Hypothalamic lipotoxicity leads to neuroinflammation and increases ghrelin sensitivity in rat

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Lipotoxicity during obesity leads to chronic systemic inflammation and type 2 diabetes associated to insulin resistance states in human and rodent animal models. Inflammation at the level of the central nervous system (CNS) is regulated by microglia. Here we determine by using an*in vitro*microglia model or rodent animal model if lipids-induced toxicity promote inflammatory cytokines release and its effects on metabolic alterations associated with Type 2 Diabetes. Primary cultures of microglia were exposed to saturated and unsaturated lipids to identify their effect on inflammatory cytokines release determined by ELISA and their correlation with the inflammatory TANK-IKKs-NF binding kinase-KB pathway evidenced by western blot analysis. We also analyzed the effect of intrahypothalamic palmitic acid (100 microM) stimulation on plasma glucose levels homeostasis and ghrelin sensitivity. We found that in contrast to oleic acid, ceramide 6 and palmitoleic acid, 100 microM palmitic acid stimulation to primary microglia culture during 24 h efficiently promotes IL1b, TNFalfa and IL6 release which correlates with TANK-IKKs-NF binding kinase-KB pathway activation. Furthermore, hypothalamic lipotoxicity stimulation in rat by intrecerebroventricular palmitic acid (100 microM) or lipopolysaccharide (0.1 microg/ml) administration do not promote changes in plasma glucose levels, evidenced by Insulin or glucosetolerant test. However, they do promote increase in food intake after ghrelin (1 microg/ml) intrecerebroventricular administration when compared to spinal fluid administration as a control. Our data demonstrate that lipotoxic damage of saturated lipids promotes microglia activation, cytokine proinflammatyory release and increase sensitivity to ghrelin hormone-sensitive food in rats.

Area: Metabolismo

ROLE OF REACTIVE OXIGEN SPECIES IN THE REGULATION OF NEUROINFLAMMATION INDUCED BY EXCITOTOXIC DAMAGE IN CEREBELLAR GRANULAR NEURONS

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Introduction: In neurological diseases and multiple conditions of nervous system injury, glutamate induces overstimulation of the ionotropic and metabotropic receptors, which promotes the entry of cations (Ca^{2+} and Na^{+}) and the production of reactive oxygen species (ROS), a phenomenon called excitotoxicity; and to which the participation of NADPH oxidases (NOX) has been associated with promoting oxidative stress. On the other hand, it is known that in several cell types of the immunological lineage, ROS regulate proinflammatory mediators, including inflammasomes, which play a central role in the generation, activation and secretion of interleukins. One of them is NLRP3 that after activation promotes the maturation of IL-1β (proinflammatory) and IL-18 (anti-inflammatory). The process of activation and regulation of the inflammatory response by inflammasomes to excitotoxic damage is not clearly understood.

Objective: Demonstrate that production of ROS from NOX during an excitotoxic damage in neurons participates in the regulation of the inflammatory response.

Methodology: Primary cultures of cerebellar granule neurons from Wistar rats of 7-8 days, were treated with glutamate (100µM for 20 minutes) after 8 days *in vitro*. Some cells were treated with a NOX inhibitor (DPI, 200 nM) or a mimetic of superoxide dismutase (EUK, 100 µM). After 1, 6, 12, 24 and 48 h of incubation, the production of ROS was determined by the administration of dihydroethidium (DHE) and the subsequent observation of the cultures by a confocal microscope. The activity of NOX and caspase-1 was determined by fluorescence spectrometry and the levels of IL-1β and IL-18 were assessed in cell supernatants by immunofluorescence and ELISA test (Affymetrix eBioscience kit).

Results: The NOX activity showed a biphasic increase in response to glutamate administration, increasing after one and 48 h of treatment, which coincided partially with the levels of ROS. Moreover, the activity of caspase-1 showed an increase over time with a maximal at 48h that was not observed in the presence of DPI and EUK, suggesting that the activity of caspase-1 is regulated by ROS produced by a NOX. Finally, the production of cytokines showed a temporary production, with an increase of IL-1β at early times, and IL-18 at longer times. This profile of cytokines production was modified by the antioxidants.

Conclusions: The production of reactive oxygen species produced by NOX appears to regulate the activation and maintenance of the cytokine processing activity of caspase-1, which could suggest the involvement of a redox regulation of NLRP3 inflammasome by NOX during the inflammatory process induced by excitotoxicity.

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Obesity induced neuroinflammation and its impact in adipose tissue morphology.

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ABSTRACT

Obesity is one of the most prevalent health problems in modern society. Physiological alterations of this disease include metabolic problems such as insulin resistance, glucose intolerance, nonalcoholic hepatic steatosis, increased accumulation of adipose tissue and cardiovascular diseases as well as alterations in the central nervous system such as increased inflammatory markers and neuronal damage in the hippocampus, cortex, and hypothalamus.

Many of the obesity induced physiological dysfunctions present an autonomic neuronal control, such as blood pressure, hepatic glucose production and mobilization of lipids from adipose tissue, suggesting an alteration in the balance between the activity of the sympathetic and parasympathetic nervous system that innervate the different organs of the body.

The adipose tissue is one of the main organs whose activity is importantly modified during obesity and it presents an autonomic modulation, through hypothalamic nuclei such as the arcuate nucleus (ARC) and the paraventricular nucleus of the hypothalamus (PVN). Previous studies have shown that obesity increases the amount of hypothalamic microglia and pro-inflammatory signals, resulting in dysregulation of the neuronal activity.

The hypothesis of this work is that the increase of the neuroinflammatory profile and neuronal damage in hypothalamic nuclei involved in the autonomous modulation of the adipose tissue could modulate the morphology and metabolism of the different adipose tissues.

The objective of this project is to determine the functional implication of the increase of the proinflammatory profile in the hypothalamus innervating fat deposits.

In the present study, we have determined that blocking microglial activity in obese male Wistar rats, through an ICV injection of minocycline, decreases glucose intolerance and the white and brown adipocytes morphology. These effects are mediated through an autonomic pathway since denervation of the white and the brown adipose tissue prevented the adipocyte hypertrophy.

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ROLE OF NOX-2 IN THE INFLAMMATORY RESPONSE TO EXCITOTOXIC DAMAGE

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*Key words:*NADPH oxidases, Excitotoxicity, Interleukins.

Introduction: The excitotoxic damage is a common phenomenon in various pathologies of the central nervous system (CNS). The mechanism of neuronal damage depends on several factors, including an increase in the intracellular concentration of calcium and the production of reactive oxygen species (ROS). Recently,several evidences point out to the NADPH oxidases (NOX), particularly NOX2, as the main source of ROS responsible for oxidative stress during this process. The NOX production of ROSin high quantities has been observed in necrosis, apoptosis and inflammation. The inflammationresulting from excitotoxic damage, can culminate in injury or the generation of a secondary damage, subsequentof a deregulated inflammatory process.

*Objective:*Characterize the role of ROS produced by NOX2 in the inflammatory response, secondary to excitotoxic damage in the striatum of mice.

*Methodology:*The excitotoxic damage was produced by intracerebral (IC)injection of glutamate (1M) in the striatum of C57-BL6 (wild type) and $gp91^{phox-}$ mice (NOX2-KO);additionally, some wild type mice were treated with recombinant mouse IL-10 (200 pg/mL) at the time of injection. Subsequently,striatum homogenateswere obtained at 1, 3, 6, 12 and 24 hours. NOX activity and the amount of active caspase 3 was performed by fluorescence spectrometry, as well as the production of interleukins 1β,4,6,10 and 12, by the ELISA method (Abcam kit). Before the mice were sacrificed, they were subjected to the cylinder and the adhesive removal test for the evaluation of motor behavior.

Results: We observed a biphasic increase of NOX activity (1hr to 12 hrs), which is not observed in NOX2 deficient animals. This increased activity correlated with the application of the cylinder test and adhesive removal, since it was observed that NOX-2 deficient animals outperformed wild type animalsafter administration of glutamate. The NOX-2 deficient animals also showed smaller amounts of activated caspase 3. As for interleukins, an increase in pro-inflammatory interleukins was observed in both groups, however, NOX-2 deficient animals showed a significant increase in the production of IL-4 and IL-10, cytokines characterized as anti-inflammatory

*Conclusions:*The absence of NOX-2 activity promotes the production of anti-inflammatory interleukins, which favor neuronal survival by decreasing caspase-3-mediated apoptotic death, which is reflected in a more effective recovery of motor performance

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Malva parviflora extract regulates the phagocytic capacity of microglial cells via a PPARγ-mediated mechanism in an Alzheimer´s disease model

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Microglia are immune cells of mesodermal origin that reach the central nervous system during development. Microglia belong to the macrophage lineage and therefore, they play a key role in responding to inflammation and to different immune challenges within the brain. In addition, recent evidences indicate a role for activated microglia in Alzheimer's disease (AD) as in this state; microglial cells release pro-inflammatory cytokines that induce neuroinflammation andcompromises microglial clearance functions.Thus, the blockage of microglia activation has been proposed as a potential therapeutic strategy in AD. Here we investigated the effects of a hydroalcoholic extract (HE) of *Malva parviflora*(*M. parviflora*) in microglia. Primary microglial cells were isolated from wild-type CD1 mice and from 5XFAD, an AD mouse model. We demonstrated that the HE of *M. parviflora* possesses anti-inflammatory properties in neonatal mice microgliaas itreversed the amoeboid phenotype (associated with activated microglia), inhibited the activation of NF-kBresulting fromLPS exposure and decreased the expression of M1 activated markers (CD86 and TNF-α) in the cortex of 5XFAD mice. Likewise, microglia cells treated with the HE of *M. parviflora* exhibited an enhanced phagocytic capacity and a multipolar morphology that correlates with decreased load of β amyloid (βA) plaques in the cortex of 5XFAD mice and with improved learning and memory.Here, we discuss the impact of the *M. parviflora* HE on PPARγactivation as a putative mechanism controlling microglia phagocytic capacity.

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Keywords: Microglial cells, Alzheimer's disease, *Malva parviflora*,PPARgamma, phagocytosis, neuroinflammation

BIOCHEMICAL PARAMETERS AND COGNITIVE IMPAIRMENT IN MULTIPLE SCLEROSIS

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ABSTRACT

INTRODUCTION. Multiple sclerosis (MS) is a progressive demyelinating autoimmune disease causingdegeneration in the patients. The disease is characterized by a number of biochemical changes that could affect different neuronal functions and in Mexican population is necessary to know how these levels could be affected in the MS. Recent studies show that hyperlipidemia is considered as a potential risk factor for develop cognitive impairment.**OBJECTIVES.** Determine the relation between the cognitive impairment and the biochemical parameters in patients with multiple sclerosis. **MATERIALS AND METHODS.**21 patients were evaluated with MSusing the cognitive test MMSE and MoCA to determine the cognitive impairment degree. We obtained a blood sample and analyzed the biochemical panel: glucose, cholesterol, triglycerides, uric acid, urea, creatinine, LDL, HDL, and VLDL. Relations were stablished using the correlations coefficient of Spearman **RESULTS.** For comparisons between the triglycerides and the cognitive tests we did not obtain significant associations MoCA(r= -0.241, p = *0.236)* nor MMSE (r= *0.155*, p = *0.450*). However, we found a relation between the diagnosis of MS and triglycerides (r= *0.447*, p= *0.22*)**. CONCLUSION.**In this study did not find the relations between biochemical parameters and cognition in the patients; however, wefound a positive correlation between the diagnosis and the lipid profile, that can be explained due the demyelinating process that occurs during this disease.

LPS-induced neuroinflammation decreases DCX+ cells proliferation in adult hippocampus

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Neurogenesis continues along the ontogenetic development of the adult mammalian brain in the subgranular zone of the dentate gyrus. Pathological conditions associated to neuroinflammation correlate with a decrease in neurogenesis. Neuroinflammation changes cellular and molecular niche components that compromise cell proliferation and survival. It still is unknown which kind of neural precursor cell may be particularly affected thus leading to reduction in the number of total new neurons in neuroinflammatory conditions. The aim of the present study was to evaluate the effects of neuroinflammation in neural precursor cell proliferative behavior. For this purpose, we administered i.p. 1 dose of 1 mg/kg LPS (Escherichia coli O127:B8 mg/kg) or saline in young adult male C57Bl/6 mice (two-month-old). Sickness behavior responses were assessed by the open field test and by changes in body weight. Our results show that LPS injection induced a reduction of 70% in locomotion as well as a loss of 7% of animals' body weight 2h and 24h post injection, respectively. The pro-inflammatory milieu in the brain was assessed through hippocampal cytokine IL-6 levels by western blot and through morphological microglia changes using an immunofluorescence confocal analysis. IL-6 levels were up-regulated even one week after LPS injection. Under control conditions resting microglia exhibited small cell bodies and a ramified morphology. After LPS injection, microglia acquired a reactive profile with enlarged cell somas, fewer and shorter processes and an increase of Ed-1 immunoreactivity. To evaluate neurogenesis, we first labeled a cohort of cells with BrdU pulses (3 i.p. injections in three days) and analyzed BrdU co-labeling with DCX+, a neuronal cell-commitment marker. Cell counting was performed in images obtained from confocal microscopy coronal sections. Our results show a decreased hippocampal neurogenesis as assessed through BrdU+DCX+ cells, which could either reflect a downregulation in the proliferative capacity or in the survival rate of neural precursor cells. We reasoned that neuroinflammation can affect: 1) proliferation of all neural precursor cell types; 2) the activation rate of neural stem cells; 3) the number of cell divisions or the cell cycle kinetics of intermediate progenitors or, 4) the neuroblast proliferation rate. To gain insight into the proliferation capacity, assessed as cell cycle re-entering capacity of the different cell populations, we first labeled a cohort cells with a short BrdU pulse (3 i.p. injections each two hours) and 24h later we estimated co-labeling with Ki67+, an endogenous proliferation marker. In control conditions, most BrdU+ or Ki67+ cells were DCX+ suggesting that these cells are the most proliferative. LPS-induced neuroinflammation reduced BrdU+ and Ki67+ cells, which shows a general decrease in proliferating cells. Proliferative deficits could further be associated to DCX+ cells since the number of Ki67+DCX+ and BrdU+DCX+ cells were diminished. Moreover, double-labeled BrdU+Ki67+ cells were diminished showing a general impaired cell-cycle re-entry capacity. Additional experiments will address if the proliferative behavior of progenitors other than DCX+ are also affected.

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"A *Malva parviflora*´s fraction prevents the deleterious effects resulting from neuroinflammation in a murine model"

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Alzheimer´s disease (AD) is a neurodegenerative disorder mainly characterized by cognitive impairment and behavior disorders. The etiology of this condition is not not well characterized; however, it is known that the inflammatory processes developed before and during the progression of this disease play a negative role on memory and learning. Based on this, new strategies to treat AD have been proposed. Among them, the use of non-steroidal anti-inflammatory drugs (NSAIDs) have shown to decrease the incidence of this disease in asymptomatic patients when administered in specific regimens. Unfortunately, the prolonged use of NSAIDs results in adverse effects, so the search for new molecules continues. In this context, the secondary metabolites of plants with anti-inflammatory activity, have become of great interest. Particularly, our group has demonstrated that the hydro-alcoholic extract of *Malva parviflora* (MpHA) has antioxidant and anti-inflammatory effects, as well as being capable of improve the cognitive deficit present in the AD model, 5XFAD mice, fed with a hypercaloric diet. However, given the amount of contained compounds in the MpHA, its chemical and pharmacological characterization is highly complex. Therefore, the present study was aimed to generate an anti-inflammatory fraction (MpF10) that constitutes a less complex mix of compounds than the MpHA. This fraction was evaluated in a murine model of neuroinflammation. MpF10 ameliorates spatial learning impairments and reduces the astrogliosis resulting from LPS exposure. Thus indicating that MpF10 recapitulates the pharmacological effect of the MpHA, and that the compounds present in this fraction represent an alternative to treat neuroinflammation.

