TORINO, 22-23 OTTOBRE 2010

SEDE: ANATOMIA UMANA
CSO M.D'AZEGLIO 52
TORINO

G.I.S.N.
XX CONVEGNO NAZIONALE

I SESSIONE: NEURODEGENERATION, NEUROREGENERATION & PHARMACOLOGICAL THERAPY

ACTIVATION OF AUTOPHAGY IN A MODEL OF RETINAL ISCHEMIA FOLLOWING HIGH INTRAOCULAR PRESSURE

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ATTIVAZIONE DELL'AUTOFAGIA IN UN MODELLO DI ISCHEMIA RETINICA

Acute primary open angle glaucoma is an optic neuropathy characterized by the elevation of intraocular pressure which causes retinal ischemia and neuronal death. Ischemia/reperfusion enhances endocytosis of both horseradish peroxidase (HRP) or fluorescent dextran into GCL-neurons 24 h after the insult. We investigated the activation of autophagy in ganglion cell layer neurons following ischemia/reperfusion, using acid phosphatase histochemistry and immunofluorescence against LC3 and LAMP1. Retinal I/R leads to the appearance of AP-positive granules and LAMP1-positive vesicles 12 and 24 h after the insult, and LC3 labeling at 24 h, and induces a consistent retinal neuron death. At 48 h the retina is negative for autophagic markers. Inhibition of autophagy by 3-methyladenine partially prevents death of neurons and reduces caspase-3 cleavage, 24 h post-lesion. Therefore, targeting autophagy could represent a novel and promising treatment for glaucoma and retinal ischemia.

PREVENTION OF ISCHEMIA/REPERFUSION-INDUCED LIPID PEROXIDATION BY *PISTACIA LENTISCUS* L. POLYPHENOL-ENRICHED ESSENTIAL OILS IN THE RAT FRONTAL CORTEX

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GLI OLII ESSENZIALI ARRICCHITI IN POLIFENOLI DI *PISTACIA LENTISCUS* L. PREVENGONO LA PEROSSIDAZIONE LIPIDICA INDOTTA DA ISCHEMIA/RIPERFUSIONE NELLA CORTECCIA FRONTALE DI RATTO

This study provides evidence for the occurrence and degree of lipid peroxidation after cerebral ischemia/reperfusion (I/R) injury and reports on the antioxidant and protective effects of dietary *Pistacia lentiscus* L.essential oils during cerebral I/R injury in Wistar rat frontal cortex. Cerebral ischemia was produced by a 20 min bilateral common carotid artery occlusion followed by 30 min reperfusion. *Pistacia lentiscus* L. essential oils enriched with polyphenols (200 mg/0, 45 ml of sunflower oil as vehicle) were administered via gavage 6 hours prior to ischemia. Rats were randomly assigned to four groups, ischemic/reperfused (I/R) and sham-operated rats treated with the vehicle or with essential oils. Different brain areas were analysed for fatty acid changes. Expression of the enzyme ciclooxygenase-2 (COX-2) was also evaluated by western blot (WB) and immunohistochemistry (IHC). A significant decrease of docosahexaenoic acid (DHA) was detected only in the frontal cortex of vehicle treated I/R rats, accompanied by a parallel increase of conjugated hexadecadienoic acid (CD16:2), a marker of peroxisomal beta-oxidation. By contrast, no significant changes were evident in I/R rats treated with essential oils as compared to either vehicle or essential oils treated sham-operated rats. WB and IHC, performed in the counterlateral hemisphere of the same animals, revealed a marked decrease of COX-2 in the frontal cortex of I/R rats treated with essential oils as compared to those treated with the vehicle only. Our data suggest that cerebral I/R induces a region specific lipid peroxidation as evidenced by the decrease of DHA, and an associated increase of peroxisomal beta-oxidation, as evidenced by the increase of CD16:2, which reveals a physiological response aimed at decreasing potentially dangerous lipid peroxidation products by increasing their catabolism in peroxisomes. We may conclude that treatment with *Pistacia lentiscus* L. polyphenol-enriched essential oils exerts a neuroprotective action during cerebral I/R, preventing lipid peroxidation and, by the modulation of COX-2 availability, reducing inflammatory response. This work was supported by Fondazione Banco di Sardegna.

THE RELEVANCE OF PTEN-INDUCED KINASE I (PINK1) IN THE FATE OF MITOCHONDRIA RELATED TO DOPAMINERGIC SURVIVAL

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L'IMPORTANZA DI PTEN-INDUCED KINASE I (PINK1) NELLA CINETICA DEI MITOCONDRI IN RELAZIONE ALLA SOPRAVVIVENZA DI CELLULE DOPAMINERGICHE

Macroautophagy is a dynamic process used to sequester and degrade entire organelles, misfolded proteins in a double membrane vesicle known as the autophagosome, which ultimately melts with a lysosome for substrate degradation. Recently, autophagy emerged as critical for neuroprotection. In fact, suppression of autophagy produces cell death *in vitro* and *in vivo*, while autophagy inducers remove protein aggregates and reduce apoptotic cell death. Recently, PTEN-induced kinase I (PINK1) was recognized to be mutated in genetic Parkinson's disease. However, the mechanisms linking PINK1 to dopamine (DA) cell death remain elusive. Distinct PINK1 isoforms are known to be active outside and within mitochondria. PINK1 interacts with the pro-autophagic protein Beclin1 and it is a powerful interactor with the protein parkin. The aim of this study was (i) to characterize whether PINK1 modulates autophagy with a special emphasis on mitochondria turn over (mitophagy); (ii) to evaluate whether these effects relate to the survival of DA cells.For this purpose, we used DA cells where PINK1 was either wt, over-expressed, mutated or silenced. These cells were studied under baseline conditions and following DA neurotoxin (methamphetamine) known to require a powerful compensatory autophagy response.We found that PINK1 wild type (wt), morphologically interacts with Beclin1 and parkin. We also found that over-expression of PINK1 increases mitophagy (with no dramatic effects in protein autophagy). This specific effects of PINK-1 on mitochondrial turnover was associated with a robust modulation of DA cell survival.

TOXIC AMYLOID BETA OLIGOMERS ACTIVATE JNK AND LEAD TO SYNAPTIC DYSFUCTION

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GLI OLIGOMERI DI BETA AMILOIDE ATTIVANO LA VIA DI TRASDUZIONE DI JNK E INDUCONO DISFUNZIONE SINAPTICA

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by cognitive impairment and memory loss. These symptoms are due to alterations of the synaptic functions induced by oligomeric forms of beta amyloid (Abeta). The molecular mechanisms by which Abeta oligomers start to induce synaptic degeneration are not well known but may involve mitogen-activated protein kinases (MAPKs). Amongst MAPKs, c-jun N terminal kinase (JNK) has been extensively studied for its role in stress stimuli and in AD pathology. To investigate the intracellular mechanisms that lead to AD synaptic pathology we set up an in vitro model of synaptic degeneration by treating hippocampal neurons from Brainbow mice with sub-toxic concentrations of synthetic Abeta oligomers. Activation of JNK signalling, following Abeta oligomers application, correlated with the reduction of the number of dendritic spines and the decrease of post-synaptic markers (PSD95, drebrin and NMDAR subunits). A strong activation of the pro-apoptotic caspase 3 was also observed specifically in the synaptic compartment, indicative of synaptic death. To study the role of JNK in the intracellular mechanisms that regulate synaptic-degeneration caused by Abeta oligomers we used the specific cell permeable JNK inhibitor peptide (D-JNKI1). Treatment with D-JNKI1 reverted the synaptic degeneration induced by Abeta oligomers and prevents the loss of PSD95 and NMDAR subunits from the post-synaptic membrane. Moreover it inhibited activation of caspase 3 in the synaptic compartment. In conclusion, JNK signalling pathway is a key player in the early events of synaptic death that characterise AD.

NEUROPROTECTION AND AUTOPHAGY INDUCTION BY A NEW ADENOSINE RECEPTORS ANTAGONIST.

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NEUROPROTEZIONE E INDUZIONE DELL'AUTOFAGIA TRAMITE UN NUOVO COMPOSTO ANTAGONISTA DEI RECETTORI ADENOSINICI.

