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ZEISS

THE OSCILLATING BRAIN TISSUE: DAY/NIGHT VARIATIONS IN THE EXTRACELLULAR MATRIX OF THE LATERAL HYPOTHALAMUS

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BACKGROUND. We recently reported that the excitatory/inhibitory wiring of hypothalamic cell bodies containing the neuropeptide orexin-A (OX)/hypocretin-1, located in the lateral hypothalamus (LH), undergo a substantial reorganization during the period of wake predominance (the night in nocturnal rodents) and of sleep predominance (daytime). This opens key questions on diurnal synaptic plasticity phenomena in different neuronal populations in basal conditions and their potential mechanisms. Such mechanisms could involve extracellular matrix (ECM) components, as ECM is reported to be among the players regulating neural plasticity. OBJECTIVES. The present study was aimed at the study of the ECM in the LH of mice in basal conditions during day and night, and in a murine model of African trypanosomiasis or sleeping sickness, a parasitic encephalitis which causes chronic neuroinflammation and leads to alteration of the sleep/wake cycle and of the structure of sleep. METHODS. Healthy adult mice in basal conditions were sacrificed during daytime and at night. A matched experimental group of mice was infected with the *Trypanosoma brucei brucei (Tbb)* parasite and sacrificed at 14 days post-infection. Fluorescent histochemical labelling with the lectin *Wisteria floribunda* agglutinin (WFA), which binds glycoprotein residues (N- acetyl-D-galactosamine) within the ECM was combined with immunofluorescent labelling of OX neurons in the LH and of GABAergic interneurons containing parvalbumin (PV) in the neocortex, where the ECM is arranged in specialized structures, the perineuronal nets (PNNs). Confocal microscopy images were analyzed, and WFA expression was evaluated with Western blotting. RESULTS. In the neocortex, PNNs were observed around the somata and processes of PV-immunopositive interneurons, in agreement with previous reports. No day/night differences in the PNN were observed, but overt oscillation of PV immunoreactivity was seen in the labelled cell bodies. In the LH, no PNNs were found around OX cell bodies, and WFA positivity was seen in the neuropil. Concerning this ECM component, striking day/night variations were observed, with a diffuse distribution at daytime, and a different phenotype, with a more compact organization and a condensation around cell bodies, at night. Western blotting showed significant difference, with higher WFA expression during night compared to daytime. Interestingly, such oscillation was not observed in the LH of the infected mice. CONCLUSIONS. The results point to day/night oscillation of ECM organization and composition in the LH, but not in PNNs in the neocortex. The findings obtained in the infected animals point out an alteration of this mechanisms during experimental sleeping sickness. Altogether the data indicate regionally selective and marked day/night variation of ECM organization and molecular composition in the LH, which could be involved in synaptic plasticity phenomena, and the disruption of this organization in a chronic neuroinflammatory pathology which leads to sleep/wake dysregulation.

THE HUMAN CEREBELLAR DENTATE NUCLEUS: NEUROCHEMICAL AND CONNECTION DATA

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The dentate nucleus (DN) is the largest and most lateral nuclei of the cerebellum and is a part of the recent phylogenetically cerebrocerebellum. Chemiocytoarchitectural studies demonstrate that the DN is principally composed by two different neuron types, the small inhibitory GABAergic neurons, mainly involved in associative circuits, and the large excitatory glutamatergic projective neurons. These last are further classified in subtypes according to their nuclear position, shape of the cell body, and dendritic branching [1]. Nevertheless, some studies demonstrate also the presence of monoaminergic and peptidergic networks of varicose fibers in the dentate nucleus [2,3] but the presence of monoaminergic and peptidergic neuronal subpopulations is still lacking.
Aim of this study is the neurochemical investigation, by immunohistochemical methods, of the presence of serotonergic, dopaminergic and neurotensiergic neuronal systems in human dentate cerebellar nucleus. Paraffin sections of human postmortem dentate nuclei were tested with different polyclonal antisera to serotonin (5-HT), dopamine transporter (DAT), and neurotensin (NT). Immunoreaction were revealed by streptavidin-biotin technique and 3, 3'-diaminobenzidine (DAB) or DAB-nickel. Results demonstrate a strong positivity for all the antigens that, in particular, possess a point-like location in the neuropil (axon terminals), and in small and large neurons as well as in the perivascular neurons in a widespread manner.
These results demonstrate the presence, in the dentate nucleus, of a 5-HT, DAT, and NT neuronal systems probably involved in local and projective circuits and, although studies have already demonstrated the role of the dentate nucleus in motor and non motor functions, at present the functional role of these monoaminergic and peptidergic neurons as well as their projective pathways are still debated [4,5]. Nevertheless, data obtained by the recent developed Diffusion Magnetic Resonance Tractography (DMRT) technique, let to hypothesize basal ganglia and limbic structures as the targets of these monoaminergic and peptidergic neuronal subpopulations [4,5,6].
Hence, the presence of different perivascular neurons subpopulations positive for 5-HT, DAT and NT suggest that this cortico-cerebellar non-traditional neuron type may play a role both in the extrasynaptic release (volume transmission) of monoamines and peptides, and in the control of the blood brain barrier permeability [7,8].

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NEURAL CELL RESPONSES TO LOW FREQUENCY ELECTROMAGNETIC FIELD EXPOSURE: IMPLICATIONS REGARDING OXIDATIVE STRESS AND NEURODEGENERATION

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Many studies have investigated on the effects of electromagnetic fields (EMF) on human health, in particular, on the extremely low frequencies used in electrical power lines (50–60 Hz), and on the radiofrequency (RF: from 3 kHz up to 300 MHz) and microwaves (MW: from 300MHz up to 3 GHz), typical of cell phone and television transmissions. In previous our *in vitro* studies, we have demonstrated that a brief exposure to EMF at 900 MHz with an amplitude modulation at 50 Hz, on rat primary astrocytes induced oxidative stress and DNA fragmentation. We also demonstrated that this is not a thermic effect. Up today the mechanism of this effect is not well clarified. We hypotized that it may be related to the increase of intracellular calcium ions levels, probably due to the hyperstimulation of the glutamate receptors, which play a crucial role in the acute and chronic brain damage including neurodegerative diseases, such as Alzheimer and Parkinson Diseases.
Furthermore, since it is known that a reduced functionality of olfactory system represents an early sign of neurodegeneration, we assessed the effect of RF electromagnetic field modulated and not modulated in amplitude on Olfactory Ensheating Cells (OECs). This cell type shows characteristics of stem cells, expresses several growth factors, is able to promote axonal regeneration and functional restoration in the injured sites of Central Nervous System. Preliminary results show that a brief exposure to EMF is able to induce a significant increase in cellular viability and a considerable difference between cell cultures exposed to RF modulate and not modulated.
Studies are now in progress to better clarify the molecular mechanisms induced by EMF on OECs, in order to highlight also the involvement of glutamate receptors.

