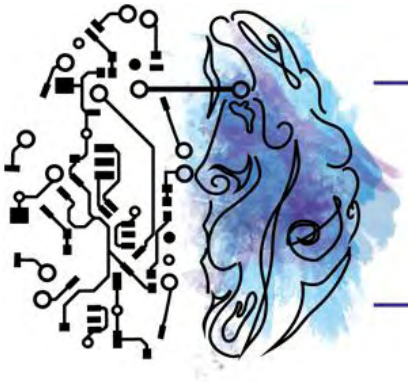

Sociedad Mexicana
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Posters Session I
Monday April 4. 17:30 – 19:30

Neuropathology
Brain Metabolism
Development
Synaptic Transmission
Signal Transduction

Diurnal variation of glutamatergic system is associated with traumatic brain injury damage.

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Introduction: Traumatic brain injury (TBI) is a major cause of both death and disability in the world; in Mexico, it is the fourth leading cause of death. In addition to the damage caused at the moment of injury, brain trauma triggers secondary injury. This is mainly mediated by the excitotoxicity induced through a massive Ca^{2+} entry into the neurons, which in turn is produced by glutamate acting through NMDA receptors. **Background**: Previous data of our laboratory showed that the damage caused by the TBI depended on the time of day that it was induced, indicating that there are processes in the brain that have a diurnal variation, which in turn would establish time periods in which a TBI cause more or less damage. **Hypothesis:** A possible way to explain our previous data is the existence of a diurnal variation of glutamatergic system in the brain, specifically of NMDA receptor expression and / or glutamate release. **Material and Methods**: Male Wistar rats (250-300 g) habituated at least 8 days under constant temperature, 12:12 light-dark cycles (lights on at 8:00 hrs.) with food and water *ad libitum* were used. NMADA expression: Control groups of rats were sacrificed at different times of day (1:00, 5:00, 9:00, 13:00, 17:00 and 21:00 hrs.; n=6). In the experimental groups, a moderate TBI was induced in motor cortex of previously anesthetized rats at different times of day (1:00, 5:00, 9:00, 13:00, 17:00 and 21:00 hrs.; n= 6) and were sacrificed 24 hours later. Immunohistochemistry, RT-PCR and western blot were used for NMDA receptor expression analysis in motor cortex. Glutamate release: microdialisys and HPLC with fluorometric detection were used to analyze glutamate at different hours of the day in non movement restricted rats (n=6). **Results**: Our data show a diurnal variation in the expression of NMDA receptor in motor cortex of TBI rats, with a minimal expression at the night hours. We also observed a diurnal variation in the release of glutamate with a peak in the night. These results indicate a probably diurnal variation of excitotoxicity caused by TBI and support our hypothesis. This allow us to develop strategies that activate neuroprotection mechanisms depending on the hour of the day that TBI happen.

Age-dependent changes in somatostatin positive neurons in different brain regions of *Calithrix jacchus* and *Tupaia belangeri*

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The neuropeptide somatostatin (SOM) is a marker of a specific subtype of gamma-aminobutyric-acid (GABA) interneurons. Decreased SOM positive interneurons are found in patients suffering from Alzheimer´s disease (AD), as well as in several transgenic mouse models of AD. Moreover, SOM levels are decreased in cerebrospinal fluid of AD patients and currently it has been proposed to be one valid biomarker of early stages of this disease. Most of AD cases are of the late-onset type, associated to life-style and risk factors occurring during adulthood. Up to date, no single animal model of late-onset AD has been accepted due to the complexity of events involved on this condition. Moreover, non-human primates promises to be a great model to develop a close to human-pathological AD condition due to the high genetic similarity between species. *Calithrix jacchus* are small new world non-human primates, with a relative short life span. It has been reported the presence of neurodegenerative markers in brain of aged *c. jacchus* (i.e. beta amyloid plaques). *Tupaia belangeri* an insectivore with a complex brain structure similar to non-human primates, presents amyloid plaques, abeta peptide accumulation, and SOM-like plaque structures in several brain regions of aged subjects. Therefore, it is proposed that these two species could represent animal models of late-onset AD type neurodegeneration. In this study we aim to analyze the number of immunoreactive SOM positive neurons of *Tupaia belangeri* (insectivore) and *Calitrhix jacchus* (non-human primate) in brain tissue at different ages. Transversal (*T.belangeri*) and coronal (*C.jacchus*) 45 µm brain sections were obtained from fixed tissue (4 % PFA) of aged, adult, and new born animals. An amplified method was used to obtain high intensity immunoreactivity signals using SOM (1:500) (Santa Cruz Biotechnology, USA). Negative control sections were always included for comparison. Confocal microscopy (Leica TCS SP8 and Zeiss Axiovert 200 LSM510 Meta-Multiphotonic) was used to obtain 20x images that were further analyzed by use of Image J 1.48 (NIH, USA). Our results showed a gauss-like shaped curved across ontogeny in CA1 region of *C.jacchus* (young vs adult $p>0.05$; adult vs old $p> 0.01$) and a similar trend in *T. belangeri* but it was non-significant. On the other hand, in subiculum we found an increased number of SOM positive neurons in aged *C. jacchus* (young vs old, $p>0.05$) and a similar trend in *T. belangeri* but it was non-significant. Our results are first to describe regional differences in SOM immunoreactivity across ontogeny in a non-human primate and in an insectivore species. This data could further corroborate with increases of abeta peptide and/or tau hyperphosporilation in same regions.