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Key words: inflammation, Alzheimer's disease, *Malva parviflora.*

Effect of valerenic acid on inflammatory mechanism in mice model of Parkinson's Disease

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Parkinson's Disease (PD) is the second most common neurodegenerative disease in the world, characterized by motor disruption, generally presented in the elderly, with neuronal damage in the *substantia nigra* nucleus of the brain. The main molecular mechanisms in the PD development are genetic mutations in the α-synuclein protein, ubiquitin cascade – bad folding proteins, mitochondrial dysfunction, oxidative stress, and neuroinflammation. Neuroinflammation is the most important determinant in the development of PD, for the exacerbated activation of glia due to α-synuclein aggregation and neuronal damage, release of reactive molecular species and proinflammatory cytokines that potentiate the neurodegeneration processes in PD. Furthermore, the finding of new strategies to prevent PD has led us to test compounds of natural products, like those found in*Valerian officinalis*, a plant used in traditional medicine to treatsleep problems and modulate inflammatory processes. One of the responsible compounds of these effects is the valerenic acid. For these reasons, the aim of this work was the assessmentof the effects of valerenic acid in a mice model of PD, through the evaluation of the motor symptoms and the proinflammatory cytokines levels: Tumor necrosis factor alpha (TNF-α), Interferon gamma (IFN-γ), the interleukins IL-1β and IL-6, in the *substantia nigra* of the mice brains. The results indicate that valerenic acid attenuates the damagingeffect of MPTP in the model of PD, with a decreased cross time in the beam walkingtest, beside an increased time in the grip hanging test andthe ability to stand on the hind limbs in the open field test. Moreover, the quantification of the cytokines showed decreased brain levels of TNF-α, IL-1β and IL-6, although IFN-γdid not show differences between groups. In conclusion, the treatment with valerenic acid in a mice model of PD has an anti-inflammatory and motor recovery effect, promising to be a good candidate as a preventive treatment for PD at the expense of further testings. We thank the financial support by FOPER-UAQ (Universidad Autónoma de Querétaro), and CONACYT, Mexico scholarship number 298061.

Immunologic characterization of pediatric medulloblastomas

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Introduction. Medulloblastoma (MB) is the most common type of pediatric malignant brain tumor, accounting for about 20% of all childhood brain cancers. MB are heterogeneous group, the histological classification defines five subgroups, including classic, desmoplastic, anaplastic, large cell and with extensive nodularity. These subgroups have different outcomes and recurrence patterns.

Actual therapy for MB consists of surgical resection, craniospinal irradiation, and chemotherapy, leading to 50-80% 5 years overall survival. However, survivors of the disease often demonstrate severe side effects. New treatments adjuvants are needed not only to cure high-risk patients but also to alleviate therapy-induced side effects. Of particular importance is the immune response to cancer cells development and progression. However, the immunobiology of MB is still poorly understood. Within the immune system cells, natural killer and CD8+ and CD4+ T cells, function as the major anti-tumor immune effector cells. Moreover, pattern recognition receptors such as toll-like receptors (TLRs) are important regulators of tumor microenvironment by induced both immunosuppressive and inflammatory cytokines production, play dual roles in promoting or combating cancer development. The aim of this study was to analyze the context immunological of pediatric medulloblastomas and to examine their association with histological subgroups and survival.

Methods. Were included biopsies from 70 pediatric patients with MB diagnostic collected between 1990 and 2011 selected from the tissue bank at the Pathology Department of the Hospital Infantil de México Federico Gómez. Tissue microarray was constructed by punching 3 spots representing each sample with a needle of 1 mm diameter using a Tissue Microarray ATA 100. Immunohistochemistry stains for CD4, CD8, NCR1, CD68, TLR7 and TLR8 was performed using the Ultra View Universal DAB Detection Kit or Alkaline Phosphatase Red Detection Kit (Ventana) and hematoxylin was employed as the nuclear counterstain. Slides were processed in a Ventana BenchMark XT processor and scanned in ScanScope CS2 (Aperio). CD4, CD8, NCR1 and CD68 positive cells were expressed per area and TLRs expression was evaluated and classified according to the level of positivity as negative, low or high.#

Results. In a total of 56 patients, the mean age was 6.6 year, and the sex ratio was 1.33:1 (male: female). We found that 7.1% of total were anaplastic MB, 50% desmoplastic/nodular and 42.9% were classic. Analyses for overall survival (OS) between the three histological groups find significant differences. The classic subgroup was a favorable factor (80% 5-year OS) and anaplastic MB was poor prognosis. The 5-year OS for patients with high expression level of TLR7 was 73.4 $\pm$ 25% and 50.0 $\pm$ 11.6% for low expression (log-rank *P* value= 0.54). Patients with high TLR8 expression OS was 85.7 $\pm$ 13.2% and 56.7 $\pm$ 14.9% for low expression (log-rank *P* value= 0.29). In conclusion, our data suggest histological subgroups are associated with survival. The association of immunological cells and TLRs expression level with subgroups of MB and survival needs further study.

Analgesic Effect of RepetitiveTranscranial Magnetic Stimulation (rTMS) in Patients With Chronic Low Back Pain

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Abstract

The objective of the present study was to assess the benefits of 1-week repetitive transcranial magnetic stimulation (rTMS) in patients with chronic low back pain (LBP). The visual analogue scale (VAS), Short Form McGill pain questionnaire (SF-MPQ), and Short Form 36 Health Survey were used to evaluate the effect of this treatment. Eighty-two patients diagnosed with LBP were divided randomly into three groups: rTMS-treated group, sham group, and physical therapy-treated group. We observed a significant reduction in VAS and SF-MPQ scores in the rTMS-treated group, but not in the sham group. Moreover, patients who received rTMS had a lower mean pain score than patients treated with physical therapy. Our study suggests that rTMS produces safe, significant, and long-term relief in patients with LBP without evident side effects. This study shows for the first time that long-term repeated sessions of rTMS decrease pain perception of LBP.

Pathological role of a polyglutamine expansion in human TATA-binding protein inSCA17modeled in *Drosophila melanogaster.*

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Spinocerebellar ataxias (SCAs) are progressive disorders in which the cerebellum and the brain stem slowly degenerate. SCAs are classified into three major groups according to their molecular etiology: 1) Ataxias related to polyglutamine expansion (polyQ); 2) Ataxias caused by repetitive expansions in non-coding regions; and 3) Ataxias produced by conventional mutations (deletions, insertions, duplications, nonsense mutations).We modeledspinocerebellar ataxia 17 (SCA17) in *Drosophila melanogaster*, also known as Huntington-4-like disease (HDL4). SCA17 is caused by an expansion of the CAG / CAA trinucleotide in the gene encoding polyQ regions in the human TBP protein (hTBP). The physiological threshold of the total glutamines present in non-pathological TBP is around 30 to 40 repetitions and a greater number of 40 causes the pathology. To analyze the neuropathological effect of hTBP with the extended polyQ (80Q) compared with wild type hTBP (34Q)we used *D. melanogaster* as a model. Our experimental strategy consisted of addressing specific expression of hTBP with polyQ expansions of 80Q using the pan-neuronal driver ELAV-GAL4. Protein aggregation in the fly brain was measured by immunofluorescence,cell death was evaluated using TUNEL and climbing abilitywas tested in adult flies.Our preliminary results, showed that hTBPprotein aggregations are age and length dependenton the flies. Confocal images showed no obvious aggregates in the brain at younger ageand the proteinswere distributed evenly throughout the whole brain. Cell death was present in greater proportion in the brain expressing hTBP80Q compared withhTBP34Q. In addition, extended polyQflies were more affected in the climbing tests compared with the 34Qexpressing flies.These results are important to understand SCA17 neuropathological progress to provide possible alternatives in the cognitive and functional deterioration of patients with this pathology.

Comparing the possible neuroprotective properties of sugarcane juice and ferulic acid in *C. elegans* and rat cortical slices

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Introduction:*C. elegans* has become an important animal model in various research fields such as neurobiology, due to its easy maintenance, genetic homology with humans, and its relatively simple nervous system composed of 302 neurons.

Sugarcane (*Saccharum officinarum*)acts as antioxidantas itcontains flavonoids such as ferulic acid (FA), a compound endowed with a strong cytoprotective activity due to both the ability to scavenge free radicals and activate cell stress response. 6-Hydroxydopamine (6-OHDA), quinolinic acid (QUIN) and ferrous sulphate ($FeSO_4$) produce a reactive oxygen species (ROS) - including hydrogen peroxide (H_2O_2), superoxide and hydroxyl radicals -, throughout different mechanisms, thus causingdifferent noxious effects on cells,including ATP depletion and cell membrane damage.

Objective: To test the possible protective effects of FA and sugarcane against the toxicity inducedby QUIN, 6-OHDA and $FeSO_4$, evidenced bysurvival rate, motor activity, fertility and oxidative damagein *C. elegans*, as well ascell viability and oxidativedamage in rat cortical slices.

Materials and methods: The *C. elegans* strain N2 (wild) was exposed to pre-treatment with FA (38 mM) or sugarcane juice for 30 minutes.The worms were then exposed to 6-OHDA (25 mM), QUIN (100 mM) or $FeSO_4$ (15 mM) for 30 minutes more. Lifespan, motor activity, fertility and lipid peroxidation were evaluated.Cortical slices were treated with FA (250 or 500 μM) or sugarcane juice for 60 minutes; subsequently, slices were exposed for 60 minutes more to 6-OHDA (100 μM), QUIN (100 μM) or $FeSO_4$ (25 μM). Cell viability and lipid peroxidation were evaluated.

Results: Nematodes treated with sugarcane showed a considerable increase in lifespan compared to 6-OHDA and QUIN.The worms treated with 6-OHDA and supplemented with FAalso exhibited these protective effects. Motor activity was improvedin all toxic models by sugarcane and FA. In general terms, worms treated with $FeSO_4$and supplemented with sugarcane, and worms treated with 6-OHDA and QUIN and supplemented with FA,showed amelioration of toxic markers.FA and sugarcane also produced cytoprotective effects against oxidative damage to membranes induced by all the toxins.Cortical slices treated with FA or sugarcane juice exhibited improvement in cell viability against QUIN and $FeSO_4$ toxicity.

Conclusion: FA and sugarcane exerted protective effects possiblyrelated with antioxidant activity against 6-OHDA, QUIN and FeSO4.These effects were evidencedin parameters of lifespan, motor activity and oxidative damage in *C. elegans*.In addition,both substances exhibited a neuroprotective effect in rat cortical slices.

Effect Of ElectricalStimulation At The St36 Acupoin In A Model Of TraumaticSpinalCordInjury in rat

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Neuropatología

Traumatic spinal cord injury (TSCI) results in devastating life long disability for patients and their families. The initial mechanical trauma is followed by a damaging secondary injury cascade involving proapoptotic signaling, ischemia, and inflammatory cell infiltration and up regulation of TNF-α and IL-β. Treatment of TSCI has improved during the last several decades due to standardized protocols for emergency medical response teams and improved medical, surgical, and rehabilitative treatments. However, TSCI continues to result in profound impairments for the individual.

Acupuncture has been used for over 4,000 years, recently, acupuncture has been described as a complementary and alternative medicine in which filiform needles are inserted at specific points on the body, called acupoints, which can subsequently be stimulated in various ways, such as through electro acupuncture (EA) Immunomodulatory effects have been reported after acupointstimulation. Anti-inflammatory effects have been reported in mouse models of inflammation associated with EA at the Zusanliacupoint (ST36). The treatment with EA at ST36 induced a nephroprotective effect associated with decreased levels of TNF-α and IL-1 in a lipo polysaccharide-induced model of acutenephritis. Recent studies have shown that EA at ST36 decreases the levels of TNF-α, IL-1-β, and IL-6 through the suppression of the Toll-like receptor 4 and nuclear factor-kappa B signaling pathway in cerebral ischemia-reperfusion injured rats.

In the present work we determined the effect of the application of EA at 30 Hz at ST36 point on inflammatory response, tissue preservation and functional recovery in a model of TSCI in rats.

The results obtained demonstrate a greater amount of tissue preserved in the group with EA at 30Hz in ST-36,compared to the group with only TSCI.

In addition, the EA at 30 Hz in ST-36 produced an inmunomodulatory effect, decreased the level of IL-6 in the spinal cord during the acutephase of LTME.

Finally, based on the BBB scale, treatment with EA at 30 Hz in ST-36 showed a favorable effect on functional recovery, compared to the untreated group.

In conclusión, in TSCI theEA at 30 Hz in ST-36 could be useful to improve this pathology.

Neuroanatomic alterations and stereotyped behaviors in the mouse C58/J. Implications for the study of autism.

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Autismspectrum disorder (ASD) is a disorder of neurodevelopment characterized by deficits in social communication and excessive repetitive behaviors. Alterations in neuronal connectivity, increased number of neurons, decreased volume of neuronal some and increase of neuropil has been described in the brains of people with ASD, however the relationship between neuroanatomic alterations and stereotyped behaviors has not been fully established. The C58/J mouse presents ASD-like behaviors. In this work we try to know if stereotyped behaviors in C58/J mouse are associated with alterations in neuronal morphology. Our results show that C58/J mice have less social interaction and greater repetitive behavior with respect to C57BL/6J mice (control group). In addition cortical neurons impregnated with Golgi stainingand evaluatedby semi-automated analysis of Shollusing sequentially the programs ImageFocus v3.0, ImageJ / fiji, NeuroImageJ and NeuroStudio,showed abnormal dendritic ramifications,which include longer and complex dendritic ramificationsand an excessive increase of dendritic spines relative to the control mice. These results together suggest that alterations in neuronal morphology may be related to some types of behavior described in ASD and support the use of the C58/J mouse as a model for studying neuroanatomic alterations associated with autism.

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Autophagy decreases oxidative stress and apoptosis in a cellular model of Parkinson's disease

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Parkinson´s disease (PD) is the most common chronic and progressive neurodegenerative movement disorder. It is characterized by the selective loss of dopaminergic neurons in the *substantia nigra*, leading to a deficiency of dopamine with subsequent motor and cognitive dysfunction. Although its etiology is still unknown, environmental exposure to herbicides including paraquat has been associated to PD development. In addition, mitochondrial dysfunction, oxidative stress (OS) and impairment of the protein degradation pathways mediated by the proteasome and autophagy, have been related with PD. Autophagy is a self-regulatory mechanism of cells and involves degradation of macromolecules and organelles, which byproducts can be re-used for cell survival. Impairment of autophagy is associated with cell death and neurodegeneration. Therefore, we wanted to determine whether induction of autophagy can protect dopaminergic neurons from cell death-induced by OS. We established the experimental cellular model of PD in the SH-SY5Y dopaminergic cells by using the herbicide paraquat, which induces OS, autophagy disruption and cell death. The effect of autophagy on PQ-mediated OS was evaluated in the cytoplasmic compartment with dihydroethidium (DHE) by fluorescence microscopy. We observed that OS induced by PQ was significantly decrease by autophagy stimulation with rapamycin. Peroxiredoxins (Prxs) are antioxidant enzymes that catalyze the reduction of hydrogen peroxide, peroxynitrite, and organic hydroperoxides. Highly oxidative environments induce Prxs hyperoxidation, with its subsequent antioxidant inactivation. We found that PQ induced Prxs hyperoxidation, while autophagy stimulation decreased this effect, which was determined by immunofluorescence and western blot. To determine the effect of autophagy on PQ-induced cell death, calpain and caspase-dependent apoptosis pathways were evaluated by fluorometry and flow cytometry, respectively. Importantly, autophagy stimulation inhibited calpain- and caspase-dependent apoptosis induced by paraquat. Our results show that autophagy stimulation has a protective role by decreasing OS and calpain- and caspase-dependent apoptosis induced by paraquat, representing a promising strategy against PD.

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Evaluation of fusion/fission proteins and mitochondrial deficit in synaptosomesin Triple transgenic mouse model of Alzheimer's Disease

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Synaptic loss is one of the early events and the best pathological correlate of cognitive impairment in Alzheimer´s disease (AD). It has been found in AD´s brain a reduced number and size of mitochondria associated with increased levels of fission proteins and reduced fusion proteins, particularly in hippocampal and cortical neurons. Mitochondrial fission and fusion play critical roles in maintaining functional mitochondria. Fusion is mediated by several proteins such as Mfn1, Mfn2 and OPA1and fission by proteins such as Drp1 and Fis1.Rates of mitochondrial fission and fusion respond to changes in metabolism. However, it is not known how factors as bioenergetics and mitochondrial dynamics may conduct to synaptic dysfunction and loss. We analyzed mitochondrial bioenergetics, mitochondrial fission/fusion proteins and ultrastructural alterations in synaptosomes of the triple transgenic mouse model of Alzheimer Disease (3xTg-AD) at different ages. We found a significant decrease in the metabolic activity of hippocampal and cortical synaptosomes from old 3xTg-AD (9-11 months) measured by reduction of MTT. Mitochondria respiratory capacity showed thatsynaptic mitochondria are coupled at all ages although a lower consumption of oxygen in the three regions examined (cerebellum, hippocampus and cortex) was observed. This bioenergetic deficit was more pronounced in hippocampal synaptosomes from the 3xTg-AD and was accompanied by a loss of mitochondrial membrane potential. Interestingly, we foundan increase in the activated form of the fission protein, p-Drp1 in hippocampal synaptosomes fromthe old 3xTg-AD mice, while the fusion protein, Mfn1 was unchanged compared to wild type animals. In addition, electron microscopy showedchanges in hippocampal synaptosomes from old 3xTg-AD consisting inenlarged synaptosomeswith swollen mitochondriawith disturbed crests andaccumulation of Aβ.These data suggestthat aging is associated with mitochondrial dysfunction at synaptic terminals that was exacerbatedin the 3xTg-AD.

