
San Benedetto del Tronto
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Consorzio Universitario Piceno
21° Convegno Nazionale del
Gruppo Italiano per lo Studio
della Neuromorfologia
G.I.S.N.
9-10 Giugno 2011
Centro Congressi Palariviera
San Benedetto del Tronto (AP)

Giovedì 9 Giugno 2011

09:30 – 12:30
SYMPOSIUM: PLASTICITY OF THE CEREBRAL CHOLINERGIC SYSTEM
SIMPOSIO: PLASTICITÀ DEL SISTEMA COLINERGICO CEREBRALE
Simposio congiunto con il 34° Congresso della Società Italiana di Istochimica
Moderatori: Prof. D Zaccheo, Prof. F. Amenta

- THE BRAIN CHOLINERGIC SYSTEM: FROM NEUROBIOLOGY TO THERAPY
G. Pepeu
Il sistema colinegico cerebrale: dalla neurobiologia alla terapia

-DEGENERATIVE DEMENTIAS. CLINICS, NEUROPSYCHOLOGY AND NEUROTRANSMITTER SYSTEMS
A.M. Fasanaro, G. Colucci, S. Carpi
Valutazione clinica delle demenze ad esordio nell'età adulta con particolare riferimento alla identificazione dei sistemi neurotrasmettitoriali coinvolti

- NEUROIMAGING AND DIAGNOSIS OF ADULT-ONSET DEMENTIAS
F. Zeccolini
Neuroimaging e diagnostica delle demenze ad esordio nell'età adulta

11:00 -11:30 Coffee break

- PRESYNAPTIC MODULATION OF CHOLINERGIC NEUROTRANSMISSION
M. Marchi, S. Zappettini, A. Salamone, G. Olivero,M. Grilli
Modulazione presinaptica della neurotrasmissione colinergica

-PRECLINICAL EVIDENCE OF AN ASSOCIATION WITH CHOLINERGIC PRECURSORS AND CHOLINESTERASE INHIBITORS IN AN ANIMAL MODEL OF CEREBRAL VASCULAR DAMAGE
S. K. Tayebati, D. Tomassoni, I. E. Nwankow, F. Amenta
Esperienze precliniche sull'associazione tra un precursore colinergico ed inibitori delle colinesterasi in modelli animali di danno cerebrale di tipo vascolare

- ASSOCIATION BETWEEN THE CHOLINESTERASE INHIBITOR DONEPEZIL AND THE CHOLINERGIC PRECURSOR CHOLINE ALPHOSCERATE IN ALZHEIMER'S DISEASE WITH ASSOCIATED CEREBROVASCULAR INJURY. FIRST RESULTS OF THE ASCOMALVA TRIAL
F. Amenta, A. Carotenuto, A.M. Fasanaro, A. Lanari, R. Rea, E. Traini.
Primi dati clinici sull'associazione donepezil/colina alfoscerato nella malattia di Alzheimer con danno vascolare. Il trial ASCOMALVA

12:30 Lunch

14:30 – 15:15
I SESSION/I SESSIONE
ANIMAL MODELS OF NEUROPATHOLOGIES
MODELLI ANIMALI DI PATOLOGIE DEL SISTEMA NERVOSO
Moderatori: Prof. M. Del Fiacco, A. Vercelli

- MRI INVESTIGATION AND HISTOPATHOLOGICAL EVALUATION OF THE BRAIN OF OLD EPILEPTIC RATS AFTER A PROLONGED PERIOD OF RECURRENT LIMBIC SEIZURES
A.Andrioli, P.Marzola, E.Nicolato, Bentivoglio M.
Dept. Neurological Sciences (DSNNMM), University of Verona, Verona, Italy
Risonanza magnetica e studi di istopatologia del'encefalo di ratti epilettici di età avanzata dopo un periodo prolungato di crisi limbiche ricorrenti

- MOTOR BEHAVIOR TESTS TO DETECT EARLY SYMPTOMS IN A MOUSE ALS MODEL.
A. Piras, V.Valsecchi, M. Boido, G. Spigolon and A. Vercelli
Test per il comportamento motorio per determinare i sintomi precoci in un modello di topi SLA.

- STUDY OF ADULT RATS DRG NEURONS AND SATELLITE CELLS INTERACTION: AN *IN VITRO* MODEL
A. Giovannelli, A. Foggetti, L. Muratori, I. Perroteau, M. Fornaro
Studio dell'interazioni tra neuroni di gangli della radice dorsale e cellule satellite: modello in vitro

15:30-17:00
II SESSION/II SESSIONE
SYSTEMATIC, CHEMICAL AND DEVELOPMENTAL NEUROMORPHOLOGY
NEUROMORFOLOGIA SISTEMATICA, CHIMICA E DELLO SVILUPPO
Moderatori: Prof. M. Del Fiacco, A. Vercelli

- DISCRETE SUBREGIONS OF THE HUMAN NUCLEUS CUNEATUS SHARE NEUROCHEMICAL FEATURES WITH THE PROTOPATHIC NUCLEI
M. Del Fiacco, M. Quartu, M.P. Serra, M. Boi.
Distinte subregioni del nucleo cuneato dell'uomo hanno caratteristiche neurochimiche in comune con i nuclei protopatici

- A PRELIMINAR REPORT ON SEROTONIN IMMUNOREACTIVE NEURONS IN THE HUMAN CEREBELLAR CORTEX
P. Flace, L. Lorusso, V. Benagiano, G. Ambrosi
Dati preliminari su neuroni immunoreattivi per la serotonina nella corteccia cerebellare di uomo

- VAMP, SNAP-25 AND SYNTAXIN IN GLUTAMATERGIC AND GABAERGIC SYNAPSES OF THE RAT CEREBELLAR CORTEX
L. Lorusso, P. Flace, A. Rizzi, L. Bosco, V. Benagiano, R. Cagiano, G. Ambrosi
VAMP,SNAP-25 e syntaxina nelle sinapsi glutamatergiche e gabaergiche della corteccia cerebellare di ratto

- DISTRIBUTION OF VGLUT-1, VGLUT-2 AND SNAP-25 WITHIN MOSSY FIBRE TERMINALS OF THE RAT CEREBELLAR CORTEX
V. Benagiano, L. Lorusso, P. Flace, A. Rizzi, G. Ambrosi
Distribuzione di VGLUT-1, VGLUT-2 and SNAP-25 in terminali di fibre muscoidi della corteccia cerebellare di ratto

- NERVOUS CONTROL OF THE HUMAN LYMPHATIC VESSELS
C. Cavallotti, M. Sabbatini, F. Mignini
Il controllo nervoso dei vasi linfatici umani

- NERVOUS CONTROL OF THE LOWER UTERINE SEGMENT (LUS)
C. Cavallotti, A. Tinelli, A. Malvasi
Il controllo nervoso del segmento uterino inferiore (SUI)

17:00 - 17:30 Coffe Break

17:30 – 18:00
THE G.I.S.N. RESEARCH GROUPS
LE UNITA' DI RICERCA DEL G.I.S.N.
Prof. Carla Ghelardini, Dr. Lorenzo Di Cesare Mannelli, Prof. Alessandra Pacini,
Dip. Farmacologia Preclinica e Clinica e
Dip. di Anatomia, Istologia e Medicina Forense, Università di Firenze

NEUROPATHIC PAIN: LOOKING FOR A DISEASE MODIFYING AGENT
Il dolore neuropatico: alla ricerca di un farmaco curativo
C. Ghelardini, L. Di Cesare Mannelli, Matteo Zanardelli, L. Bonaccini, A. Pacini
Moderatori: Prof. F. Amenta, Prof. D. Zaccheo

18:00 - 19:00
CONSIGLIO DEI SOCI DEL G.I.S.N.