Adenosine receptors A2A (A2A ARs) are purinergic receptors largely expressed in dopamine (DA)-rich areas of the central nervous system. In particular, they are abundant within basal ganglia, where they modulate the activity of various neurotransmitters, including DA. Despite the lack of knowledge on their fine physiological mechanisms, it is worth to mention that A2A AR antagonists prevent neuronal death and dyskinesia. In the present study we investigated: (i) whether a novel A2A AR antagonist (ATBI-10) protects DA containing neurons against toxic stimuli. (ii) whether specific mechanisms are involved in these phenomena. In order to evaluate neuroprotection we selected DA cell model such as PC12 and SH-SY5Y cell lines. We measured the effects of ATBI-10 on 1-methyl 4-phenylpyridinium (MPP+) and methamphetamine (Meth)- induced cell death. Moreover, in the same experimental conditions we compared the effects of ATBI-10 with the gold standard A2A ARs antagonist ZM241385 or, *vice versa* we used the gold standard for A2A ARs agonist. In addition we administered adenosine. We documented the neuroprotective efficacy of ATBI-10 in both Meth and MPP+ toxicity, being mostly effective against Meth toxicity (complete prevention). This was replicated using ZM241385 while it was suppressed by the administration of NECA or adenosine. In light of the key role of autophagy in modulating the survival of DA neurons we investigated the association between A2A AR and the autophagic pathway. We found that antagonism at A2A ARs increases (increased LC3-II levels) while A2A agonism decreases autophagy.

ANXIETY AND COGNITIVE PERFORMANCE: A POPULATION STUDY IN WISTAR RATS.

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ANSIA E PRESTAZIONI COGNITIVE: STUDIO DI POPOLAZIONE IN RATTI WISTAR.

Human subjects display a great variability in the predisposition to respond anxiogenically to stimuli, i.e. trait anxiety. This susceptibility has been studied in rodents through the creation of selected strains for anxiety-like behaviour, to obtain extremes anxiety traits. Moreover, anxiety has been shown to variously affect cognitive performance both in humans and selected rodents strain. However, interindividual differences in basal anxiety level in naïve rats and how they may affect cognitive functioning have been poorly investigated. Therefore, the aim of this study is to provide an evidence of the huge interindividual differences in anxiety levels in naïve Wistar rats and demonstrate how they can affect a widely used cognitive test. Primarily, we run a population study, testing 182 Wistar rats for their anxiety-like behaviour in Elevated Plus Maze (EPM), which can provide a measure for animals "basal" anxiety level. Anxiety level was inferred by the amount of time spent by each rat in the open arms and in the centre of the EPM apparatus. Secondly, 60 rats, among those previously exposed to EPM, performed a Novel Object Recognition test (NOR), used to assess cognitive abilities in general, particularly recognition memory. NOR provided an evaluation of recognition memory through measure of the discrimination between a familiar and a novel object. Basing on EPM data, we obtained a population, which matched a Gaussian distribution and we identified three main groups: a high anxiety group (HA), a medium anxiety group (MA) and a low anxiety group (LA). The performance of the three groups in the NOR was compared, and the results showed a significant difference between the HA group and the MA and LA group in the discrimination of novelty. First and foremost, these data show that a large interindividual variability in basal anxiety level in naïve animals. Moreover, we found that anxiety level significantly affect animals performance on the cognitive task. Therefore we claim the need to consider interindividual differences in emotionality (e.g. anxiety) in general, and the need to assess anxiety level while studying rats cognitive abilities.

NEUROANATOMIA E NEUROETICA

FILL THE GAP: FMRI AND 2PHOTON IMAGING CENTRAL NERVOUS SYSTEM

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Progress in intensive care has led to an increase in the number of patients who survive severe acute brain injury. Some of these patients permanently lose all brainstem functions (brain death), whereas others emerge from coma progressing through different stages before fully or partially (minimally conscious state) recovering consciousness, but most of them remain in a state of wakefulness without awareness (vegetative state). Clinical practice has shown the challenges in identifying signs of these patients' perception of environment and themselves, and misdiagnosis between vegetative state, minimally conscious state and locked in syndrome are very common. Bedside clinical assessment of residual brain functions in these patients is based only on inferences made from their motor responses, that can be limited or inconsistent. To date, the vast majority of studies have stressed the importance of etiology (traumatic or ischemic-anoxic) to determine prognosis of disorders of consciousness, but there is insufficient attention to their physiopathology and consequently there are no standards of care for targeted pharmacologic interventions, because of the lack of evidence-based studies. Integration of different neuroimaging techniques could help to find the connections between molecular neuronal mechanisms and specific connectivity patterns of activation in the brain of these patients, filling the gap between clinic, functional magnetic resonance imaging and neuroscience knowledge, improving the understanding of consciousness and related disorders. In particular, resting state functional connectivity studies allow the investigation of neuroglial networks without requiring subject's collaboration, only analyzing the intrinsic spatial properties of fMRI signal, in terms of its distribution coherence within the brain. These approaches let us challenging to parallel human brain network findings with molecular data achieved by 2-photon microscopy experimental studies.

II SESSIONE
ANIMAL MODELS OF NEUROPATHOLOGIES: I. SMA

TIME-COURSE AND MECHANISMS OF MOTONEURON DEATH IN SMAII MICE

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PROFILO TEMPORALE E MECCANISMI DELLA MORTE DEI MOTONEURONI NEI TOPI SMAII

Spinal muscular atrophy (SMA) is a recessive autosomal neuromuscular disease, due to the deletion of the telomeric survival motoneuron gene (SMN1), which leads to motor impairment, muscle weakness and atrophy, and premature death caused by motoneuron degeneration. However, the causes and the pathways of motoneuron death still remain elusive: at most, some authors hypothesized apoptosis in the earlier stages followed by necrosis at the later stages of SMA. Moreover, most studies focus on lower motoneurons, thus neglecting upper motoneurons. In order to clarify the occurring mechanisms, we have employed the murine model of SMA II (the intermediate form of SMA), which by day 5 determines motor dysfunction until death approximately at 13 days. In different set of experiments, we have collected brains and spinal cords from SMA II and wild type pups at postnatal day 9 (P9) and P13 for neuron counts and immunohistochemisty. In the meanwhile, throughout their lifespan, SMAII mice underwent a battery of motor tasks and were assessed daily for body weight and motor performance. We found a progressive worsening in most behavioral tests, closely related to motoneurons loss. Following Nissl staining, we have performed stereological counts to evaluate the number of upper and lower motoneuron. Our results confirm the reports of a progressive decrease in lower motoneuron number in SMA II mice compared with controls, but also highlight a parallel decrease in upper motoneurons. Immunohistochemistry against cleaved Caspase-3 put in evidence labeled motoneurons,

thus suggesting an involvement of the apoptotic mode of cell death. In addition, LC3 positive vesicles were observed in SMAII lower motoneurons, implicating an upregulation of phagocytosis. Therefore, apoptosis and autophagy are involved in motoneuron death in SMAII mice, and understanding these mechanisms could be relevant in identifying new therapeutic strategies to protect motoneurons and maybe delay the disease progression. Supported by Girotondo Onlus grants.

MOTOR NEURON DEGENERATION IN A GENETIC MODEL OF SPINAL MUSCLE ATROPHY (SMA)

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LA DEGENERAZIONE DEI MOTONEURONI IN UN MODELLO GENETICO DI ATROFIA MUSCOLARE SPINALE (SMA)

Spinal muscle atrophy (SMA) is an autosomal recessive neurodegenerative disorder which consists in the loss of motor neurons. This leads to progressive limb and trunk palsy. SMA is classified in three subtypes depending on disease onset and severity: type I (severe), type II (intermediate) and type III (mild). The pathophysiology of SMA is still unknown and no effective treatment is presently available. The genetic defect underlying SMA consists in mutations of the survival motor neuron gene (*SMN*), which is present in two copies, *SMN1* and *SMN2*. Experimental models of SMA are puzzling due to the embryonic lethality of mice knockout for the *Smn* gene. *Smn-/-* Mice expressing various compensatory copies of the human *SMN* genes have been recently developed providing valuable models to study SMA. In this study a transgenic type III SMA mouse model was characterized. The genotype (*SMN1 -/-*, *SMN2 +/+*, *SMN1A2G +/-*) allowed a long disease course to evaluate both behavioural and morphological deterioration. We used a variety of tests such as Rotarod test, Paw Grip Endurance test and stride length test. Morphological studies were carried out at light microscopy using common histological staining procedures. All mice presented a progressive muscular worsening evidenced by the loss of the limb extension reflex and the Paw Grip Endurance test failure. Analysis of the spinal cord of these transgenic type III SMA mice at lumbar level shows severe cell death involving motor neurons within the lamina IX. Moreover the size of spared motor neurons is augmented in SMA mice. These latter findings are very similar to those we described in the transgenic G93A mouse, a model for amyotrophic lateral sclerosis (SLA) characterized by a defect in autophagy. Studies are in progress to investigate the actual mechanism of motoneuron death in SMA.