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CHANGES IN CIRCADIAN RHYTHM BY ARSENIC ACCELERATE AMYLOID PATHOLOGY IN THE 3XTG-AD MODEL.

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Worldwide, every year there is an increase in the number of people exposed to inorganic arsenic (iAs) via drinking water. Human populations present impaired cognitive function as a result of prenatal and childhood iAs exposure, while studies in animal models in similar conditions demonstrate neurobehavioral deficits, accompanied by protein and enzyme alterations typical of the Alzheimer´s disease (AD). It has been suggested that the disruption of the circadian rhythm could enhance neurodegeneration, by promoting inflammation and synaptic damage. Cermakian et al., 2011, demonstrated β-amyloid peptide expression in AD patients can facilitate BMAL1 degradation in neuronal cells suggesting that AD-related processes could directly influence the cellular clock function. In order to determine whether iAs promotes the alterations of the circadian rhythms and cognitive deficit characteristic of AD, we analyze the effect of iAs over 3xTg-AD, which represents a model for the development of amyloid plaques, neurofibrillary tangles and behavioral alterations. Male and female 3xTg-AD mice (25-30 g) were divided into 2 groups: 1) controls, without arsenic in drinking water; and 2) exposed to 3-ppm sodium arsenite in drinking water from gestation until 6 months post-birth. We investigated the behavior phenotype on a test battery, including locomotor activity and contextual fear conditioning. Immunohistochemical studies were performed in the brain to detect AD markers (BAM and total tau) and c-Fos activation. IL-6, Nrf-2, the clock gene Per2, melatonin receptor was evaluated by RT-PCR and ROS production. 3xTg-AD mice exposed to iAs presented alterations in their circadian rhythm and, specifically, males showed higher locomotor activity during the day and shorter free running periods prior to the onset of AD-pathology. Females had a slightly decrease in activity levels during their active phase compared to controls. iAs-exposed mice exhibited a significant difference in freezing time when compared to the control group. Immunopositivity to amyloid in sections of frontal cortex resulted in meaningful effects of the treatment. Moreover, results indicated increased oxidative stress, which was accompanied by an activation of inflammatory responses, as indicated by higher levels of mRNA coding for IL-6 and Nrf-2. These findings confirm that iAs exposure disturbs the circadian rhythm of locomotor activity, accompanied by the exacerbation of cognitive deficits and degenerative markers that are characteristic of AD.

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Keywords: Amyloid Beta (βA), Circadian rhythm, Interleukin 6 (IL-6), c-Fos Activity.

Profile of the release of amino acid neurotransmitters in rodent is depending of diurnal variation: Microdialysis study.

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Introduction: The main biological processes are periodic oscillations that are observed at all levels of organization of organisms. This periodicity is fundamental in biological systems because conferred advantage to an external environment that is cyclic.In humans maintaining these rhythms is required to generate appropriate responses to a cyclical environment, if this rhythmicity is lost or altered disorders and increased vulnerability to various diseases and harmful events occur. In the rat, for example traumatic brain injury causes further damage if it is induced in the dark hours compared to the hours of light. The balance of responses injury and neuroprotection could be depends of diurnal variations. **Aim:**Due to the relevance to understand the patron in the release of the different neurotransmitters, we propose to evaluate the extracellular levels of the aminoacids neurotransmitters in the brain cortex by microdialysis technique. **Methods:** Male Wistar bred-in-house rats (280–320 g, n=6). Rats were anesthetized, a guide cannula was implanted,into the cranium over the frontal cortex and, rats were allowed to recover for 3 days. On the experimental day 4th, a microdialysis probe was inserted into the guide cannula. Animals were placed in an individual container system for rodents (BAS). The probe was then connected to a micro-perfusion pump and continuously perfused. Dialysates collection (60 µL sample every 30 min) started after a 120 min stabilization period. All the microdialysis experiment during 72 hrs, the collection in this period was each 4 hrs.Measurement of extracellular neurotransmitter levels by High Performance Liquid Chromatography (HPLC). Statistical analysis consisted of using analysis of variance (ANOVA), followed by post hoc Tukey's test and Fisher post hoc analysis was used to analyze differences between groups. **Results:**Our results show the diurnal variation in the cerebral levels of nineteen amino acids,GABA, Glycine, Glutamate, phenylalanine, aspartate, glutamine, show peeks in the dark hours, this data is very interesting because could be open a new windows of investigation about the why is the biological importance of this variation and offer new possibilities of pharmacological and physiopalogical investigation. PAPIIT IN223417 and CONACYT 152510

Atomoxetine (ATX) in differentiated SH-SY5Y cells alters mitochondrial function

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Attention-deficit/hyperactivity disorder (ADHD) is the most common neurobehavioral disorder in childhood and is characterized by inattention, impulsivity and hyperactivity. ADHD is the most frequently diagnosed condition in children, affecting more than 5 million Mexicans. Atomoxetine (ATX) is a non-stimulant drug used in the treatment of ADHD, is a selective norepinephrine reuptake inhibitor. It has been shown that ATX has additional effects beyond the norepinephrine reuptake inhibition, affecting several signal transduction pathways and alters gene expression, for example: it was demonstrated that ATX acts as an NMDA receptor blocker; inhibits G-protein-activated inwardly rectifying K^+ channels; up-regulates BDNF expression in the prefrontal cortex, thus influencing synaptic plasticity and cognitive function; increases the expression of GABA A receptor subunits as well as ubiquinol–cytochrome c reductase complex core protein 2 and synaptosomal-associated protein of 25 kDa, which is an ADHD candidate gene and an important vesicle protein involved in axonal growth, synaptic plasticity and regulation of neurotransmitter release (Prog Neuro-Psychopharmacol Biol Psych 40:221, 2013; Brit J Pharmacol 160:283, 2010; Neuropsychopharmacol 35:1560, 2010; Pharmacol Res 62:523, 2010). Therefore, we have studied whether ATX affects mitochondrial function in differentiated SH-SY5Y cells exposed over a range of concentrations. Our data showed that concentrations between 1 to 50 µM of ATX in differentiated SH-SY5Y cells, produced alterations on the mitochondrial mass, membrane potential, complexes of the mitochondrial respiratory chain and autophagy. Thus, depending of the ATX concentration used, there are alterations on mitochondrial function, indicating that ATX produces additional effects beyond the norepinephrine reuptake inhibition.

Characterization of the circadian system in a Triple Transgenic model for Alzheimer's disease (3xTg-AD).

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Albeit the substantial number of reports regarding the alteration in physiological parameters in clinical and experimental studies of Alzheimer´s disease (AD), it is not known if the circadian system is equally affected in this malady as the cortex and the hippocampus. The aim of this project was to gain more understanding on the progressive modificationsin the 24 h-cycles of circadian locomotor activity, as well as in the structural alterations in the suprachiasmatic nucleus (SCN) of the 3xTG-AD mouse model of the AD. Male subjects of non-transgenic and transgenic mice were studied at 3, 8 and 13 months of age, to characterize early, intermediate and advanced stages of the neurodegenerative process. Comparisons were also done with the wild type mouse C57BL/6J. The next physiological studies were done: 1) Daily locomotive activity under different protocols of photic stimulation (light-dark cycles, jet-lag and continuous darkness) to finely typify the circadian system of the 3xTG-AD mice; 2) electroretinograms under photopic and scotopic conditions to evaluate the functionality of the neural communication in the retina. The cellular and histological integrity of the SCN was evaluated by the presence of the β-amyloid deposits (Aβ) as well as the hyperphosphorylated Tau protein (pTau) (both markers of the AD onset). So far, our results indicate very subtle modifications in the 24 h rhythms of locomotor activity under the different light-dark protocols, being more relevant the shortening of the period by almost 40 min in the transgenic mice. In contrast, electroretinograms done at 8 months old mice indicate a alteration in the electrical recording, suggesting a clear degenerative process in the retina of the 3xTG-AD mice. A preliminary conclusion is that the SCN and its underlying circadian activity is just modestly affected in the transgenic mice.

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Effect of Resveratrolon glucose transporter 3 expression in ischemia

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Cerebral ischemia is characterized by a violent diminution of the blood circulation to the brain. Ischemia activates several mechanisms involved in neuronal damage such as excitotoxicity. N-methyl-D-aspartate (NMDA) glutamate receptors are highly permeable to Ca^{2+} ion channels whose over-activation induces death exclusively in neurons. The extracellular glutamate concentration is finely regulated through the astrocytic Na^{+}-dependent glutamate transporter. Therefore, the preservation of the transmembrane Na^{+} gradient signifies an enormous energy cost for the astrocyte. Glucose transporters, GLUT-1 and GLUT-3, are the most abundant in the mammalian brain associated to the high level of expression in astrocytes and neurons, respectively. Under ischemic conditions brain cells regulate GLUT transporters expression in order to compensate its energetic deficiency**Objective.**In this work, we describe the effect of resveratrol on neurons, astrocytes, and endothelium under ischemic conditions. **Methods.**We utilize an in vivo occlusion of the middle cerebral artery (MCAO) model of ischemia.Wistar rats were subjected to MCAO for 2 h followed by 24 h of reperfusion. Resveratrol was given (1 mg/kg, in 50 % ethanol; *i. v.*) at the onset of reperfusion. We use an in vitro model of energetic stress created by transient glutamate excitation and oxygen and glucose deprivation (OGD) in different cultured cells. Neurons were cultured from cerebral cortex of rat embryos (E17-18)a used after 10 DIV. Astrocytes were cultured from neonatal cortex (3 days) and used after 10 - 21 DIV. Endothelium cell line (HBEC-5i), were used at 90% of confluence. Cultures were stimulated with 100 µM glutamate for 10 minutes followed bydifferent times recovery (from 1 to 3h). GLUT expression was evaluated by qPCR, western blot, and immunofluorescence assays. **Results and discussion**. We observed that MCAO increasedGLUT3 expression in the rat brain, while resveratrol prevented this alteration. In the other hand, we proved thatexcitotoxicity did not modify GLUT3 expression on neurons and astrocytes. Similarly resveratrol did not have any effect on these cell types. NF-κB pathway activation promotes transcription and translocation of GLUT3 to the membrane in conditions of OGD which simulates ischemia, although the pathway has not been clearly described, therefore it is possible that OGD is involved in changes observed on the entire brain. Our results suggest that resveratrol differentiallyalter the signaling pathways of transcription or translation of GLUT3 in brain cells in order to support neuronal energetics under stress conditions such as cerebral ischemia.

Neuropathology

ERβ-PKCα interaction plays an important role in medulloblastoma development

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Medulloblastoma, which arises in the cerebellum, is the most common malignant pediatric brain tumor according to the World Health Organization (WHO).The prognosis of patients depends on several factors due to the high histological and molecular heterogeneity of the medulloblastoma subgroups. It is known that estrogen receptor beta (ERβ) is important for development and maturation of cerebellar granular cells. Therefore, it is a possible factor associated to medulloblastoma biology.*In silico* studies have shown that protein kinase C alpha (PKCα) is able to phosphorylate ERβ with high probability. Furthermore, both proteins are expressed in medulloblastoma biopsies and it is known that ERβ and PKCαactivation increases proliferation of medulloblastoma cell lines.

The aim of this work was to study the ERβ and PKCα participation in medulloblastoma development as well as their interaction to regulate cellular processes such as proliferation using amedulloblastoma cell line.

To study if PKCα activation induces ERβ phosphorylation, cell cultures were treated with tetradecanoylphorbol acetate (TPA), a PKC activator, and samples were processed for co-inmuno precipitation and western blot technique. We observed a basal association between ERβ and PKCα with a significant increase after 5 minutes of treatment. In the same way, the treatment with TPA induces a significant increase in the ERβ phosphorylation from 5 to 30 minutes of treatment. Moreover, immunofluorescence showed that TPA induces the translocation of PKCα to the nucleus, where ERβ is mainly located. The treatment of the cells with the ERβ agonist diarylr propionitrile (DPN) 0.1 and 1 nM increases proliferation of the medulloblastoma cell line Daoy after 72 hours of treatment. The latter was determined by trypan blue exclusion method and BrdU assay.

Our results suggest that PKCα may play an important role in ERβ activation and consequently in medulloblastoma development.

Dihydroprogesterone promotes the growth of human glioblastoma cells

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5α-dihydroprogesterone (5α-DHP), the primary metabolite resulting from the major metabolic pathway of progesterone (P4), is known to be an important endogenous progestogen and neurosteroid. 5α-DHP is synthesized in high concentrations in both the uterus and the central nervous system.P4 has been shown to promote the progression of astrocytomas, the most frequent and aggressive brain tumors. However, the role of 5α-DHP in these tumors is unknown.Considering that 5α-DHP bindsto boththe intracellular progesterone receptor (PR) and the membrane progesterone receptors (mPRs), it is possible that this metabolite also favors the progression of astrocytomas.We studied the effects of different concentrations of 5α-DHP (1 nM, 10 nM, 100 nM and 1 μM) on the number of U87 and U251 cells derived from a high-grade human astrocytoma (glioblastoma) using a trypan blue dye exclusion assay. Each day during five consecutive days, cells were harvested and counted. We observed that 5α-DHP increased the number of both U87 and U251 cells, from day 3 until day 5 of treatment.The highest effect was seen in U251 cells with 10 nM 5α-DHP, and in U87 with 100 nM. These data suggest that 5α-DHP induces the growth of human glioblastoma cells. This work was financially supported by Consejo Nacional de Ciencia y Tecnología (Conacyt, CB250866).

Alterations of striatal cholinergic system in autism model in the rat

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The **A**utistic **S**pectrum **D**isorder (ASD) is a group of pervasive developmental disorders characterized by: deficits in social interactions, communication disorders,repetitive behavior and cognitive rigidity.Recent data have shown that the striatal cholinergic system, participates in the re-orientation of attention, which is affected in a model of ASD in rats.One of the components of the striatum (NStr) that participates in cognitive processesis the cholinergic interneurons (CIs), which are the main source of acetylcholine (ACh) in the NStr. In this sense, recent studies shown that the ACh levels are decreased in the animal models of ASD, in bothprefrontal cortex and the dorsal NStr, due to an increase in the levels of the acetylcholinesterase enzyme (AChE, an enzyme that degrades ACh), at least for prefrontal cortex. However, at the NStr level we unknown whether this is the only cause or others (e.g. decrease of enzyme that synthesizes the ACh, the choline acetyltransferase; ChAT or even in the number of CIs could be affected). Likewise, despite the importance of these CIs in cognitive processes, little is known if they are affected in ASD. Because of this, is our interest to study the striatal cholinergic system in rats exposed to valproic acid (VPA), as a model of autism, conducting behavioral and immunohistochemical tests for the enzyme ChAT.In the present investigation we shown that the striatal CIs, decreased in number and it was more important at the level of the **D**orso**L**ateral **S**triatum (DLS). This significant decrease in number of striatal CIs would have a direct impact on the levels of acetylcholine at the DLS. The decreased of ACh in the DLS, could affect the activity of the striatal microcircuit due to the lack of muscarinic and nicotinic acetylcholine receptors activation, which is in agreement with the increased levels of attention presented by the rats exposed to the VPA.

Diurnal variation of minocycline administration effect on behavioral recovery after a Traumatic Brain Injury in rats

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Introduction: Traumatic brain injury (TBI) is a relevant problem; both for research and health care, the economic impact of this event is high, thus offering neuroprotection processes that reduce the consequences for patients, as for the health system is of paramount importance. This work aims to address the problem of TBI, specifically regarding the neuroinflammation process that occurs after a TBI in addition to offering an effective neuroprotectionstrategy through the use of minocycline, an antibiotic that has recently been reported to have an anti-inflammatory and anti-apoptotic effects in the central nervous system (CNS), through the inhibition of microglial activation but also taking into account the possible diurnal variation of activation of the immune system in the brain, in order to offer a more effective therapeutic window. **Methods**: We used Wistar rats weighing 250-300 gr that were housed in single plexiglass cages with food and water available *ad libitum* at room temperature 21 ± 2°C and were given one week to acclimate before the experiments. All rats were maintained on a 12:12 light cycle with lights turn on at 08:00 for one group(normal cycle) or 20:00 for the other group (inverted cycle), each group were divided in five sub-groups(control, TBI+vehicle sacrificed 24 hrs after, TBI+vehicle sacrificed 72 hrs after, TBI+minocyclinesacrificed 24hr after and TBI+minocyclinesacrified 72hrs after), both TBI and sacrificewas performed at 13hrs(normal cycle) or 1hrs (inverted cycle), n=6 for each group.The rats were anesthetizedand a severeTBIwasinduced witha calibrated pneumatic piston on the exposed skull to impact the motor cortex previously determined by a stereotaxic device. Behavior response was analyzed using a Hunter´s scale and a cylinder test and the evaluations were made before and after TBI. The results were graphed and analyzed with ANOVA statistical test. **Results:** Hunter's scale showed no statistical differences between vehicle vs. minocycline groups on either cycle, but a tendency is showed towards better scores resulting in lesser neurological damage at 24 hrs and specially at 72 hrs in groups treated with minocycline posterior to TBI. Cilinder´s test showed more evident results, revealing statistical differences between the vehicle vs.minocycline groups, both at 24 hours and at 72 hours, on either cycle.We conclude that minocycline administration results in better behavioral recovery in inverted cycle, however we need to corroborate with the immunological profile of microglia. The Project was supported by PAPIIT IN223417.