Gli argomenti all'ordine del giorno sono i seguenti:
1. Comunicazioni del Presidente e del Segretario Generale;
2. Approvazione del Conto Consuntivo del Bilancio 2010;
3. Approvazione del Bilancio di Previsione 2011;
4. Sede e data XXII Convegno Nazionale del G.I.S.N.;
5. Domande di ammissione di nuovi Soci;
6. Varie, ed eventualmente sopravvenute

20:30 Cena Sociale

Venerdì 10 giugno 2011

9:30 – 10:30
III SESSION/III SESSIONE
TROPHIC FACTORS, NEUROMEDIATORS AND RECEPTORS
FATTORI TROFICI, NEUROMEDIATORI E RECETTORI
Moderatori: Prof. R. Pellitteri, Prof. G. Panzica,

- EFFECT OF THIOCTIC ACID AND α-GLYCERYL PHOSPHORYL-CHOLINE ON ASTROGLIAL CELL PROLIFERATION AND DIFFERENTIATION IN PRIMARY CULTURE
V. Bramanti , D. Bronzi, S. Grasso , G. Malfa , D. Tomassoni , B. Tomasello , M. Renis , F. Amenta, R. Avola
Effetto dell'acido tioctico e dell' α-gliceril fosforil colina sulla proliferazione e differenziazione di cellule astrogliali in coltura primaria

- REDUCED HYPOTHALAMIC BDNF LEVELS IN WNIN OBESE MUTANT RATS
J. K. Sinha, N. V. Giridharan, M. Raghunath
Riduzione dei livelli di BDNF nell'ipotalamo di ratti mutanti obesi WNIN

- MICROGLIA RESPONSE TO SYSTEMIC OR CENTRAL EXPOSURE TO LIPOPOLYSACCHARIDE
J.M. Gemechu, G. Grassi-Zucconi, M. Bentivoglio, A. Andrioli
Dept. of Neurological Sciences (DSNNMM), University of Verona, Verona, Italy
Risposta della microglia alla somministrazione sistemica o centrale di lipopolisaccaride

- CXCL12/CXCR4 LIGAND-RECEPTOR PAIR IS INVOLVED IN GLIO-VASCULAR INTERACTIONS DURING BRAIN DEVELOPMENT
D. Virgintino, M. Rizzi, M. Errede, F. Girolamo M. Strippoli, L. Roncali
La coppia ligando-recettore CXCl12/CXCR4 e' coinvolta nelle interazioni glio-vascolari nel corso dello sviluppo del cervello

10:30 - 11:00
LETTURA STORICA
PATRIOT SCIENTISTS DURING THE *RISORGIMENTO*
M. Bentivoglio
Scienziati patrioti nel risorgimento
Moderatori: Prof. Marina Del Fiacco, Prof. G. Ambrosi

11:00- 11:15 Coffe Break

11:15 – 11:45
FINANZIAMENTI EUROPEI ALLA RICERCA NEUROBIOLOGICA
Nicola Bergonzi
Agenzia Per la Promozione della Ricerca Europea (APRE)
Moderatore: Prof. F. Amenta

11:45 – 13:00
IV SESSION/IV SESSIONE
NEURODEGENERATION, AND PHARMACOLOGICAL THERAPY
NEURODEGENERAZIONE, NEURORIGENERAZIONE E TERAPIA FARMACOLOGICA
Moderatori: Prof. D. Virgintino, Prof. S.K. Tayebati

- NEURAL STEM CELL RESPONSE TO INFLAMMATORY DEMYELINATION IN A MODEL OF PROGRESSIVE MULTIPLE SCLEROSIS
M.Strippoli, G.Ferrara, M.Errede, M.Rizzi, C.Coppola, L.Roncali, R.Perris, D.Virgintino, F.Girolamo
Risposta delle cellule staminali neurali alla demielinizzazione infiammatoria in un modello di sclerosi multipla progressiva

- OLFACTORY ENSHEATHING CELLS PROTECT CORTICAL NEURONS CULTURES EXPOSED TO HYPOXIA
R. Pellitteri , A. Russo, S. Stanzani and D. Zaccheo
Le cellule gliali olfattive proteggono i neuroni corticali in coltura esposte ad ipossia

- EFFECTS OF EARLY AND PROLONGED SYSTEMIC DELIVERY OF ROLIPRAM AFTER ACUTE SPINAL CORD CONTUSION
S. Geuna, L.M. Costa, J. E. Pereira, S. Raimondo, G. Ronchi, E. Nikulina, M. T. Filbin, A. S.P. Varejão
Efficacia della somministrazione sistemica, precoce e prolungata di rolipram dopo lesione contusiva acuta del midollo spinale

- THERAPEUTIC EFFECTS OF COMBINATION OF MESENCHYMAL STEM CELL AND NEURAL PRECURSOR CO-TRANSPLANTATION WITH ENRICHED ENVIRONMENT HOUSING IN A MURINE MODEL OF SPINAL CORD INJURY.
M.Boido, A.Niapour, H.Salehi, E.De Amicis, A.Vercelli
Effetti terapeutici della combinazione del trapianto di cellule staminali mesenchimali e precursori neurali e dell'alloggiamento in ambiente arricchito in un modello murino di trauma spinale.

- CENTRAL NERVOUS SYSTEM CHANGES IN A MODEL OF COMPRESSIVE NEUROPATHY: THIOCTIC ACID ENANTIOMERS ACTIVITY.
E. I. Nwankwo, F. Amenta, L. Di Cesare Mannelli, A. Pacini, L. Bonaccini, C. Ghelardini, S. K. Tayebati and D. Tomassoni.
Alterazioni del sistema nervoso centrale in un modello di neuropatia compressiva: attivita degli enantiomeri dell'acido tioctico.

13:00
Chiusura del Convegno

SYMPOSIUM: PLASTICITY OF THE CEREBRAL CHOLINERGIC SYSTEM
SIMPOSIO: PLASTICITÀ DEL SISTEMA COLINERGICO CEREBRALE
Simposio congiunto con il 34° Congresso della Società Italiana di Istochimica

THE BRAIN CHOLINERGIC SYSTEM: FROM NEUROBIOLOGY TO THERAPY

G. Pepeu
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The brain cholinergic system is formed by neurons located in the cholinergic nuclei of the brain stem and midbrain, in the forebrain cholinergic nuclei, and by the striatal interneurons. The brain stem and midbrain neurons project mainly to the thalamus, hypothalamus and the forebrain nuclei. These in turn project to the cerebral cortex, hippocampus, amygdala and olfactory bulb forming a diffuse cholinergic network. The cholinergic neurons are characterized by specific features: cholinoacetyltrasferase coupled to a high affinity choline uptake mechanism for synthesizing acetylcholine (ACh), the presynaptic vesicles storing it, and the dependence on NGF. ACh released in the synaptic cleft acts on different subtypes of muscarinic and neuronal nicotinic receptors and is inactivated by acetylcholinesterase and butirylcholinesterase . Activation of the central cholinergic neurons induces arousal and plays a role in attention and memory formation. Striatal cholinergic neurons regulate motor activity. The brain cholinergic neurons undergo moderate degenerative changes during aging and mild cognitive impairment, and severe degeneration in Alzheimer's disease (AD) and other neurodegenerative diseases including alcoholic dementia and Parkinson's disease. The cholinergic hypofunction associated with neurodegenerative disease and schizophrenia, as well as that induced by cholinergic receptor blockade, result in cognitive impairment. Attempts to treat the cognitive deficits in AD and schizophrenia, and to improve memory in aging, have been made in the last 30 years by using cholinesterase inhibitors (ChEIs), receptor agonists, choline precursors, and by increasing NGF levels. Consistent, albeit temporary, results have been only obtained with ChEIs, and new nicotinic agonists seem promising.

DEGENERATIVE DEMENTIAS. CLINICS, NEUROPSYCHOLOGY AND NEUROTRANSMITTER SYSTEMS

A.M. Fasanaro, G. Colucci, S. Carpi

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Dementia is becoming a common disease in the Western word and the number of cases is estimated to increase in the next years. Alzheimer's disease is the most common form of dementia in the elderly, accounting for 50%-56% of cases diagnosed clinically or at autopsy. Alzheimer's disease is characterized by deterioration of memory and other cognitive domains, and leading to death 3-9 years after diagnosis.

It is important for clinicians to recognize early signs and symptoms of dementia and to identify modifiable risk factors and early disease markers. Identification of neurotransmitter systems most affected in single patients may contribute to establish the most appropriate treatments. This identification is possible using sophisticated imaging techniques or to some extent with carefully done neuropsychological evaluation.

This work summarizes the main clinical and neuropsychological correlations of neurotransmitter systems involvement in adult-onset dementia disorders and how this may be used for proper pharmacotherapeutic approaches.

NEUROIMAGING AND DIAGNOSIS OF ADULT-ONSET DEMENTIAS

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"Dementia" is the generic term for diseases in which memory problems are associated with the failure of other mental functions as to make the person dependent. Alzheimer's disease is the most common form of dementia (50%). Vascular dementia is also very common (18%). They meet often combined forms. The age is the greatest risk of contracting the disease. However, memory problems are not always a symptom of an early dementia! The mental faculties are altered with age, the rate of assimilation of information is slower and it affects the learning ability and memory. This is the reason why older people are apt to forget and think they are affected by the onset of dementia. Through neuropsychological testing and imaging, but are unable to distinguish clearly between memory disorders associated with aging, by the onset of dementia. Vascular dementia is the second level of frequency (18%). It is caused by arteriosclerosis of the blood vessels of the brain, which leads to a slowing of movement. This results in the death of tiny areas of the brain when it comes to micro-infarctions or entire regions when it comes to major disturbances of the circulation (cerebral infarction). MRI is the method of micro-level to distinguish many heart attacks that might otherwise go unnoticed. Main symptoms: sudden appearance of cognitive disorders related to vascular problems, mood swings, erratic evolution and gradual worsening of the disease. Currently, neuroradiology plays an important role in supporting the diagnosis of dementia. Over the last decade there has been a great increase in the number of published studies applying neuroimaging techniques to the study of degenerative dementia, particularly Alzheimer disease.