INTERACTION BETWEEN HISTONE DEACETYLASE AND MICRO-RNA IN A MOUSE MODEL OF SPINAL MUSCULAR ATROPHY (SMA)

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INTERAZIONE TRA LE ISTONE DEACETILASI E I MICRORNA IN UN MODELLO MURINO DI ATROFIA MUSCOLARE SPINALE

Proximal spinal muscular atrophy (SMA) is a common autosomal recessive neurodegenerative disease of spinal α-motor neurons, and is the leading cause of death among infants and neonates with and incidence of 1/6000-1/10000 (Pearn, 1978). Symptoms include weakness and atrophy of both proximal limb and respiratory muscles. The primary cause of SMA is the deficiency of survival motor neuron (SMN) protein due to loss or mutation of the *smn1* gene (Lefebvre et al., 1995). Humans also bear a second copy of the gene, *smn2*, which differs from *smn1* chiefly by a C to T transition in exon 7. The majority of *smn2* transcripts lack exon 7 and encode a less functional protein. As the copy number of *smn2* increases, the severity of SMA symptoms decreases and the age of onset is delayed (Wirth et al., 2006). SMN is a widely expressed protein, highly conserved through evolution, with important roles in diverse aspects of RNA metabolism, including pre-RNA splicing, transcription, and metabolism of ribosomal RNAs. In addition, SMN may play other roles in motor neurons since they are uniquely susceptible to SMN protein deficiency, but the mechanism of selective disruption of the motor neurons in mice and humans remains unknown. In the last decade, histone deacetylase inhibitors have been

shown to activate the *smn2* promoter, likely throught direct modification of the acetylation state of histones at the promoter (Kernochan et al., 2005). Recently, suberoylanilide hydroxamic acid (SAHA) significantly ameliorated the severe SMA phenotype in mice models, in terms of lifespan, improvement in motor function, increased number of motor neurons and increased size and occupancy of post synaptic sites of neuromuscular junctions and muscle fibers (Riessland et al., 2010).
Therefore, we aim to investigate the molecular mechanisms activated by histone deacetylase inhibitors in a mouse model of intermediate SMA.
First, we measure the expression of the microRNA-206 precursor, a skeletal muscle-specific microRNA which seems very important for the correct signaling between the motor neuron unit and the muscle fiber at the synaptic junction in a mouse model of amyotrophic lateral sclerosis (Williams et al., 2009). Our results show a different expression of the miRNA-206 precursor in muscle fibers of wild type animals compared to knock-out mice for the *smn1* gene. Then, we will measure histone deacetylase 4 (HDAC4) mRNA transcript levels to correlate them with the expression of miRNA-206. Finally, we are going to test histone deacetylates inhibitors on our mice and to test their phenotype after the treatment with several behavioral tests.

III SESSIONE
NEUROINFLAMMATION

PRO-INFLAMMATORY CYTOKINES AND THEIR RECEPTORS EXPRESSION IN A MOUSE MODEL OF PARKINSON'S LIKE DISEASE

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ESPRESSIONE DI CITOCHINE PROINFIAMMATORIE E DEI RISPETTIVI RECETTORI IN UN MODELLO SPERIMENTALE DI PARKINSONISMO

Upregulation of inflammatory response in the brain is associated with a number of neurodegenerative diseases, including Alzheimer's and Parkinson's disease (PD), amyotrophic lateral sclerosis, multiple sclerosis. In particular PD is a common neurodegenerative pathological state characterised by the degeneration of dopaminergic neurons in the substantia nigra (SN) pars compacta, determining reduced dopamine levels in the caudate-putamen (CP) which lead to movement malfunction. Despite intensive research, the cause of neuronal loss in PD is poorly understood. To study the specific cause of PD, researchers have used a variety of toxins as agents to bring about damage to the dopaminergic neurons, such as 6-hydroxydopamine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), paraquat and rotenone. Among these, the MPTP poisoning leads to symptoms very closely matched to many features of Parkinsonian syndrome in both humans and animals. In this study we investigated, in an experimental MPTP mouse model of PD, the expression of pro-inflammatory cytokines interleukin (IL)-1 β, tumor necrosis factor (TNF)- α and IL-6 and their receptors in the SN and CP, in order to evaluate their involvement in this neurodegenerative disease. In MPTP-treated animals we observed a significant increase in IL-1 β, TNF- α and IL-6 mRNA expression levels both in the SN and CP in comparison with untreated mice. In addition, both mRNA and protein levels of IL-1RI, TNF- α RI and IL-6R were significantly enhanced in the SN of MPTP-treated mice in comparison to controls, whereas no significant differences were observed in the CP between treated and untreated mice. Overall, these results indicate a possible role of both pro-inflammatory cytokines and their receptors in the pathogenesis of PD.

MIGRATORY BEHAVIOUR OF DENDRITIC CELLS IN THE MOUSE BRAIN CORTEX IN NORMAL AND PATHOLOGICAL CONDITIONS BY MULTIPHOTON MICROSCOPY

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IL COMPORTAMENTO MIGRATORIO DELLE CELLULE DENDRITICHE NELLA CORTECCIA CEREBRALE DI TOPO IN CONDIZIONI NORMALI E PATOLOGICHE ANALIZZATO MEDIANTE MICROSCOPIA A MULTIFOTONI

Dendritic cells (DCs) have an immune surveillance role in the central nervous system. It is well known that in normal conditions they maintain the immuno-tolerance by T-cell suppression and that their presence is limited to the meninges, choroid plexus and rarely to the brain parenchyma. During inflammation, DCs infiltrate the brain parenchyma, enhance their antigen processing capacity and promote the initiation of immune responses by T-cell activation. Information on DCs *in vivo* in the CNS is very limited. We are investigating the dynamic behavior of the DCs in the mouse brain cortex *in vivo* in normal conditions and in a model of chronic neuroinflammation represented by infection with the parasite *Trypanosoma brucei* (*Tb*), the causative agent of African trypanosomiasis. This disease evolves in humans and experimental animals in two stages: an early stage, in which *Tb* invade peripheral organs through the hemolymphatic system; and a late meningoencephalitic stage with severe consequences on nervous system functions. In order to visualize dynamic cell processes in healthy mice and in mice infected with the non-human pathogenic subspecies *Tb brucei*, a little craniotomy was performed leaving

the dura mater unperturbed and the bone flap was replaced by a coverglass. This chronic implantation provides an excellent optical access for multiphoton acquisition *in vivo*, allowing high-resolution imaging over time. Blood vessels were visualized by iv injection of TRITC-conjugated dextran. Four-dimensional reconstruction (x,y,z,t) of transcranial images were analyzed by Imaris software to track the migratory route of DCs, their position with respect to the vasculature over time, and their movement features (crawling, rolling, etc.). The initial results of this study show that in the healthy brain DCs are mainly localized in the meninges, they are motionless and exhibit a round shape. In addition, with the progression of *Tb brucei* infection DCs have been observed to penetrate the brain parenchyma from the meninges, their number increases and their motility is enhanced. At an advanced phase of the infection, phagosome- like structures are also observed in the brain parenchyma. The data suggest an involvement of DCs in pathogenetic mechanisms and severity of *Tb* brain infection. *In vivo* experiments with fluorescent *Tb brucei* are in progress to visualize in the brain potential interactions between the parasite and host immune cells represented by DCs.