THE NEUROANATOMICAL FEATURES OF THE LOCUS COERULEUS IN NEURODEGENERATION

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The pontine nucleus Locus Coeruleus (LC) is part of the so-called isodendritic core of the brain stem reticular formation and is the main source of noradrenaline in the brain. LC is placed in the upper part of the pons, and is mainly formed by medium-sized neurons containing neuromelanin. Each one of two symmetrical LC nuclei is formed by up to 60.000 neurons in humans, which send axons profusely branching and innervating the entire cerebral and cerebellar cortices. By releasing noradrenaline through "bouton en passage" it modulates the activity of several cortical areas. In particular, LC modulates electroencephalogram activity, sleep/wake cycle, memory consolidation, and other cognitive functions, mainly related to attention, alerting and novelty orienting.
A significant LC cell loss has been shown in Parkinson's Disease (PD) and in cases of severe Alzheimer's Disease (AD) dementia, in *post-mortem* studies.
Exciting histological data suggest a very early involvement of LC in the pathogenesis of AD: accumulation of phospho-tau deposits in the axons of LC neurons seems to precede their occurrence in limbic regions in Mild Cognitive Impairment (MCI, the prodromal phase of Dementia) or even in pre-MCI stages. LC impairment seems to accelerate beta amyloid plaques deposition and neuroinflammation.
Recently, specific 1,5 and 3,0 Tesla Magnetic Resonance Imaging (MRI) protocols and post-processing analysis have been developed in order to detect neuromelanin-containing LC neurons *in vivo* in controls and in PD patients. In the presentation, after introducing the state of the art of the neuroanatomical features of LC in degenerative diseases, we report existing data on the MRI characterization of LC in PD and controls, as well as more recent evidences in AD and MCI. The potential role of these MRI data in helping to disclose the pathogenesis and contributing to the correct diagnosis in patients is discussed.

METHAMPHETAMINE AND PRION PROTEIN BRIDGING DRUGS OF ABUSE AND NEURODEGENERATION

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The cellular prion protein (PrPc) is physiologically expressed within selective brain areas of mammals. Despite not being fully elucidated, a physiological role of PrPc as a co-chaperone resembling that described for alpha-synuclein has been recently suggested. Conformational changes in the secondary structure of PrPc lead to a pathogenic isoform known as scrapie prion protein (PrPsc), which is characterized by high aggregation propensity, high insolubility and protease resistance. Prion protein's metabolism greatly depends on autophagy and ubiquitin proteasome cell-clearing systems. Methamphetamine (METH), which is a widely abused drug, produces severe neurotoxic effects mainly represented by an abnormal dopamine (DA) activity, which is in turn associated with neurotoxicity and protein misfolding. These effects produce a compensatory increase of chaperones while clogging cell-clearing pathways. When the clearing systems are clogged, high levels of PrPc may lead to its misfolding into PrPsc. Thus, in the present study we explored whether METH administration modifies the amount of PrPc and whether this triggers the appearance of PrPsc. To such an aim, we analyzed the effects of METH and DA administration in PC12 and striatal cells by using SDS-PAGE Coomassie blue, immune-histochemistry and immune-gold electron microscopy. To prove whether METH administration produces PrPsc aggregates, we used antibodies directed against PrP following exposure to proteinase K or sarkosyl, which digest folded PrPc leaving aside PrPsc. We found that METH triggers PrPsc aggregates in DA-containing cells while METH is not effective in primary striatal neurons, which do not produce DA. In the latter cells, exogenous DA is needed to trigger PrPsc accumulation similarly to what happens in DA containing cells under the effects of METH. The present findings, while fostering novel molecular mechanisms involving prion proteins, indicate that, cell pathology similar to prion disorders can be mimicked via a DA-dependent mechanism by a drug of abuse.

LINKING ACETYLATED α-TUBULIN TO NEURODEGENERATION IN PARKINSON'S DISEASE: AN IMMUNOHISTOCHEMICAL STUDY

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Context • Parkinson's disease (PD) is characterized by specific neuropathological hallmarks: i) the loss of dopaminergic neurons in *substantia nigra pars compacta* (SNpc), which project primarily to the *corpus striatum,* and ii) the presence of intra-neuronal aggregates called Lewy bodies (LBs) and Lewy neurites (LNs). PD etiology is not fully understood, but a combination of genetic and environmental risk factors has been proposed to take part in the pathogenesis. Among the multiple hits, microtubules dysfunction is emerging as a key player in triggering neurodegeneration in PD experimental models. Interestingly, the unbalance in tubulin posttranslational modifications has been reported as an early event specifically associated with dopaminergic neurons in MPTP mice model and the treatment with a microtubule stabilizer, epothilone D, exerts a neuroprotective effect on the dopaminergic neurons. This strongly suggests that the destabilization of microtubules could play an important pathogenic role in PD. Objective • The aim of this work was to investigate microtubule dysfunction in *post-mortem* human brains from PD patients. We focused on the distribution of acetylated α-tubulin, a modified form of tubulin that is strictly associated to microtubule stability, and its correlation with Lewy bodies pathology. Design • We analyzed different brain regions of PD patients at Brake stage VI (n=8) and age- and sex-matched control subjects (n=3). Results • Our results showed that acetylated α-tubulin accumulates in neuronal cell bodies of PD patients, compared to controls, in those regions that are commonly interested by LBs, such as dorsal motor nucleus of vagus, *substantia nigra*, nucleus basalis of Meynert and *putamen*. The analysis of the interplay between α-synuclein and acetylated α-tubulin revealed that neurons strongly positive for acetylated α-tubulin rarely accumulate LBs. Conclusions • This is the first study that points out alterations of acetylated α-tubulin in PD patients, thus proposing its pathogenetic role. Funding • Fondazione Grigioni per il Morbo di Parkinson (Milano).

MICROGLIA IN BASAL CONDITIONS: DAY/NIGHT VARIATIONS?

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Context • Microglia constantly scan brain microenvironment by their highly motile processes in normal and pathological conditions. Main microglia phenotypes can be distinguished in rodents: the ramified “resting” (i.e. "surveillant") microglia and the activated, hypertrophic microglia, which can reach an “amoeboid” phenotype. This microglial dynamics is critical for brain homeostasis and for defense processes. While microglia activation in various pathological conditions has been extensively investigated, microglia plasticity in normal, unchallenged conditions remains to be explored. This is of critical importance since, in basal conditions, transient microglia-synapse interactions seem to play a key role in synaptic activity regulation directly and/or by microglia-microvesicle (MV) release, which is dependent on neuronal activity. Moreover, we recently observed a daily reorganization of axosomatic synapses in orexin/hypocretin-containing neurons, which reside in the lateral hypothalamus (LH) and play a key role in wakefulness stability, but a participation of microglia in this process remains to be assessed. Objective • On this basis, the present study was aimed at the investigation of microglia plasticity in the LH, its possible involvement in diurnal changes of the synaptic wiring of orexinergic cell bodies, as well as MV release in the cerebrospinal fluid (CSF). Methods • Adult male CX3CR1-GFP transgenic mice, in which microglial cells are tagged with green fluorescent protein (GF), and Sprague-Dawley rats were sacrificed at two time points (night and day) in antiphase. Diurnal changes in the microglial morphology and microglia-synapse interactions were investigated in the transgenic mice. Triple immunofluorescence in confocal microscopy was used to visualize orexinergic cell bodies, and varicosities containing the vesicular GABA transporter (VGAT) or the vesicular glutamate transporter (VGluT)2 as presynaptic markers combined with postsynaptic scaffold proteins (gephyrin or the postsynaptic density protein-95 -PSD95-, respectively). The rats were used for the study of MVs of microglia/macrophage origin in the CSF using flow cytometry and fluorescent staining of isolectin B4. Results • 3-D reconstructions of the GFP-labelled microglia in the LH indicated a trend towards day/night changes in microglial cell morphology, which at night (when nocturnal rodents are predominantly awake) turned out to be endowed with more ramified processes than at daytime (the period of sleep predominance). Interactions between microglial processes and OX neurons have been observed during both day and night, and the study of the microglia-synapse contacts is currently in progress. Moreover, quantification of myeloid MVs in the CSF showed a significantly higher amount of MVs during night than day. Conclusions • The findings point to *i)* a trend towards plastic diurnal changes of microglia in the LH with more extended and branched processes during the animal’s wakefulness, and *ii)* a significant increase of release of MVs of microglial/macrophagic origin in the CSF at night, and therefore in relation with neuronal activity during the animal's period of wakefulness.