Protective effects of cannabinoid-profile agents against the synergistic toxicity of glutaric acid and quinolinic acid on in vitro models

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Introduction: Glutaric acidemia type I is a genetic metabolic disease, caused by mutations in the GCDH gene. Deficiencies in this protein result in incomplete amino acid degradation and accumulation of toxic intermediate metabolites, mainly glutaric acid (GA). These toxins elicit a wide array of damaging phenomena in the CNS, including excitotoxicity and oxidative stress; however, accumulation of organic acids is not enough to explain all the toxic events in the disease. Quinolinic acid (QUIN) is a neuroactive metabolite of the kynurenine pathway and a potential excitotoxin. When this pathway is altered, QUIN can be elevated to toxic levels. Though a synergistic toxic effect between these two agents has previously been reported in other in vitro models, it still requires characterization.

Many stimulants of the ECS, including the endogenous anandamide (AEA), have shown neuroprotective potential in different toxic models. The increase on the levels of AEA, both by direct application and by regulation of its metabolism, has been shown to exert neuroprotective effects.

Objective: To test the synergistic effect of glutaric acid and quinolinic acid in brain cotical slices and primary neuronal cultures, as well as the potential neuroprotective effect of annandamide and URB597 in the synergistic toxic model.

Materials and methods: Brain slices were preatreted for 1h with anandamide (10 and 15 µM) and then treated with toxic and subtoxic concentrations of GA and QUIN. In order to test the level of toxicity and protection, cell viability was evauated through the MTT assay.

Primary cortical neurons obtained from Wistar rats were pretreated for 24 h with anandamide (10 µM) and then treated with both toxic (100µM QUIN and 1mM GA) and subtoxic (50µM QUIN and 500µM GA) concentrations of QUIN and GA (independently or in combination). Cell viability was evaluated by crystal violet assay.

Results: Slices trated with sub-toxic concentrations QUIN or GA *per se* showed no significant decrese in viability; however, the co-application of both tratments caused a significant loss of MTT reduction. Pretreatment with 10 µM AEA significantly protected against decreased viability induced by both toxic agents, either *per se* or in combination. In cell cultures quinolinic acid (100µM) has shown signifacant toxicity. Cell viability in cells pretreated with 10µM AEA have shown a significant reduction in viability loss.

Conclusions: GA and QUIN can exert a synergistic toxic effect when co-applied to cortical brain slices. AEA exerted a protective effect when applied as a pretreatment, against the toxic effects of QUIN and/or GA in both brain slices and cell cultures.

Mifepristoneimprove the efficacy of temozolamide in glioblastomaassociated with DNA damage repair and Apoptosis

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Glioblastoma multiforme is the most common primary central nervous system tumor. The standard treatment is surgery, followed by chemotherapy (Temozolamide) and radiotherapy. However, the response to this treatment has not been sufficient with an average survival of patients 10-12 months. Therefore it is important to have new strategies that modify molecular targets and consequently increase therapeutic efficacy, decrease resistance and remission of the disease. Recent studies have shown that Mifepristone (Mif) increases the cytotoxicity of various antineoplastic. The aim of the present study is to investigate whether Mifepristone can modulate the growth of glioma tumors treated with Tz, as well as the study ofthe molecular mechanisms involved in the response to therapy such a apoptosis and repair to DNA damage proteins.The effect of Mifepristone combined with Temozolamide was evaluated in an orthotopic model of glioblastoma. C6 cells were implanted in Wistar rats and the weight was followed throughout the study, after 2 weeks post-implantation the rats were arranged in four groups including: A) Sham, B) Control (without treatment), C) Tz, D) Mif and E)Tz-Mif. Cell proliferation was evaluated by PET/CT images using 18F-FLT and KI-67; hematoxylin and eosin method was used to observe the morphology. For the evaluation of apoptosis and repair to DNA damage were performed western blot using the corresponding antibodies (Bcl-2, Bax and caspase 3and the DNA repair enzyme O6-methylguanine DNA methyltransferase MGMT). Our results showed an accelerated decrease in the weight, hypercellularity, mitosis and necrosis in control and Tz groups indicating rapid tumor proliferation, while in the rats with Tz-Mif the weight was maintained throughout the study and few Ki-67 positive cells were observed; these results were comparable with PET/CT images, showing a decrease of 18F-FLT uptake. In western blot we observed an increase in the levels of Bax and CL-caspase 3 and a decrease in Bcl-2 levels, as well as a decrease of MGMT in the group of Tz-Mif. Our results suggest that the combination of the antihormonal agent with the standard chemotherapy could improve the efficacy of temozolamide in glioblastoma.

Characterization of chemical biomarkers in a novel *in vivo* model of ictogenesis

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Purpose. Epilepsy is a common neurological disorder in which the random nature of seizures poses difficult research challenges. We recently developed a novel *in vivo* model of ictogenesis, allowing experimental modulation of the risk of temporal lobe seizures. In the present study, we used this model to search for biochemical changes associated with increased seizure risk.

Methods. Male Sprague-Dawley rats with intraperitoneal pilocarpine were prepared to generate Epileptic (n=15) and Control (n=15) animals. A cannula implanted into *nucleus reuniens* was used for local KCl or PBS injection while another cannula was implanted into left hippocampus for microdialysis experiments. During intracerebral microdialysis experiments, KCl or PBS were injected (120 mM or 1X, respectively; 0.1 µl/min over 5 min) into the *nucleus reuniens* of freely moving rats, a process recently shown to increase the risk of seizures up to three-fold. This injection was a total of 9 times, with 15 min between injections, comprising 180 min. Dialysates were collected before (6 collections), during (27 collections) and after (4 collections) KCl or PBS injections, derivatized immediately, and analyzed by liquid chromatography-mass spectrometry (LC-MS) to assess the extracellular concentrations of 24 different neurotransmitters.

Results. The majority of Epileptic animals had seizures during the KCl injections. The LC-MS analysis revealed that pilocarpine produced significant differences in baseline levels of several neurotransmitters: decreased levels of adenosine (79%), homovanillic acid (55%), and serotonin (86%), and increased levels of choline (286%), glutamate (66%), phenylalanine (67%), and tyrosine (51%). During the KCl injection into *nucleus reuniens*, which increased the risk of seizures, there was an additional significant change from baseline in several neurotransmitters. Both the difference in baseline concentrations (between animals with and without pilocarpine-induced seizures) and the difference during the KCl injection showed complex interactions between multiple neurotransmitters, an effect that we quantified using stepwise logistic regression.

Conclusion. Our results are the first to show how the pilocarpine model alters the basal hippocampal extracellular concentrations of 24 different neurotransmitters at the same time. In addition, we have identified several neurotransmitters that are altered during a time period of increased seizure risk. These neurotransmitters, and the interaction between them, are candidates for future experiments investigating the basic mechanisms of ictogenesis, search for biomarkers associated with that risk, and potentially develop and optimize more effective antiseizure therapies.

Synthesis and characterization of the SiO_2/DA microimplant for evaluation in a hemiparkinsonism rat model

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Parkinson's disease (PD) was first described in 1817 by James Parkinson in his essay "Resting Tremor" as "agitating paralysis", which is now known as a neurodegenerative disorder and is the second most common worldwide. In the literature, PD has different associated causes, highlighting the loss of dopaminergic cells in the substantia nigra pars compacta (SNpc), causing a remarkable increase in the emission of inhibitory impulses from the reticulated part of the substantia nigra and the medial part of the pale globe towards the thalamus, consequently reduction of excitation of the motor cortex [1].

Patients affected with PD are commonly in advanced age presenting motor affections such as resting tremor, bradykinesia and muscular rigidity, in addition to non-motor like cognitive dysfunction, neuropsychiatric problems and sleep disorders. On the other hand, it should be mentioned that the symptoms appear when between 60 and 70% of the cells present in the SNpc have died, although a totally effective treatment has not been generated against this disease.

The most frequently used treatment for PD is the L-DOPA decarboxylase inhibitor, however it is expensive and causes an exacerbation effect in approximately 3 to 5 years.

According to the literature, the neurotransmitter dopamine (DA) is a unstablemolecule due to the sources of reactive oxygen $(ROS)_3$, which causes spontaneous oxidation *in vitro*, producing the appearance of neuromelanin, which is highly cytotoxic.

In the development of new technologies for drug delivery and transport, the sol-gel process has emerged as a platform for the stabilization and co-gelation of drugs for the treatment of different diseases. Among the research platforms, SiO_2 has been outstanding in recent years due to its structural characteristics, such as surface area modification, biocompatibility [2] and chemical, thermal and low cost stability.

For this reason, this work describes the development of a drug release system through the synthesis of a microimplantof silicon dioxide synthesized by the sol-gel method, which encapsulates the dopamine. This microimplant named SiO_2/DA, which will allow to transport the DA to the striatum to be used as a treatment in the PD in tests *in vivo*. Thismicroimplantwill be characterized by Infrared Spectroscopy (IR) and Electronic Microscopy of Transmission (TEM). The behavioral evaluation will be performance by test of exploration, locomotion in an open field and spinning behavior.

Keywords: Parkinson'sDisease, Dopamine, Silica

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Retinal and visual cortex identification of $A\beta_{pE3-42}$ and $A\beta pE_{11-42}$ peptides in Alzheimer disease and normal aging

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized primarily by memory loss. There are pathological changes in the brain such as marked atrophy and significant neuronal damage, accompanied by abnormal accumulation of amyloid beta peptide (Aβ) and the formation of neurofibrillary tangles of the hyperphosphorylated Tau protein [1]. Aβ peptides are formed from the processing of the Amyloide Precursor Protein (APP), with Aβ40 and Aβ42 being the most abundant. Besides of these peptides, recent studies have identified different amyloid species like N-truncated peptides such as N-terminal AβpE3-42 and AβpE11-42. In both cases formed by the enzyme Glutaminyl cilase (Qc) in the brain of AD patients. Some studies also show that AβpE3-42 and AβpE11-42 are more propense to aggregate, and can function as a seed for Aβ1-42[3] that increases its toxicity. There is no clear information available about how these modified species are eliminated in the brain and other areas of the central nervous system. There are reports that mention that 60% of AD patients, display visual function deffects and it has been demonstrated the presence of amyloid peptide deposits in these patients.[3] However, if N-truncated species are present in the retina and if there are changes in their production as well as on the clerance mechanisms of them during the development of AD pathology and normal aging has not yet been demonstrated.

well as the enzymes involved in its elimination as Prolil Endopeptidase (PreP)[2] **Aim.** To identify the accumulation of AβpE3-42 y AβpE11-42 peptides, as well as to evaluate changes in the expression of Qc and on the enzyme Prolil Endopeptidase, involved in the production and elimination ob N-truncated peptides respectively in cells of the antero-posterior visual pathway. thus stablishing a relationship between AD's neuropathology and the peptide's aggregation. **Methods** Double immunofluorescence (IF) assays (; Aβ3-42, Aβ11-42 1-42) were performed on histological slides of visual cortex and the retina from 18 months old (18M) triple transgenic mice for Alzheimer's disease (3xTgDA) (18 months old) and old mice (18 M). Wild type and 4 M old mice were used as control gropus respectively. To confirm changes in the expression of these enzymes, WB and QPCR-RT techniques were performed .**Results**. The results shown an increase in the accumulation of AβpE11-42 and AβpE3-42 peptides in the retina and visual cortex of transgenic mice as well in 18 M old mice compared to control groups. In the same way, differential expression of enzymes involved in the production and degradation of N-truncated peptides were identidfied. **Conclusion** Our results demonstrate for the first time the detection and identification N-truncated peptides in structures such as retina and optic nerve in a model of AD as well as the expression of differential levels of the enzymes involved in its processing, allowing us to obtain a broader perspective of what occurs in patients with this pathology.

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Role of autophagy in the protective effect of the ketone body Beta-hydroxybutyrate against ischemic injury induced bythe occlusion of the medial cerebral artery

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Cerebral vascular disease (CVD) including cerebral ischemiais the second cause of death and the third cause of disability worldwide. In Mexico, the rate of mortality caused by CVD has been estimated in 292/100 000 inhabitants. The most common cause of CVD in humans is the occlusion of the medial cerebral artery (MCAO). At present, there is no effective treatment to prevent neuronal damage caused by CVD, thus the investigation of new therapeutic strategies to reduce ischemicbrain injury is of great relevance. It is well known that ischemic neuronal damage involves energy deficitand oxidative stress. However, the role of autophagy is still controversial. Autophagy is a lysosome-dependent degradation process involved in the recycling long-lived proteins and damaged cellular components, however when it is impaired, it can contribute to cell death. We have previously reported that the ketone body beta-hydroxybutyrate (D-BHB) can substitute for glucose as an alternative fuel during glucose deprivation in cultured neurons and severe hypoglycemia in vivo, preventing oxidative stress and neuronal death. In addition, we recently reported that D-BHB stimulates the autophagic flux and prevents neuronal death in cortical cultures. In the present study we have investigated whether the protective effect of D-BHB against neuronal death induced by MCAO correlates with the stimulation of the autophagic flux in the cortex and the striatum. The autophagic flux was evaluated at different times after MCAO in control animals and animals treated with D-BHB by the conversion of LC3-I in LC3-II, which indicates autophagosome formation, and by the decrease in p62 content, a protein involved in autophagic degradation, by immunoblot. Ischemic injury was evaluated by the determination of the infarct volume and neuronal death by Cresyl violet and Fluoro-Jade B staining. The neurological outcome was determined by monitoring motor alterations and animal survival. Results indicate that animals treated with D-BHB show a significant reduction in the infarct volume and the number of dead cells in the parietal cortex. They also show decreasedmotor alterations and increased animal survival. These results correlated with a partial decrease in LC3-II and p62 content in the striatum and the cortex of stroked animals treated with D-BHB, suggesting that the ketone body stimulates the autophagic flux.
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Effect of Transcranial Magnetic Stimulation in a model of rat´s Hemiparkinson

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Transcranial magnetic stimulation (TMS) is a non-invasive therapy that can alter the excitability of the cerebral cortex and other parts of the brain. TMS has been used as a treatment in different neuropsychiatric and neurodegenerative pathologies, although its mechanism of action has not been fully elucidated. In patients with Parkinson's disease (PD), TMS induces some recovery of motor and cognitive function, as well as an increase in dopamine. It is known that the frequency of TMS stimulation plays a critical role in the induced response; so, frequencies greater than 5 Hz typically induce facilitation, whereas those less than or equal to 1 Hz induce plasticity inhibition. Accordingly, the following question arises: What effects has the transcranial magnetic stimulation of different frequencies on the animal model of PD? Thus, the aim of the study was evaluated motor behaviors related to the animal model of PD, induced by low (1 Hz) or high frequency (5 Hz) EMT.
Methods: Hemiparkinson model was used in adult male Wistar rats (200-220 g). The model consists of lesioning with 6-OHDA the right nigrostriatal pathway. The lesion was evaluated 10 days later with the apomorphine-induced rotation test. The following experimental groups were formed (n = 4 in each): 1) control (non-injury); 2) false injury (sham); 3) false injury + EMT; 4) sham + false stimulation; 5) injured (Lx); 6) Lx + EMT and 7) Lx + false stimulation. The EMT was performed with the EMAGPRO12 device and an eight-shaped coil. The device allows to provide magnetic stimuli to rodents, in the same way that an EMT does in humans. The stimulation parameters selected were 1 and 5 Hz, 10 min/day for 30 days. Stimulation was initiated 15 days after the injury. Before and the day after the last day of stimulation, the behavioral tests performed were: balance bar, curling, open field and elevated plus maze.