Allopregnanolone promotes proliferation in human glioblastoma cells.

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ABSTRACT

Allopregnanolone (3α-THP), one of the main reduced progesterone (P4) metabolites, is a known neuroprotective and myelinating agent. It has been reported that 3α-THP induces proliferation of neural progenitor cells, oligodendrocyte progenitor and Schwann cells. It has been shown that P4 favors the progression of astrocytomas, the most frequent and aggressive primary brain tumors. However, little is known about the actions of its metabolites and specifically, of 3α-THP in these tumors. We determined the expression of the two isoforms of 5α-reductase (5α-R1 and 5α-R2), the main enzyme implicated in P4 metabolism and formation of 3α-THP; in U87 cells derived from a human astrocytoma of the highest grade (glioblastoma). Besides, we studied the effects of different concentrations (1 nM-1 µM) of 3α-THP on the cell number and proliferation of U87 cells. We observed that 5α-R1 was predominantly expressed in U87 cells. 3α-THP increased the number of U87 cells in a dose dependent manner from day 3 until day 5 of treatment. The highest effect was seen with 3α-THP 1 µM. Although 3α-THP 10 nM also increased cell proliferation at day 3 in a similar way as P4 (10 nM). Interestingly, the co-treatment of P4 and finasteride (a potent 5α-R inhibitor), decreased cell proliferation. These data suggest that 3α-THP mediates P4 effects and induces proliferation in human glioblastoma cells.

Amyloid-β protein-induced actin cytoskeletal reorganization in human neuroblastoma cells is mediated by RhoA and prevented by NSAIDs

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NSAIDs are well known by their anti-inflammatory properties. Their role as neuroprotective compounds, particularly against amyloid-β (Aβ) toxicity, has been only recently addressed. Interestingly, Aβ neurotoxicity is at first accompanied by the reorganization of the actin cytoskeleton. The actin cytoskeleton plays a pivotal role in many neuronal morphological changes that occur during plastic events mainly through the induction of filopodia, which are the principal precursors of dendritic spines, drive the force for axonal growth and cone advance and form the basis of structural integrity in synapses. In early steps of Alzheimer´s disease synapse maintenance is defective. Thus, in the present work we analyzed if the effects of Aβ in cell viability and actin reorganization were modulated by NSAIDs indomethacin and ibuprofen in a model of differentiated human neuroblastoma cells. For viability studies we measured MTT reduction in the presence and absence of the active Aβ peptide 25-35 (20 μM) and together with indomethacin (50 μM) and ibuprofen (50 μM). To determine the reorganization of the actin cytoskeleton we studied fluorescence phalloidin stain by inmmunohistochemistry in the absence or presence of amyloid and NSAIDs and with the RhoA inhibitor, Y27632. Aβ induced dramatic changes in actin reorganization consisting of neurite retraction and of the induction of filopodia structures and stress fibers. These changes were prevented by NSAIDs ibuprofen and indomethacin and by the RhoA inhibitor suggesting that Aβ targets RhoA altering the actin cytoskeleton and that NSAIDs may have a protective effect.

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An enriched environment improves glucose homeostasis and reduces inflammation in the hypothalamus of obese mice

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The hypothalamus is the structure that regulates food intake and energy expenditure to keep energy balance. Recent studies have shown that obesity induces a low grade inflammatory state characterized by the activation of the JNK and IKK pathways in different tissues, accompanied by the expression of inflammatory cytokines. In the hypothalamus, this inflammatory process impairs both insulin and leptin signaling. Accordingly, the inhibition of JNK1 or IKKβ in hypothalamic neurons leads to a reduction in food intake and weight gain, and increases insulin sensitivity. Interestingly, an enriched environment prevents the development of obesity by increasing BDNF levels in the hypothalamus and also regulates the activation of the immune system. Nonetheless, it is not known whether this effect involves inhibition of the JNK and IKK pathways nor whether an enriched environment is capable of reestablishing hypothalamic function in obese mice that already present alterations in the glucose metabolism.