P66SHC DELETION REDUCES NEURODEGENERATION IN A MPTP-INDUCED ANIMAL MODEL OF PARKINSON'S-LIKE DISEASE

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P66shc is a vertebrate protein, one of three isoforms encoded by the ShcA locus. The other two isoforms, p46shc and p52shc, are involved in the regulation of Ras activation. Despite the high similarity at molecular level of p66shc, p52shc and p46shc, p66shc functionally differentiates from the other ShcA isoforms. Indeed, p66shc is involved in the regulation of intracellular redox balance and oxidative stress levels: it have been shown that the amount of reactive oxygen species decreases in p66shc-depleted cultivated cells, as well as p66shc-/- mice show diminished levels of both systemic and intracellular oxidative stress. Parkinson's disease (PD) is a neurological disorder characterized by a significant loss of dopaminergic neurons in the substantia nigra (SN) resulting in reduced striatal dopamine .Many evidences demonstrate that oxidative stress is involved in neurodegeneration and disease progression. Neurodegeneration in the SN is associated with a massive astrogliosis and excessive microglial activation, which in turn augments oxidative stress on dopaminergic neurons. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes the selective degeneration of mesencephalic dopaminergic neurons. MPTP-treated animals are widely used as models of PD. The robust glia activation found in MPTP-treated mice make this model particularly suitable for the study of oxidative stress involvement in PD.
In the present study we have used this parkinsonism model in p66shc knockout mice (P66shc-/-) to analyze oxidative stress role, mediated by p66shc, in neurodegeneration.
We observed by HPLC that p66shc deletion significantly reduced dopamine loss in the striatum of MPTP-treated mice. In these mice, astroglia resulted less activated at both caudate-putamen and SN level, suggesting a reduced alteration of nervous tissue. Also microglial cell activation was significantly reduced in p66shc-/- respect to wild type MPTP treated animals. This reduction was related to a possible neuroprotection since we observed a limited decline of tyrosine hydroxylase immunoreactivity and protein expression in the in both striatum and SN of p66shc-/- following MPTP injection.

UNRAVELING THE ROLE OF NURR1 IN A MURINE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Context • The nuclear receptor related 1 protein (Nurr1) belongs to the steroid nuclear hormone receptor class, but it is considered an orphan receptor since its activity is not regulated by ligands. The role of Nurr1 is still debated, but it seems implicated both in neuroprotection and immunomodulation in different neurodegenerative diseases, as Parkinson's disease and multiple sclerosis: indeed by cooperating with the CoREST complex, Nurr1 can repress the activity of the pro-inflammatory transcription factor NF-kB, therefore playing an anti-inflammatory role. Neuroinflammation is a pathological hallmark of many neurodegenerative diseases including amyotrophic lateral sclerosis (ALS). Objective • Here we aimed to unravel the role of Nurr1 in ALS, in order to identify a new therapeutic target. Design • By following the progression of disease in SOD1 G93A mice (the most widely used animal model of ALS), we have correlated the Nurr1 expression with the severity of the disease. The behavioral tests (rotarod and paw grip endurance test) have been employed to identify three different phases of the disease (pre-symptomatic, early and late symptomatic). Results • RT-PCR showed that Nurr1 mRNA expression is strongly up-regulated in the presymptomatic phase and at the onset of the pathology and decreases in the last phase. Moreover Nurr1 activates its target gene, BDNF, and probably blocks the transactivation of some NF-kB target genes (as iNOS). We also evaluated the Nurr1 expression by immunofluorescence reactions, in order to clarify which cells are specifically involved in this pathway: Nurr1 is stably expressed in neurons in WT, whereas in TG is strongly upregulated in astrocytes, in particular in the early symptomatic stage. No positivity has been detected in microglia. Conclusions • Our overarching hypothesis is that Nurr1 activation aims to modulate neuroinflammation and to protect motor neurons, at least at the onset of disease. Our observations, in association with the pharmacological modulation of Nurr1, could clarify the role of Nurr1 in such devastating disease.

β2-V287L MUTANT NICOTINIC ACETYLCHOLINE RECEPTOR INFLUENCE ON SYNAPTIC BALANCE AND CHOLINERGIC INNERVATION IN A MURINE MODEL OF AUTOSOMAL DOMINANT NOCTURNAL FRONTAL LOBE EPILEPSY (ADNFLE)

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Context • ADNFLE is a sleep-related epilepsy, characterised by focal hyperkinetic seizures, often arising in the frontal lobe during stage II of sleep. The ADNFLE families often bear point mutations on genes coding for subunits of the nicotinic acetylcholine receptors (nAChRs). Objective • We investigated the relationship between mutant heteromeric nAChRs and ADNFLE, by using a conditional murine model expressing the β2-V287L nAChR mutation. Design • We studied the expression of choline acetyltransferase (ChAT) in the prefrontal cortex (PFC), somatosensory cortex (SS), thalamus (TH) and three cholinergic nuclei involved in the sleep-wake cycle regulation. Next, we considered the total expression of vesicular glutamate transporters 1 and 2 (VGLUT1 and VGLUT2) and the number of VGAT+ (vesicular GABA transporter) and VGLUT1+ synaptic terminals contacting parvalbumin-positive (PV+) interneurons in the PFC. These analyses were performed on adult mice expressing β2-V287L (TG), as compared to the control (CTRL) littermates. Results • We estimated the number of ChAT-positive neurons in the cholinergic nuclei by using stereological counts, but no significant differences were found. Instead, the cholinergic innervation showed a significant increase in the PFC, SS and TH of P90 TG mice. Our preliminary data also showed that the expression of VGLUT1 tends to increase in layer V of TG mice, accompanied by an altered distribution of VGAT+ terminals on PV+ neurons. Conclusions • Our results demonstrate that β2-V287L mutation seems to locally alter the expression of ChAT, VGLUT1, VGAT and further studies are needed to clarify the role that these alterations may play in ADNFLE.

MICROTUBULE DEFECTS IN PROGRESSIVE SUPRANUCLEAR PALSY

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Context • Progressive Supranuclear Palsy (PSP) is a sporadic neurodegenerative disease, characterized by frequent falls, supranuclear vertical gaze palsy, pseudobulbar palsy and rigidity of the neck. To date, its etiopathogenesis remains elusive and there are not available treatments. PSP is considered a tauopathy, since its hallmark is the accumulation of the hyperphosphorylated form of the microtubule stabilizing protein Tau. Microtubules are key elements of cytoskeleton and they are fundamental in many cellular functions, such as maintenance of cell shape, cell migration and intracellular transport. Thus, the detachment of tau from microtubules, due to its hyperphosphorylation, could trigger the destabilization of this crucial component of the neuronal cytoskeleton and lead to cell death in PSP patients. Objective • Here we intended to evaluate if microtubule dysfunctions occur in PSP disorder. Our study was focused on bone marrow mesenchymal stromal cells (MSCs) and *post-mortem* brain tissues from PSP patients. Design • We characterized MSCs isolated from ten patients affected by PSP and analysed three *post-mortem* brain tissues from PSP patients. Results • We found that MSCs from patients showed altered morphology and growth. Interestingly, these undifferentiated cells display the imbalance in α-tubulin post-translational modifications, namely acetylation and tyrosination, and defects in microtubule stability. Looking at the distribution of acetylated α-tubulin in *post-mortem* brain tissues, we unraveled the substantial and area-specific accumulation of this modified form of tubulin in neuronal cell bodies. Conclusions • Our results provide the first evidence that defects in microtubule regulation and stability occur and are detectable in a non-neural compartment in PSP patients. Moreover, our preliminary results in *post-mortem* brain tissues strongly suggest that microtubule dysfunction could distinguish neurons of PSP patients. Funding • Fondazione Grigioni per il Morbo di Parkinson (Milano).