Results: There were no significant differences in any behavioral tests for TMS or false stimulation in the control and sham groups. Both groups of injured animals receiving EMTs of 1 Hz or 5 Hz showed significant differences in the equilibrium bar (increased travel time) and the elevated plus maze tests (greater% of entrances and spend time in the open arms) versus lesioned animals. The other behavioral tests do not show significant differences in these groups.

TMS produces electrical currents in cortical regions beneath the stimulation coil that can change excitability of cortical and subcortical structures depending on the frequency of the stimulation applied. However, in the animal model of hemiparkinson that we developed, both frequencies used to stimulate (1 and 5 Hz) induce similar changes in the balance and a reduction of the anxiety of the animals.

Conclusion: The behavioral tests used in the EP model are not conclusive to differentiate the effects of high versus low frequency EMT. Other frequencies must be tested, in addition to correlating the behavioral data with immunohistochemical and expression of specific neuronal markers involved in the response to EMT stimulation.

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FEEDING RESTRICTION CONFERS AN ANTI-OXIDATIVE EFFECT IN RAT HIPPOCAMPUS AFTER SEIZURE INDUCTION.

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Introduction: Several reports on experimental models and epilepsy patients have demonstrated that oxidative stress has a crucial role in epilepsy because it contributes to the etiophatogenesis of seizures and neuronal cell death. On the other hand, metabolic-based diets such as ketogenic diet (KD) and calorie restriction (CR) have shown to have an anti-oxidative effect on several models of neurological or neurodegeneration diseases. We recently described that feeding restriction (FR) had an anticonvulsant effect that was mediated by metabolic and epigenetic changes; thus, the aim was to investigate whether FR could have an anti-oxidative effect in *status epilepticus* (SE)-induced hippocampus. **Objective:** To analyze the anti-oxidative effect of FR in hippocampus after the induction of SE by pilocarpine injection. **Methodology:** Male Wistar rats weighing 220 g were divided into four groups (*Ad libitum* (AL), FR, AL+SE, and FR+SE). AL animals had free access to food and water. FR schedule consisted of allowing rats to feed only for two hours daily for 21 days with free access to water. The pilocarpine seizure model consisted of a pre-treatment of lithium chloride (3mEq/kg i.p.). Eighteen hours after LiCl injection, animals were injected with scopolamine (1 mg/kg s.c.) and 30 min later, animals received a pilocarpine injection (60 mg/kg s.c.) to induce SE for 90 minutes and immediately attenuated with diazepam injection (5 mg/kg). AL animals followed the same procedure without pilocarpine injection. All animals were sacrificed during an acute time-course (3, 8 and 24 h) and their hippocampi were used to measure malondialdehyde (MDA) levels, a subproduct of lipid peroxidation. Moreover, frozen brain slices were obtained to measure superoxide ion in CA1, CA3 and dentate gyrus of hippocampus by fluorescent microscopy using dihydroethidium (DHE) on rats 24 h after pilocarpine injection. **Results:** Preliminary results showed that pilocarpine-injected rats had significant high levels of MDA compared with those of AL animals or FR-treated animals throughout the time-course. Interestingly, rats subjected to FR and SE-induced showed a slight decrease of MDA levels during the time points of 3 and 8 hours. However, after 24 h post-SE, MDA levels were statistically reduced compared with those of ALSE rats. Furthermore, animals subjected to FR and SE had a reduction of fluorescence of DHE in all regions of hippocampus compared with that of pilocarpine-injected rats. **Conclusion:** Our results suggest that FR had an anti-oxidative effect on pilocarpine seizure model by reducing MDA levels and superoxide ion presence in the hippocampus 24h post-SE. Even though, we do not know the precise mechanism which FR exert this effect, it is important to note that FR has similar effects that other diets such as KD o CR whereby could be used as a new alternative therapy. This project was supported by CONACyT 239594 grant.

Keywords: Status epilepticus, oxidative stress, lipoperoxidation, reactive oxygen species, feeding restriction.

Recurrent moderate hypoglycemia enhances brain injury induced by the hypoglycemic coma and leads to memory decline

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Most Type 1 Diabetes Mellitus (TIDM) patients who are under intensive insulin therapy suffer from repetitive episodes of moderate hypoglycemia (RMH), which increase the risk for severe hypoglycemia (SH). SH can progress to the coma state, which induces neuronal death in vulnerable brain regions such as the cortex, the hippocampus and the striatum by a mechanism involving oxidative damage. However, the consequences of RMH on neuronal damage and cognitive function are not well understood, nor its effect on a subsequent period of hypoglycemic coma. The purpose of the present study was to investigate whether RMH can exacerbate neuronal damage and cognitive decline induced by a short (7-10 min) coma period in an in vivo model. Rats received an injection of insulin (6.5 insulin units, IU) during 7 consecutive days leading to moderate (40 mg/dl glucose) hypoglycemia. At day 8 animals received 32 IU to induce the hypoglycemic coma and were rescued with glucose after 7-10 min. Neuronal death and oxidative damage were assessed 24 after the coma by histological analysis and immunocytochemistry. Reduced glutathione (GSH) and glycogen content in brain was also assessed. Cognitive function was evaluated5, 7 and 15 days after the coma, in two memory tests. Results show that previous RMH exacerbates oxidative damage and neuronal death induced by the hypoglycemic coma in the parietal cortex and the striatum but mainly in the hippocampus. These changes correlated with a severe decrease in GSH, glycogen super compensation and a significant spatial and contextual memory deficit. Treatment with the antioxidant N-acetyl-Cysteine reduced neuronal death and cognitive decline in the hippocampus. Results demonstrate that previous RMH enhances brain vulnerability to acute hypoglycemia by a mechanism involving decreased antioxidant defense and oxidative damage. They also highlight the relevance of an adequate control of moderate hypoglycemic episodes in TIDM.

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Área: Neuropatología.

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"Alpha-mangostin attenuates inflammation induced by systemic LPS administration in C57BL/6J mice and ameliorates memory deficits in a transgenic mouse model of Alzheimer´s disease"

Neuroinflammation is an important featurein the pathogenesis and progression of several neurodegenerative diseases such as Alzheimer´s disease, Parkinson´s disease, multiple sclerosis and amyotrophic lateral sclerosis. Neuroinflammation is characterized by reactive astrocytes and microglia, besides the increase in levels of pro-inflammatory cytokines in the brain. For that reason, the use of anti-inflammatory compounds has been proposed as an alternative for the treatment of neurodegenerative diseases. Currently, numerous studies are carried out with natural and synthetic compounds aimed to prevent or decrease the neuroinflammation involved in thesedisorders. Recently, alpha-mangostin (α-MG), a natural polyphenolic compound derived from the pericarp of mangosteen fruit, has gained interest because of its multiple properties including anti-bacterial, anti-oxidative, anti-inflammatory and anticanceractivity, as well as acetylcholinesterase and Aβ peptide aggregation inhibitor capacity. Based on previous information, in this study we evaluated the anti-inflammatory effect of α-MG onneuroinflammation induced by peripheral LPS administration in C57BL/6J mice and its capacity to ameliorate memory deficits in a triple-transgenic mouse model of Alzheimer´s disease (3xTg-AD).

First, weobserved that α-MG treatment diminished diarrhea and conjunctivitis signs observed in mice after the LPS administration.Then, we found that α-MG attenuated the increase in serum and brain IL-6 protein levels in LPS-treated mice. In addition, we demonstrated that α-MG is capable to reduce the increase in brain COX-2 levels induced by LPS. Interestingly, we found that increase in brain COX-2 levels occurred in vascular endothelial cells.

On the other hand, we investigated theα-MG effect on glial activation induced by peripheral LPS administration. We have shown that α-MG significantly decreased TSPO expression, a glial activation marker, in both cortex and hippocampus from LPS-treated mice. Moreover, we found thatTSPO expression was increased in vascular endothelial cells from LPS-treated mice but not in mice fed with α-MG prior to LPS challenge.

After documenting the anti-inflammatory effect of α-MG in the brain, we tested the effect of α-MG on behavior in 12-month-old 3xTg-AD mice. We found that α-MG-treated mice performed better than vehicle-treated mice in a novel object recognition test. Probably, this effect is associated to the anti-inflammatory activity from α-MG, however, othermechanisms may be involved.

In summary, our results show that α-MG can attenuate neuroinflammation induced by peripheral LPS administration in C57BL/6J mice by reducing brain IL-6, COX-2 and TSPO levels. In addition, weshown that α-MG attenuates vascular endothelial cell activation induced by LPS-treatment, which are important players in brain inflammation.
Finallyα-MG is able to ameliorate memory impairment in a triple-transgenic mouse model of Alzheimer's disease.

The activation of membrane progesterone receptors promotes the migration and invasion of human glioblastoma cells

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Glioblastomas are the most frequent and aggressive brain tumors in humans, which arise from glial cells or glial precursor cells. They mainly occur in adults between 45 and 65 years-old, are highly invasive, and the survival rate after diagnosis is lower than two years. Several factors are involved in glioblastoma development including sex hormones such as progesterone (P_4), which is a steroid hormone with multiple functions in the central nervous system. P4 exerts its effects through two different mechanisms of action: the classical and the non-classical one. In the first mechanism of action, P4 interacts with its intracellular receptor (PR), which is a ligand-activated transcription factor. The second mechanism of action involves the activation of specific P4 membrane receptors (mPRα-ε) that trigger diverse signaling pathways that induce the formation of second messengers, modifications in the conductance to specific ions as well as the activation of different kinases.

It has been observed that P_4 induces the migration and invasion of human glioblastoma cells through its interaction with PR. However, the treatment of glioblastoma cells with PR antagonists partially inhibits the effects of the hormone. Besides, both mPRα and β subtypes have been described to be expressed in human glioblastoma cell lines (U87 and U251). This suggests that P_4 should exert its effects through other signaling pathways such as those regulated by mPRs. In this work, we used the selective mPRs agonist 10-ethenyl-19-norprogesterone (Org OD 02-0, 10 and 100 nM) to treat U87 and U251 cells and evaluate their migration and invasion capabilities with a scratch-wound and transwell assays, respectively. We observed that mPRs activation with 10 and 100 nM of Org OD 02-2 significantly increases the migration of U251 and U87 cells from 3 to 24 h. When evaluating cell invasion, we observed that only the concentration of 100 nM increases invasion in both cell lines. Our results show that P_4 promotes migration and invasion processes in U87 and U251 glioblastoma cell lines through the activation of mPRs.

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Focal Cerebral Ischemia Induces Early Protein and Genetic Expression Changes.Cytoplasmic and MitochondrialProteomicStudy.

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Ischemic stroke is the second cause of death and third in disability worldwide remaining as the major socioeconomic burden. Focal cerebral ischemia (FCI), which is caused by reduced blood supply to the brain tissue, produces biochemical, metabolic and genetic alterations leading to cellular injure. To assess protein and genetic changes, FCI was induced in Wistar rats by middle cerebral artery occlusion (MCAO) during 15 min and 1h with and without 24h of reperfusion;striatum, hippocampus and cerebral cortex 2DE proteomic map was done; spots with protein expression changes were selected and identified by mass spectrometry. In cytoplasm it was founded: NDKA, PEBP-1, Actin cytoplasmic 2 and SOD1 lower protein expression and14-3-3γ higher protein expression changes in cerebral cortex; in mitochondria: lower expression of Prohibitin and Elongation factor Tu was founded in hippocampus, and higher expression of α-Syn, β-Syn, Aconitate hydratase, and 14-3-3γisoform X1 were observed, in striatum and hippocampus. NDKA (related with neurodegenerative disorders and oxidative stress), PEBP-1 (HCNP precursor), 14-3-3γ (involved in apoptosis pathway), α-Syn (related with neurodegenerative disorders), protein expression changes was detected by immunohistochemistry; mRNA expression changes were founded by PCR real time. 14-3-3 antagonizesapoptotic signals binding to pro-apoptotic phosphorylated Bad protein in the S112, S136and S155motifs, which showed an increase in early FCI (S136 and S112 [30%] and S155 [7%]) anddiminishedin FCI/RP, assessed by western blot. Hematoxylin-eosin stain of four micrometer-thick sections of paraffin embedded tissue shows cellular shrinkage, nuclear pyknosis, cytotoxic and vasogenic edema. Here we show that brain response to FCI take place since the first minutes of MCAO, showing protein and genetic expression changes both in cytoplasm as well as in mitochondria. These proteins are involved in several metabolic and biochemical pathways.Analysis of protein and genetic changes in FCI and the study ofsignaling pathway were they participate, could give us more options for new therapies investigation.

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ACTIVATION OF AMPK BY RESVERATROL HAS A NEUROPROTECTIVE EFFECT

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Cerebral ischemia results from occlusion of a major cerebral artery.Decrease of cerebral blood flow restricts the delivery of substrates, predominantly oxygen and glucose, with the consequent decline in energy metabolism. This reduction involves a sequence of molecular events such as excitotoxicity that inducesneuronaldeath. Interestingly, cellulardeath can be prevented by modulating the energetic state of brain on animals subject to ischemiaand treated with resveratrol (RSV). RSV is an antioxidant that exerts a neuroprotective effect on cerebral ischemia through various mechanisms that have not been clarified. Notably, RSV activates AMP-activated kinase (AMPK), an enzyme whose function is closely associated with activation of ATP generating pathways.**Objective**.To investigatethe effect of RSV on AMPK activation and autophagy induction on*in vitro* model of energetic stress induced by transient glutamate excitation.**Methods.**Rat primary neuronal cultures (E17) of 7-8 days *in vitro*were stimulated with glutamate (100 µM)for 10 min. RSV (100 µM) was administered at the start of recovery. Then, analyses were performed after different times of recovery. Cell viability was assessed by MTT reduction and LDH release. Production of superoxide radicals was measure based on NBT reduction.Mitochondrial calcium buffer capacity was analyzed with the fluorescent probe Fluo-4 AM. AMPK activationwas assessed by phosphorylation status and induction of autophagy through LC3 (I and II) levels measured by Western blot. **Results.**Excitotoxicity reduced viability and increased superoxide radicals production and the number of cells unable to buffer cytoplasmic calcium, showing that mitochondrial function was altered. Excitotoxicity increased AMPK phosphorylation level after 20 min of recovery and RSV induced an additional increase on it. RSV exerted a protective effect that was prevented after AMPK pharmacological inhibition with compound C. RSV partially preventedthe mitochondrial loss of buffer capacity, besides, it induced LC3-II levels at short recovery times. **Discussion.**RSV has a beneficial effect againstexcitotoxicity that depend on activation of cell survival pathways linked toAMPK activation. Our results suggest that neuronal activation of AMPK by RSV is associated to autophagy activation; recycling of organelles and biomolecules might increase the energetic supplies required by the cells under stress and that may lead to the restoration of energy metabolism.

DNA methylation and gene expression of astroglia before, during and after oxygen and glucose deprivation

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Epigenetic mechanisms such as DNA methylation are a well known radical modifier of genetic expression in cancer and neurodegenerative diseases, mainly through a substantial transcription regulation in active and inactive promoters and modifing transcription elongation and splicing in CpG islands located intra and intergenically. It is also widely recognized that astrocytes, the main regulator cell in the brain, play a crucial role in neurovascular-related disorders like ischemic stroke and malignant tumours, however in molecular resolution there are few works that describes the overall genetic and epigenetic programme of these complex phenomena, and events like reperfusion damage remains only partially understood. We established the relationship between DNA methylation and gene expression in a hypoxia model in an astrocyte-like human grade I non-tumorigenic glioblastoma via RNA-seq and Methylated DNA Immunoprecipitation sequencing (MeDIP-seq) analysis. In general, we identified several genomic features including promoters and enhancers whose methylation levels change not only during oxygen and glucose deprivation (OGD) but also after eight hours of recovery, along with statistically significant differences in both high and low CG promoters addresing house-kepping and ubiquitous genes and cell lineage-specific genes. Moreover, these DNA methylation remodeling were correlated, in both OGD and recovery, with gene expression of several genes and these organization of the transcriptome and methylome is different in both normoxia and OGD as well as in recovery. These results could elucidate the overall transformation of cells in terms of transcription and DNA methylation in patological occurrences involving ischemia and disclose the reperfusion stage damage to genomic scaled, uncompletely described until now.