PROGRESSION OF *TRYPANOSOMA BRUCEI* AND T CELL INVASION OF THE BRAIN PARENCHYMA IN THE RAT

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PROGRESSIONE DELL'INVASIONE DI *TRYPANOSOMA BRUCEI* E LINFOCITI T NEL PARENCHIMA CEREBRALE DEL RATTO

Human African trypanosomiasis (HAT) is a severe disease caused by *Trypanosoma brucei* (*Tb*). The first stage of the infection evolves into the second stage when *Tb* invades the brain parenchyma crossing the blood-brain barrier (BBB). No detailed information of the timing and progression of the BBB crossing by lymphocytes events are available. Parasite neuroinvasion and infiltration of CD4+ and CD8+ T cells were here investigated in rats infected with *Tb brucei (Tbb)* by immunocytochemistry and quantitative analyses from day post-infection (dpi) 1 to 24. The analyses were correlated with two parameters previously considered robust symptoms of second stage: body weight loss and the onset of sleep-onset rapid eye movement (SOREM) episodes detected by means of continuous telemetric recording. In our rat infection model the disease has a duration of about 35 days. The findings revealed that parasite neuroinvasion starts relatively early, with parasite detection in the brain parenchyma at dpi 9 and a considerable number of parasites from dpi 12 onward. Comparison of parasites in the parenchyma of anterior *versus* posterior brain regions showed a prevalence of parasite density in the posterior hypothalamic regions, and a temporal increase much more evident anteriorly than posteriorly, where marked parasite density exhibited instead an oscillation. Lymphocyte infiltration initiated at the time of parasite neuroinvasion and showed a quantitative progression. Body weight loss was consistently observed only at an advanced stage of infection associated with high lymphocyte infiltration. SOREM episodes started early post-infection and their number and duration did not reflect the amount of parasites in the brain parenchyma. Altogether the findings show that *Tbb* and T-cell entry in the brain parenchyma is a process which starts relatively early after infection and develops over time with regional differences of parasite invasion, suggesting regional differences of BBB permeability during the infection. The study elucidates temporal and regional features of *Tbb* neuroinvasion and accompanying lymphocyte infiltration and points to the importance of inflammatory cells in functional alterations.

OLIGODENDROCYTE PRECURSOR RESPONSE IN CEREBRAL CORTEX OF EAE MICE

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RISPOSTA DEI PRECURSORI DEGLI OLIGODENDROCITI NELLA CORTECCIA CEREBRALE DI TOPI EAE

Although several studies carried out on demyelinating lesions of spinal cord in experimental autoimmune encephalomyelitis (EAE) have described the response of cells of the oligodendrocyte (OL) lineage to the disease, relatively little is known about the involvement of these cells in cerebral cortex pathology as a consequence of the difficulty of revealing signs of demyelination in standard models, MOG-induced, of EAE. In our analysis we decided to firstly analyse the putative involvement of the cortex during EAE and then the possible response of the OLs to the events of demyelination. A broad panel of cell-specific and myelin-specific markers were used to reveal and quantitatively evaluate, in healthy brains and during early (6 days post onset; dpo) and late (30 dpo) EAE, (1) levels of cortex myelination, (2) activation of macrophage/microglia cells, (3) antigenic phenotype and (4) proliferation and/or shortage of OLs. (1) On MBP/MOG immunolabelled sections, a significant reduction of myelinated nerve fibres was observed already at 6 dpo in the supragranular and granular layers and in subcortical white matter. Myelin reduction was more evident in subpial layers and extended towards the deeper layers in restricted cortex areas. (2) Activated $CD45^{low}$/C11b-reactive microglia and a sparse infiltrate of $CD45^{high}$-macrophage-like cells were detectable throughout all the cortex, without any evident relation with the vasculature. The described results prompted us to further investigate the cells of the OL lineage which were (3) identified as $A2B5^+$ 'glial restricted progenitors' (GRPs); $NG2^+/O4^-$ oligodendrocyte precursor cells (OPCs)/polydendrocytes; $NG2^+/O4^+$ pre-OLs; $NG2^-/O4^+$ pre-myelinating OLs; and $CNPase^+/MBP^+/MOG^+$ mature OLs. (4) In EAE $A2B5^+$ GRPs and $NG2^+$ OPCs were found to be increased during the early phases of the disease to then diminished in the late phase together with $NG2^+/O4^+$ pre-OLs and mature OLs. The prematurative cell type represented by $NG2^-/O4^+$ pre-myelinating OLs, although stable in number in the late disease, did not seem to be able to sustain the depleted compartment of myelinating OLs hence impairing remyelination of the damaged cortex areas.

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LETTURA MAGISTRALE

MORPHOLOGICAL BASES OF THE LEUCOCYTE/ENDOTHELIAL CELL INTERACTIONS IN EXPERIMENTAL EPILEPSY.

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BASI MORFOLOGICHE DELLE INTERAZIONI TRA LEUCOCITI E CELLULE ENDOTELIALI NELL'EPILESSIA SPERIMENTALE

Structural brain alterations have been considered to play the key role in the etiology of different neurological diseases, and in particular in epilepsy. We recently proposed that, instead, structural alterations observed in this neurological condition may be independent to the altered neuronal excitability . In particular, we suggested that we should focus the attention also to attention to non-neuronal cells. In recent years growing evidence suggest that astrocytes, microglia, blood leukocytes and blood-brain barrier breakdown are involved in the pathogenesis of epilepsy. In particular, leukocyte-endothelium interactions and eventually subsequent leukocyte recruitment in the brain parenchyma seem to represent key players in the epileptogenic cascade. In conclusion, we want to emphasize the necessity to shift the attention to single cell type, like neurons, to the more complex cell network present in the brain, to better elucidate anatomical correlates of brain pathologies.

IV SESSIONE
SYSTEMATIC, CHEMICAL AND DEVELOPMENTAL NEUROMORPHOLOGY

TESTOSTERONE REGULATES ADULT NEUROGENESIS IN RAT SVZ

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IL TESTOSTERONE REGOLA LA NEUROGENESI NELLA SVZ DEL RATTO ADULTO

Adult neurogenesis is a process occurring within the Central Nervous System in the olfactory bulbs, in the subventricular zone (SVZ), and in the dentate gyrus. Steroid hormones are supposed to play a role in regulating this phenomenon, therefore we studied here the activational effects of Testosterone (T) on the proliferation of neuroblasts in the SVZ. To this aim, we analyzed three groups of 5 adult male rats: castrated rats (CX), castrated and treated with T (CX+T), and controls rats (CN). All the animals received two intraperitoneal injection of BrdU and were sacrificed one day after the last BrdU administration to investigate the proliferation within the SVZ. The rate of neurogenesis was evaluated through the counting of immunocytochemically stained BrdU-positive cells. For the quantitative evaluation, we analysed three sections per animal, taken at rostral (Bregma 2.20 mm), intermediate (Bregma 1.60 mm) and caudal (Bregma 1.20 mm) levels. Our data revealed a significant decrease in the area covered by BrdU-positive elements in CX animals in comparison to both CX+T and CN. This effects is however limited to the intermediate level of SVZ. Moreover, we divided the SVZ in three areas: dorsal area, medial and lateral wall of the lateral ventricle and we made a stereological analysis on six sections *in serie* for animal. In lateral wall of the lateral ventricle, quantitative analysis revealed that the estimated number of BrdU-positive cells, in the CX group, is statistically lower in comparison to the other two groups. In conclusion, present data suggest that the decrease of circulating levels of T caused by castration induces a decrease of the rate of neurogenesis in specific parts of the SVZ.

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MORPHOLOGICAL FEATURES AND VIABILITY OF OLFACTORY ENSHEATHING CELLS IN DIFFERENT CULTURE CONDITIONS.

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CARATTERISTICHE MORFOLOGICHE E SOPRAVVIVENZA DELLE CELLULE GLIALI OLFATTIVE CRESCIUTE IN DIFFERENTI CONDIZIONI DI COLTURA.

