COMPARTMENT OF HUMAN COLON: EXPRESSION AND FUNCTION IN NORMAL COLON AND DIVERTICULAR DISEASE

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Background. A large body of evidence supports that prostanoids, generated by cyclooxygenase isoforms (COX-1, COX-2), contribute to the control of intestinal motor functions and to intestinal motor alterations in functional/inflammatory gut dysmotility. Despite this, the neuromuscular distribution pattern of these enzymes and the role played by COX pathways in human intestinal motility disorders are unknown.

Aim. To examine the localization and function of COX-1 and COX-2 in colonic neuromuscular tissues dissected from patients with diverticular disease (DD), a common intestinal disorder characterized by abnormal bowel motility.

Patients and Methods. Full thickness colonic samples were collected from 8 patients with a diagnosis of DD, undergoing elective left hemicolectomy. Morphological and immunohistochemical studies were carried out on paraffin cross-sections: the neuromuscular compartment, with particular regard to myenteric ganglia, was examined for HuC/D positive neurons and S100β positive glial cells, as well as COX-1 and COX-2 immunoreactive cells. Functional studies were executed on longitudinal muscle strips in organ bath and the effects of COX inhibitors on excitatory neuromuscular activity were studied. Data from DD patients were compared with findings on normal colonic wall from 8 healthy controls undergone to left colon resection for uncomplicated colorectal neoplasia.

Results and Conclusions. The myenteric glial cell/neuron ratio of all DD patients was in the range of control values, but COX expression in neuromuscular tissues was not. In fact, the normal pattern of COX-1 expression in myenteric neurons and COX-2 in non neuronal cells and longitudinal muscle was altered in DD patients. In all pathological samples a pronounced reduction of both the immunoreactive isoforms was observed within the myenteric cell population but the COX-2 neuromuscular levels were maintained. Preliminary functional data have demonstrated a loss of modulation by both COX isoforms on cholinergic excitatory pathways obtained in colonic muscle strips isolated from DD patients. These findings provide the first evidence that the normal pattern of COX distribution is significantly altered in myenteric ganglia of DD patients. All together the immunohistochemical and functional results may account for the colonic dysmotilty responsible for painful abdominal symptoms in patients with DD.

MECHANOSENSITIVITY IN THE ENTERIC NERVOUS SYSTEM

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The enteric nervous system (ENS) contains sensory neurons that enable it to function in isolation. Mechanotransduction mechanism remains vastly unknown despite the evidence that ENS neurons appear to respond to sustained distension. Up to now, the most common theory postulate mechanosensitivity as a property of specialized intrinsic primary afferent neurons (IPANs). This concept has been challenged from studies revealing mechanosensitivity in a different class of neurons and outlining a possible multifunctional role of neurons in the ENS. We aimed our research to further investigate in the ENS the existence and the properties of the neurons responding to mechanical stimuli that mimic contractile activity rather than using sustained stretch. Using a fast Neuroimaging technique based on a voltage sensitive dye we identified mechanosensitive myenteric neurons which fired action potentials in response to ganglion deformation. Experiments were performed in guinea pig and mouse fresh preparation from myenteric plexus ileum and in organotypic culture of ileum segments with DiI retrograde tracing. Two techniques were applied to deform ganglia and neurons: von Frey hairs and intraganglionic injections of small volumes of Krebs solution. Both stimulation techniques, in both species, revealed similar results: around 25% of all neurons responded. The discharge pattern suggests that mechanosensitive neurons behave like rapidly adapting mechanosensors that respond to dynamic changes. We therefore suggest referring to these neurons as Rapidly Adapting Mechanosensitive Enteric Neurons (RAMEN). Deformation evoked spike discharge is not changed by synaptic blockade with hexamethonium or ω-conotoxin, defunctionalisation of extrinsic afferents with capsaicin or muscle paralysis with nifedipine, suggesting direct activation of RAMEN. We could show that RAMEN differ from IPANs for neurochemical code and electrophysiological properties.
This work provides strong evidences that mechanosensitivity is a feature of different classes of enteric neurons supporting the concept of multifunctional mechanosensitive neurons which may fulfill sensory, integrative and motor functions. Gut diseases associated with sensorimotor dysfunction are daily bread for pathologists, thus it appears of crucial importance to understand mechanosensitive pathways in the ENS.

A MORPHOLOGICAL ANALYSIS OF HORSE (*Equus caballus*) SPINAL GANGLION NEURONS.