OXALIPLATIN-INDUCED BLOOD BRAIN BARRIER IMPAIRMENT: MORPHOLOGICAL AND MOLECULAR PATHWAY BY AN IN VITRO MODEL OF RAT BRAIN MICROVASCULAR ENDOTHELIAL CELLS.

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Context • Oxaliplatin is a well known chemotherapeutic organic compound especially used for metastatic colorectal cancer [1]. Despite its beneficial effects in tumour reduction, it leads to the pathogenesis of neuropathic pain, thus forcing physician for dose reduction and the therapy discontinuation [2]. While neurons and glial cells are well known to be implicated in these functional changes [3], very little is known about the chemotherapic agent interaction with the bloob-brain barrier (BBB). Main hypothesis • Even if it has been demonstrated that barrier hinders the chemotherapy's entry into the central nervous system [4], we postulated that oxaliplatin may enter the endothelial cells of the BBB vessels and trigger a signaling pathway that induce the disassembly of the tight junctions (TJs), the critical components of the BBB integrity. Experimental design • To ascertain our hypothesis and define the mechanistic pathway involved in the effects of oxaliplatin, we used biochemical and morphological assays to monitor the activation of the postulated pathway components and to assess changes in the molecular and functional phenotype of a rat brain endothelial cell line (RBE4), a widely accepted in vitro model for the study of the BBB [5]. Results • Our data show that exposing RBE4 cells to oxaliplatin caused a significant and rapid increase in ROS production which, in turn elicits a non-apoptotic ER-stress (as determined by the GRP78 overexpression, a known marker of this condition), and subsequent caspase-3 activation. This latter event leads to the proteolytic cleavage and thus the opening of pannexin-1 (panx-1), a large transmembrane channel that allows an ATP massive spillage. By extracellular enzymes, ATP is converted in adenosine which binds to its receptors inducing an alteration of BBB permeability. Although our results showed that the release of ATP is independent of panx-1 opening, the measured significant increase in extracellular ATP concentration is associated with a dislocation of the TJ Zonula occludens-1 and with F-actin stress fiber formation. Conclusion • To conclude, our results clearly show the oxaliplatin capability to impair the BBB permeability, indirectly acting on cytoskeleton organization and tight junctions, engaging the non-apoptotic role of ER stress and caspase 3 activation.

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NUCLEOLIN AS A POTENTIAL REGULATOR IN GLIOBLASTOMA NEOVASCULARIZATION

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Glioblastoma multiforme (GBM) is the most fatal brain cancer. The malignancy and drug resistance primarily reflect the existence of abundant disorganized microvessels with aggressive endothelial cells and pericytes and a population of proliferating glioma astrocytes. Understanding the mechanism that controls GBM stemness is important to benefit the prognosis of GBM patients. Nucleolin (NCL), which is responsible for ribosome biogenesis and RNA maturation, is overexpressed in GBM. However, the role of NCL in GBM development and drug resistance is still unclear. In this study, NCL has been studied in surgical samples from 26 GBM and 4 control adult subjects compared to 5 developing human brain specimens. NCL expression profiles reveals distinct subcellular localizations: tumor cells show cytoplasm/plasmamembrane and nucleoplasm NCL, whereas neurovascular cells show distinct nucleolar and nucleoplasm profile. Each cellular NCL subpopulation may play a different role in glioblastoma growth and neovascularisation. NCL is a promising marker for the GBM diagnosis and prognosis as hallmark of neurovascular cells and could predict chemotherapeutic efficiency against GBM neovascularization.

REPARATIVE EFFECT OF ANNEXIN A1 ON BRAIN MICROVESSELS DURING METABOLIC IMBALANCE

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The blood-brain barrier (BBB) is a complex structure primarily formed by endothelial cells sealed by tight junctions (TJ) which controls the regulation and maintenance of central nervous system (CNS) microenviroment homeostasis. The BBB integrity could be regulated by annexin A1 (AnxA1), a molecule belonging to glucocorticoid anti-inflammatory proteins and normally expressed in brain microvascular endothelial cells. Several studies demonstrated that metabolic imbalance is associated with alterations in BBB structure and function together with moderate inflammation and the activation of microglia cells and an increased incidence of cognitive impairment. To better understand the influence of metabolic imbalance on BBB function and the possible role of AnxA1, we analyzed, by immunofluorescence confocal microscopy, expression and distribution of TJs proteins, claudin-5 and occludin, vascular basal lamina (BL) molecule, laminin, and protein AnxA1 in brain microvessels of wild type (WT) and AnxA-knock-out (ANXA KO) mice fed with chow diet (CHOW) and with a high fat diet (HFD), and after administration of human recombinant AnxA1. The results showed a reduced expression of TJ proteins and a reduced laminin content in WT and AnxA KO HFD mice. After AnxA1 admistration, BBB-microvessel features seem to recover suggesting a protective effects of AnxA1 molecule. These findings confirm that during metabolic imbalence the BBB is damaged and suggest a potential effect of ANXA1 on BBB repair.

MODULATION OF THE ENDOCANNABINOID SYSTEM BY PLANT BIOACTIVE COMPOUNDS IN THE PHYSIOLOGICAL RESPONSE TO TRANSIENT COMMON CAROTID ARTERY OCCLUSION AND REPERFUSION.

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Context •The transient global cerebral hypoperfusion/reperfusion, triggered by induction of Bilateral Common Carotid Artery Occlusion followed by Reperfusion (BCCAO/R), has been shown to stimulate early molecular changes that can be easily traced in brain tissue and plasma, and that are indicative of the tissue physiological response to the reperfusion-induced oxidative stress and inflammation. Objective • To probe the possibility to prevent the molecular changes induced by the BCCAO/R with dietary natural compounds known to exert anti-inflammatory activity, such as the phytocannabinoid beta-caryophyllene (BCP) and the polyphenolic compound resveratrol (RVT), also recently reported to help preserving the neuronal structural integrity. Design • For both BCP and RVT, the endocannabinoid system (ECS), fatty acid profile, lipoperoxides, PPAR-alpha, cyclooxygenase-2 (COX-2) were assessed in the frontal and temporal-occipital cerebral cortex by means of HPLC, Western Blot and immunohistochemistry; in the case of RVT markers of synaptic plasticity, such as synaptophysin, syntaxin-3 and post-synaptic density protein-95 (PSD-95) were also investigated. For each substance two groups of adult Wistar rats were used, sham-operated and submitted to BCCAO/R. In both groups, 6 hours before surgery, half of the rats were gavage-fed with a single dose (40 mg/per rat in 300 µl of sunflower oil as vehicle) of either BCP or RVT, while the second half were pre-treated with the vehicle alone. Setting • Surgery was performed at the animal house of the DiSB, while experimental procedures were conducted at the laboratories of Physiology and Neuroanatomy (Cittadella Universitaria, Monserrato, CA, Italy). Results • After BCCAO/R, both BCP and RVT prevented the BCCAO/R-induced increase of lipoperoxides in the frontal cortex, while only BCP affected their plasmatic levels. More interestingly both compounds deeply affected the endocannabinoid system (both ligands and CB receptors), increased PPAR-alpha receptors and spared levels of docosahexaenoic acid (DHA) in BCCAO/R rats. Interestingly, after RVT-treatment the increase of CB1 and CB2 mirrored that of synaptic plasticity markers, such as synaptophysin and post-synaptic density protein-95 (PSD-95). No such changes were observed in the temporal-occipital cortex. Conclusions • Collectively, BCP or RVT, acting preferentially as agonists for CB2 and PPAR-alpha, respectively, modulate the ECS and PPAR-alpha system, and prevent the lipoperoxide increase, a hallmark of the BCCAO/R-induced oxidative stress. Furthermore, RVT-pre-treatment enhances the relative levels of both pre- and post-synaptic proteins in the frontal cortex. It is suggested that natural compounds such as BCP and RVT may be used as a dietary supplement of choice to control the physiological response to the hypoperfusion/reperfusion-induced oxidative stress.