Olfactory Ensheathing Cells (OECs) represent a particular type of glial cells which support the continuous neuronal turn-over of the olfactory district and ensheath olfactory axons from the epithelium to the olfactory bulb. *In vitro*, OECs, as source of neurotrophic factors, promote axonal growth supporting axonal extension. In *in vivo* studies, some authors have shown that OECs form myelin promoting remyelination of damaged axons, expressing a myelin constituent. Consequently, OECs transplantation appears to be a promising treatment for spinal cord injury (SCI). On the contrary, the functional recovery of SCI is limited. This might be ascribed to the microenvironment at the lesion site, which shows deprivation of growth factors, nutrients and oxygen. To evaluate the effects of these factors, in the present study we used an *in vitro* approach by growing OECs in condition of hypoxia and serum deprivation. OECs were prepared from neonatal mouse olfactory bulbs and grown in different conditions: some OECs were grown both with DMEM/FBS and DMEM/FBS with GDNF. Other OECs were grown both with serum-free DMEM/F12 medium and DMEM/F12 added with GDNF. All these conditions were repeated with an anoxic insult, inverting coverslips with OECs in their wells and thus lowering the concentration of oxygen. After a week, the cells were processed for immunocytochemical procedure. Our results show that both serum and oxygen deprivation induce a reduction of the cellular survival. In particular the combination of both insults resulted in a more pronounced effect compared with controls. Moreover, we considered the effect of the growth factor GDNF, and observed that it exerted a positive influence only when OECs were grown in serum-free

DMEM/F12, whereas it increased their ability to form clusters. GDNF exerted no positive effect on OECs grown in anoxic conditions both in the presence and in the absence of serum. The morphological features of OECs present differences depending on the growth conditions: in controls they exhibited both star and spindle shape, while grown in serum-free medium formed clusters. The cells exposed only to anoxic insult showed a flat shape, but when exposed to both insults formed clusters of small size cells. This model allows us to simulate secondary events during SCI. The observation that GDNF alone does not rescue OECs from anoxic insult but only from serum deprivation suggest that OECs transplantation would be helpful to promote functional recovery in SCI if combined with a cocktail of trophic factors.

EVALUATION OF BEHAVIOURAL PERFORMANCE IN A RAT MODEL OF PRENATAL CEREBRAL MALDEVELOPMENT

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VALUTAZIONE DELLE PERFORMANCE COMPORTAMENTALI IN UN MODELLO DI MALFORMAZIONE CEREBRALE PRENATALE NEL RATTO.

Malformations of cortical development (MCD) are one of the most common causes of neurological disabilities including autism, mental retardation and epilepsy. To mimic a disruption of cortical formation in rodents, methylazoxymethanol (MAM) and thalidomide (THAL) are often used to affect two key processes on the brain development, neurogenesis and vasculogenesis respectively. Rats prenatally exposed to MAM and THAL show a particular type of brain, altered both in morphology and in functionality, when they observed at early postnatal days. These malformations consist in abnormal ventricular size, hemispheric asymmetry, gliosis in regions of focal leakage of blood brain barrier, abnormal hippocampal connectivity and mossy fibers sprouting. While morphological abnormalities gradually disappeared at adult stages, alterations in connectivity and sprouting of specific cerebral areas are not reversible, and persisted well into adulthood. Based on these results, we hypothesized the possibility that these animals will have an altered pattern of motor behaviours and cognitive abilities. The aim of this study is to characterized the behavioural phenotype of rats prenatally exposed to MAM and THAL. Pregnant Sprague-Dawley rats were injected with 7,5 mg/kg of MAM and 15mg/kg of THAL at day 14/15 of gestation, while the control animals were injected with saline. Male and female pups were tested in the Surface Righting test and in the Negative Geotaxis test at 2, 4, 6, 8, 10, 12, 14 and 16 post-natal day (PND), in order to investigate their motor functions and coordination pattern. From PND20 to PND51 animals were subjected to Novel Object Recognition test (PND20 and PND45), to the Enriched Open Field test (PND27 and PND51), and to Elevated Plus Maze test (PND34), to evaluate their recognition memory abilities, their exploratory activity and their anxiety level respectively. Body weight was measured right after each experimental session. The results of this preliminary study allowed us to examine behavioural features of MAM-THAL rats, that can provide indications for a possible classification of this model into a specific pathological profile of disease.

SYNAPTOBREVIN, SNAP-25 AND SYNTAXIN ARE DIFFERENTLY EXPRESSED BY GLUTAMATERGIC AND GABAERGIC AXON TERMINALS OF THE RAT CEREBELLAR CORTEX

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SINAPTOBREVINA, SNAP-25 E SINTAXINA SONO ESPRESSE IN MODO DIFFERENZIALE DAI TERMINALI GLUTAMATERGICI E GABAERGICI DELLA CORTECCIA DEL CERVELLETTO DI RATTO

The study aimed to describe the distribution of the synaptobrevin, a SNARE protein localized on the vesicle membrane (vSNARE), and SNAP-25 and syntaxin, SNARE proteins localized on the presynaptic membrane, within the glutamatergic and GABAergic axon terminals of the rat cerebellar cortex. The co-localization of synaptobrevin-2 or SNAP-25 or syntaxin-1a with vGLUT-1 and vGLUT-2, as concerned the glutamatergic synapses, and with GAD-65/67, as concerned the GABAergic synapses, was revealed by double labelling light microscope immunohistochemical techniques. Results and discussion. Axon terminals which co-localized vGLUT-1 with synaptobrevin-2 or SNAP-25 or syntaxin were widely distributed in the molecular, Purkinje and granular layer, consistent with the hypothesis that the release of glutamate by the terminals expressing vGLUT-1 require the presence of the above SNARE proteins. On the contrary, among the glutamatergic terminals expressing vGLUT-2, only those localized in the granular layer co-localized also synaptobrevin-2, SNAP-25 and syntaxin. Finally, the co-localization of GAD-65/67 and synaptobrevin-2 or SNAP-25 or syntaxin was observed only occasionally, in terminals forming the pinceau on the inner pole of Purkinje neurons. Conclusion. Synaptobrevin-2 or SNAP-25 or syntaxin characterized only a part of glutamatergic axon terminals of the cerebellar cortex. All the glutamatergic terminals expressing vGLUT-1, namely those belonging to parallel fibres, in the molecular layer, and mossy fibres, in the granular layer, expressed also these SNARE proteins. The same proteins where not detected in the glutamategic teminals of climbing fibres expressing v-GLUT-2. The GABAergic terminals in the molecular layer, belonging to stellate and Purkinje neurons, and in the granular layer, belonging to Golgi, Lugaro and candelabrum neurons, never expressed above SNARE proteins. The only GABAergic terminals which occasionally co-localized GAD-65/67 and synaptobrevin-2, SNAP-25 and syntaxin were those belonging to the basket neurons.

AXON DIAMETERS, CONDUCTION VELOCITIES AND COMMUNICATION DELAYS BETWEEN CORTICAL SITES: A NOVEL COMPUTER-ASSISTED APPROACH TO STUDY CORTICAL DYNAMICS IN PRIMATE BRAIN

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DIAMETRI ASSONALI, VELOCITA' DI CONDUZIONE E ASINCRONIE NELLA COMUNICAZIONE TRA AREE CORTICALI: UN NUOVO APPROCCIO COMPUTERIZZATO PER LO STUDIO DELLE DINAMICHE CORTICALI NEL CERVELLO DI PRIMATE

The connectivity between brain sites is anatomically and functionally established through bundles of axons (tracts) whose diameters and lengths determine the temporal profile of activation of each site in response to a given stimulus. Conduction delays can be estimated on the basis of tract length and of the diameter of axons within each tract, which in turn determines their conduction velocity. In particular, the comparison of axon diameter and length of tracts originating from cortical areas with different position along the antero-posterior axis of the brain can give an estimate of conduction delays existing between different cortical and subcortical sites, which is an important determinant of cortical dynamics. In the present work we used biotinylated dextran amine (BDA) injections to trace axon fibers originating from 7 different cortical sites (prefrontal, premotor, motor, somatosensory, parietal, striate and peristriate visual) in 5 long-tailed macaques (*Macaca fascicularis*). For each labeled system of fibers,

we identified clusters of axons reaching short or long distance targets in the ispilateral hemisphere (intracortical fibers), deep nuclei (cortico-thalamic fibers) or in the contralateral hemisphere (callosally-projecting fibers) in BDA-reacted coronal sections using the Neurolucida software. Axon diameters were measured in regions of maximal density of labeled axons and axonal profiles were approximated to circles whose size was incremented in steps of 0.07μm. Approximately 12,400 axon diameters were measured. Tract trajectory and length were three-dimensionally reconstructed and measured after the visualization of labeled axons in approximately one hundred serial section along the z axis and 3D solid reconstruction. Linear regression analysis was used to evaluate whether a relation exists between axon diameter and i) length of tracts, ii) degree of myelination at the site injected and iii) the somatic diameter of the projecting neurons. For each site of injection, conduction velocity was determined on the basis of axon diameter distribution as measured in the corresponding tracts; conduction delays were estimated by multiplying conduction velocity with tract length. In conclusion this approach allowed a precise estimate of conduction velocities within the brain and provided a promising tool to model intrahemispheric and interhemispheric delays and to explore their role on cortical dynamics.