Studying Cu(II) interactions with amyloid-β peptide and Prion Protein: Insights into the molecular battle for Cu(II) in Alzheimer Disease

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In the brain, Cu(II) is an essential cofactor and neuromodulator[1] of synaptic activity. Neurodegenerative diseases, such as Alzheimer Disease (AD), are associated with disruption of Cu homeostasis. AD is characterized by an extracellular deposition of amyloid-βpeptide (Aβ)aggregates[2]. Amyloid plaques contain high concentrations of Cu[3].At physiological pH, Aβcoordinates Cu(II) with high affinity and forms two different Aβ-Cu(II) coordination modes[4].Although the interaction between Aβ and Cu(II) has been associated withneurotoxicity, the neurotoxic mechanisms are not well understood. Recently, another Cu-binding protein—theCellular Prion Protein (PrP^C)—hasbeen implicated in Aβtoxicity[5]. PrP^Ccan coordinate six Cu(II) ions[6] andtheseinteractions are important for itsneuromodulation and neuroprotective properties[5].Earlyreports proposed that Aβcan disrupt the interaction between PrP^C and Cu(II)[5]; however, it is not clear howthis interactionmight occur.In this study, we evaluated the ability of Aβ to compete for Cu(II) ions with eachbinding site inPrP^C, using electron paramagnetic resonance and circular dichroism. Our results showthat Aβcancompete for Cu(II) with PrP^C. The formation of new species with spectroscopic features that are distinct fromAβ-Cu(II) and PrP-Cu(II) complexes was observed.These results provide a new perspective about Aβ-Cu/PrP complexes that might be relevant to understandAD pathogenesis.This research has been funded by CONACYT (grants **#**221134 and fellowships to Y.P. and L.P.O.).

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Antinociceptive effect of ferulic acid in painful diabetic neuropathy rats

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Painful diabetic neuropathy (PDN) is one of the most common and debilitant consequences of diabetes mellitus. Up to 34% of diabetic population suffers from pain, and the relief of this is infrequent and unsatisfactory. Recently, some reports indicate that ferulic acid (FA) diminishes tactile allodynia and reduces vincristine-induced thermal and mechanical hyperalgesia. The aim of this study was to assessment the antinociceptive, antiallodynic and antihyperalgesic effect of FA on PDN rats. Diabetes was induced in female Wistar rats (220-240 g) by a single streptozotocin injection (50 mg/kg, i.p.). Acute and chronic effect of FA (0.3-100 mg/kg. i.p.) was determinate in chemical hyperalgesia, thermal hypoalgesia and, tactile allodynia on PDN rats. To determinate the mechanism of action involved in the antinociceptive effect of FA, animals were administrated with L-NAME, methiothepin, WAY-100635, BRL- 15572, SB-224289 y SB-69955. Results showed that acute and chronic treatment with FA reduced nociception and tactile allodynia in PDN rats, whereas that, thermal hypoalgesia was reduced by chronic administration of FA. Moreover, antinociceptive effect of FA, on the formalin submitted rats, was prevented by the administration of methiothepin, WAY-100635, SB-224289, BRL-15572 y SB-699551, but not L-NAME administration. As a conclusion, data suggest that acute and chronic treatment with FA prevents and reverts the PDN. In addition, the antinociceptive effect of FA involves the serotonergic pathway through specific activation of 5-HT$1_{A/B/D}$y 5-HT5_{A}receptors.

The Indirect Pathway of the Basal Ganglia Contributes to the Switch Between Action Sequences.

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The ability to learn and generate sequential actions is a behavioral mechanism used by all organisms as part of the skills to ensure their survival. Pathologies affecting the functionality of basal ganglia nuclei, such as Parkinson's, Huntington's and other motor control disorders, are related to deficiencies in the ability to initiate, execute and switch between motor programs. The study of the physiology, pharmacology and anatomy of the basal ganglia has allowed to propose models to explain the contribution of these on the selection of actions and execution of movements. In functional terms, these models agree that the activity of the direct pathway facilitates execution of the desired motor programs.On the other side, the contribution of the indirect pathway can be summarized from two experimental evidences: 1) the activation of this subcircuit inhibits movement and 2) this pathway shows an increase in activity before the start of an action sequence. These evidencesraise the question: Why is the indirect pathway activated before the beginning of a sequence of actions?

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The aim of this work is to probe if theactivation of the indirect pathway before the start of an action sequence leads the switch between two action sequences. We hypothesized that the activation of the indirect pathway facilitates the first sequence's stop to promote the transition to the second sequence. To test our hypothesis, we designed a training program to train subjects to switch between action sequences [chain of fixed ratio sequences of lever presses FR4→FR4→Reward] on two different schemes: stimulus-response (SR) and self-paced (SP) sequences. Throughin-vivo extracellular recordings and photostimulation-assisted identification of neuronal populations (PINP)in dorsomedial striatum from anA2a-Cre mice, we identified 1) indirect pathway's activity that correlates with the start, execution, switch and the end of a chain of sequences.2) We identify that at least, 15% of the photo-identified neurons were capable to distinguish between SR and SP conditions. 3) Through optogenetic inhibition of the indirect pathway's neurons we identified that inhibition of this pathway in the self-paced condition increase the delay between the sequences and affects the correct execution of the chain.

These results show that the activity of the indirect pathway of the basal ganglia system is required to perform a chain of action sequences and suggest that the activity of this pathway is specifically required to allow the transition among action sequences.

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Human Neural Stem Cells Culture in Poly-D-lactic- Acid (PDLA) 3d Printed Scaffolds for Spinal Cord Injury.

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RESUMEN

Central nervous system (CNS) lesions have a growing social and economic importance in developed and underdeveloped countries, one of these conditions is spinal cord injury (SCI). Medullary lesion (SCI) is a CNS disorder for which there are no successful treatments, for this reason, in recent years new alternatives have been explored to treat SCI, such as cell therapy, however, the fact of injecting cells is ineffective due to the low survival of cells during treatment. Therefore, new approaches have been used proposing a combination of cellular grafts with biomaterials. Currently a variety of materials have been studied, as it is the poly-lactic acid (PLA). We generated PLA scaffolds with different pore sizes (300 µm, 400 µm and 500 µm) by 3D printing and subsequently analyzed by scanning electron microscopy and we found that have well-defined structures and pores. In addition, it was evaluated its suitability for growing human neural stem cells (HNSC) cultures. The aim of this study is to evaluate the effects that could have a pore size on survival and cell growth. Cell viability tests were performed at 3, 7 and 11 days of cellular differentiation, we obtained that PLA does not decrease viability and even, scaffolding with pores of 400 µm and 500 µm offer benefits to neural cultivation. As for cell proliferation, we obtained that the 500 µm scaffold has beneficial effects. We conclude that PLA scaffolds do not generate toxicity or cell death in the HNSC, therefore, the PLA could be a candidate material for SCI therapy.

Green fluorescent protein expression in mouse brain structures delivered with an intranasally administered non-viral vector.

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Abstract

Intranasal administration has been well-studied and offers the possibility to deliver larger molecular weight biologics, such as proteins, viral vectors, non-viral vectors as plasmids, nanoparticles and recombinant particles, to the brain and treat a variety of diseases in the central nervous system. The predominant challenge in this field is finding effective vectors that are capable of crossing the blood-brain barrier. In this study, we investigated whether a non-viral vector, the plasmid (pIRES-hrGFP-1a), could cross the blood-brain barrier, reach brain cells and express green fluorescent proteinafter intranasal administration.Micereceived 25 µg of pIRES-hrGFP-1a intranasally, and PCR, RT-PCR and immunohistochemical techniques were performed after 24 hr to detect pIRES-hrGFP-1a, GFP mRNA and green fluorescent protein, respectively. The pIRES-hrGFP-1a crossed the blood-brain barrier and was mainly detected in the olfactory nerves (20%) and hypothalamus (16%). In contrast, GFP/18S-expressing mRNAs were detected mainly in the olfactory bulbs (95%), frontal cortex (71%) and amygdala (60%).Green fluorescent proteinwas strongly detected in the olfactory bulb, hippocampus, frontal cortex and brainstem. Detection of pIRES-hrGFP-1a and green fluorescent proteinin brain structures suggest that intranasal administration of pIRES-hrGFPis a forward and practical strategyfor crossing the blood-brain barrier and reaching the brain. We concluded that pIRES-hrGFP-1a could be considered a good candidate for future gene therapy studies, opening the possibility for cloning relevant genes with therapeutic purposes and targeting them to the brain.

GABAR-Taurine interaction in the process of proliferation of neuralprogenitor cells from the sub-ventricular zone of the mouse brain.

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Degenerative diseases of the nervous system have become a public health problem worldwide. Cell replacement therapy with neural precursor cells has been of great importance because it offers an opportunity to stop and repair the degenerative process due to the proliferative and differentiating activity that this cells go through which under pathological conditions could help to repair the damage. Currently, research in this field is mainly focused on determining the factors and signals that allow the maintenance of cellular viability and / or facilitate the regulation of proliferation and differentiation processes to ensure a correct adaptation of cells to the new environment.Experimental evidence, suggest the participation of different neuro-active molecules, such as taurine, in the processes of proliferation and differentiation of neural progenitor cells from the sub-ventricular zone. However, it is not at all clear how the mechanism by which taurine participates by modulating the proliferation processes of the progenitor cells from the sub-ventricular zone. The aim of this study, was to determine whether taurine, through interaction with GABAergic receptors present in neural progenitor cells, modulates the proliferation process. Our results showed an increase in cell proliferation in the presence of 10 mM taurine. The application of bicuculine and picrotoxin,in the presence of taurine,generated a decrease in the cellular number of 39.1% and 47.4% in the control with GABA. On the other hand, the CGP55485 (inhibitor of the metabotropic receptors)did not have a significant effect on the cellular number in the presence of taurine. The incorporation of BrdU assay showed a significant decreaseoncell proliferation under these conditions,confirming the previous results. These results suggest that the effect of taurine, in the proliferation process of neural progenitor cells from sub-ventricular zone, is partially mediated by ionotropic GABA receptors. The characterization of these mechanisms will allow us to know moreabout the mechanisms that regulate the proliferation processes in these cell types, which would help us to understand the events of neurogenesis. In addition to this, it would allow us to develop pharmacological strategies, which will be aimed at the modulation of proliferation processes in neural progenitor cells.

The mRVG29 complex has the ability to deliver DNA molecules to murine brain.

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Introduction: Neuronal damage and glial dysfunction are present in neurodegenerative diseases. The neurodegenerative process does not depend on a single type of cell. Glial dysfunction can trigger neurotoxicity in a number of different ways. Neuroglia has a fundamental role in protecting the CNS through multiple homoeostatic mechanisms. A peptide-based delivery system may be specific when directed towards a particular cell type for the delivery of therapeutic molecules. Our research group has developed a peptide-based delivery system that include RVG29 with a mutation in acetylcholine receptor binding site, our systemmRVG29 has been proved to have a great efficiency in the delivery of DNA as cargo moleculeto neuronal phenotype and astrocytes *in vitro* but it is not able to transfect oligodendrocyte precursor cells.

Objective: To evaluate the ability ofmRVG29to deliver DNA molecules to murine brain.

Material and methods:mRVG29, a karyophilic peptide (KP) and a plasmid encoding fluorescent green protein (pGL) were bound by electrostatic charges to form RVG complex. To evaluate the transfection ability of mRVG29 complex *in vivo*, mRVG29 complex (100 ng) were injected into the cerebral cortex, striatumand hippocampus of C57BL/6 mice by stereotactic surgery. The mice were sacrificed 4days post-surgery and their brains were prepared for histological analysis. The expression of reporter gene (GFP) in brain cells was analyzed by immunohistochemistry in cortex, striatum and hippocampus. The GFP expression in astrocytes, oligodendrocyte and microglia was detected by immunofluorescence.

Results: The results *in vivo* shows that the GFP expression is detected 4 days post-transfection in the cerebral cortex, striatum and hippocampus of different types of cells. In contrast of what we found in our previous report, we found GFP expression in oligodendrocytes*in vivo*. We also found gene reporter expression in astrocytes and microglia. The results *in vivo* show that mRVG29 complex has the ability to transfect, neurons, astrocytes, oligodendrocytes and microglía.

Conclusion: The mRVG29 complex has the ability to transfect most cell types in the murine brain. The transfection ability of this novel mRVG29 peptide makes it an excellent candidate as a therapeutic gene delivery vector in neurodegenerative diseases.

"Injectable Nano-Network for Curcumin/Dopamine release in treating hemiparkinsonism induced in the rat"

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Parkinson's disease (PD) is a chronic and progressive neurological disorder, which affects the central nervous system, causing programmed cell death of dopaminergic neurons located in the substantia nigra pars compacta (SNpc). Dopamine (DA) is a fundamental chemical so that the movement of the body is performed correctly. Currently there are several therapies, such as pharmacology, deep brain stimulation and cell replacement therapies, but none has been able to cure the disease, prevent its progression or have any proven neuroprotective effect.

In recent years, the curcumin, a polyphenolic compound in the spice turmeric, has been shown to have anti-inflammatory and antioxidant properties, and is being extensively investigated to treat various neurodegenerative diseases, such as the PD. Although a specific cause is not known for this disease, its development is mainly associated with inflammatory processes and a high oxidative stress, which could be counteracted by curcumin, avoiding the addition of α-synuclein, Fibrillation, and inhibition of monoamine oxidase B.

In the present study, we propose the use of a microimplant based on a polymer nano- network, self-degradable and regulated by electrostatic charges and able to release dopamine/curcumin by diffusion. The project proposes a form of drug delivery that can be released in a chronic and dosed form in a preventive and advanced phase that is biocompatible with the organism and can stop or delay the progression of the induced hemiparkinsonism in the rat with 6- OHDA.

Keywords: Curcumin, Parkinson's disease, Dopamine, Oxidative Stress, Nano-network.

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Neuropathology

Allopregnanolone promotes changes in the gene expression profile of human glioblastoma cells

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ABSTRACT

Glioblastomas are the most frequentand aggressive astrocytic tumors. In such malignancies, the steroid hormone progesterone (P4) promotes proliferation, invasion, and migration. Besides, glioblastoma biopsies and cell lines express the enzymes that metabolize this steroid. Allopregnanolone (3α-THP), a reduced metabolite of P4, activates different mechanisms of action. Recently, we have reported that 3α-THP promotes cell proliferation in a similar manneras P4. In the present study, we used microarrays to evaluate changes in the gene expression profile of the human glioblastoma cell line U87. For this purpose, we treated U87 cells with vehicle (DMSO 0.01%), 3α-THP (10 nM), P4 (10 nM), and Finasteride (F, 100 nM, an inhibitor of the enzyme 5α-Reductase, which isfundamentalin thesynthesis of 3α-THP from P4). After 72 h of steroids treatment, microarray analysis showed that 3α-THP modified the expression of 114 annotated genes, while F changed 41 genes. We selected10genes whose products are cytoskeleton components, transcription factors, and proteins implicated in the maintenance of DNA stability and replicationin order to validate changes in gene expressionby RT-qPCR. Our results indicate that six genes wereup-regulated by 3α-THP, two of themwere also up-regulated by F. Two other genes were up-regulated by P4, however, such effect was blocked by F. The remaining genes were regulated by the combined treatment of 3α-THP and F. Together these findings suggest that P4 and its reduced metabolite3α-THPregulate different set of genes that canpromote the progressionof glioblastomas.

Maturation, survival and activation of adult hippocampal neurons born after a focal excitotoxic damage in dentate gyrus: evaluating anatomo-functional recovery.

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Adult hippocampal neurogenesis is a plasticity mechanism that occurs throughout life in the adult brain. The generation of functional and integrated new neurons in the hippocampal circuit takes around 8 weeks in physiological conditions. The activation of adult-born neurons has been reported at 3 week-old by the expression of Immediate Early Genes (IEGs') such as c-fos, also in physiological conditions. For years it has been of interest to evaluate the possible participation of adult-born neurons in cognitive processes and in pathological conditions like brain damage. Given that neurogenesis is highly increased after several types of damage, it has been suggested that adult hippocampal neurogenesis may work as a compensatory or repair mechanism. We have previously shown that a morphological reorganization as well as the functional (behavioral and electrophysiological) restoration of lost hippocampal dentate gyrus (DG) function occurs along time after a focal DG lesion. In this research now we evaluate if adult-born neurons after damage: 1) mature normally; 2) survive at two different time points and 3) activate in response to a DG-memory task, the contextual fear conditioning (CFC). For this purpose we induced a focal excitotoxic damage with kainic acid (0.75mM/µl) in the DG of adult young male rats and evaluated at two different time points: 1) learning and memory in a contextual fear memory task; 2) neuronal proliferation and maturation and; 3) c-fos expression in adult-born neurons after the task performance. We observed that at 10 days post-lesion (dpl) but not at 30dpl, memory retrieval is impaired, which correlates with the previously observed anatomical disorganization. At 10dpl there is an increase in the number of young new neurons (BrdU+/DCX+) in the granular cell layer surrounding the lesion and in the hilus. Moreover, the number of mature new neurons (BrdU+/NeuN+) is higher than in the sham group, suggesting a possible acceleration in the maturation process after the lesion. We also observed an increase in the number of young and mature new neurons prone to activate (BrdU+/DCX+/c-fos+ and BrdU+/NeuN+/c-fos+) in the granular cell layer. The activation of new neurons (showed by c-fos expression) at an immature state (10dpl) may suggest an acceleration in the maturation process or a modification in the activation threshold of these neurons after damage in response to a contextual fear memory task. At 30dpl the number of mature new neurons (BrdU+/NeuN+) is higher after damage than in the sham group showing that adult-born neurons survive at least one month after pathological conditions, and the number of mature new neurons prone to activate (BrdU+/NeuN+/c-fos) is also increased after damage, interestingly this effect is only observed in the GCL but not in the hilus. This activation is only found after memory retrieval, given that in non-conditioned animals the number of BrdU+/NeuN+/c-fos+ new neurons is significantly fewer than after memory retrieval. Our results show that the DG is able to reorganize itself after damage and that the morphological and functional recovery is associated with an increase in activating new neurons, suggesting that adult hippocampal neurogenesis may be a possible mechanism for reorganization given that adult-born neurons not only mature and survive after damage but also activate in response to a memory task.