PRESYNAPTIC MODULATION OF CHOLINERGIC NEUROTRANSMISSION

M. Marchi[1,2,3] , S. Zappettini[1], A. Salamone[1], G. Olivero[1] and M. Grilli[1]
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Presynaptic muscarinic and nicotinic acetylcholine receptors in the CNS are present on cholinergic and non cholinergic nerve terminals. They may interact with other metabotropic or ionotropic receptors and produce integrated responses which , in turn , generates antagonistic or synergistic effects. The cross-talk between receptors represents an important mechanism of neurotransmission modulation and plasticity. It can occur by direct physical interactions as in the case of G protein-coupled receptor heterodimerization, or it may involve intracellular pathways. The facilitatory or inhibitory action of one receptor might therefore depend on the function of the other receptors co-existing on the neuron. Recent studies have shown that this phenomenon also concerns the muscarinic and the nicotinic receptor subtypes[1]. The understanding of these interactions may allow a better evaluation not only the pharmacological effects of cholinergic drugs but also the normal physiological role of the natural neurotransmitter acetylcholine. This presentation will focus on the co-existence and the functional interaction between the release regulating presynaptic nicotinic or muscarinic receptors and other receptors co-existing on the same axon terminals.

1. Marchi M. and Grilli M., Progress in Neurobiology, 2010, 92:105-111.

PRECLINICAL EVIDENCE OF AN ASSOCIATION WITH A CHOLINERGIC PRECURSORS AND CHOLINESTERASE INHIBITORS IN AN ANIMAL MODEL OF CEREBRAL VASCULAR DAMAGE

S. K. Tayebati[1], D. Tomassoni[2], I. E. Nwankow[1] and F. Amenta[1]

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Cholinergic hypofunction is a trait of Alzheimer's disease (AD)and of other forms of dementias such as vascular dementia (VaD) and Lewy bodies dementia (LBD). The acetylcholinesterase (AChE)/ cholinesterase (ChE) inhibitors (Is) are one of the two classes of drug approved for AD treatment. ChE-Is are licensed for the symptomatic relief of mild to moderate AD. These drugs are used out of label as a therapeutic approach for treating cognitive disorders other than AD such as VaD and LBD. A main problem deriving from their use is that the benefits of AD symptomatic treatment are modest and not long lasting. It is also thought that the magnitude of benefit of this class of drugs can elicit is apparently marginal and difficult to detect and to measure clinically. Moreover, widespread use of AChE/ChE inhibitors may be accompanied by serious adverse events. Cholinergic precursors represent an old approach to treat cholinergic dysfunction, but the first drugs proposed did not show clinical benefit on symptoms of AD. Actually, evidence for an enhancement of ACh biosynthesis by choline or lecithin is faint. The same is not true for cytidine 5'-diphosphocholine and choline alphoscerate for which a modest improvement of cognitive dysfunction in dementia disorders is documented. The association of ChE-Is with phospholipids involved in choline biosynthetic pathways was proposed to further enhance cholinergic neurotransmission compared to single compounds. It could represent a strategy to provide a stronger cholinergic challenge in dementia. This study reviews the cholinergic hypothesis of geriatric memory dysfunction and discusses based on original finding the neurochemical bases of the association between ChE-Is and cholinergic precursors. Evidence of a possible neuroprotective. effect of the association in animal models is also presented.

ASSOCIATION BETWEEN THE CHOLINESTERASE INHIBITOR DONEPEZIL AND THE CHOLINERGIC PRECURSOR CHOLINE ALPHOSCERATE IN ALZHEIMER'S DISEASE WITH ASSOCIATED CEREBROVASCULAR INJURY. FIRST RESULTS OF THE ASCOMALVA TRIAL

F. Amenta[1], A. Carotenuto[1], A.M. Fasanaro[2], A. Lanari[3], R. Rea[1], E. Traini[1]

[1]Clinical Research Unit, School of Pharmacy,University of Camerino, Camerino; [2]Alzheimer's Evaluation Unit and Involutive Brain Diseases, National Survey of Hospital A. Cardarelli, Naples; [3]Unit of Neurology, Hospital C. Poma Mantova, Italy. E-mail: francesco.amenta@unicam.it

Here we summarize the first results of the ongoing clinical trial on the "Effect of association between a *c*holinesterase inhibitor (ChE-I) and choline alphoscerate on cognitive deficits in Alzheimer's disease associated with cerebrovascular impairment" (ASCOMALVA). This trial wants to assess if association between the ChE-I donepezil at the daily dose of 10 mg and choline alphoscerate at the daily dose of 1,200 mg/day was accompanied by changes in Mini Mental State Evaluation (MMSE), Basic Activities of Daily Living (BADL), Instrumental Activities of Daily Living (IADL) and Neuropsychiatric Inventory (NPI). This latter included evaluation of severity and of caregiver stress measures (NPIF and NPIS).

At the moment this double-blind trial has completed the 6 months observation of 70 patients of the 210 planned . Patients were aged between 56 and 86 years (mean 75 $\pm$ 10 years) and were included in the protocol with a MMSE score between 15 and 23. Patients should suffer from ischemic brain damage documented by neuroimaging (MRI and CT scan), with a score ≥ 2 in at least one subfield (white matter or basal ganglia) according to the new rating scale for age-related white matter changes (ARWMC). Recruited patients were then randomly allotted to an active treatment group (donepezil + choline alphoscerate) or to a reference treatment group (donepezil + placebo). The 70 patients so far analyzed were treated for 6 months and were examined at recruitement and after 3 and 6 months of treatment. Protocol plans to prolong treatment for 24 months and to check patients at 3, 6, 9, 12, 18 and 24 months after being enrolled in the trial.

Patients allotted to the reference treatment group showed a slight time- dependent worsening of MMSE, IADL and NPIS scores and no changes in the BADL and NPIF scores were noticeable. Treatment with donepezil plus choline alphoscerate improved compared to donepezil alone the different items analyzed except the BADL that was slightly worsened.

The above results suggest that association of the cholinergic precursor choline alphoscerate to the standard treatment with a ChE-I may represent a therapeutic option to prolong beneficial effects of cholinergic therapies in Alzheimer's disease patients with concomitant ischemic cerebrovascular disease.

I SESSION/I SESSIONE
ANIMAL MODELS OF NEUROPATHOLOGIES
MODELLI ANIMALI DI PATOLOGIE DEL SISTEMA NERVOSO

MRI INVESTIGATION AND HISTOPATHOLOGICAL EVALUATION OF THE BRAIN OF OLD EPILEPTIC RATS AFTER A PROLONGED PERIOD OF RECURRENT LIMBIC SEIZURES

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Temporal lobe epilepsy (TLE) is a neurological condition characterized by recurrent limbic seizures. In the pilocarpine model of TLE in rats, which reproduces features of TLE in humans, the initial status epilepticus is followed by a seizure-free period after which the animals develop spontaneous recurrent seizures (SRS) for the rest of their life. Magnetic resonance imaging (MRI) techniques applied to the brain in both humans and animals can provide non-invasive assessments *in vivo* and are widely used to obtain information of CNS structure and function. In the present study we used structural and functional MRI to investigate brain alterations in epileptic rats at an advanced age to determine the outcome of untreated TLE. Rats of 3 months of age were subjected to treatment with pilocarpine which was followed by status epilepticus; after a period of 15-45 days the animals developed SRS and were allowed to survive for 18-20 months. At the chosen end time-point, structural MRI acquisitions were performed in transversal and coronal sections of epileptic and age-matched control rats using high resolution T2-weighted sequences (Rapid Acquisition with Relaxation Enhancement, RARE) with slice thickness=1 mm and in-plane space resolution= 0.0137cm/pixel. Functional MRI acquisitions were performed by measuring the cerebral blood volume (CBV) and flow (CBF). T2-weighted image analysis of the epileptic animals consistently showed marked reduction of cortical thickness (especially of the parietal, piriform and entorhinal cortices) and hippocampal volume, with enlargement of ventricles and cerebral atrophy; damage in thalamic nuclei were also frequently found. CBV and CBF maps of the epileptic animals also revealed alterations and regional hypoperfusion compared to controls. The epileptic rats were sacrificed immediately after the imaging sessions, and histopathological brain features were investigated. These analyses confirmed brain degenerative alterations, which were especially evident in hippocampal, cortical and thalamic regions. This study presents for the first time imaging data in experimental epilepsy after a very long period of SRS, providing evidence of marked damage in various brain regions when seizures are not cured.
Supported by EC grant EPICURE (LSHM-CT-2006-037315)

MOTOR BEHAVIOR TESTS TO DETECT EARLY SYMPTOMS IN A MOUSE ALS MODEL.