ARE OLIGODENDROCYTE PROGENITORS ALL BORN EQUAL? A LESSON FROM A MICROCEPHALY MODEL

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It is well established that neurons are highly heterogeneous, in terms of function, morphology, gene expression, developmental origin and vulnerability to disease. The study of glial cell biology is instead quite far from this level of understanding, despite the occasional observation of distinct functional properties and the identification of distinct embryonic sources for subsets of astroglia and oligodendroglia. Whether specific glial cell subpopulations differ in molecular features or in their ability to contribute/respond to pathological conditions is still not understood. We tackled this issue by studying a mouse model of microcephaly, where the germinal ablation of Citron-kinase (Cit-K, a citoskeleton regulator involved in cell division and DNA repair; Di Cunto et al., 2000 Neuron; Bianchi et al., 2017 Cell Rep) triggered distinct responses in dorsal and ventral telencephalic oligodendrocyte progenitors (OPCs). Namely, dorsally generated OPCs of the cerebral cortex underwent depletion by apoptosis within the second week after birth. In contrast, ventral OPCs of the striatum and hypothalamus persisted and displayed a senescent phenotype. Such differential sensitivity was not associated to distinct levels of DNA damage in dorsal and ventral Cit-K KO OPCs, but rather to a distinct capability to set up Nrf2-mediated antioxidant defenses. Notably, neither dorsal nor ventral OPCs did progress along oligodendrogenesis, as shown by lack of both pre-myelinating and myelinating cells in the entire Cit-K KO forebrain. In vivo and in vitro experiments showed that such additional differentiation defect largely depended on cell-extrinsic factors, thereby indicating that the germinal Cit-K deletion results in environmental conditions that hamper oligodendroglia maturation and myelination.

These data provide novel evidence of the molecular and functional heterogeneity in postnatal OPC subsets and suggest that dorsal and ventral OPCs may be differentially vulnerable to pathological conditions associated with DNA damage and oxidative stress.

AXO-GLIAL INTERPLAY IN OLIGODENDROCYTE SPECIFICATION AND MYELINATION: ROLE OF JNK1

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The C-Jun N-terminal kinase (JNK) pathway participates in several physiological and pathological mechanisms by phosphorylation of downstream effectors. JNK is expressed in three isoforms, which may play different roles. In fact, JNK1 exerts pleiotropic roles during brain development such as control of regional apoptosis, of microtubule dynamics during dendrite morphogenesis and cortical interneuron migration. Moreover, JNK1 KO mice show alterations of the corpus callosum suggestive of myelin defects. Therefore, we investigated the role of JNK1 in the development of myelinated tracts. In particular, we focused on oligodendrocyte (OL) development. The somatosensory cortex of JNK1 KO mice was reacted with anti-PDGFRalpha antibodies to label oligodendrocyte precursor cells (OPCs) and with anti-myelin binding protein (MBP) antibodies to label the mature, myelinating ones. Immunohistochemical and cell counting analyses revealed a significant increase in the density of OPCs at P7 and P15 in KO mice compared to WT, with no changes in their distribution throughout the supragranular and infragranular cortical layers. Furthermore, JNK1 KO mice at both early postnatal and adult ages showed a lower extent of MBP expression, both in infragranular and in supragranular layers, indicative of reduced myelination. Inspection of the staining suggested that MBP expression was also altered in the corpus callosum. Based on these data, we analysed more deeply the structure of myelinated axons and examined the nodes of Ranvier by labelling for contactin associated protein 1 (CASPR), one of the proteins of the adhesion complex that mediates their assembly. We found that JNK1 KO mice display a higher density of nodes and that the nodes are longer compared to the WT. With the aim to assess cell autonomous defects of JNK1 KO oligodendrocytes accounting for this phenotype, we performed in vitro cultures of rat OPCs treated with DJNKi (a specific inhibitor of the three isoforms of JNK that partly mimics JNK1 KO). The Neural/Glial antigen-2 (NG2) and Ki67 immunohistochemical analysis revealed a different cell morphology and a higher proliferative rate of the OPCs treated with the inhibitor, compared to the non-treated ones. Our findings suggest for the first time that JNK1 takes part in oligodendrocyte development and in the axo-glial interplay. Further experiments will be devoted to examine the ultrastructural alteration of JNK1 KO myelin and nodes and to disentangle the relative contribution of JNK1 in oligodendrocytes or neurons to the observed phenotype.

MICROGLIA-DERIVED EXTRACELLULAR VESICLES REGULATE PROLIFERATION AND DIFFERENTIATION OF OLIGODENDROCYTE PRECURSOR CELLS

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In multiple sclerosis, microglia (MG) have a key role in neuroinflammation. They participate in mechanisms of damage and, in response to injury, can acquire distinct phenotypes with opposite effects. The acquisition of different phenotypes depends on the nature and duration of environmental signals, and plays distinctive roles in remyelination. The 'proinflammatory' phenotype (M1) is associated with a persistent inflammation that inhibits myelin regeneration, while the 'proregenerative' one (M2) promotes remyelination by both secretion of anti-inflammatory factors and phagocytosis of myelin debris. We recently established that conditioning with mesenchymal stem cells (MSCs) is particularly effective in directing MG toward proregenerative functions. However, the modes of action of MG in fostering or inhibiting CNS repair are only partly known. In this study we aimed at exploring whether the distinct effects of MG phenotypes on remyelination may be mediated by extracellular vesicles (EVs), which are released from MG and can transfer multiple information. Furthermore, EVs have recently been proposed as transporters of toxic agents in neurodegenerative diseases, suggesting a role in both diffusion and progression of the pathology. In vitro analysis revealed that EVs produced by M1 cells tend to inhibit proliferation of oligodendrocyte precursor cells (OPC) whereas those released by both M2 and M1+MSC MG tend to increase OPC proliferation. Moreover, exposure to EVs derived from M1, M2 and M1+MSC MG, but not resting cells, promoted OPC differentiation, as indicated by an increase in the fraction of cells positive for the myelin protein MBP. Quantification of linear MBP-positive segments extending along axons of dorsal root ganglion cells indicated that EVs produced by M1+MSC MG promote myelin deposition more efficiently than EVs released by M2 MG. To validate these results and extend the analysis to remyelination models, we are investigating whether EVs released from different phenotypes of MG may promote OPC recruitment and remyelination in the lysolecithin mouse model of focal demyelination. Preliminary results unveil EVs as important players in microglia-OPCs cross-talk and indicates that the phenotype acquired by MG greatly impacts the recruitment/differentiation of OPCs.

EFFECT OF GROWTH FACTORS ON CYTOSKELETAL PROTEINS, CYCLIN D1, ERK EXPRESSION AND DNA LABELING IN ESTRADIOL-PRETREATED ASTROGLIAL CELL CULTURES

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In the present research have been studied the interactions between the "competence" growth factor basic fibroblast growth factor (bFGF) and/or estrogen 17β-estradiol and the "progression" growth factors epidermal growth factor (EGF), insulin-like growth factor-I (IGF-I), and insulin (INS) on DNA labeling and also cyclin D1, extracellular signal-related kinase 1/2 (ERK1/2), cytoskeletal proteins (i.e. glial fibrillary acidic protein (GFAP) and Vimentin) expression in astroglial cell cultures under different experimental conditions. Pretreatment for 24 hr with bFGF and subsequent exposure for 36 hr to estradiol (E2) and EGF, IGF-I, or INS stimulated DNA labeling in the last 12 hr, especially when the cultures were treated with progression growth factors. bFGF pretreatment and subsequent treatment with E2 for 36 hr stimulated DNA labeling. The 36-hr E2 treatment alone did not significantly decrease DNA labeling, but contemporary addition of E2 with two or three growth factors stimulated DNA labeling remarkably. When E2 was coadded with growth factors, a significantly increased DNA labeling was observed, demonstrating an astroglial synergistic mitogenic effect evoked by contemporary treatment with growth factors in the presence of estrogens. Cyclin D1 expression was markedly increased when astrocyte cultures were pretreated for 36 hr with E2 and subsequently treated with two or three competence and progression growth factors. A highly significant increase of ERK1/2 expression was observed after all the treatments. GFAP and vimentin expression was markedly increased when the cultures were treated with two or three growth factors. In conclusion, our data demonstrate estradiol-growth factor cross-talk during astroglial cell proliferation and differentiation in culture.