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Horse spinal ganglion (SG) neurons were morphometrically and neurochemically characterized, and dark and light SG neurons sized. The statistical analysis of relative histograms confirmed the presence of two widely accepted categories, i.e., small and large neurons. The phenotypical characterization was carried out by four well known neuronal markers: calcitonin gene-related peptide (CGRP), substance P (SP), neuronal nitric oxide synthase (nNOS) and isolectin B4 (IB4) from *Griffonia simplicifolia*. All four possible triple staining combinations were performed, with the following results: (1) most SG neurons were triple-stained; (2) SP-IR SG neurons showed the largest percentages of co-localization with the other markers studied; (3) CGRP-IR and IB4-labeled SG neurons showed the largest individual percentages stained, suggesting their involvement in different functions compared to the other cytotypes identified; (4) nNOS-IR neurons were more represented than in small rodents--consistently with data obtained on sheep. It is plausible to hypothesize different functions for sensory nitrergic neurons of small and large mammals; (5) IB4 was widely co-localized with both CGRP and SP, showing that IB4 cannot be employed to differentiate between peptidergic and non-peptidergic horse SG neurons.

Tracer studies performed injecting Fast Blue (FB) tracer in the ileo-cecal junction, combined with neurochemical studies, confirmed the wide involvement of CGRP and SP in visceral sensation, and showed that in horse, similarly to sheep and contrarily to small rodents, IB4 SG neurons are widely involved in visceral innervations.

TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE-1 (TRPV1) IN THE HUMAN TRIGEMINAL GANGLION AND SPINAL NUCLEUS

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The transient receptor potential vanilloid type-1 (TRPV1 or VR1) is expressed by sensory neurons and activated by capsaicin, heat, acidic and basic deviations from homeostatic pH, and inflammatory mediators, with depolarisation leading to burning pain. TRPV1 activation also leads to release of sensory neuropeptides calcitonin gene-related peptide (CGRP) and substance P (SP) which, in turn, activate their effector cell receptors and contribute to the process of neurogenic inflammation and sensitization of nociceptors. Data in the literature report on the occurrence of TRPV1 expressing primary sensory neurons in several animal species. However considerable discrepancy exists among different studies and data on human tissue are mostly on spinal ganglion neurons. In view of the possible involvement of TRPV1 in painful neuropathies such as mouth burning syndrome, headache and deep-tissue craniofacial pain disorders, we undertook the analysis of the occurrence of TRPV1 in the trigeminal sensory system and here relate on the localization of TRPV1 immunoreactivity in the human trigeminal ganglion and spinal nucleus.

Autoptic specimens from pre- and full-term newborns and adult subjects were examined.

Several commercially available antibodies against TRPV1 were tested by western blot, immunofluorescence and avidine-biotin-peroxidase complex (ABC) immunohistochemistry in human and rat tissue. Among them, only one, which in human brain homogenates western blot test revealed a single band slightly below 83 kDa, yelded a reliable immunostaining in human specimens.

In the trigeminal ganglion, with higher frequency in the newborn than in adult specimens, a subpopulation of predominantly small- to medium-sized neurons showed a distinct immunostaining in the perikaryon, sometimes extending to the proximal process; immunostained fiber tracts run isolated or in thin bundles in between the neurons and in fascicles across the ganglion. Coexistence with the most abundantly occurring neuropeptide CGRP was observed sporadically. Centrally, TRPV1-like immunoreactive material labelled extensive fiber tracts and punctate terminal-like elements distributed in the spinal tract and in lamina I, inner lamina II and deep magnocellular part of the spinal trigeminal nucleus, where it was codistributed with SP and CGRP. Neuronal cell bodies with peripheral membrane-like immunostaining were also detectable in the superficial layers of the nucleus. The results obtained will be discussed and compared to the available data in literature.