A. Piras, V. Valsecchi, M. Boido, G. Spigolon and A. Vercelli
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Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disease that causes degeneration and death of upper and lower motoneurons, leading to weakness, spasticity, muscle atrophy and death due to respiratory failure within 2-5 years.
The etiology of ALS remains unknown, but familial 2% ALS cases are due to a point mutation in the gene coding for Cu^{2+}/Zn^{2+} superoxide dismutase (SOD1).
Alteration of the activity of this enzyme determine an oxidative stress imbalance correlated with protein and lipid structure damages.
In our laboratory, we studied mice carrying the mutant protein (most notably the G93A mutant SOD1) with high transgene copy number, reported to exhibit the first symptoms by postnatal day 90 (P90) and to die by P130. Affected males were identified by PCR; the expression of the transgene was measured by RT-PCR. In fact, disease onset correlated with the amount of transcript rather than to gene copy number. To study the onset and progression of motor symptoms, we used a battery of tests starting from the pre-symptomatic phase (6 weeks): scoring of motor deficits, weighing, performance on the rotarod task and paw grip endurance (PaGE). The animals were assessed weekly, and twice a week when symptoms became evident. The first days of tests were considered training. Neurological tests evaluated the presence of abnormalities, such as tremor or dragging of lower hindlimbs, but it was not always reliable, since sometimes the shift from a degree of weakness to the other was not linear. Weight generally decreased significantly only in late stages of the disease, i.e. when mice became unable to feed. Rotarod and PAGE tests, able to measure motor function abilities, were the most sensitive to detect the onset of the symptomatic phase. In conclusion, the association of several tests can detect the very early phase of the disease, thus allowing to test treatments very early in the history of the disease.

STUDY OF ADULT RATS DRG NEURONS AND SATELLITE CELLS INTERACTION: AN *IN VITRO* MODEL

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DRG neurons have a single axon that forms a T-shaped bifurcation; the long branch extends towards the periphery and forms the sensory endings in the skin, muscle, viscera and other organs, and the short branch enters the spinal cord.

The satellite glial cells (SGCs) are the main type of glia housed in the DRG. These cells usually wrap around individual sensory neurons, thus forming a complete envelope.

Little is known about SGC physiology and their interactions with neurons.

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. Improve our understanding about the correlation neuron-glia is an aspect very important in treatment of sensory fibers regeneration after nerve injury. Therefore, the aim of this study is to define an in vitro model to exam the relationship between cocultured satellite cells and neurons and show the importance of glial cells and their factor for axonal regrowth.

DRG cells, when dissociated and cultured in SFM medium, are able to form circle structures in which SGC arrangement was very interesting.

The present study focuses on the mechanisms that undergo the formation of these circular complexes, in particular, in order to investigate the contribution or requirement of neurotrophic factors, such as NGF, BDNF and NT3, in the arrangement of these structures, an *in vitro* analysis was performed.

Time lapse microscopy was used to study the cell-cell interaction and their ability to build up the complex geometrical aggregation.

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.

II SESSION/II SESSIONE
SYSTEMATIC, CHEMICAL AND DEVELOPMENTAL NEUROMORPHOLOGY
NEUROMORFOLOGIA SISTEMATICA, CHIMICA E DELLO SVILUPPO

DISCRETE SUBREGIONS OF THE HUMAN NUCLEUS CUNEATUS SHARE NEUROCHEMICAL FEATURES WITH THE PROTOPATHIC NUCLEI

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The brainstem cuneate (CN) and gracile (GN) nuclei receive innocuous tactile and proprioceptive primary somatosensory information and postsynaptic visceral nociception via the dorsal columns. The existence of still underappreciated interspecies differences has been suggested between rodents and primates. Early studies in our laboratory showed that, at variance with observations in laboratory animals, the neuropeptide substance P is detectable by immunohistochemistry in dense plexus-like nerve fibres and terminals localized to discrete subregions of the human CN. Here we report our findings aimed at a further neurochemical characterization of the human dorsal column nuclei.

Tissue distribution of a number of neuropeptides, trophic factors and neuroplasticity-associated proteins was analyzed by means of immunohistochemistry in postmortem specimens of medulla oblongata from subjects aged 21 gestation weeks to 78 years, with no signs of neuropathology.

Immunoreactivity to the neuropeptides substance P, calcitonin gene-related peptide, leucine- and methionine-enkephalin, somatostatin, galanin, and peptide histidine-isoleucine, to trophic factors belonging to the glial cell-derived neurotrophic factor and related receptors, and to the neuroplasticity-associated proteins growth-associated protein-43 and polysialylated-neural cell adhesion molecule labelled neuronal elements in restricted areas of the CN, located along its dorsal edge or embedded in the white matter of the cuneate fasciculus. Multiple immunolabelling in the same or adjacent sections showed that the examined substances, with respect to one another, were distributed as in the superficial layers of the spinal dorsal horn and trigeminal subnucleus caudalis. By contrast, the immunoreactivity detectable in the GN was sparse and not gathered in definite subregions.

The results obtained show that, at variance with that of laboratory mammals, including primates, the human CN contains one or more clear-cut subregions with neurochemical features similar to those of the protopathic relay nuclei and whose superficial layers may constitute a “gelatinous subnucleus”. The origin as well as the functional involvement of such innervation remains to be elucidated.

A PRELIMINAR REPORT ON SEROTONIN IMMUNOREACTIVE NEURONS IN THE HUMAN CEREBELLAR CORTEX

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INTRODUCTION. Immunohistochemical studies in various species have evidenced, in all layers of the cerebellar cortex, serotonergic nerve fibres with varicosities (1,2,3), originating from serotonergic neurons localized in the brain stem reticular formation and cerebellar nuclei (3, 4, 5). Furthermore, biochemical and pharmacological studies have revealed presence of different types of serotonin receptors in the cerebellar cortex of various species, including man, suggesting a functional role of serotonin neurotransmitter in cerebellar cortex circuitry (6, 7, 8). Data on the presence of serotonergic neurons in the cerebellar cortex are not yet available. Aim of this study was to ascertain, using immunohistochemistry, whether an intrinsic serotonergic neuronal system exists in the human cerebellar cortex.

MATERIAL AND METHODS. Fragments of human post-mortem cerebellar cortex were taken at autopsy 24h after death, fixed in an aldehyde solution, embedded in paraffin, sectioned into 5 μm sections, and submitted to LM immunohistochemical techniques using a rabbit anti-serotonin polyclonal antibody. To control the specificity of the antibody, we used as a positive control fragments of duodenum and medulla of rat, which were subjected to the same immunohistochemical procedure.

RESULTS. The controls of specificity revealed positivity for serotonin in the epithelial cells in the duodenum mucosa and neurons of the medullar raphe nuclei. In the cerebellar cortex, the immunoreactivity for serotonin was detected within neuronal bodies distributed in all layers of the human cerebellar cortex. In the molecular layer, it was detected in subpopulations of stellate and basket neurons; in the Purkinje neuron layer, in a subpopulation of Purkinje neurons; in the granular layer, in subpopulations of granules and large neurons (e.g.,. Golgi, candelabrum, Lugaro). Serotonin immunoreactivity was also observed in neuronal processes distributed throughout the cortex and extending in the white matter, and in putative terminals in the spaces of Held in the granular layer.

CONCLUSION. The present study reports the first demonstration of the existence of an intrinsic serotonergic neuronal system in the cerebellar cortex. Therefore, in addition to already demonstrated extrinsic serotonergic fibres, coming from outside the cerebellar cortex, intrinsic serotonergic fibres exist, originating from neuronal bodies localized in the cerebellar cortex. These data suggest that serotonin may play an important role in neurotransmission and neuromodulation mechanisms of the human cerebellar cortex.

REFERENCES

1. Takeuchi et al, Cell Tissue Res 226:1-12, 1982; 2. Bishop et al, J Comp Neurol 235:301-321, 1985; 3. Kerr & Bishop, J Comp Neurol 304: 502-515, 1991; 4. Bishop & Ho, Brain Res 331: 195-207, 1985; 5. Walker et al, J Comp Neurol 276: 481-497, 1988; 6. Hannon & Hoyer, Behav Brain Res 195: 198-213, 2008; 7. Li et al, Brain Res 617: 87-95, 1993; 8. Strahlendorf et al, Eur J Pharmacol 201: 239-242, 1991.

VAMP, SNAP-25 AND SYNTAXIN IN GLUTAMATERGIC AND GABAERGIC SYNAPSES OF THE RAT CEREBELLAR CORTEX

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The distribution and functional role of glutamate and GABA in the synapses of the mammalian cerebellar cortex is known. On the contrary, the molecular mechanisms underlying processes of trafficking and release of vesicles containing glutamate and GABA in the cerebellar synapses are still poorly understood. In order to investigate these mechanisms, we studied, using double light microscopy immunohistochemical techniques, the expression of synaptosomal associated protein of 25Kda (SNAP 25), syntaxin and vesicle associated membrane protein (VAMP)/sinaptobrevin, in glutamatergic and GABAergic synapses of the rat cerebellar cortex. The glutamatergic axon terminals were revealed by antibodies against the glutamate vesicular transporters vGlut-1 and vGlut-2, the GABAergic axon terminals, by antibodies against the isoforms 65 and 67 of the glutamic acid decarboxylase (GAD).
Experiments of double labelling for vGluT-1 and VAMP or SNAP-25 or syntaxin evidenced a large number of punctate elements (axon terminals) which expressed these co-localizations, but also some elements which do not expressed them. Experiments of double labelling for vGluT-2 and VAMP or SNAP-25 or syntaxin revealed total absence of co-localizations in the molecular/Purkinje layers and presence of them in punctate elements distributed in the granular layer. Double labelling for GAD and VAMP or SNAP-25 or syntaxin showed absence of co-localization, with the exception of some puncta localized on the deep pole of the body of Purkinje neurons.
These results indicated that VAMP, SNAP-25 and syntaxin are considerably involved in the regulation mechanisms of the cerebellar cortex synapses. VAMP, SNAP-25 and syntaxin are largely represented in the glutamatergic synapses, although they lack in the synapses formed by terminals of climbing fibres and of subpopulations of parallel and mossy fibres. Most of GABAergic synapses of the cerebellar cortex lack VAMP, SNAP-25 and syntaxin, the only type of GABAergic synapses containing VAMP, SNAP-25 and syntaxin being represented by that between basket and Purkinje neurons.