Modifications in cytoskeletal and astrocyte proteins content in prefrontal cortex in a murine model of autism (C58/J strain)

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Introduction

Autism Spectrum Disorder (ASD) has been recognized as a complex brain disorder with high heritability. Two common characteristics are present in this disorder: impairment of social interaction and communication, and restricted and repetitive behaviours. Alterations in neuritogenesis, elongation of axons and dendrites, and greater spine densities in ASD patients, particularly found in brain structures associated to memory and learning as prefrontal cortex, suggest connectional changes and disturbances in plasticity in the autistic brain. The cytoskeleton has a pivotal role in regulating the structure and dynamics of dendrites, spines and axon outgrowth. Also it is essential for stabilization and remodelling of synaptic connections along with synaptic and astrocyte secreted proteins. Hence, some modifications of cytoskeletal and astrocyte components could be involved at a molecular level in the mentioned abnormalities.
The objective of this research was to analyse changes in the content of cytoskeletal proteins β-actin, cofilin, synaptopodin, α-tubulin, MAP2A, Tau and astrocyte secreted protein thrombospondin-1 in prefrontal cortex of an autistic murine model corresponding to the C58/J strain.

Methodology

Prefrontal cortex from C58/J and C57 BL/6 (wild type) mice was dissected. Samples were processed for Western Blot technique. To identify proteins we used specific antibodies against each one.

Results

α-tubulin content showed no change in prefrontal cortex between both strains, however its associated proteins as MAP2 and Tau presented clear differences. We observed six Tau isoforms with molecular weights between 20-100 kDa in prefrontal cortex of wild type mice strain (C57BL/6). Two of them were selected: 80 and 60 kDa as these isoforms were presented in both strains. The four remaining isoforms content was not analysed since it completely disappear in autistic prefrontal cortex. The 80 kDa Tau isoform content in prefrontal cortex of autistic mice (C58/J) was not different compared to the WT strain (C57 BL/6), but the 60 kDa isoform and its phosphorylated state content showed a decrease in the autistic brain area compared to the WT one. Furthermore the MAP2A protein content was lower in prefrontal cortex of autistic mice compared to WT strain. Similar to α-tubulin, the level of actin protein was uniform in WT and autistic prefrontal cortex, instead, the content of phosphorylated actin-associated protein cofilin showed a decrease in the autistic prefrontal cortex. Besides, synaptopodin content, another actin-associated protein enriched in dendritic spines, was similar in prefrontal cortex of autistic mice and WT strain. Finally, the protein content of astrocyte secreted protein thrombospondin-1 showed a decrease in the autistic mice brain area, although the GFAP protein content was not different between both strains.

Conclusions

Our work showed important changes in 60 kDa Tau/phospho-Tau isoform, MAP2A and phosphorylated-cofilin content, as well as differences in the astrocyte-secreted protein thrombospondin-1 in prefrontal cortex between wild type (C57 BL/6) and autistic animals (C58/J). These differences in autistic mice could be associated with disturbances in neuronal cytoskeleton dynamics of ASD.

Effects of pair bonding on cell proliferation in *Microtus ochrogaster.*

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Microtus ochrogaster (prairie vole) is a rodent that establishes a solid social organization. These mammals establish a pair bonding and both males and females display parental behaviors toward the pups and nestle cares. These complex behaviors could be associated with plastic neuronal changes. In our lab we are interested in studying the plastic changes associated with the formation of pair bonding in adult voles.

The aim of this study is evaluate the effects of pair bonding on cell proliferation in subventricular zone (SVZ) and rostral migratory stream (RMS) in male voles. To achieve this goal, we obtain 21 adult males that were randomly distributed in three different groups: 1) Control: animals that were not exposure to female sensorial cues; 2) Exposed: male voles were exposed to a sexually receptive female but they had not physic contact, and 3) Pair bonding: male rodents that mate. Behavioral test last 6 h. To identify potential new cells, animals were injected with the DNA synthesis marker BrdU during behavioral test. Animals were sacrificed 48 h latter and their brains were processed to immunohistochemistry assays. We performed double-immunostaining for BrdU or endogenous cell proliferation marker Ki67, whereas to evaluate if the new cells express markers of immature neurons we used Doublecortin. We analyzed at least three sagittal slides per animal using confocal microscopy. To quantify the number of proliferating neuroblasts, we divided SVZ and RMS in three regions (ventral, anteromedial, dorsal; and anterior, media and posterior, respectively). Now, we are analyzing immunolabeling images from experimental animals using Image J software, and finally these data will be tested with static analysis.

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Dentate gyrus neurogenesis induced by pair bonding in prairie voles

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Neurogenesis in the adult is a plastic neural process recapitulated in specific brain areas like the hippocampus dentate gyrus (DG). This process can be modulated by mating which favors pair bonding formation in Microtus ochrogaster.The plastic neuronal process involved in pair bonding are not fully known. In the present study, we evaluated if pair bonding formation induces neurogenesis in the DG. For this purpose we use 48 Microtus ochrogaster, 24 males and 24 females. Females were bilaterally ovariectomized and 2 weeks later treated with estradiol benzoate (0.5 µg/femaledaily for 4days). Voles were aleatory assigned to one of the following groups; 1) pair bonding, subjects that copulate with a conspecific; 2) exposure, those animals who were exposed to sensory signals of a conspecific and 3) control, subjects that were not exposed to sexual cues. During the behavioral tests, that lasted 6h, all animals receivedthree i.pdoses (100mg/kg) of the DNA synthesis marker 5-Bromo-2`-deoxyuridine (BrdU). Fifteen days later, all animals were sacrificed and perfused. Brains were sliced in the coronal plane at the level of the DG and processed for immunohistochemistry to detect BrdU positive cells. Our results show significant differences in the dorsal DG of pair bonding males vs male control group ($p<0.05$). There were no differences in this region in females. No differences were found in the ventral DG in females or males. In conclusion, pair bonding induces neurogenesis in males dorsal DG, but has no effect on females DG.

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Effect of the decrease of prefrontal serotonin on the theta activity of the prelimbic-amygdala circuit, expressed during spatial reversal learning, in rats.

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The flexible behavior required for acquisition and establishment of reversal learning is supported by the function of the prefrontal cortex and is sensitive to the integration of emotional information that is processed by the amygdala. Prelimbic (PL) cortex and basolateral amygdala (BLA), present a reciprocal synaptic connection that forms the PL-BLA circuit, which is responsible for generating complex behavior to adapt to changes in the environment. The serotonergic system exerts an important modulating function on the PL-BLA circuit function in the establishment of reversal learning. In the present work, the lesion of the prefrontal serotonergic (5-HT) terminals was performed using the neurotoxic 5,7-dihydroxytryptamine, to evaluate the effect of the lesion on the acquisition of a spatial reversal learning task in the Morris water maze, as well as about the theta activity that underpin such learning. Male Sprague Dawley rats were used, lesion of 5-HT terminals was induced and a recording electrode was placed in the PL cortex and another in the BLA. Evaluation of spatial learning and memorytask, as well as of reversal learning, was carried out and the electrical activity was recorded simultaneously cerebral. Injury of 5-HT terminals reduced the concentration of prefrontal serotonin to 90%, which did not affect spatial learning. The decrease of the prefrontal serotonin during spatial learning favored the decrease of theta low frequency activity, and the increase of theta activity in the high and maximum frequency in the PL cortex. Likewise, in BLA the lesions of 5-HT terminals of the cortex induced a decrease of theta low frequency activity and an increase of theta high frequency activity. On the other hand, during the acquisition of reversal learning the decrease of the prefrontal serotonin did not affect the theta activity in PL cortex, opposite to what was observed in the BLA, where it was produced in the high frequency theta activity.The present results suggest thatthe lesion of serotoninergic afferent endings, leading to an important decrease in serotonin content in PL cortex, induces disinhibition of the prefrontal electrical activity increasing of PL and of BLA theta activity, during the expression of cognitive processes and possibly long term functional alteration of brain structures connected with the PL.

IDENTIFICATION IN MALE RATS, BY MANGANESE ENHANCED MAGNETIC RESONANCE, OF THE NEURAL CIRCUITS CONTROLLING SEXUALLY MOTIVATED BEHAVIORS., **GaytánTocavénLorena, Ortiz-RetanaJuan, Martinez-GascaDeisy, AlcauterSolorzanoSarael, Paredes Raúl. Instituto de Neurobiología UNAM**

Sexual behavior is a motivated behavior, which is of paramount importance for the survival of many species. Several research groups have identified, using different techniques, the brain structures involved in the control of sexual behavior which include: the accessory olfactory bulb, the bed nucleus of the striaterminalis, the medial amygdala and the preoptic area of the anterior hypothalamus, (De Olmos et al., 1978, Scalia, Winannss., 1975).

Two motivated behavior which are crucial for the expression of sexual behavior are sexual incentive motivation and partner preference. The possible circuits controlling these behaviors have not been studied. In the sexual motivation incentive test, where no physical contact is possible, the time and frequency of visits to the incentive zone of a sexually experienced male (SE) or a sexually receptive female (RF) are measured.In the partner preference test the subjectscan interact with the stimulus animalsquantifying the sexual interaction and the time spent in each compartment.So the aim of this project is to determine by manganese enhance magnetic resonance imaging (MEMRI) the different neural circuits, activated in partner preference and sexual incentive motivation.
The use of MEMRI allows mapping the brain of the animal in vivo where manganese ions (Mn^{2+}) pass through the blood brain barrier and can enter into excited cells via voltage-gated calcium channelsidentifying brain regions activated by a particular behavior (Takeda et al., 2003). In the present experiments,$MnCl_2$ (16 mg/kg) was administered 24h before the behavioral tests and immediately thereafter the subjects were placed in a Bruker 7T MR scanner.
Our preliminary results show that manganese at 16 mg/kgdoes not produced unspecific effects evaluated by the use of a running wheel and a rota-road test.Sexual behavior was not affected by the administration of manganese at 16 mg/kg. With this dose, we obtained a good contrast for MRI analysis. We are now analyzing the images to determine the circuit activated after sexual behavior, partner preference and sexual incentive motivation.
Technical assistance Francisco Camacho, supported by CONACYT 253631, Fronteras 374 and DGAPA IN210215.

Neural Correlates of Value-Based Decision-Making During Spatial Navigation in the Rat

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All animals navigate through space in order to obtain rewarding stimuli. Hungry rats readily learn specific, complex navigation behaviors in maze environments to obtain food rewards. The hippocampal formation (HF) is a critical and well-studied structure for processing spatial information (e.g. O'Keefe & Nadel 1978). The ventral tegmental area (VTA) widely distributes dopamine (DA), and provides reward prediction error (RPE) signals (e.g. Schultz et al 1997). Anatomical evidence indicates that a microcircuit connects HF place cells to VTA by way of the lateral septum (LS; e.g. Lou et al 2011), providing a functional pathway for spatial information to interact with reward signaling as a hungry rat learns to exploit a maze for food rewards. To determine how the brain functionally connects spatial information to the RPE system, we are performing several complementary experiments. Extracellular electrophysiology (ephys) in the LS confirms the presence of place-like cells in this structure, bolstering the argument that the HF-LS-VTA circuit can connect spatial navigation information to RPE signaling. Ongoing experiments employ ephys in the HF, VTA and nucleus accumbens (NAcc; a primary target of the VTA RPE signal). Fast-scan cyclic voltammetry (FSCV) in the NAcc provides direct measurement of dopaminergic RPE signaling arising from the VTA. I have developed a novel technique to combine FSCV and ephys simultaneously, allowing examination of the interaction of neural firing, LFPs and bulk dopaminergic signaling for the first time.

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Functional and anatomical segregation of sensorimotor cortex layer 5 neurons projecting subcortically

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Neocortex Layer 5 of mammals contains populations of neurons that send information product of intracortical processing to many cerebral regions. However, the way in which the intracortical activity generates a segregation of the different outputs related to specific targets is unclear. The main objective of this study was to determine if the layer 5 projection neurons are functionally segregated. To address this question, we combined the use of retrograde neural tracers and two photon microscopy using transgenic mice expressing the calcium indicator CGaMP6f in layer 5 pyramidal neurons. In this way, we analyzed the functional relationships between 719 pairs of hodologically identified neurons (corticospinal) and 622 pairs of non-identified neurons in brain slices of the sensorimotor cortex. The results shows that the functional correlations in both, time and frequency domain, are significantly higher among corticospinal neurons than with unidentified pyramidal neurons. This functional segregation implies a hierarchical organization of subsystems that drives in a parallel way subcortical neural circuits, suggesting a new level of sensorimotor integration.

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In vivo two-photon calcium imaging of primary visual cortex in a genetic mouse model of autism

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Autism spectrum disorder or ASD is a neurodevelopment disorder whose hallmarks are characterized by social deficits, language impairment and repetitive behaviors. Have been reported that patients with ASD shown differential activity in cortical regions,for instance increased in neuronal activity in visual processing brain areas and atypical visual perception compared with no autistic people.

The causes of these alterations remain unclear, but many studies demonstrate strong genetics basis correlatedto ASD. Some identified mutated genes in ASD are related to synaptic function and structure, because of thissome ASDs are called “synaptopathies”. An example of this is the Phean-McDermid syndrome (PMS), which is caused by a deletion of *shank3* gene in one allele in the chromosome 22. *Shank3* encodes for the SHANK3 protein, a postsynaptic scaffolding protein that is present in glutamatergic synapses. However, is unknown the neuronal consequences related to haploinsufficiency of *shank3* in the brain, specifically in visual areas.

Thus, our goal isstudy the neural activity in the primary visual cortex (V1) of a genetic mouse model of PMS, which has a deletion of shank3 genein one allele, like in humans. We hypothesize that the haploinsufficiency of *shank3*might affect the neural activity.

In order to demonstrate our hypothesis, we analyzed the neural activity in layers 2/3 of V1 of heterozygous ($SHANK3^{+/-}$) and wild-type ($SHANK3^{+/+}$) awake mice inhead-fixed mode, while visual stimuli are presented. We recorded neural activity *in vivo* using genetically encoded calcium indicator GCaMP6fby two-photon imaging through a cranial window.

Our results shown differences in the neural activity evoked by visual stimulation between $SHANK3^{+/-}$ and $SHANK3^{+/+}$mice. First, we found a bigger proportion of responsive cells in heterozygous mice. Also, we found that there is not any preference for a particular stimulus in heterozygous mice whereas wild-type mice shown preference for a particular orientation of visual stimulus. Finally, we found that neurons of heterozygous mice are more selective to orientation but no to direction of the visual stimuli.Altogether, this data demonstrated by the first time that visual processing might be different in subjects with autism, in this case due to a haploinsufficiency of shank3.

Neurochemical, structural and behavioral modifications after chronic modulation of Wnt signaling in the adult hippocampus.

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Wnt signaling plays a potential role in synapse function in the adult brain. Despite the functional role of the Wnt signaling pathway in adult neural circuits, there is currently no evidence regarding the relationships between Wnt signaling modulation and hippocampal structural changes *in vivo*. Thus, we analyzed the effect of chronic infusion of Wnt agonists, Wnt7a and Wnt5a, and antagonist, Dkk-1, through anosmotic mini-pump on the rat hippocampus. Wnt7a and Wnt5a increased the number of perforated synapses and the density of pre-and postsynaptic proteins, such as Bassoon and PSD-95, associated with synapse assembly.The observed increase in PSD95 protein was consistent with the data obtained by Western blot quantitation, and was produced particularly after Wnt5a treatment. On the other hand, Dkk-1 infusion was associated with a decrease in PSD-95 content. Wnt7a and Wnt5a agonists also had an effect on the formation of new born granule neurons,assessed through doublecortin (DCX) staining in the DG. The chronic treatment with the Wnt7a agonist increased the number of DCX-positive cells by twofold, while Wnt5a produced a threefold increase in the number of DCX-positive cells in the crest of the DG. Additionally, the DCX-positive cells of the animals treated with the Wnt5a agonist and the Dkk-1 antagonist exhibited a very disorganized neurite growth patterns. The activation of Wnt canonical pathway measured by total β-catenin levels and its active form was produced by both Wnt agonists compared with control PBS and Dkk-1 infused animals. Wnt7a and Wnt5a also produced a decrease in tau phosphorylation at the Ser199/202, while the appearance of the PHF-1 (phospho-Ser396/404) form of tau was more pronounced in the Dkk-1 group. Furthermore, the canonical antagonist, Dkk-1, inhibited spatial memory consolidation. Thus, the present study elucidates the potential participation of Wnt signaling in the remodeling of hippocampal synapses that may underlie plasticity events *in vivo*.