V SESSIONE
PNS: FROM ANATOMY TO DISEASE

PRIMARY CO-CULTURE OF ADULT DISSOCIATED DORSAL ROOT GANGLIA CELLS: STUDY OF NEURON-GLIAL CELLS INTERACTION

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COLTURE PRIMARIE DI CELLULE DISSOCIATE DI GANGLI SPINALI ADULTI: STUDIO DELL'INTERAZIONE TRA NEURONI E CELLULE GLIALI

The interactions between neurons and glial cells could be indispensable to repair a nervous ending damaged, not only with the myelination by the Schwann Cells (SC) out of the ganglia but also within this organ to support an efficacy sprouting. The demonstration that the presence of satellite glial cells (SGC), with their factors, could arise the capability of neurons to sprout and to reach a target, would be important for an hypothetical treatment. Indeed the recreation of the same environment at nerve lesion level, could favourite the crossing of the axons from the proximal portion towards the distal portion of the lesion, reaching the right target. In order to investigate the contribution or requirement of neurotrophic factors (such as NGF) to peripheral sensory axon growth and to examine the relationships between SGC and DRG, an *in vitro* analysis was performed. DRG cells, when dissociated and cultured in presence of NGF, are able to form circle structures in which SGC arrangement was very interesting, and the neurothrophic factor seemed to speed up the aggregation. Time lapse microscopy was used to study the cell-cell interaction and their ability to build up the complex geometrical aggregation. Our attention focused on these geometric cluster of cells. Further investigation were carried out by co-culturing DRG neurons, SGC (from the same dissociation) and a SC population, to verify if SC participated and/or lead to the circles formation. Finally, in order to understand the function of this geometrical organization, we performed an immunocitochemical analysis. In particular we investigated the presence of new-generated synapses. The results showed that SC didn't seem to have a particular role in these construction, they looked uniformly spread on the surface, abundant within the circle as out of it. The expression of presynaptic vesicle proteins has been confirmed. These results lead to the hypothesis that adult DRG cells, as suggested for embryonic cells, maintain the ability in culture to aggregate forming a geometrical cluster that resemble the ex-novo formation of a ganglionic micro-environment. Does mean that adult ganglionic cells, when plated in culture, have got a organ-organization memory which led cells to aggregate, restoring the interactions which originally linked neurons and glial cells in vivo. Moreover, the presence of newly formed functional communication within DRG neurons and glial cells, finding that would need to be strongly confirmed by performing further investigations, may open many interesting new research projects.

PHRENIC NERVE BRANCHING IN RAT THYMUS

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DIRAMAZIONI DEL NERVO FRENICO NEL TIMO DI RATTO

The anatomical innervation of the thymus is rather complex, because innervation reaches it by multiple nerve pathways. A minor component of thymus nerve supply is represented by the phrenic nerve, a somatic nerve originating in the cervical plexus. The thymus innervations by the phrenic nerve is an old acquisition from embriogenic and anatomical studies. This finding about thymus anatomy is refereed in several texts and treatises. Nevertheless only scanty and no detailed studies can be found about intraparenchymal phrenic nerve thymus innervations in the literature. In the present work we have studied the phrenic nerve thymus distribution by specific monolateral or bilateral surgical or chemical removal of thymus innervation. Histochemical techniques were used to visualize ACHE-positive nerve or cathecolaminergic-positive nerve fibers. The intraparenchymal branching of the phrenic nerve has been visualized, following specific denervation, as an AChE-positive nerve component exclusively distributed in the intraparenchymal subcapsular region of thymus lobules. The phrenic nerve access the intraparenchymal subcapsular region directly through the connectival capsula of thymus lobules. Instead in subcapsular zone other parasympathetic and orthosympathetic nerve fibers have been observed branching only in perivascular site. We hypothesize that some nerve fibers of orthosympathetic system running into phrenic nerve to reach thymus parenchyma; these phrenic nerve fibers might be the fibers originally innervating the embryonal ectodermic areas from which the subcapsular zone of thymus lobule has been developed (in fact some visceral component of cutaneous somatic innervation is performed by cholinergic nerve fibers). These fibers should be represent the intraparenchymal nerve fibers particularly deputed to interact with subcortical environment of thymus lobe where it is known are resident immature ($CD4^-$ and $CD8^-$, double negative) thymocytes. Some AChE-positive nerve profiles have been found in the subcapsular region mainly in close proximity to the thymic epithelial cells. These findings stimulated us to further evaluation of the functional involvement of the phrenic nerve fibers in the cortico-lympho-epitelial complex, in which thymic epithelial cells interact with immature lymphocytes. A further step will be to confirm our hypothesis by study embryonal development of thymus innervation.

JNK ACTIVATION FOLLOWING SCIATIC NERVE LESION IN ADULT MOUSE

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ATTIVAZIONE DI JNK IN SEGUITO A LESIONE DEL NERVO SCIATICO IN TOPI ADULTI

There is great interest in discovering new targets for pain therapy since current tools for analgesia are often only partially successful. Although JNK activity in both DRGs and spinal cord is responsible for neuropathic pain condition produced by peripheral nerve injury, the relative contribution of each different JNK isoform is unclear. Moreover, intrathecal infusion of a specific JNK peptide inhibitor, DJNKI1, has been demonstrated to play a protective role on the onset and the maintenance of mechanical allodynia, a characteristic behavioral response for neuropathic pain. However, neuropathic pain was not permanently prevented, because allodynia occurred once the drug administration was terminated. Here, using a knock-out (KO) approach, we showed that sciatic nerve transection (SNT)-induced mechanical allodynia, measured by an automatic von Frey apparatus, is markedly attenuated in all KO mice for the different JNK isoforms. A time course study showed that within 24 hours from SNT in all mice the mechanical nociceptive thresholds decreased by 30-40% ipsilaterally, compared to the contralateral hindpaw. Only in wild types (wt) mechanical allodynia persisted for the 30-day

observation period. By 72h, JNK1, JNK2 and JNK3 KO began to progressively increase the paw withdrawal latencies. In a separate group of wt, in order to compare the KO for individual JNK isoforms to that of the knockdown for all JNKs, the inhibitor DJNKI1 was i.p. injected 30' before SNT. DJNKI1 treatment almost completely blocked mechanical allodynia onset at 24h and, even if reduced, the anti-allodynic effect was still present at day 30 post-SNT. Finally, in sections of injured DRGs of KO mice and age-matched wt, we analysed the expression by IHC of the growth associated protein (GAP) 43 and the Calcitonin Gene Related Peptide (CGRP), both regulated by nerve injury. The DJNKI1 treatment of wt allowed us to investigate the involvement of JNK on the expression of the above mentioned proteins. SNT induced GAP43 up-regulation that was prevented by DJNKI1 treatment. The number of GAP43-IR neurons on the ipsilateral side was unchanged in JNK1 and JNK3 KO. DJNKI1 also reversed SNT-induced CGRP down-regulation. 72h post-SNT, we found a marked decrease in the percentage of CGRP-IR neurons in wt and JNK3 KO. These results indicate that the inhibition of JNK pathway interferes with the onset and maintenance of mechanical allodynia after SNT, but JNK inactivation has also an effect on axonal sprouting following peripheral nerve injury. Moreover, sensory axon regeneration may require the activation of different JNK isoforms.