DISTRIBUTION OF VGLUT-1, VGLUT-2 AND SNAP-25 WITHIN MOSSY FIBRE TERMINALS OF THE RAT CEREBELLAR CORTEX

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Mossy fibres (MF) originate from neurons localized in brainstem and spinal cord and project to cortex of all cerebellar lobes. MF end in the granular layer, where they synapse on granule dendrites; GABAergic terminals from Golgi, candelabrum and Lugaro neurons modulate these synapses, so constituting synaptic complexes (glomeruli). Most of MF are excitatory and use glutamate as chemical neurotransmitter; MF containing aspartate, norepinephrine, acetylcholine and serotonin have been also described. In the present study, using techniques of multiple labelling in immunofluorescence, we analyzed in the granular layer of rat cerebellar cortex the distribution of immunoreactivity to VGluT-1 and VGluT-2, glutamate transporters which specifically characterize glutamatergic terminals, and SNAP-25, a protein localized on the membrane of synaptic terminals and involved in the regulation of glutamate release.

Experiments of multiple labelling for VGluT-1, VGluT-2 and SNAP-25, revealed axon terminals, lying in large numbers in the interstitial spaces among granule bodies, which displayed different patterns of expression of the immunoractivities: (1) terminals expressing only V-GluT-1 immunoreactivity; (2) terminals expressing co-localization of V-GluT-1 and SNAP-25; (3) terminals expressing only V-GluT-2 immunoreactivity; (4) terminals expressing co-localization of V-GluT-2 and SNAP-25; (5) terminals expressing only SNAP-25 immunoreactivity; (6) terminals expressing co-localization of V-GluT-1 and V-GluT-2; and (7) terminals expressing co-localization of V-GluT-1, V-GluT-2 and SNAP-25.

This study indicates that most of MF terminals express VGluT-1 and/or VGluT-2, markers of the presence of glutamate, and SNAP-25, which is associated with mechanisms of glutamate release. However, subpopulations of MF terminals which do not co-localize VGluT-1 and VGluT-2 and/or do not express SNAP-25 also exist. The MF terminals negative for VGluT-1 and VGluT-2 and positive for SNAP-25 could be represented by the non-glutamatergic contingents of them.

NERVOUS CONTROL OF THE HUMAN LYMPHATIC VESSELS

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In this paper the autonomic nerve fibres of the human lymphatic vessels were analysed. Classical histological, chemical and/or biochemical techniques for the analysis of nerve fibres within the wall of the human lymphatic vessels were used. The following experimental procedures were performed: 1) drawing of tissue containing lymphatic vessels; 2) cutting of tissue; 3) morphological staining of samples; 4) staining of nerve fibres for Ache; 5) fluorescence microscopy for the staining of ANF; 6) staining of NPY-like immune reaction; 7) Biochemical dosage of nor-adrenaline; 9) quantitative analysis of images (Q.A.I); 10) statistical analysis of data. Our results are reported in fig 1, fig 2 and table 1. As can be seen The human lymphatic vessels of large diameter (thoracic duct and lymphatic vessels of the lung) were provided with numerous adrenergic and cholinergic nerve fibres. Moreover a few nervous fibres have as neurotransmitter the peptide NPY. Biochemical dosage of nor-adrenaline shows that this drug is well represented I the wall of the major human lymphatic vessels.

The lymphatic vessels were studied in the last years by many Authors in different organs from a morphological to clinical point of view. Some morphological and functional studies on nervous fibres and lymphatic vessels hypothesised a close relation between these two structures in different human organs and tissues. After this hypothesis there was a growing interest for the morphological and/or functional links between nervous fibres and lymphatic vessels, but only few papers, focused on this topic, were published. Nevertheless an increased knowledge of the nervous control of the lymphatic vessels is more and more required to clarify the structural basis of many disorders affecting lymphatic circulation.

Figure 1. Acetylcholinesterase-positive nervous fibres, with plexiform distribution, within the wall of two adjacent lymphatic vessels.

Figure 2. Catecholaminergic nerve fibres within the wall of two adjacent lymphatic vessels. The fluorescence is scattered with zones of higher intensity (major concentration of mono amines) and zones of lesser intensity (decreased concentration of neurotransmitters).

NERVOUS CONTROL OF THE LOWER UTERINE SEGMENT (LUS)

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The human uterus shows three distinctive anatomic and functional regions: the cervix (including the lower uterine segment or LUS), corpus and fundus. The Lus, normally, has not a clear delimitation. In fact it is well defined only during the parturition. The cervix and LUS are lined by a columnar epithelium, which is frequently removed in surgical curettage. This uterine region can be recognized by two properties: 1) its stroma is different from endometrial fibromuscular stroma of other uterine regions, 2) its epithelium does not participate in the cyclic changes of the functional uterine endometrium. Nevertheless cervix and LUS are richly innerved by autonomic nerve fibers and these fibers are provided with numerous neurotransmitters.

Materials and Methods: during autopsies small specimens of the uterus were collected in a total of 14 dead women (35.6 ± 4.9 years old). In this group four are pregnant and ten none. The samples were harvested in three different regions of the uterus (fundus, corpus and cervix). All samples were cut in slices and immune-chemically stained for nerve fibers the contain the following neurotransmitters: 1) Adrenaline; 2) Nor-adrenaline; 3) Dopamine; 4) 5-OH-triptamine; 5) Acetyl-choline; 6) Enkefaline; 7) Substance P; 8) vaso-intestinal-peptide; 9) Oxitocine; 10) Prolattin. After staining morphological-metrical quantification of each neurotransmitter was performed

Results: Our results demonstrate that samples coming from uterine fundus, corpus and cervix are differently provided with each studied neurotransmitter. In particular, the cervix (including LUS) shows an high amount of both nerve fibers and related neurotransmitters while the uterine corpus and fundus posses a lower number of nerve fibers and lower amount of neurotransmitters.

In fact, many authors demonstrated that the pregnant uterus don't shows an increase of nerve fibers and blood vessels. Nevertheless in the uterine cervical blood vessels and myometrial smooth muscle fibers of pregnant women we found an increased number of nerve fibers immune-reactive for SP and VIP.

THE G.I.S.N. RESEARCH GROUPS
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NEUROPATHIC PAIN: LOOKING FOR A DISEASE MODIFYING AGENT

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Neuropathic pain is an unpleasant, abnormal signalling associated with injury or malfunction in the peripheral or central nervous system (CNS). These widespread disorders are induced by metabolic insult or trauma, autoimmune diseases, infections, drug or toxin exposure, and by cancer or viral chemotherapy. Neuropathies are extremely difficult to treat and actual therapies are generally palliative and include conservative non-pharmacological therapies, drugs and more invasive interventions. Available drugs for pain improvement are not able to revert the nervous alteration or to induce tissue regeneration [1]. On the other hand, many growth factors for the nervous system do not relieve pain. NGF, the prototypical neurotrophic factor, maintains the survival of sympathetic and sensory neurons as well as neurite outgrowth but it also exerts profound biological effect on nociceptors that express high-affinity NGF receptors [2]. Therefore, a great deal of interest has evolved around the research of compounds able to decrease hyperalgesia and to induce, at the same time, neuroprotection or neuroregeneration.