PERIPHERAL MOTOR ACTION OF GLUCAGON LIKE PEPTIDE-1 THROUGH ENTERIC NEURONAL RECEPTORS

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Background: Glucagon-like-peptide-1 (GLP-1) is a proglucagon-derived peptide expressed in the enteroendocrine-L cells of small and large intestine and released in response to meal ingestion. GLP-1 has been reported to exert inhibitory effects on gastrointestinal motility through vagal afferents and central nervous mechanisms, whereas no data are available about a direct influence on gastrointestinal smooth muscle cells. The present aims were to investigate the effects of GLP-1 on the spontaneous and evoked mechanical activity of mouse duodenum and colon and to identify the presence and distribution of its receptor (GLP-1R) in the muscle coat.
Methods: Organ bath technique and immunohistochemistry were used.
Results: GLP-1 failed to affect spontaneous mechanical activity. It caused in circular smooth muscle of both intestinal segments a concentration-dependent reduction of the electrically-evoked cholinergic contractions, without affecting the longitudinal muscle responses. The GLP-1 inhibitory effect was significantly antagonized by exendin (9-39), an antagonist of GLP-1R. In both intestinal preparations, GLP-1 effect was not affected by guanethidine, a blocker of adrenergic neurotransmission, but it was significantly reduced by N⌉-nitro-L-arginine methyl ester, inhibitor of nitric oxide (NO)-synthase. GLP-1 failed to affect the contractions evoked by exogenous carbachol in both segments. Immunohistochemistry demonstrated GLP-1R expression in the enteric neurons, some of which co-expressing neuronal NO synthase (nNOS) or acetylcholine transferase (ChAT). Furthermore, some nNOS-IR neurons appeared surrounded by GLP-1R-IR nerve varicosities.
Discussion and Conclusion: The present *in vitro* study demonstrates, for the first time, that activation of GLP-1 peripheral receptors can exert an inhibitory effect on motility of small and large intestine, independently by the neural extrinsic control. Furthermore, by immunohistochemistry, we presently show that GLP-1R is expressed by the enteric neurons, either in duodenum or colon, some of these neurons co-expressed nNOS and ChAT, some nNOS neurons received GLP1-IR varicosities. Taken together, immunohistochemical and physiological data suggest that GLP-1 is able to modulate negatively the excitatory cholinergic neurotransmission in mouse duodenal and proximal colonic circular muscle, by acting on specific prejunctional GLP-1R, and this inhibitory effect seems to be mediated by a nitrergic pathway.

MODIFICATIONS TO α–TRANSDUCIN AND NEUROPEPTIDE IMMUNOREACTIVITIES EVOKED BY FEEDING IN THE PIG GI TRACT

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Taste receptors (TR1s, TR2s) for sweet and bitter along with related G-proteins (α-transducin and α-gustducin) have been localized in the GI tract mucosa of several mammals. Taste signalling molecules are now conceived to be part of a wide chemosensing system contributing to sense luminal contents. In the pig model (resembling the human gut) we established whether feeding changes, i.e. fasting and re-feeding, were able to affect: 1) the expression of α-transducin throughout the GI tract; 2) neuropeptide coding of the cells expressing α-transducin; and 3) the spatial relationship between α-transducin cells and nerves supplying the gut. Pig stomach-to-rectum specimens (n= 12; 45 days of age), subdivided in 3 groups, i.e. control (C), fasted for 24h (F) and re-fed (R), were fixed in 4%-paraformaldehyde, embedded either in paraffin and processed for single and double labelling immunofluorescence with antibodies to: α-transducin, chromogranin-A (CgA), gastrin/cholecystokinin (Gas/CCK), somatostatin (SOM) and PGP9.5. The highest density of α-transducin-immunoreactive (IR) cells has been shown in the pylorus (C: 191±22, F: 99±28 and R: 112±51) and in the cardial mucosa (C: 79±46, F: 29±13 and R: 57±10), whereas in the intestine the highest α-transducin cell density was observed in the duodenum of C (54±22) vs. F and R (41±20, 14±6, respectively). The density of α-transducin-IR cells decreased along the large intestine. The percentage of α-transducin/CgA-IR cells was reduced in the GI tract of F and R vs. C. In the jejunum, α-transducin/CCK cells decreased in F and R vs. C. Gas/α-transducin and SOM/α-transducin colocalizations have never been detected. 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 α-transducin cells. In conclusion, changes to α-transducin expression in neuropeptide-containing cells following F and R provide a basis to the concept that these cells participate to luminal chemosensing in the GI tract. The identification of chemosensory molecule plasticity in response to fasting and nutrients in specialized GI cells could open novel therapeutic strategies in feeding behaviour or metabolic disorders.

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V SESSION
ANIMAL MODELS
OF NEUROPATHOLOGIES

ALTERATIONS OF THE SUPRACHIASMATIC NUCLEUS IN A RODENT MODEL OF THE HUMAN PATHOGENIC PARASITE *TRYPANOSOMA BRUCEI* INFECTION

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The parasite *Trypanosoma brucei (Tb)* is the causative agent of human African trypanosomiasis (HAT) or sleeping sickness, and the subspecies *Tb gambiense (Tbg)* is currently responsible for most of the reported cases. *Tb brucei* (*Tbb*), a non-human pathogenic parasite subspecies, has been widely used, for safety reasons, in experimental rodent models of the disease. *Tbg* infection leads in humans to a complex neuropsychiatric syndrome, hallmarked by disturbances of the sleep-wake cycle and sleep structure, which are in part replicated in the rat model of *Tbb* infection. Sleep-wake cycle disruption during HAT has recalled attention on the hypothalamic suprachiasmatic nucleus (SCN), which plays a role of master circadian pacemaker in the mammalian brain. Structural neuronal changes have not been, however, up to now documented in the SCN of *Tbb*-infected laboratory rats, in which gene expression changes, glial activation, and functional alterations have instead been reported.