STUDY OF PERIPHERAL NERVE REGENERATION AFTER TRAUMATIC LESION

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STUDIO DELLA RIGENERAZIONE DEL NERVO PERIFERICO IN SEGUITO A LESIONI TRAUMATICHE

Recently much interest has been dedicated to the perspective of improving peripheral nerve repair and regeneration after traumatic lesion and, similarly to many other fields of regenerative medicine, great expectations have risen within the general public to the potential clinical application of tissue engineering in the treatment of damaged nerves. However, in spite of the scientific advancements, applications to the patients is still very limited and it appears that to optimize the strategy for the tissue engineering of the peripheral nerves in the clinical view, more basic science research is needed and neuroscientists have to strive for a new level of innovation which will bring together (in a multi-translational approach) the main pillars of tissue engineering, namely 1) Microsurgery, 2) Transplantation (of tissues, cells and genes), 3) Material science, 4) Physical therapy. In this presentation, we will focus on an example of successful translational research in tissue engineering, namely nerve reconstruction by muscle-vein-combined nerve scaffolds, on which we have carried out a series of experimental and clinical studies, as well as on some of the pitfalls which may arise in this research field.

VI SESSIONE
ANIMAL MODELS OF NEUROPATHOLOGIES: II. OTHER MODELS

BRAIN MORPHOLOGY IN OBESE ZUCHER RAT: A MODEL OF METABOLIC SYNDROME

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The metabolic syndrome (MetS) known also as "syndrome X" is a disorder characterized primarily by the development of insulin resistance. The current thought is that insulin resistance and subsequent hyperinsulinemia, originating from abdominal obesity, induces a number of disturbances. Several clinical trials have linked MetS and its individual components to an increased risk of cerebrovascular disese, cardiovascular disease and all-cause mortality. Obesity is suggested to be a risk factor for Alzheimer's disease and vascular dementia and is associated with impaired cognitive function in population-based investigations. The obese Zucker rat (OZR) represents a model of type 2 diabetes exhibiting a moderate degree of arterial hypertension and increased oxidative stress. It is thought that

OZRs correspond to early-stage cardiovascular complications associated with MetS. To clarify the possible relationships between MetS and brain damage, the present study has investigated brain microanatomy in OZRs compared with their littermate controls lean Zucker rats (LZRs). Male OZRs and LZRs 12 weeks old were used. Animals had brain removed and processed for analysis of nerve cell number, glial-fibrillary acidic protein (GFAP) immunoreactive astrocytes, expression of phosphorylated neurofilament (NFP) immunoreactive axon in frontal cortex, hippocampus and striatum. Synaptophyisin immunoreactivity, chosen as a marker of synaptic vesicle activity was assessed as well. A lower density of nerve cell profiles was observed in the frontal cortex and in the hippocampus of OZRs compared to LZRs. In the frontal cortex of OZRs a significant increase in the number of GFAP immunoreactive astrocytes was also noticeable. Similar findings were seen in the hippocampus, where an increased number of GFAP immunoreactive astrocytes was detected in the CA1 subfield and in the dentate gyrus of OZRs compared to the LZRs. OZRs developed as animal model of type 2 diabetes, may also represent a model for assessing the influence of MetS on brain and for clarifying possible relationships with neurological phenomena reported in obese individuals. The pattern of astrogliosis characterized in the present study suggests the occurrence of degenerative changes in OZRs, the extent and characterization of which merit further studies.

ROLE OF N-ACETYLASPARTATE (NAA) AND N-ACETYLASPARTYLGLUTAMATE (NAAG) IN EARLY STAGE OF PILOCARPINE-INDUCED STATUS EPILEPTICUS

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RUOLO DELL'N-ACETILASPARTATO (NAA) E DELL'N-ACETILASPARTILGLUTAMMATO (NAAG) NELLO STADIO PRECOCE DI STATO EPILETTICO INDOTTO DA PILOCARPINA

Approximately 50 million people worldwide are affected by epilepsy, one of the most common neurological conditions. Magnetic resonance imaging (MRI) techniques provide accurate anatomical definition, but despite their high resolution, these techniques fail to visualize the pathological neocortical and hippocampal changes in a sizable number of patients with focal pathologies. Further, visualized lesions on MRI may not all produce seizures. One of the keys to the understanding of the epileptogenic zone lies in the recognition of the metabolic alterations that occur in the setting of epileptic seizures. Magnetic resonance spectroscopy (MRS) is a valuable tool that can be used to study the metabolic changes in the brain of animal models of epilepsy. The present work shows a comparison between 1H-MRS and T2 maps on pilocarpine-induced *status epilepticus* (SE) in adult Wistar rats, in early stages of the acute phase when the initial precipitating injury, triggering epileptogenesis, occurs . Rats were scanned 5 and 15 minutes after pilocarpine injections and from 5 minutes to 4 hours after SE-onset , in order to investigate differences in T2 relaxation as well as in the metabolites levels in three brain regions (Motor Cortex, Medial Thalamus and Hippocampus). These regions were chosen because recent evidences pointed out a critical role of extra-hippocampal regions in the etiopathogenesis of pilocarpine-induced epilepsy. T2 values show significant difference between control and pilocarpine-treated animals only 240' after SE-onset in the Motor Cortex. In the other regions of the brain, no significant change in T2 can be observed at any investigated time point. LCModel quantification on 1H-MRS spectra shows a statistically significant decrease of (NAA+NAAG)/(GPC+PCh) in the Motor Cortex in the treated rats, compared to controls, starting from the first investigated time point (5' after pilocarpine injection). No significant alteration of (NAA+NAAG)/(GPC+PCh) was observed in Medial Thalamus and Hippocampus. The present results suggest that NAA+NAAG changes, detected by MRS, may precede morphological lesions, detected by T2, in the pilocarpine model. Moreover separate concentrations of the neuronal compounds values of NAA and NAAG were determined by HPLC method in order to investigate distinctly the importance of this two amino acids. 1H-MRS and T2 relaxation maps constitute two non invasive techniques, which can allow a deep investigation in the early epilepsy stages, as proved in the present work. We are hereby proposing that 1HMRS is able to identify differences in NAA+NAAG in

the cortex since early stages, before any alterations observed by T2 maps. Results show that 1H-MRS is a potential valuable tool in developing drug targeting experiments on this model of pilocarpine-induced epilepsy and for humans studies. This work also show the possible leading role of NAA+NAAG in the early stage of epileptrogenesis.

JNK PATHWAY INVOLVEMENT DURING KAINATE INDUCED EXCITOTOXICITY

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RUOLO DI JNK NEL DANNO DA ECCITOTOSSICITA' INDOTTO DA CRISI EPILETTICHE

Kainate (KA) is a convulsive agent that causes epileptic seizures with delayed neuronal damage in the limbic system. Excitotoxicity by KA activates complex signal transduction events, one of which consists in the c-Jun N-terminal kinase (JNK) pathway. JNK function is strictly dependent on its intracellular localisation. In particular JNK activation in mitochondria seems to play an important role in cell death and its inhibition by the peptide DJNKI1 represents a useful tool against neurodegeneration induced by seizure activity. We have previously shown that KA causes massive cell death in the hippocampus: in Nissl-stained sections, stereological counts showed a significant decrease in neuronal density in all CA fields, both at 1 and 5 days after seizures, which was partially prevented by DJNKI1 treatment. These results were confirmed by counts of degenerating neurons in CA3 in FluoroJade B-stained sections. Following these data, we analyzed the activation pattern of JNK isoforms in the mitochondria and the effects of DJNKI1 administration following KA-triggered excitotoxicity. We performed systemic administration of KA (15 mg/Kg) on adult male Sprague Dawley rats and we collected the hippocampus after 3, 6 and 12 hours. WBs on mitochondrial fraction of the hippocampus revealed that, in untreated rats, basal activity mostly depended on JNK1 isoform, but, following KA, JNK3 became the dominant activated isoform. Moreover, DJNKI1 completely prevented the mitochondrial increase in JNK3 activation as well as the cytochrome c release and PARP activation. Also, we observed the decrease of the ratio Bcl-2/Bax after KA and, most importantly, the increase of this ratio in DJNKI1 treated animals, confirming that JNK is a central mediator of apoptosis and neurodegeneration. Our study points out the role of mitochondrial JNK in excitotoxic-induced neuronal death and the capability of DJNKI1 as a neuroprotective drug.