Pain is the common symptom due to variable nervous tissue alterations leading to different neuropathies. Traumas induce morphological changes evident also further than the lesion site. Damage to peripheral nerve are able to induce a decrease in myelin thickness, axon diameter and number of fibers in the proximal and, at a lesser extent, in the distal part of the nerve. An important inflammatory component is present as oedema and macrophagic infiltrate [3]. Moreover, in a model of peripheral neuropathy induced in the rat by loose ligation of the sciatic nerve (Chronic Constriction Injury; CCI) the activation of the apoptosis cascade has been described both in proximal and distal portion of the nerve. A mitochondrial damage alters membrane permeability, cytochrome C is released in the cytosol and triggers the signal up to the fragmentation of the genome [4]. Animal treatment with Acetyl-L-Carnitine (ALCAR; 100 $mgkg^{-1}$ i.p. twice daily for 14 days), but not with L-Carnitine or Gabapentin, prevents apoptosis induction. ALCAR is also able to prevent hyperalgesia and both the anti-apoptotic and the anti-hyperalgesic effects are reverted by the nicotinic receptor (nAChR) antagonist mecamylamine [5]. On the other hand, in the same model, acute administration of the alpha7 nAChR agonist PNU-282987, 10 and 30 mg kg^{-1} p.o. (15 days after ligation), is able to reduce hyperalgesia in a methyllicaconitine-reversed manner. This alpha7 nAChR agonist exerts no analgesic effects. Chronic PNU-282987 treatments, 30 mg kg^{-1} once a day for 7 days and 10 mg kg^{-1} for 28 days are able to decrease pain perception. Repeated treatments with PNU-282987 reduce the presence of oedema and macrophagic infiltrate and, on the coronal sections of the nerve, a significant higher myelin sheath, axonal diameter and number of fibers are observable [3].

In other neuropathies, like those chemotherapy-dependent, the effect of neurotoxicity is lesser pronounced and detectable and a smir of signs diffuse from the peripheral (PNS) to the central nervous system has been highlighted. Characteristically, an important component of oxidative stress is present both in the PNS and in the CNS. Both neuropathy groups show as common characteristic a glial activation in CNS. Astrocytes and microglia have well-documented roles in pain [6]. Although astrocytes and microglia in the CNS each have unique roles in the modulation of neuronal function [7], they have some overlapping actions. Both cells types are key mediators of the CNS

innate immune response. Growing evidence ascribe to glia pathological effects as neuronal hyperexcitability and chronic inflammation. Spinal microglia have been recognized as pivotal in the initial phases of neuropathic pain, whereas astrocytes may be involved in the maintenance [8,9]. On the other hand glia has a number of housekeeping function, among them neuroprotection [10,11]. It is important to consider that both astrocytes and microglia are necessary for the homeostasis of the environment surrounding neurons, and also for the regulated clearance of apoptotic cells.

A major challenge that new drug-development strategies for the treatment of neuropathic pain face is targeting the pathological and pain trigger actions of astrocytes and microglia without altering their protective and recuperative roles. In order to evaluate the glia role in neuropathic pain, we recently described a decrease of the anti-hyperalgesic growth factor Artemin a member of the glial cell line-derived neurotrophic factor (GDNF)-related family in the CCI model. The neuroprotective compound ALCAR is able to normalize this expression level as well as to induce Artemin expression in sham rats [12]. Topic of debate is whether this ALCAR effect is due to a nicotinic mechanism. nAChR signalling has a major role in the glia-neuron network, in particular alpha7 nAChR is expressed in microglial cells [13] and its activation attenuates the pro-inflammatory response of microglial cultures [14]. Also astrocytes express this receptor subtype but their possible functional role is poorly understood [15]. In conclusion, glia is strongly involved in neuropathic pain as well as in neuroregenerative mechanism and the stimulation of the glia-expressed alpha7 nAChR shows anti-hyperalgesic and neuroprotective effects. Further understanding of the molecular mechanisms that underlie the effects of glia on neuropathy processing and more evidence about the importance of nAChRs in the signal switch between pain and regeneration should lead to the development of new and more efficient approaches for the clinical management.

References

1.Di Cesare Mannelli L, Ghelardini C, Toscano A, Pacini A, Bartolini A. Neuroscience 165:1345-1352, 2010

2.Pezet S, McMahon SB. Annu Rev Neurosci 29:507-538, 2006

3.Pacini A, Di Cesare Mannelli L, Bonaccini L, Ronzoni S, Bartolini A, Ghelardini C. Pain, 150:542-549, 2010

4.Di Cesare Mannelli L, Ghelardini C, Calvani M, Nicolai R, Mosconi L, Vivoli E, Pacini A, Bartolini A. Eur J Neurosci 26:820-827, 2007

5.Di Cesare Mannelli L, Ghelardini C, Calvani M, Nicolai R, Mosconi L, Toscano A, Pacini A, Bartolini A. J Neurosci Res 87:200-207, 2009

6.Pocock JM, Kettenmann H. Trends Neurosci 30:527-535, 2007

7.Haydon PG. Nature Rev Neurosci 2:185-193, 2001

8.Marchand F, Perretti M, McMahon SB. Nat Rev Neurosci 6:521-532, 2005

9.Scholz J, Woolf CJ. Nat Neurosci 10:1361-1368, 2007

10.Glezer I, Simard AR, Rivest S. Neuroscience 147:867-883, 2007

11.Milligan ED, Watkins LR. Nat Rev Neurosci 10:23-36, 2009

12.Vivoli E, Di Cesare Mannelli L, Salvicchi A, Bartolini A, Koverech A, Nicolai R, Benatti P, Ghelardini C. Neuroscience, 2010. PMID: 20302919

13.Carnevale D, De Simone R, Minghetti L. CNS Neurol Disord Drug Targets 6:388-397, 2007

14.Suzuki T, Hide I, Matsubara A, Hama C, Harada K, Miyano K, Andrä M, Matsubayashi H, Sakai N, Kohsaka S, Inoue K, Nakata Y. J Neurosci Res 83:1461-1470, 2006

15.Ono K, Toyono T, Inenaga K. Neuroscience 154:994-1001, 2008

III SESSION/III SESSIONE
TROPHIC FACTORS, NEUROMEDIATORS AND RECEPTORS
FATTORI TROFICI, NEUROMEDIATORI E RECETTORI

EFFECT OF THIOCTIC ACID AND α-GLYCERYL PHOSPHORYL-CHOLINE ON ASTROGLIAL CELL PROLIFERATION AND DIFFERENTIATION IN PRIMARY CULTURE

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Thioctic acid plays a crucial role as antioxidant and metabolic component of some enzymatic complexes involved in glucose metabolism of different cell types. α-glyceryl phosphoryl-choline (α-GPG) is a semi-synthetic derivative of phosphatidylcholines representing, among acetilcholine precursors, a relatively new cholinergic drug.
In the present study, we evaluated the expression of some proliferation and differentiation markers in 15 DIV astrocyte cultures treated with 50 μM (+)thioctic acid or (+/-) thioctic acid and/or α-GPC (10μM).
Immunocytochemical analysis showed that astroglial cells cultures treated with (+)thioctic acid or (+/-) thioctic acid and/or α-GPC were GFAP positive.
Western blotting analysis showed that GFAP and vimentin expression increased after treatment with (+)thioctic acid compared to control ones; nevertheless, not significant modification of GFAP and vimentin expression was observed in α-GPC-treated astrocyte cultures. In addition, the treatment with (+)thioctic acid and α-GPC both togheter induced an enhancement of vimentin expression compared to control ones, but not significant modification of GFAP expression in (+)thioctic acid plus α-GPC treated cultures compared to control ones was found.
The treatment with (+)thioctic acid alone or in combination with α-GPC induced, also, an highly significant enhancement of cyclin D1 expression, a well known proliferation marker, in astrocyte cultures compared to untreated control ones; nevertheless, not significant modification of cyclin D1 expression in α-GPC-treated astrocyte cultures was observed.
Alkaline Comet assay analysis showed that the treatments with (+)thioctic acid or (+/-) thioctic acid did not increase the DNA fragmentation, conversely a genoprotective effect was observed.
Moreover, are in progress in our laboratory other experiments concerning the evaluation of well known proliferation, differentiation and apoptotic biomarkers, such as Ornityne decarboxilase, MAP-Kinase, PARP in (+)thioctic acid and/or α-GPC-treated astrocyte cultures.
This in order to evaluate the neuroprotective role played by these metabolic and antioxidant molecules, in astroglial compartment, during interactive cross-talk between glial and neuronal cells, after brain lesions or damages.

REDUCED HYPOTHALAMIC BDNF LEVELS IN WNIN OBESE MUTANT RATS

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Brain-derived neurotrophic factor (BDNF) plays many roles in the central nervous system, encompassing neuronal development, synaptic plasticity, and cognitive functions. It has been reported that BDNF reduction in hypothalamic nuclei may affect the strength of synaptic connections and dendritic spine density, leading to altered behaviors. Such studies in rodents have correlates in humans affected by WAGR (Wilms' tumor, aniridia, genitourinary anomalies and mental retardation, with obesity) contiguous gene syndrome whose gene deletions extend into the *BDNF* locus. Furthermore, BDNF heterozygous mice develop an eating behavior disorder leading to obesity, and fat BDNF heterozygous mutant (FBH) mice are leptin- and insulin-resistant. This indicates a possible cross-talk between the BDNF and insulin growth factor 1 signaling pathways that may be involved in the control of various physiological processes, including food intake and metabolism, as well as ageing. The WNIN obese (WNIN-Ob) mutant rat model developed at the National Center for Laboratory Animal Sciences (NCLAS; NIN, Hyderabad) from the Wistar rat inbred colony (WNIN) exhibit hyperphagia, increased cholesterolemia, triglycerdemia, and leptinemia, with decreased lifespan. This is the first inbred rat mutant of its kind developed from an inbred laboratory stock and is currently the heaviest rat (≅1.5 kg) of its kind in the world. In the present study we investigated the BDNF level in the hypothalamus and cerebral cortex of WNIN-Ob rats. Tissue samples from these brain regions of male WNIN-Ob rats of 4-6 months of age were examined using the BioPlex system. This is a highly sensitive multiplex suspension array technique which utilizes the principle of capture sandwich immunoassay to measure protein levels in tissue extracts and biological fluids. A significant decrease in BDNF concentration compared to WNIN control rats was found in the hypothalamus. In addition, BDNF levels were significantly decreased in the plasma, and were also decreased in the cerebrospinal fluid. No changes in BDNF level were instead detected in the cortex of WNIN-Ob rats, pointing to a regional selectivity of BDNF alteration in the hypothalamus. Such alteration could be involved in the obesity of these rats, and could also be potentially implicated in their decreased longevity. The expression and activity of TrkB receptor and downstream signaling molecules in WNIN-Ob rats of different ages will be examined in future studies to decipher ageing-related alterations of the BDNF signaling pathway in this rodent model.