DISTRIBUTION OF RAD21 IMMUNOREACTIVITY IN MOUSE AND HUMAN GUT NEURONS

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Objective: RAD21 is a double-strand-break repair protein and a critical component of the cohesin complex with key roles in several cellular functions including transcriptional regulation. A mutation in RAD21 is associated with chronic intestinal pseudobstruction (CIPO). This study investigated the distribution of RAD21 immunoreactivity (IR) in enteric and sensory neurons in order to investigate how RAD21 mutations might contribute to gut sensory-motor dysfunction.
Methods: Colocalization of RAD21-IR with markers for subsets of neurons was examined in mouse and human small intestine and mouse sensory dorsal root ganglia (DRG).
Results: RAD21-IR was found in a subset of neuronal cell bodies and nerve fibers in myenteric and submucosal plexuses of mouse and human small intestine, as labeled by PGP9.5 and HuC/D. Within the myenteric plexus this pattern was found in 61.56±0.9 and 144.7±4.1 neurons/field for RAD21 and HuC/D, respectively ($n=4$, $P < 0.05$, t-test). The percentage of neurons expressing cytoplasmic RAD21 was not different between adult and 10 day old mice. RAD21-IR did not colocalize with neuronal nitric oxide synthase (nNOS). A subset of choline acetyl transferase (ChAT) positive neurons were RAD21-IR. RAD21-IR was also present in neurons that were ChAT and nNOS negative. RAD21-IR was not detected in the cytoplasm of interstitial cells of Cajal, glial cells or fibroblast-like cells which were positive for Kit, GFAP and PDGFRα respectively. Many neuronal cell bodies and nerve processes in mouse DRG displayed RAD21-IR
Conclusions: In mouse and human small intestine, RAD21 was detected in subsets of ChAT+/nNOS- and ChAT-/nNOS- enteric neurons and in sensory neurons of mouse DRG. Cytoplasmic localization of RAD21 in subsets of peripheral neurons may contribute to the enteric neuropathy found in patients with mutations in RAD21 that are linked familial CIPO.
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SINTHETIC TORPOR INDUCES A REVERSIBLE HYPERPHOSPHORILATION OF TAU PROTEIN IN CENTRAL AND ENTERIC NERVOUS SYSTEMS

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The synthetic torpor (ST) is a torpor-like state artificially induced in rats (a non-hibernating mammal) by the inhibition of the Raphe Pallidus (RPa), a key neural structure driving thermogenesis. In hibernating mammals, Tau protein is widely hyperphosphorilated (P-Tau) during torpor bouts and the process is completely reversed following the returning to euthermia. P-Tau is a hallmark for many neurodegenerative diseases, defined as tauopathies. In the present work, the occurrence of P-Tau and its eventual dephosphorilation was assessed during the ST and after 6h of recovery at euthermia, respectively. Male Spague-Dawley rats were implanted with a cannula targeted to the RPa and injected (100nl) with the $GABA_A$ agonist muscimol (1mM), once per hour for 6 hours, inducing ST. A control group was injected with saline. At the end of the experimental protocol, each animal was perfused with paraformaldehyde solution and the brain and the intestine were extracted. Brains were sliced 35µm thick by a cryostat. Sections were stored at -80 °C until analysed. Myenteric and submucosal plexus whole-mount preparations of the small intestine and colon were processed for immunohistochemical analysis. To assess P-Tau, the monoclonal mouse anti AT-8 antibody was used. Results showed that during the ST there was a marked P-Tau expression throughout the brain and in both plexuses of the enteric nervous system. During the recovery condition, P-Tau reversed completely into the non-phosporilated form within the brain and in most enteric neurons, although a subpopulation of these neurons showed a persistence of high P-Tau expression. Taken together, these data indicate that also in non-hibernating mammals P-Tau may be reversed in both central and enteric neurons, suggesting resilience to neurodegenerative processes. The reasons why some enteric neurons display less efficiency to Tau dephosphorylation remain to be deciphered; nonetheless ST emerges as a valuable tool to better understand the molecular mechanisms leading to degeneration in tauopathies.

QUANTITATIVE CHANGES OF ENTERIC NEURONS CORRELATE WITH CLINICAL FEATURES IN PATIENTS WITH SEVERE DYSMOTILITY

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Context: Severe gastrointestinal symptoms are often associated with markedly perturbed enteric motility, a finding related to underlying enteric neuropathies. Current methods used to demonstrate enteric neuropathies are mainly based on classic qualitative histopathological/immunohistochemical evaluation. This standard approach, however, is hampered by data interpretation, inter-observer variation and lack of expertise among pathologists. Objective: We assessed quantitatively the enteric innervation in patients with severe dysmotility (SD) and correlate the data with clinical features. Methods: Jejunal full-thickness biopsies were collected from 32 well characterized SD patients (16-77 years; 22 F); and from n=8 controls (47-73 years 4F). A symptom questionnaire was fulfilled prior to surgery. Patients were subdivided according to a previous qualitative histopathological evaluation: n=10 with an apparently normal (AN) neuro-muscular layer; n=14 with inflammatory (INF) changes throughout the neuromuscular layer; and n=8 with degenerative neuro-muscular alterations (DEG). Myenteric (MP) and submucosal (SP) neurons were stained using neuron specific enolase antibody and neuronal cell bodies/ganglion were counted in at least three sections by 3 independent and skilled operators. Mean numbers of neuronal cell bodies/ganglion were analyzed by student's t-test and the correlation with symptoms/signs via Spearman correlation test. Results: The final concordance among the 3 operators was 80%. MP and SP neuronal cell bodies were decreased in SD *vs.* controls ($P<0.001$). Also MP and SP neurons decreased in AN, INF and DEG *vs.* controls ($P<0.0001$ in MP and $P<0.05$ in SP). Furthermore INF and DEG showed less MP (but not SP) neuronal cell bodies compared to AN ($P=0.0224$ and $P=0.0044$). Both the reduced MP and SP neuronal cell bodies correlated with abdominal distension/pain, early satiety, constipation and gastroparesis ($P<0.05$). Conclusions: The proposed method, showing a low discordance rate (20%), identified an overall 50% decreased of MP and SP neuronal cell bodies implying a critical loss of the neuronal mass. The 50% neuronal reduction correlated with a variety of symptoms / signs of SD patients. Notably, quantitative neuronal abnormalities can be demonstrated in patients with AN histopathology.