We here examined the SCN after *Tbg* ip inoculation in *Mastomys* rats, which represent an animal species susceptible to this infection. The results were compared with those observed in *Mastomys* rats infected with *Tbb* and with non-infected respective controls. Qualitative and quantitative analyses were pursued at 4 and 8 weeks post-infection, when trypanosomes of both subspecies were numerous in the brain parenchyma. Glial fibrillary acidic protein (GFAP) immunoreactivity, as marker of astrocytes, was significantly increased in the SCN, especially in its ventrolateral portion, of *Tbg*-infected animals with respect to controls. GFAP immunoreactivity was also significantly increased in the SCN of *Tbb*-infected rats at 4 weeks post-infection compared with controls. Interestingly, the number of SCN neurons did not show significant changes in the *Tbb*-infected animals, whereas it was significantly decreased in *Tbg*-infected ones at 8 weeks post-infection. Neuronal loss was not evident in other brain structures (e.g. the hippocampus) of the same animals. Immunostaining of two main SCN neuropeptides, arginine-vasopressin and vasoactive intestinal polypeptide, was downregulated in both *Tbg*- and *Tbb*-infected animals, and such decrease was significantly enhanced in the *Tbg*-infected ones. The findings indicate that, during African trypanosome infection, the degree of astroglial activation, neuronal loss and neuropeptide expression in the SCN is dependent from the parasite subspecies and is especially marked after infection with human pathogenic parasites. The data thus provide novel evidence that African trypanosomes may target selectively the brain biological clock.

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GRAFT OF EMBRYONIC AND ADULT STEM CELLS IN AN EXPERIMENTAL MODEL OF SPINAL CORD HEMISECTION

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Spinal cord injury (SCI) can determinate neurological deficits below the injury site, producing a functional damage to local neurons and axons fibres, and many secondary events (glial activation, inflammation, oxidative stress, glial scar formation, cell death). These disorders are difficult to manage and contribute to poor quality of patient's life.
Serotonergic raphespinal projections promote functional recovery after SCI, but spontaneous regeneration of most severed axons is limited by the glial cyst and scar that form at the lesion site.
In the present study we examined whether stem cell transplantation could offer a promising approach for inducing regeneration through the damaged area, comparing the effects of transplantation of embryonic neural precursors (NPs) and adult mesenchymal stem cells (MSCs).
Spinal cord hemisection was performed at the L2 neuromer in adult mice. Two weeks post-injury, we transplanted NPs or MSCs into the cord, caudal to the hemisection site (L3 neuromer). Injured mice without a graft served as controls. In order to value the functional recovery, mice underwent a battery of motor tasks (Basso Mouse Scale, posture, grip test, foot-fault test and hindlimb flexion).
Twenty-eight days after transplantation, animals were sacrificed and analyzed for survival of grafted cells, for effects of engraftment on the local cellular environment and for the sprouting of serotonergic axons.
Both types of stem cells survived several weeks and were integrated into the injured host spinal cord; moreover NPs were able to express neuronal markers (5-HT, MAP-2 and NeuN), but were negative for the astrocyte marker GFAP. All transplanted animals displayed an increased number of 5-HT-positive fibres caudal to the hemisection, compared to untreated mice. Finally stem cell transplantation near the lesion site significantly improved functional recovery in animals with SCI.
These results point to a therapeutic potential for such cell grafting: both cell types probably could deliver trophic and immunomodulatory factors in proximity of lesion site, inducing axonal regeneration.
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CLINICAL AND PATHOLOGICAL EFFICACY OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS IN THE EXPERIMENTAL MODEL OF AMYOTROPHIC LATERAL SCLEROSIS.

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Amyotrophic lateral sclerosis (ALS) is a degenerative disease of the central nervous system, in which motoneurons are selectively affected. Most cases are sporadic, whereas mutations of the enzyme SOD1 accounted for the familiar forms.

Recently several studies investigated the migratory, immune-regulatory properties as well as the neural differentiation potential of mesenchymal stem cells (MSCs). These cells can be isolated from different tissues: the bone marrow (BM-MSCs) and the adipose tissue (ASCs) are the most frequent sources. The results so far obtained are encouraging and promising for their future therapeutic applications in inflammatory and degenerative nervous diseases.