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MICROGLIA RESPONSE TO SYSTEMIC OR CENTRAL EXPOSURE TO LIPOPOLYSACCHARIDE

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The response of microglia to a variety of insults to the brain is known since the discovery of microglia as resident cells of the brain parenchyma. In recent years, it has been definitely ascertained that microglia represent the main cell type of the brain immune response, undergoing a temporally and spatially regulated process of activation in response to different kinds of stimuli. The present study has been based on recent literature evidence on microglia polarization towards a "classical activation" (M1, potentially neurotoxic) or "alternative activation" (M2, potentially neuroprotective) in response to challenges. The regulation of this polarized process is, however, still largely unknown. We here investigated the induction of molecules which characterize the M1 and M2 microglia response to systemic (ip) or intracerebroventricular (icv) administration of the endotoxin lipopolysaccharide (LPS). These challenges elicit different inflammatory responses of the brain parenchyma; in particular, leukocyte infiltration in the brain occurs after icv but not after ip LPS exposure. Young adult mice were subjected to ip or icv LPS injection and sacrificed at different time points during the first 24h, compared with matched saline-treated control mice. Analyses with the pan-T cell marker CD3 confirmed the occurrence of T cells in the brain parenchyma after icv but not after ip LPS injections. Immunohistochemical phenotyping of microglia was pursued to reveal major histocompatibility complex class II (MHCII) antigen, a key molecule in M1 activation, and chitinase 3-like 3 (YM1), part of the M2 molecular repertoire. In first 24h after LPS exposure, MHCII-immunolabeled microglia were observed after both ip and icv injections, whereas no YM1-immunopositive microglia were observed in either LPS-treated group. Interestingly, MHCII immunolabeling peaked at 6h after ip LPS injections and at 24h after icv LPS injection, indicating a different temporal regulation of this event. In addition, mice subjected to ip LPS injection were examined after 5, 7 or 10 days: MHCII immunosignal persisted, although weaker, until day 7; YM1-immunopositive microglia were observed at days 7 and 10. Analyses at the same time points after icv LPS injection are at present in progress. Altogether the findings point out dynamic processes of microglia activation and its polarization over time. Further experiments will elucidate the range of differences in polarized microglia responses after inflammatory challenges which involve different dynamics of T cell recruitment to the brain.

CXCL12/CXCR4 LIGAND-RECEPTOR PAIR IS INVOLVED IN GLIO-VASCULAR INTERACTIONS DURING BRAIN DEVELOPMENT

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We have investigated glio-vascular interactions in developing cerebral cortex of human foetuses at midgestation, specifically examining the role that CXCL12/CXCR4 ligand-receptor pair plays in regulating brain vascularization and microvessel sprouting. During brain development radial glia cells (RGCs) are uniquely situated by virtue of the contact that they maintain with both the ventricular and pial surfaces. Since the classical studies of Pasko Rakic (reviewed in Rakic P., Brain Res. Rev., 2007 and in Nat. Rev. Neurosci., 2009), RGCs have been recognized to contribute to cerebral cortex architecture and recently have been also demonstrated to act as a significant source of neural cells. Our hypothesis is that CXCL12, a potent migration and differentiation factor released by RGCs, may signal *via* its receptor CXCR4 to coordinate neuroblast migration and brain expansion with vessel growth, elongation and branching throughout glio-vascular interactions. The results demonstrate that vascular cells, endothelial cells and pericytes, grow in from the pial surface, closely associated with $CXCL12^{+}$ RGC fibers and with giant, transitional forms of cortex astrocytes. Deep in the cortex, at sites of active microvessel branching, together with the expression of CXCL12 by RGC fibers and perivascular astrocytes, the ligand-receptor pair has been also detected on sprouting endothelial cells and pericytes. These observations indicate that during normal brain development glio-vascular CXCL12/CXCR4-mediated effects may contribute to the paracrine and possibly autocrine control of microvessel growth.

LETTURA STORICA

PATRIOT SCIENTISTS DURING THE *RISORGIMENTO*

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We celebrate this year the 150th anniversary of the unification of Italy: in 1861 the Kingdom of Italy was proclaimed in Turin by a parliament formed by elected representatives from all parts of Italy (except Venice, which remained under Austrian rule until 1866, and Rome, under papal control until 1870). It is important to recall, in this context, that during the *Risorgimento*, science and science popularization represented a key element for the maturation of a national identity by promoting, in the formation of individuals as well as of the public opinion, ideas of freedom, unity and tolerance. It should also be recalled that a number of patriot scientists have been very active during the *Risorgimento*, fighting also in battlefields, and contributed to the making of the country. For example, Raffaele Pirìa (1814-1865), chemist, fought in 1848 in the battle of Curtatone and Montanara. While working as a research fellow in Paris, Pirìa had extracted in 1838 the chemical compound salycilic acid, thus making fundamental contributions to the history of aspirin. Agostino Bertani (1812-1886), physician, collaborated with Giuseppe Mazzini and Giuseppe Garibaldi in the movement for Italian liberation and was very active during key events of the *Risorgimento* (such as the insurrection in Milan in March 1848); he was also one of the strategists of the March of the Thousand. Pio Foà (1848-1923), a Garibaldi's volunteer during the Third Independence War and a soldier in battle of Bezzecca in 1866, was a student of Cesare Lombroso at the University of Pavia and of Friedrich D. von Recklinghausen in Strasbourg. Foà published pathological studies of neuropsychiatric diseases of asylum patients; he became Senator in 1908. Giulio Bizzozero (1846-1901) mentor of Camillo Golgi ((1843-1926) engaged in the Third Independence War and was Senator since 1890. Camillo Bozzolo (1845-1920), a pathologist who fought in the Third Independence War, has left seminal descriptions of haematological (multiple myeloma) and infectious (*Dyplococcus* pneumonia) diseases; he became Senator in 1910. At the opening of the academic year 1895-96, Golgi, then Rector of the University of Pavia, referring to the conquest of Rome in 1870, stated "*a Voi, che svolgete la svolgete la vostra attività nel campo degli studii, ora più che mai si impone l'obbligo di moltiplicare gli sforzi per far fruttare il lavoro di preparazione compiuto da quelli che vi hanno preceduto.. Essi hanno lottato e lottano per ottenere mezzi di studio e di insegnamento...*". This is a vibrant message, worth remembering nowadays.

IV SESSION/IV SESSIONE
NEURODEGENERATION, AND PHARMACOLOGICAL THERAPY
NEURODEGENERAZIONE, NEURORIGENERAZIONE E TERAPIA FARMACOLOGICA

NEURAL STEM CELL RESPONSE TO INFLAMMATORY DEMYELINATION IN A MODEL OF PROGRESSIVE MULTIPLE SCLEROSIS

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The ventricular and subventricular zone (VZ and SVZ) and the subpial layer I of the cerebral cortex contain neural stem cells that can generate both neurons and glia, even in adulthood. A population of glial fibrillary acidic protein (GFAP)-expressing cells in the SVZ (B cells) is the primary source of precursors cells. They produce a transit-amplifying cell population (C cells) that in turn gives rise to neuroblasts (A cells). Platelet-derived growth factor receptor-α (PDGFR-α) is an additional marker for B stem cells that has been recently identified in the adult brain where it regulates cell commitment to oligodendrocyte lineage. In an animal model of progressive Multiple Sclerosis, the chronic subtype of experimental autoimmune encephalomyelitis (EAE), we observed an early glial response to the inflammatory demyelination characterized by proliferation of PDGFR-α/NG2 oligodendrocyte precursor cells (OPCs), a population widely distributed within the adult brain, as an attempt to generate new remyelinating oligodendrocytes. In late stage of disease, demyelination progression was associated with reduction of PDGFR-α/NG2 OPCs and SVZ PDGFR-α/GFAP B cells, and also of subpial PDGFR-α stem cells, whereas the remaining subpopulation of SVZ GFAP-expressing B cells appeared unmodified. The proliferation potential of the oligodendrocyte precursors seems to be exhausted or blocked by persistent stimuli derived by inflammatory cells within the neuropil or circulating in cerebrospinal fluid. A better understanding of the pathogenic mechanisms of the oligodendrocyte lineage impairment in the chronic EAE could be useful for comprehension of remyelination failure in chronic inflammatory brain disorders.