SEVERE INTESTINAL DYSMOTILITY: CORRELATION BETWEEN SMALL BOWEL MANOMETRIC PATTERNS AND HISTOPATHOLOGICAL FINDINGS IN FULL-THICKNESS SMALL BOWEL BIOPSIES

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Background & Aim: Intestinal manometry is the current standard for the evaluation of small bowel motility. Patients with severe digestive symptoms and abnormal motility pattern can be diagnosed either with chronic intestinal pseudo-obstruction (CIPO), when there are clinical and radiological symptoms/signs mimicking mechanical intestinal obstruction, or enteric dysmotility (ED), when these signs are absent. Both CIPO and ED can be associated with enteric neuromuscular abnormalities shown by histopathological analysis of small bowel full-thickness biopsies. Neither intestinal manometry nor small bowel neuro-muscular pathology are fully standardized diagnostic methods and the correlation between these two diagnostic methods is unknown. In this study we prospectively compared small bowel manometric abnormalities with subsequent histopathological findings in small bowel biopsies.
Materials and Methods: Thirty eight patients (32F and 6M; 16 to 65 yrs) presenting with severe and chronic clinical manifestations of gut dysmotility were investigated with standard stationary intestinal manometry and full thickness intestinal biopsy. Mechanical obstruction and macroscopically recognizable gut lesions were previously excluded by a thorough diagnostic workup. Manometric diagnosis of abnormal motility was established using previously published criteria (*Gut 1987; 28:5-12*). Intestinal specimens were processed for evaluation by traditional staining and a panel of immunohistochemical markers for muscle, interstitial cells of Cajal, glia and neurons. Histopathological patterns were identified according to the London classification (*Gut 2010; 59:882-7*). Manometric finding concordance was obtained in thirty five and those were compared with the corresponding neuromuscular findings at histopathology.
Results: Twenty one patients (15F; age range: 16-77 yrs) had CIPO, whereas 17 (13F; 24-61 yrs) had ED. Of the 35 patients with manometric concordance, 21 had neuropathy, 3 myopathy, 3 occlusive manometric pattern, and 3 indeterminate manometric patterns; 5 had non typical manometric abnormalities. At histopathology, 10 patients (7F; 24-61 yrs) showed an apparently normal small bowel histopathology, while 5 patients (3F; 32-70 yrs) showed enteric neuropathy, 4 (3F; 29-53 yrs) showed features of myopathy, 4 (3F; 16-57 yrs) a lymphocytic neuro-myositis and 12 (9F; 22-77 yrs) mast cell infiltration. Patients with abnormal intestinal manometric patterns (neuropathic, myopathic or obstructive) had abnormal histopathological findings in 73% of cases. However, manometric patterns did not match with the specific neuromuscular findings. Among patients with a neuropathic pattern, histopathology was abnormal in 62% and of these only 23% had an enteric neuropathy, whereas 62% had neuromuscular inflammation and 15% enteric myopathy. Patients with a myopathic or obstructive pattern all had abnormal histopathology, however none of them with signs of enteric myopathy.
Conclusion: Intestinal dysmotility detected by manometry is often associated with abnormal neuromuscular findings in full thickness biopsies. Although there is no correlation between the manometric patterns and the histopathological findings, an abnormal manometric study predicts a higher probability of abnormal histopathology and underscores the decision to obtain a transmural intestinal biopsy.

THE ENTERIC NERVOUS SYSTEM IN OBESE ZUCKER RATS AS AN ANIMAL MODEL OF METABOLIC SYNDROME

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The interaction between brain and gut has been long recognized. This bidirectional interaction plays an important role not only in gastrointestinal function but also in shaping higher cognitive function such as feelings and subconscious decision-making. Disturbances of this system have been associated with a wide range of disorders, including obesity. Previous studies have highlighted increased intestinal permeability and signs of intestinal inflammation both in rats and in mice with obesity induced by high-fat diet. In addition, other findings have reported neuronal plasticity in obese animals prior to the development of pathological changes in the histological features or abnormal mucosal functions. In Obese Zucker rats (OZRs) modifications in the glucidic profile of the intestinal mucosa and secretory products were found.
To investigate the impact of obesity/metabolic syndrome on key intestinal functions and phenotype, we have studied the intestinal tracts of OZRs at two different age, compared to the lean control rats (LZRs). Distinct portions of the small intestine (duodenum, jejunum, and ileum) were analyzed by the immunochemical and immunohistochemical approach. Sections were treated with anti-HUCD (for studying the neural network), glial fibrillary acidic protein (GFAP) (for evaluating astrocytes elements), neuronal nitric oxide synthase (nNOS) and Vesicular acetylcholine transporter (VAChT) to characterize the different type of enteric neurons.
Data on body weight showed an increase in the OZRs of different age. The blood pressure was higher in the OZRs compared to the LZRs of 20 weeks of age. The values of glucose and insulin increased in OZRs of different ages compared to the LZRs. The OZRs showed a remarkable increase in triglycerides and total cholesterol. These data indicate in OZRs a condition of dis-metabolism similarly to metabolic syndrome.
Enteric neurons were detected by cytoplasmic and/or nuclear HuC/D immunostaining. In duodenum, jejunum, and ileum of 20-week old OZRs, myenteric neurons displayed an inhomogeneous HuC/D staining and several cytoplasmic vacuoles, with a scant cytoplasmic HuC/D expression. Immunoreaction with anti-GFAP showed a clear localization of glial cells around the body neurons of myenteric ganglia. The reaction increased around the neurons in 20-weeks-old OZRs compared age-matched LZRs. Obesity correlates with a reduction in nNOS neurons. In fact, the section of OZRs incubated with the anti-nNos antibody, revealed a reduction of positive neurons in ganglia and reduction of nNOS reactive fibers. In OZRs, the network of VAChT-positive nerve fibers and cell bodies was more evident than in LZRs at 20 weeks of age.
The above data showed that OZRs display a progressive decrease in myenteric neurons in the duodenum, jejunum, and ileum that coincides with transient inflammation of the gastrointestinal tract. These observations suggest that obese rats have altered intestinal motor control, attributable to a loss of ganglionic neurons, potentially related to the activation of astrocytes cells and inflammatory process.

CEREBROVASCULAR ALTERATIONS AND SYNAPTIC PLASTICITY MODULATION IN RATS DEVELOPING OBESITY

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The systemic effects, such as increased fat mass, hypertension, insulin resistance and metabolic disorders, have been identified as factors that may lead to cognitive dysfunction. For these reasons obesity represents a risk factor for the development of cerebrovascular disease and cognitive decline.

To investigate the possible relationships between obesity and nervous system changes, neuronal, glial and synaptic vesicular markers were studied in the brain of rat developing obesity (diet-induced obesity dio) after 17 weeks of hypercaloric diet compared to the control rats with not fat diet (chow). Food consumption, fat mass content, blood pressure and blood parameters were checked out. Behavioural tests were used to estimate cognitive performance, while rt-qpcr, immunochemical and immunohistochemical analysis were performed to evaluate modulation of specific cerebrovascular parameters.

The obese phenotype starts to develop after 5 weeks of high fat diet exposure and body weight was higher in dio rats compared to chow during the treatment. Systolic blood pressure, glycaemia and insulin were higher in dio rats only after 17 weeks of high fat diet. No changes in total cholesterol and triglycerides were observed. Furthermore increase of thiobarbituric reactive substances and of oxidated proteins, was observed in the serum and brain of dio rats compared to chow rats. The open-field test revealed, in the older dio rats, a decrease of cumulative distance traveled, their number of rearings and increas-ing the total immobility time. In the passive avoidance test older dio rats showed a reduc-tion of retention latency time, while the morris water maze revealed that rats performance (escape latency time, time spent for recognizing the position of the platform inside the pool the day of test) worsen in dio rats compared to the chow rats.

In the frontal cortex and hippocampus of older dio rats compared to age-matched chow a reduction of neurofilament expression was revealed in the hippocampus of older dio rats without decrease of the number of neurons. A modulation of acquaporin (aqp-4) and glucose transport (glut-1), with an increased expression of the glial-fibrillary acid protein was found immunochemical and immunohistochemical analysis. A modulation of transient receptor potential (trp) channels and synaptic components was highlighted in cerebral areas of dio rats after 17 weeks of high fat diet.
Based on these data, obese rats developed astrogliosis, neurodegeneration, synaptic alterations possible related to impaired learning and memory tasks. Further studies are needed to better clarify the neuronal modifications and the implication of specific neurotransmitter systems in obesity.