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VII SESSIONE
TROPHIC FACTORS, NEUROMEDIATORS AND RECEPTORS

MORPHOLOGICAL CHANGES INDUCED BY NEUROPEPTIDE IN VITRO STIMULATION OF HUMAN SALIVARY GLANDS

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MODIFICAZIONI MORFOLOGICHE INDOTTE DALLA STIMOLAZIONE IN VITRO CON NEUROPEPTIDI DI GHIANDOLE SALIVARI DELL'UOMO

Salivary secretion is controlled by the synergistic activity of the two autonomic nervous system sections. The stimulation of parasympathetic muscarinic receptors causes a copious secretion of water and electrolytes, whereas the stimulation of sympathetic β-adrenergic receptors produces a small amount of secretion rich in exocytosed proteins (1). Morphological studies on salivary glands show that a dramatic sequential reorganization of the luminal acinar cell membrane accompanies exocytosis (2) and that stimulation of either β-adrenergic or muscarinic receptors induces two distinct exocytosis-coupled-to-endocytosis mechanisms (3). Besides the classical cholinergic and adrenergic neuromediators, numerous peptides, collectively designated as non-adrenergic non-cholinergic (NANC) agonists, are known to participate with different effectiveness in the secretory mechanisms and hence influence the composition of saliva (4,5). Using microfilament fluorescence staining and ultrastructural analysis, we described the dynamic changes induced in the rat parotid gland by in vitro stimulation with a number

of neuropeptides, and compared them to those elicited by □ -adrenergic and muscarinic agonists (6). In this study, using the same methodological approach, we report that Substance P, contrary to what seen in the rat, fails to induce the morphological changes associated with granule exocytosis or fluid secretion in the human parotid gland and that Vasoactive intestinal polypeptide and Peptide histidine-methionine elicit changes indicative of secretory activity in the human submandibular gland. The results being in agreement with the outcome of other methodological approaches, these procedures may contribute effectively to functional studies on the regulation of gland activity and secretory mechanisms. Considering the high interspecific variability, which advises against the extrapolation to man of data obtained in other species, it may be particularly useful in human tissue analyses, as it may overcome the obvious limitations of experimental treatments.

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DISTRIBUTION OF VASOACTIVE INTESTINAL PEPTIDE, NEUROPEPTIDE Y, SUBSTANCE P AND NEUROTENSINE IN NORMAL HUMAN THYMUS.

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DISTRIBUZIONE DEL PEPTIDE INTESTINALE VASOATTIVO, DEL NEUROPEPTIDE Y, DELLA SOSTANZA P E DELLA NEUROTENSINA NEL TIMO UMANO NORMALE

Human thymus of healthy subjects has been studied in order to visualize the morphological distributive pattern of several neuropeptides such as vasoactive intestinal peptide, neuropeptide Y, substance P and Neurotensine with the aim of underline their relation in neuroimmunomodulation in human thymus in physiological conditions. Morphological and morphometrical analysis following immuno-histochemical staining for the above neuropeptides were performed. In normal thymus the distribution of these neuropeptides is localized near the nerve fibres and thymus cells in different lobular zones. In subcapsular parenchyma numerous substance P positive nerve fibres were observed and a specific localization of neurotensine positive fibers in cortico-medullary and medullary zone of thymus lobules was shown. The perivascular and parenchymal distribution of analysed neuropeptides are well in accordance with supposed regulatory function of nerve and cells secreting neuropeptides into thymus. Thymus is widely innervated by sympathetic and parasympathetic nerve fibres that enter the thymus along with blood vessels and branch into the cortex, the capsular and septal system, the cortico-medullary junction and the medulla. Previous works observed that nervous fibres in thymus are widely involved in the complex T-lymphocytes maturation process. Experiments performed in our laboratories demonstrated that adrenergic and cholinergic nerve fibres of the thymus change after administration of immune-stimulating drugs. Indeed today the existence of cross talk between the nervous system and immune system is a matter of fact. Inside the human thymus, a population of substance P positive epithelial cells has been discovered, localized in the subcapsular zone. All these observations demonstrate the presence in the thymus gland of an original microenvironment in which nerve fibres and neuropeptides together have a fundamental functional relevance on a correct T-lymphocyte maturation and differentiation.

DISTRIBUTION OF VASOACTIVE INTESTINAL PEPTIDE, NEUROPEPTIDE Y, SUBSTANCE P AND NEUROTENSINE IN THE HUMAN THYMOMA.

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DISTRIBUZIONE DEL PEPTIDE INTESTINALE VASOATTIVO, DEL NEUROPEPTIDE Y, DELLA SOSTANZA P E DELLA NEUROTENSINA NEL TIMOMA UMANO.

We have studied the role of some peptidergic nerve fibres in the human thymoma, in order to add new clinical data on thymoma neuroimmunomodulation. In the human thymoma the distribution of neuropeptides positive nerve fibres is quantitatively reduced while the cells immune-positive to VIP and substance P are quantitatively increased in comparison with thymus of the healthy subjects. The modification of neuro-peptides pattern in thymoma suggest a role of the studied neurotransmitters in some autoimmunity diseases (Myastenia Gravis frequently associated with Thymoma). The human thymus provides critical signals for the expansion of T-cell precursors and terminal differentiation of a limited number of mature, non auto-reactive T-cells.
The thymus is under the control of the autonomic nervous system (sympathetic and para-sympathetic nerve fibres). Moreover, somatic nerve fibres reach the thymus through the phrenic nerve. Neuropeptides are known to play a role of neurotransmitters or co-transmitters in peripheral nervous system , for example vasoactive intestinal peptide has been observed to be frequently associated to cholinergic nerve fibres while neuropeptide Y is frequently associated to catecholaminergic nerve fibres. Morphological and Immune-histochemical observations were carried out on samples of human thymoma harvested during surgery. Our results provide direct evidence of the presence in the human thymoma of the following neuropeptides: Neurotensin, Neuropeptide Y, Vasoactive intestinal polipeptide and Substance P. The presence of specific neuropeptides in the human thymus suggests that a special neurotransmission is required for the induction of its immune role; furthermore, also the development and maturation of thymus cells is under the control of the autonomic nervous system. Our research aims to develop our knowledge on the neuropeptides of the human thymoma in order to better understand their role in pathological conditions.

NEUROPEPTIDE S INCREASES CONDITIONED REINSTATEMENT OF ETHANOL SEEKING BY ACTIVATION OF HYPOTHALAMIC HYPOCRETIN SYSTEM: NEUROANATOMICAL EVIDENCES

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IL NEUROPEPTIDE S AUMENTA LA RICADUTA NELLA RICERCA DELL'ALCOL ATTIVANDO IL SISTEMA IPOCRETINERGICO IPOTALAMICO: EVIDENZE NEUROANATOMICHE

The association of ethanol's reinforcing effects with specific environmental stimuli is thought to be a critical factor for relapse risk in alcoholism. Here we describe a significant role of Neuropeptide S (NPS) system on reinstatement of ethanol-seeking induced by environmental stimuli previously associated with ethanol and predictive of its availability in rats. Results showed that intracerebroventricular (ICV) injection of NPS (1.0 and 2.0 nmol/rat) did not modify ethanol self-administration. In the reinstatement experiments, ICV NPS treatment (1.0, 2.0 and 4.0 nmol/rat) resulted in a significant increase of ethanol seeking elicited by ethanol-associated cues. We also found that ICV NPS administration activates c-Fos expression in different brain areas and in particular in Hypocretin-1/Orexin-A (Hcrt-1/Ox-A) immunoreactive neurons in the lateral hypothalamus (LH). Site-specific NPS injection (0.1 and 0.5 nmol/rat) into the lateral hypothalamus also reinstated extinguished responding to ethanol, an effect that was selectively blocked with the Hcrt-1/Ox-A receptor selective antagonist SB334867. Overall results provide compelling support for the role of the NPS receptor system in the regulation of ethanol-related behaviours and indicates that activation of the NPSR facilitates Hcrt-1/Ox-A system activity in the LH that, in turn, appears responsible for the marked augmentation of conditioned reinstatement of ethanol-seeking.