OLFACTORY ENSHEATHING CELLS PROTECT CORTICAL NEURONS CULTURES EXPOSED TO HYPOXIA

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Numerous *in vitro* studies report that neurons are susceptible to hypoxia producing immediate cell damage or death. These effects could be prevented by high levels of growth factor. A source of growth molecules are Olfactory Ensheathing Cells (OECs) that represent a unique population of glial cells that, in the olfactory system, support the continuous neuronal turn-over and ensheath olfactory axons. *In vitro*, OECs, as source of neurotrophic factors and adhesion molecules, promote axonal growth; *in vivo* they form myelin promoting remyelination of damaged axons. Moreover, OECs utilization in transplantation appears to be a promising treatment for spinal cord injury (SCI). In this study, we used an *in vitro* approach to examine the effect of OECs on cortical neurons exposed to hypoxic insult measuring cellular damage and neuronal loss. Co-cultures of OECs from olfactory bulbs of postnatal rats (P1) and neurons from rat embryonic cerebral cortex (E15) were successfully established; hypoxia was obtained by inverting coverslips and cells processed immunocytochemically after a week using polyclonal antibody PGP 9.5 as neuronal marker. Furthermore, some neuronal cultures were added with glial cell line-derived neurotrophic factor (GDNF) for a week, to tentatively rescue cells from oxygen deprivation. Both neuronal cultures and co-cultures were grown in DMEM/FCS. Some coverslips of cortical neurons both in normal and hypoxic condition were considered as controls. Conditioned medium from OECs cultures was used to feed some cortical neurons coverslips in both conditions.

Our results show that in co-cultures of cortical neurons and OECs the number of neurons was significantly increased in comparison with control cultures. Moreover, these neurons exhibited a dense axonal outgrowth. OEC-conditioned media did not stimulate the neuronal survival. When the cultures were exposed to hypoxic insult the neuronal survival was very low both in controls and in GDNF-treated neurons. In co-cultures and in OEC-conditioned media cultures an increased neuronal survival was observed. These data suggest that OECs have the capacity to promote the survival and growth of cortical neurons *in vitro* exposed to hypoxic condition exerting a protective influence. This effect could be mediated by multiple growth factors secreted by OECs. As some experiments *in vivo* have shown that traumatic SCI is often accompanied by secondary insults such as ischemia or hypoxia, our results suggest that OECs might be considered a possible approach for restoration in traumatic injury.

EFFECTS OF EARLY AND PROLONGED SYSTEMIC DELIVERY OF ROLIPRAM AFTER ACUTE SPINAL CORD CONTUSION

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The selective phosphodiesterase type 4 inhibitor Rolipram is a promising putative therapeutic agent for the treatment of acute spinal cord injury (SCI). In this study, we evaluated, in a standardized rat experimental model of traumatic spinal cord injury, whether early and prolonged systemic delivery of Rolipram can improve post-traumatic outcome.

In 16 adult female rats, a moderate contusion injury (200 kdyn) was produced at the T10 vertebral level using the PSI Infinite Horizon impactor®. Animals were randomly assigned and blindly divided into 2 groups of 8 rats that received rolipram (3.18 mg/kg/day) dissolved in DMSO or to a control group of 8 animals that received only DMSO using an osmotic mini-pump. The pumps were implanted subcutaneously and removed after 2 weeks under isoflurane anaesthesia (5 min). At week 7 post-injury, the animals were sacrificed, the spinal cords were removed, fixed in paraformaldehyde and embedded in paraffin. Blocks were then serially cut (at 10-μm nominal thickness) perpendicular to the main spinal cord axis for a length of 20 mm (10mm upstream and 10 mm downstream from the crush site). For stereological assessment, design-based sampling and the Cavalieri method together with the unbiased point counting grid method were used to estimate the following parameters: lesion length, lesion volume and area of spared white matter at the lesion epicenter.

Results show that spared white matter at the lesion epicenter is significantly greater in the Rolipram group (461,313 ± 56,390 μm^2) than the control group (301,168 ± 181,333 μm^2). On the other hand, no significant differences were found in the lesion volume and lesion length between groups. These results suggest that early and prolonged systemic delivery of Rolipram is likely to have a positive effect by preventing white matter damage after acute spinal cord contusion injury.

-THERAPEUTIC EFFECTS OF COMBINATION OF MESENCHYMAL STEM CELL AND NEURAL PRECURSOR CO-TRANSPLANTATION WITH ENRICHED ENVIRONMENT HOUSING IN A MURINE MODEL OF SPINAL CORD INJURY.

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Stem cells have the ability to give rise to undifferentiated stem cells and to differentiate into committed mature cells, can support regeneration after an insult, show an anti-inflammatory potential and can interact with the host immune system: for all these reasons they seem good candidates in neuroregenerative medicine, since CNS has a limited capacity for self-repair after trauma or disease.

We developed an experimental model of spinal cord injury (SCI), reproducing in the mouse the damage to local neurons and to axons fibers observed in human patients. In previous experiments we demonstrated the therapeutic potential shown by mesenchymal stem cells (MSCs) and neural precursors (NPs): here we intend to test the eventual synergistic effect obtained by the combined graft of both stem cells.

We performed a spinal cord compression at vertebral T13 in adult mice and 2 weeks after SCI we injected a cell cocktail (2/3 NPs and 1/3 MSCs, for a total of 100.000 cells) directly into the lesion cavity. After the graft all the transplanted mice were housed in enriched environments in order to stimulate the locomotor activity of animals. Injured mice without graft served as controls: some of them were put in enriched cages, the others in conventional cages. In order to evaluate the functional recovery, mice underwent a battery of motor tasks. Three weeks after graft/saline, animals were sacrified and analyzed for effects of engraftment on the glial scar formation, astroglial activation, cellular and axonal damage.

Results relative to the control groups suggest that the additional physical exercise determined by enriched environment assures an improved recovery compared to normal environment: the glial cyst volume reconstructed with Neurolucida software appears smaller and the inflammation (in terms of GFAP-fluorescence intensity) appears reduced in the animals housed in enriched cages. These results correlate with the behavioural improvement shown by the motor/sensory tests.

Moreover the combination of exercise with stem cell graft further improves its therapeutic effectiveness, determining better histological results and consequently an higher recovery.

Therefore we propose that MSCs, delivering trophic and immunomodulatory molecules, can modulate the neuroinhibitory environment of the injured spinal cord and promote the integration of NPs: these positive results can be additionally enhanced by locomotor training.

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CENTRAL NERVOUS SYSTEM CHANGES IN A MODEL OF COMPRESSIVE NEUROPATHY: THIOCTIC ACID ENANTIOMERS ACTIVITY.

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Oxidative stress is a situation of imbalance between the production of free radicals leading to potential tissue damage. It has been suggested that excessive oxidative stress may have a relevant role in the development neurological disorders. Peripheral neuropathy is on of these disorders characterized by myelin suffering and axonal degeneration related to an increase of oxidative stress. Thioctic or alpha-lipoic acid (ALA) is a naturally occurring compound with an antioxidant activity. It has two optical isomers designated as (R+) and (S-). Naturally occurring ALA is the (R+)-thioctic acid, but synthetic ALA is a mixture of (R+) and (S-).

The purpose of the present study was to assess if compression of sciatic nerve, induced by loose ligation of it, is accompanied by an increased oxidative stress and central nervous system changes. This study has also investigated the role of ALA enantiometers treatment to prevent peripheral nerve damage related to oxidative stress. Loose ligation of the right sciatic nerve was performed in spontaneously hypertensive rats (SHR), used as a model of increased oxidative stress, and normotensive Wistar-Kyoto rats (WKY) used as a reference group. Animals with sciatic nerve ligation were left untreated or were treated intraperitoneally for 14 days with racemic-ALA (25 and 50 mg/Kg/day), (R+)-ALA(25 mg/Kg/day), (S-)-ALA (25 mg/Kg/day) and pregabalin (50 mg/Kg/day).

Brain of spontaneously hypertensive rats (SHR) develops astrogliosis and neuronal damage. Loose ligation as well lead to the increase of astrogliosis probably related to the increase of oxidative stress in this animal model of compressive neuropathy. Treatment for 15 days with (R+)-thioctic acid decrease number and size of astrocytes. The number of neurons was not affected by antioxidant treatments.

The above data reveal the occurrence of microanatomical changes in the central nervous system as a consequence of peripheral nerve damage and confirm the protective antioxidants role of thioctic acid. The more pronounced effect of (R+)-thioctic acid, demonstrated in this study, may have consequences worthwhile of being investigated in further preclinical and/or clinical studies.