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Neural basis of bimanual coordination

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Many of our daily movements require bimanual control, typing on a keyboard, tying the shoelace or using the cutlery; their accuracy execution requires spatial and temporal coordination. Previous evidence has shown that sensorimotor cortex (M1/S1) is involved in bimanual movements and sends broad bilateral projections to its counterpart and the striatum. However, the role of sensorimotor projections in bimanual coordination remains unclear. We hypothesized that striatum integrates bilateral information from sensorimotor cortices to coordinate motor outputs during bilateral movements. To investigate the participation of cortico-striatal projections in bimanual coordination, we created a task to accurately quantify kinematic parameters of coordinated bimanual movements in rodents. We found that rats naturally develop coordinated bimanual movements, with highly spatially and temporarily correlated trajectories. Unilateral striatal lesion causes slowness of the contralateral paw and consequently spatial and temporal uncoupling. Further analysis will clarify if the striatum is required for executing a coordinate motor output or if it only provides kinematic control. Ongoing optogenetic in anesthetized rats indicate that both, unilateral photoactivation/photoinhibition of sensorimotor cortex modify the neuronal activity on the contralateral cortex. Future experiments using the same technology in freely moving animals executing bimanual movements will help to unravel the participation of specific circuits.

Área: 6. Plasticidad Celular y Circuitos Neurales

Title: Phosphorylation of tau protein modulates hippocampal theta activity and prevents epileptiform activity

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Abstract

Tau hyperphosphorylation at several sites including those close to their microtubule domain region (MDr) is considered a key pathogenic event for Alzheimer´s disease (AD) development. However, at very early disease stage, phosphorylation increase in theMDrdomain of tau protein was found to promote neuroprotection by preventing epileptiform activity.Mechanistically, our data showed that phosphorylation of tau protein can modulate the hippocampal theta activity by reconfiguring the hippocampal circuitry response. Overall, our work confronts the leading AD hypothesis that postulate the fosforilación of tau protein as the pivotal event leading to neurodegeneration.

Keywords: Tau, phosphorylation, theta activity, epileptiform activity, receptor overexcitation, and compensatory mechanism.

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The cortico-striatal contribution to a chain of action sequences.

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The study of switch between actions sequences is important to understand the obsessive-compulsive disorder, in which the capacity to switch between action sequences is lost.

It's has been suggested that the basal ganglia (BG) receives an internal signal from different cortices in order to start/stop action sequences, e.g. from the supplementary motor area (in rodent M2) and prefrontal cortices. Specifically these cortical areas project to the striatum, the main input to the basal ganglia system.To date, it is not understood how thedifferent corticesmay guide the striatal activity and therefore the switch between actions sequences.

To address this question we 1) developed a task in which animals do a chain of two fixed ratio sequences. Thetask consists of two kinds of trials: stimulus-response trials (S-R; where animals are guided to switch between two sequences) and self-initiated trials (S-I; where animals switch between sequences without any guide). 2) We recorded the neuronal activity from secondary motor cortex (M2) and Prelimbic cortex (PL) while animals perform the switch between action sequences.3) To test the cortico-striatal contribution we implemented optogeneticinhibition in cortico-striatal terminals.

As preliminary results we found that:1) At the beginning/end of S-R trials PL cortex increased its activity more than M2 cortex.2)M2 cortex activity is larger one second before the beginning of the second sequence.3) In S-I trials PL cortex activity is larger than M2 cortex at the beginning/end of S-R trials.4) The inhibition of M2→striatal terminals decreased the latency to start and the length of the first sequence in S-R trials. 5) The inhibition of PL→striatal terminals decreased the latency to start a sequence, the number of presses and the length of the first sequence only in S-I trials.

These results suggest that PL cortex activity encodes the boundaries of the two action sequences (both in S-R and S-I trials), but more importantlyin S-I trials, while M2 cortex activity encodes the transition between action sequences only in S-R trials and is more important during the S-R trials.Further analyses are necessary to confirm these preliminary conclusions.

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Pallidal Gaba B receptors activation: effects on the firing pattern of thalamic reticular nucleus.

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The external segment of globus pallidus (Gpe) is an integrative hub within basal ganglia (BG) circuits and their abnormal activity has been implicated on physiology and pathophysiology of motor and cognitive disease. Ionotropic GABA A receptor mediated effects upon Gpe electrical activity, however electrophysiology studies have demonstrated that GABA also activate GABA B receptor and regulate its firing pattern. Recently was reported that Gpe controls electrical activity of thalamic reticular nucleus (nRT) by pallido-reticular pathway.

Neurons of nRt are GABAergics and modulate corticothalamic (CT) and thalamocortical (TC) circuits by two types of spiking modalities: burst and tonic firing. Burst firing synchronizes rhythmic oscillation in the CT-TC fiber and the lost of these function has been associated with absence seizure epilepsy and schizophrenia. Electrical activity of the nRT depends on both, intrinsic properties of membrane and its neuronal connections.

In this framework the presence of pallido-reticular loop suggests that pallidal GABA B receptor participates in functional activity of nRT by adjusting its electrical activity, thus in this assay, the spontaneous electrical activity of nRt was analyzed by extracellular unit recording in anesthetized rats and under pharmacological activation of pallidal GABA B receptor and the firing pattern of nRT was analyzed by the burst index (BI).

We found that activation of pallidal GABA B receptor modifies the spontaneous firing rate of nRt neuron without changing the firing pattern. Infusion of agonist GABA B receptor decreases the spiking rate of nRt neurons 100 % relative to basal values, nevertheless BI remained without changes with intrapallidal application of agonist GABA B receptor; this effect was associated with a 90%decrease of Gpe spiking rate by intrapallidal administration of agonist GABA B receptor. This data suggest that pallidal GABA B receptor activation has effects on spiking activity of nRt.

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Topic: Electrophysiology/Neural Circuits.

Molecular characterization of the mutant *buc-1* in the nematode *Caenorhabditis elegans*

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C. elegans is a genetic model that has triggered a series of breakthrough discoveries in molecular and genetic biology. Its nervous system represents an advantage over other models due to its simplicity, small number of neurons and easy genetic manipulation that help understand the functions of genes that construct a given phenotype in animals of different taxonomic levels. Here, we generated a *buc-1* mutant strain by chemical mutagenesis using ethyl methanesulfonate. The strain was selected following the analysis of 1490 haploid genomes. The mutant´s nomenclature refers directly to its locomotive deficiency: "backing uncoordinated" (*buc*). This strain develops the basic activities of its behavior with less efficiency than the wild-type strain, although it is able to reproduce and thus, *buc-1*presents altered locomotion, as it twists during reverse locomotion and whenever it is not moving. Genetic mapping using SNP´s found that *buc-1* maps in the center of chromosome IV.Furthermore, it was identified that the affected gene in the *buc-1* mutant was *lgc-43.*This gene encodes for a membrane ion channel that is related to nematode locomotion.A comparison of the sequence to the wild-type gene sequence resulted in the identification of four SNP's of which three were not relevant due to their homology (leucinefor methionine, leucinefor valine and asparagine for histidine) but one of them exchanged the polarity of the amino acid (serine for isoleucine), constituting the most important evidence to date of the defect responsible for the *buc-1* mutant strain. Finally, pharmacological tests with aldicarb, levamisole and ivermectinwere carried out. These tests helped us to understand the mechanism involved in the*buc-1* mutant, concluding that there is an above-normal release of the neurotransmitter acetylcholine, however, there are no postsynaptic defects.

Paced mating increases the expression of μ opioid receptors in the ventromedial hypothalamus

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Mating induces a positive affective state which is blocked by the systemic administration of naloxone, a specific opioid antagonist. Opioids are released in the medial preoptic area (mPOA) and other brain regions during sexual behavior and mu opioids receptors are activated in males that copulate until ejaculation. The aim of the present study was to determine if mating increases the expression of μ opioid receptor (MOR) in areas involved in the control of sexual behavior in male rats. We used ninety sexually experienced Wistar male rats that were randomly assigned to one of the following groups (n=10 each): a) Paced (P), males were allowed to mate, pacing the sexual interaction; b) Non Paced (NP), males were allowed to mate without pacing the sexual interaction; c) Control (C), males were able to hear, see and smell a sexually receptive female, but no physical contact was possible. Males were sacrificed by decapitation 4, 8 or 12 h after the behavioral tests. The mPOA, ventromedial hypothalamus (VMH), amygdala (AMY), olfatory bulbs (OB) and the cortex (CTX) as control were dissected. After RNA isolation and cDNA synthesis, expression of the MOR was determined by qPCR in duplicates. No significant differences were found among P, NP and C groups in the expression of MOR in the mPOA and the AMY independently of the time of sacrifice. In the VMH, the expression of MOR increased in the P compared to the C and NP groups at 4h. No significant differences were found in this area at 8 and 12h. In the CTX and OB, expression of the receptor was not detectable. Interestingly, we found that the expression of MOR varied at the different times of sacrifice. In conclusion, our results showed that the expression of MOR varied in the different brain areas depending on the time of the day. Independently of this variation, paced mating in males induced an increase in MOR in the ventromedial hypothalamus 4 h after mating, further supporting the contention that opioids are involved in the control of sexual behavior.

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“Effect of treatment with levetiracetam on neurotransmission dentate gyrus of rats with Temporal Lobe Epilepsy "

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Temporal lobe epilepsy (TLE) is characterized by the appearance of epileptic foci in limbic structures, particularly in hippocampal formation. Three fundamental pathophysiological features have been described in this condition: the imbalance of the inhibition-excitation system, hyperexcitability and neuronal hypersynchrony. Levetiracetam (LEV) is an anticonvulsant drug with a unique profile of activity that results in a decrease of spontaneous recurrent seizures (SRZ). Nevertheless, the exact mechanism by which this drug reduces SRZ is still under investigation, but is clear that levetiracetam´s mechanism of action may involve effects on neurotransmitter release because of its primary target, the SV2A protein. It is not clear, however, whether these effects encompass excitatory or inhibitory neurotransmission, or both, since SV2A is ubiquitously expressed in nearly all types of synaptic vesicles.

Therefore, the objective of the present study was evaluating the effects of LEV on the inhibitory and excitatory neurotransmission of dentate gyrus (region with major synaptic changes). For this purpose, male Wistar rats were divided into the following groups: a) control, b) control + LEV c) epileptic and d) epileptic + LEV. ELT was generated through the systemic administration of lithium and pilocarpine, the epileptic condition of the animal was determined after the behavioral analysis of videotapes. One group of animals received LEV treatment for one week (300 mg / kg / d) through osmotic minipumps. The animals were implanted with a microdialysis cannula in the dentate gyrus, then 14 dialysate samples were collected using the microdialysis technique. The amino acids contained in the dialysates were quantified by high performance liquid chromatography (HPLC) accoupled with fluorometric detection. The extracellular concentration of each amino acid (GABA, glutamate, aspartate, glutamine, taurine and glycine) was measured both basal as well as in the presence of a depolarizing stimulus that consisted of the application of a solution with a high potassium content (100 mM).

Conclusion: The epilepsy condition causes an imbalance between the excitatory and inhibitory neurotransmitter systems in the dentate gyrus and apparently, the treatment with levetiracetam through the increase the concentration of neurotransmitter GABA tends to restore the lost balance; mechanism by which it could also avoid epileptic seizures.

An Order of Magnitude Analysis of Inositol tris-phosphate Diffusion at the Nanoscale in a Model of Peri-synaptic Astrocyte Projection.

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Astrocytes were conceived for decades only as supporting cells of the brain. However,the observation of Ca2+ waves in astrocyte synctitia, their neurotransmitter receptor expression and gliotransmitter secretion suggested a role in information handling, conception thathas raised some controversies. **Synaptic Neuron-Astrocyte metabotropic communication mediated by Inositol tris-phosphate**(**SN-AmcIP3**)is supported by different reports. However, some models contradict this idea and Ca2+ stores have been found 1000±325 nm apart from the Postsynaptic Density in the Perisynaptic Astrocyte Projections (PAP's), suggesting that SN-AmcIP3 is extrasynaptic. However, this assumption does not consider IP3 Diffusion Coefficient (*Dab*), that activates IP3 Receptor (IP3R) releasing Ca2+ fromintracellular stores. Here we idealized a model of a PAP (PAPm) to perform an order of magnitude analysis of IP3 diffusion using a transient mass diffusion model. This analysis shows that IP3 forms a concentration gradient along the PAPm that reaches the steady state in milliseconds, three orders of magnitude beforeIP3 degradation. The model predicts that IP3 concentration near the Ca2+ stores may activate IP3R,depending upon Phospholipase C (PLC) number and activity. Moreover, the PAPm supports that IP3 and extracellular Ca2+ entry synergize to promote global Ca2+ transients in PAP's. Thus, the model presented here indicates that SN-AmcIP3 is not limited by Ca2+ stores position in PAP's.

Localization of the MCTP proteinheterologously expressed in HEK-293 cells

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MCTP (Multiple C2 Domain and Transmembrane Region Protein) is a protein that presents three C2 domains that binds to Ca^{+2} and two transmembrane regions. This protein is similar to others proteins important in synaptic function such as synaptotagmins and ferlines. MCTP is widely conserved both vertebrates and invertebrates, and is expressed in brain, heart, kidney, liver, lungs and muscle of rat. *Danio rerio* genome present four mctpgenes (1a,1b,2a,2b), data from our laboratory shows that zebrafish mctp genes are expressed in early development and adulthood in brain and heart. Recently, itshas been reported that MCTP is a calcium sensor protein that localizes to the membranes of the endoplasmic reticulum (ER) in motoneurons of *Drosophila melanogaster,* this protein also can be involved in the homeostatic stabilization of neurotransmission. Despite of this, the subcellular localization of MCTP protein still remains unclear because another studies report its presence in others organelles. Therefore, the main objective of this work was to determine the localization of MCTP through a heterologous expression in HEK-293 cells. For this purpose, we built plasmidswith each of the four zebrafish MCTP genes fused to fluorescent proteins as GFP and mCherry. These plasmids were transfected with others plasmids that express orgenelles marker´s proteins as: ER, early endosomes, late endosomes, recycle endosomes, Golgi apparat, lysosomes and plasmatic membrane. Then, 24 hours after transfection we identified the subcellular localization of MCTP by fluorescent confocal microscopy. Finally, our results suggest that MCTP is localized in ER, early endosomes, late endosomes and recycle endosomes. These observations can open new questions about the function of MCTP protein.

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Effect of silver nanoparticles on the permeability of the brain blood barrier.Role of metallothioneins

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Work area: GLIA

Abstract:

The aim of this work was to evaluate the effect on the permeability of male rat brain blood barrier (BBB)and the mechanisms associated with the exposure to AgNPs(<10 nm). Male Wistar rats were divided into 4 groups: Control, Zn (27 mg / kg), AgNPs(15 mg / kg) for 24 h and Zn (27 mg / kg) 24 h + AgNPs (15 mg / Kg)for 24 h.The permeability of the BBB was evaluated using the Evans blue extraction technique and the brain tissue morphological changes and metallothioneins(MTs) expression were observed by western blot analysis. We found that rats exposed to AgNPs showed degeneration of nerve tissue and increased BBB permeability in contrast to the control rats and Zn-treated rats, whereas a pre-administration of Zn reversed the deleterious effects of AgNPs; as well it was observed that the expresion of MTs was increased in the group of rats treated with Zn and AgNPS, whereas those receiving a pretreatment of Zn and subsequent AgNPs showed a decrease in MTs levels. These results suggest the protective role of Zn against BBB damage induced by AgNPs. Several trials are yet to be performed to corroborate the role of MTs and Zn in the effects induced by AgNPs on the Central Neurvous System (CNS). The development of this project will allow us to understand the effects promoted by nano materials such as AgNPS exert on the CNS, particularly inthe damage of BBB, as well as the participation of MTs as a possible protective agent, at this level.