CONJUGATED LINOLEIC ACID (CLA) REDUCES NEUROINFLAMMATION BY MODULATING CNS FATTY ACID METABOLISM

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Context • Conjugated linoleic acid (CLA) is an unusual fatty acid widely present in our diet because, even though in variable amounts, is present in meat and dairy products from ruminants. CLA possesses several biological activities in different tissues, although the study of its possible effects on CNS is in its infancy. Objectives • To evaluate the activity of CLA on fatty acid metabolism and related metabolites that are ligands of PPAR-alpha in cultured astrocytes, rat midbrain slices, human plasma and cerebrospinal fluid (CSF) from women with a defect in peroxisome metabolism, the X-linked adrenoleukodistrophy (X-ALD), which is characterised by a sustained neuroinflammation. Design • After administration of CLA, the profile of fatty acids and their metabolites were measured by LC-MS and proinflammatory marker expression by RT-PCR in astrocytes, midbrain slices and human plasma and CSF. Results • In astrocytes, CLA incubation decreased arachidonic acid formation and increased DHA/EPA ratio, regarded as a marker of PPAR alpha activation, and affected TNF alpha and IL-beta 1 levels. Administration of CLA in women with X-ALD was found to improve somatosensory evoked potentials, and to increase DHA/EPA ratio in both CSF and plasma with paralleled decrease of IL-6 in CSF. In rat midbrain slices, incubation with CLA increased the biosynthesis of brain specific PPAR-alpha ligands, Palmitoylethanolamide (PEA) and Oleoylethanolamide (OEA), provided with well-known antinflammatory properties. Conclusions • Our results show that CLA modifies fatty acid metabolism, biosynthesis of PEA and OEA, thereby positively modulating neuroinflammatory responses, by inducing CNS PPAR alpha activation. Thus, dietary intake of CLA-enriched dairy products may be envisaged as nutritional coadjutant in the prevention/treatment of neurodegenerative diseases with a relevant neuroinflammatory component.

MORPHOLOGICAL DIFFERENCES IN D1 AND D2 MEDIUM-SIZED SPINY NEURONS IN NUCLEUS ACCUMBENS SHELL.

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Changes of previously existing neural networks requires a high degree of plasticity through fast formation and elimination of synapses, but the retaining of basic pathways demands a protection against changes induced by improper neuronal activity. Both requirements must be guaranteed by different levels of plasticity so that, during new encoding, basic information can be protected from being restructuration.
The complexity of ventral striatum functions suggests that the variety of action of the dopamine (DA) in the control of motivated and addictive behaviours is due to the segregation of D1Rs and D2Rs DAergic receptors in two distinct groups of medium sized neurons.
In this work we described the differences in spines morphology and density between the spiny neurons of the shell expressing D1 (D1-MSN) and D2 (D2-MSN) receptors. In particular, long thin spines seem to characterize D1-MSNs, while in D2-MSNs the most represented seem to be the mushroom spines
These differences can be the basis of understanding the mechanisms of learning motivated behaviour of the dopaminergic mesolimbic pathway.

SEXUAL DIMORPHIC FEATURES IN THE OLFACTORY BULB OF MICE

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Context • Main Olfactory bulb (MOB) and Accessory olfactory bulb (AOB) interneurons modulate many social and reproductive behaviors as mating choice and maternal behaviors. Those circuits are developed postnatally and continuously shaped by adult neurogenesis. Our previous studies, among others, showed that direct genistein (GEN) administration in the first postnatal week results in alterations of neuronal circuits involved in the above mentioned behaviors and may perturb the onset of sexual dimorphisms in different neuronal populations. Objective • Identify whether olfactory bulb interneurons or neurogenic processes display a sexual dimorphism and whether it may be altered by postnatal GEN administration. Treatment • Pups were fed daily with either GEN (50 mg/kg) or estradiol (E_2, 50 microg/kg) or vehicle (sesame oil) from postnatal day 1 (P1) to P8 and were analyzed P90. Analysis • We identified different MOB/AOB interneurons based on their location and the expression of specific markers. Moreover, we analyzed a subset of adult generated cells by the incorporation of the thymidine analogue 5-bromo-2 -deoxyuridine (BrdU), administrated in one single injection one month before sacrifice. Results • Few differences were highlighted between sexes in control animals, limited to very specific subpopulations, in specific regions. Compared to females, males displayed a lower cell density of Calbindin positive cells in the AOB, of BrdU labeled cells in the medial part of the MOB and a higher cell density of Parvalbumin positive cells in the internal plexiform layer of the MOB. E_2 treatment abolished those dimorphisms. Interestingly, GEN mimicked E_2 treatment in Parvalbumin cells, while it had an opposite effect on BrdU positive cells. Conclusions • 1) there are species specific differences on the organization of MOB/AOB system between mice and rats. 2) Only a subset of MOB interneurons, belonging to specific circuits are sexually dimorphic. 3) E_2 and GEN treatment may affect few subpopulations of interneurons in a sex-dependent way 4) Those treatments have only a minor effect on adult generated cells.

TART-CHERRIES SUPPLEMENTATION COULD MODIFY BRAIN ALTERATIONS IN A ANIMAL MODEL OF OBESITY?

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Tart cherries (Prunus cerasus L.) are a rich source of anthocyanins, phytochemical flavonoids found in red-, blue- and purple-pigmented fruits and vegetables. Anthocyanin-rich plant extracts can modify lipid metabolism in vitro and reduce hyperlipidemia in vivo. Dyslipidemia, hypertension, impaired glucose tolerance, insulin resistance often accompanied obesity, in which the accumulation of adipose tissue and metabolic changes increase the incidence of cardio- and cerebrovascular diseases.

This study evaluates the potential positive effects of tart-cherries extract and seeds powder on rats with Diet-Induced Obesity (DIO). These rats were studied for 17 weeks of hypercaloric diet with the supplementation of tart cherries seeds powder (DS) and seeds powder plus tart cherries extract, containing 1mg of anthocyanins (DES). DIO rats were compared to the control rats with standard diet (CHOW). We measured food consumption, fat mass content and fasting glycemia, insulin levels, cholesterol, and triglycerides, in order to determine the systemic effects of high-fat diet. In the brain, hippocampus and frontal cortex were analyzed, immunochemical, immunohistochemical and qRT-PCR techniques to determine neuronal and glial alterations.

DIO rats increased significantly their body weight, insulin and glucose levels, systolic blood pressure, after 17 weeks of high-fat diet, in comparison to CHOW.

No difference in body weight was found in DS and DES rats compared to age-matched DIO rats. Supplementation of tart cherries with DS and DES induce a decrease in the blood pressure and the glycemia. Furthermore, the serum levels of thiobarbituric reactive substances decreased without changes of the antioxidant properties.

Immunochemical and immunohistochemical analysis showed that tart-cherries supplementation reduces the glial-fibrillary acid protein (GFAP) in the hippocampus and in the frontal cortex of DIO rats. Moreover, the expression of neurofilament (NF) decreased in frontal cortex and hippocampus DIO compared to CHOW. No differences were found in DS and DES rats compared to control DIO rats. On the other hand tart cherries supplementation is not able to modify the modulation of Synaptophysin and ions channels expression in DIO rats.

These preliminary findings indicate that supplementation with tart cherries, although did not reduce the body weight in DIO rats, prevent the development of related risk factors. Further studies are needed to better clarify the benefits of tart cherry supplementation on the brain, and the possible use of obesity-induced cerebrovascular disease.