The aim of this study is to evaluate the efficacy of the administration of ASCs in an experimental model of ALS (SOD1 G93A). Mice treated with ASCs and a control group were clinically tested during disease and their spinal cord histo-pathologically analyzed at sacrifice.

The preliminary results showed a significant improvement of motor performances of treated mice as compared to controls, whereas we did not observe any increase in survival. Histologically we observed a significant reduction in the microglial and astrocytic reaction in the spinal cord. By using ASCs expressing GFP, we found that these cells were able to penetrate into spinal cord of SOD mice; by double immunohistochemistry with neural phenotypic markers, we found no evidence of neural differentiation of ASCs present in the central nervous system. However, these cells in culture produced detectable levels by ELISA technique of the neurotrophins bFGF, BDNF and PDGF.

These encouraging data represent a starting point for the clinical application of new treatment protocols for ALS.

NEURONAL DEATH FOLLOWING SEIZURE ACTIVITY: MOLECULAR MECHANISMS AND PREVENTION

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Systemic injections of kainic acid (KA) induce epileptic seizures with delayed neuronal damage in the limbic system, especially in the hippocampus. KA excitotoxicity activates complex signal transduction events that trigger apoptotic cell death. The c-Jun N-terminal kinase (JNK) pathway plays an important role in cell death, and the peptide DJNKI1, a competitive JNK inhibitor, represents a potent neuroprotectant.To analyze the role of JNK in excitotoxic neuronal death in epilepsy, seizures were induced on adult male Sprague-Dawley rats by i.p. injection of KA with or without DJNKI1 i.p. administration, 2h after KA treatment. KA caused massive cell death in the hippocampus: stereological counts showed a significant decrease in neuronal density in all CA fields, both at 1 and 5d after seizures, which was partially prevented by DJNKI1 treatment (up to 53% of neuronal survival). No signs of oedema were detected by 3D reconstruction of hippocampal volume using the Neurolucida Software. Excitotoxic neuronal cell death was accompanied by glial cell activation, particularly in and around the regions encompassing dying neurons. Quantitative analysis of GFAP immunostaining was performed using the Scion Image Software and marked gliosis was seen 5d after KA. Although this process was slightly decreased in DJNKI1 treated animals, no significant differences were observed between the groups. A marked neuronal phospho-c-Jun labeling, as target of JNK, could be observed starting from 3h after KA. DJNKI1 treatment also reduced the positivity for P-c-Jun in the hippocampus. Analysis of purified mitochondria revealed that DJNKI1completely reversed cytochrome c release and the kainate-induced alteration of mitochondrial JNK translocation and activation.

Therefore, JNK is a key molecule in epilepsy-induced neuronal cell death and represents a promising target for pharmacological prevention of seizure-induced cell death, which can be achieved by DJNKI1 administration.

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EARLY SUBPIAL DEMYELINATION AND OLIGODENDROCYTE LINEAGE CELL RESPONSE IN CHRONIC EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Multiple Sclerosis (MS) is a chronic inflammatory disease of CNS characterized by multifocal destruction of myelin and loss of axons and oligodendrocytes. Experimental autoimmune encephalomyelitis (EAE), an animal model of MS, has been extensively used to study the MS pathogenesis and treatment options. Although MS and EAE are regarded as white matter (WM) diseases, grey matter (GM) demyelination was also described in MS and EAE models. The study focused on the responses of endogenous glial progenitors, oligodendrocyte precursor cells, pre-oligodendrocytes and immature oligodendrocytes to early demyelination in myelin oligodendrocyte glycoprotein (MOG_{35-55}) induced chronic EAE. The study was performed by immunohistochemistry and confocal microscopy in adult mouse cerebral cortex, 20 days after MOG immunization, one week after onset of tail weakness, and 6-10 days before evidence of severe clinical symptoms. Anti-mature oligodendrocyte marker antibodies (MOG, MBP) were used to detect areas of brain demyelination, and anti- A2B5, NG2, O4 and CNPase antibodies were used to identify growing/differentiating cells of the oligodendrocyte lineage. At the disease early stage, when plaques of demyelination were undetectable in brain WM, diffuse demyelination appeared in the GM, especially in the cortex layer I. Like MS intracortical lesions, EAE cortical demyelination was not associated with leukocyte dense infiltration. The response to the cortex demyelination was represented by increment in number and immunoreactivity of all the developing cells of the oligodendrocyte lineage. The results suggest that subpial demyelination is an early event during EAE course, that probably precedes development of typical WM demyelinated lesions. The cortical demyelination could be a signalling for generation of a pre-oligodendrocyte larger population.