We found that an enriched environment decreased basal glucose levels and increased glucose tolerance and insulin sensitivity, although it did not alter weight gain. We also observed reduced cell infiltration to the adipose tissue, a marker of inflammation. In the hypothalamus we observed a reduction in the levels of IKKβ and p65, as well as a reduction in the phosphorylation of JNK. Current experiments are aimed to determine the impact of an enriched environment on proinflammatory cytokine levels in the hypothalamus from obese mice. Together, our results show that an enriched environment can reduce the activation of different inflammatory pathways and improve glucose homeostasis in a model of obesity.

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Arsenic exposure modifies the expression of RAGE and β-amyloid in rat brain

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Exposure to inorganic arsenic (As_i) affects millions of people around the world. Chronic arsenic exposure affects the nervous system and it is associated with cognitive deficits and peripheral neuropathies. Cellular alterations induced by arsenic involve increased generation of reactive species that induce severe cellular alterations such as DNA fragmentation, apoptosis, and lipid peroxidation, one of the major causes of cell membrane damage that eventually leads to cellular degeneration. Also, arsenic exposure is associated with alterations of chemical transmission and demyelination. Recently, a new hypothesis has emerged and focuses on the involvement of As_i exposure in the possible development of Alzheimer disease (Gong, 2010; Zarazúa et al. 2011). This study evaluates the effects of chronic exposure to of As_i (3 ppm by drinking water) on the production and elimination of β-Amyloid (Aβ) in a Wistar rat model.

Male Wistar rats were exposed to 3 ppm of arsenic in drinking water from fetal development until 4 months of age. Protein expression of Aβ and receptor for advanced glycation end-products (RAGE) has been detected with immunoblot, low-density lipoprotein receptor-1 (LRP) with immunoprecipitation, RAGE and LRP expression through mRNA assay and the enzymatic activity of β-secretase (BACE1) was evaluated by a FRET-derived assay. Our results indicate that As_i exposure increases cerebral levels of Aβ and RAGE. However, BACE1 enzyme activity showed no significant changes. Arsenic exposure did not affect the levels of mRNA coding for RAGE, but contributed to the increased of receptor expression at protein level. mRNA coding for LRP receptor remained unchanged. In this work, we provide the first evidence of the effects of arsenic exposure on the processing of APP and cerebral amyloid clearance in an *in vivo* model.

Keywords: Inorganic Arsenic (Asi), Sodium Arsenite ($NaAsO_2$) Amyloid Precursor Protein (APP), Amyloid Beta (Aβ), Receptor for Advanced Glycation Endproducts (RAGE), low-density lipoprotein receptor-1 (LRP.

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BDNF transfection and dopamine D3 receptor agonist treatment induces motor coordination and dendritic spines recovery with preservation of TH+ neurons in SNc in a Parkinson's disease model by Mn inhalation in rats.

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ABSTRACT. Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons of the substantia nigra pars compacta (SNc), and the development of motor symptoms (bradiscinesia, muscular rigidity, resting tremor and postural changes) that incapacitates the patient to perform daily activities. To date, the treatments, such as levodopa, reduce symptoms but do not cure the disease; after 4 or 5 years of levodopa treatment adverse motor disturbances appear,such as involuntary movements (dyskinesias). Recent research has reported the relationship between dopaminergic neurons and Brain-Derived Neurotrophic Factor (BDNF). BDNF is synthesized by dopaminergic neurons and is responsible for D3 receptors synthesis during development and maintains its expression in the adult brain. Activation of these receptors by specific agonists protect neurons from degeneration, thus, it is considered that there are a synergistic relationship between BDNF and D3 receptors, it assumed that this relationship would be use as a neuroprotective therapy. At present PD animal models are not ideal, although exhibit some of the characteristic features of the disease no one mimics the alterations observed in the disease. Manganese mixture inhalation model (Mn), manganese chloride ($MnCl_2$) and manganese acetate Mn $(OAc)_3$ was used in this report. The animals (male Wistar rats) were exposed to the mixture one hour 3 times a week, and the motor tests used (open field, rotarod and runway) were conducted to measure the performance as well as the progressive damage at 3 and 6 months of exposure. Subsequently the D3 agonist treatment (7-OH-DPAT) by a microdiffusion pump and BDNF gene transfection for dopaminergic neurons in the SNc were administered and then we evaluated its effect measuring the animals motor performance, and if the recovery is associated with TH+ neurons and dendritic spines preservation in the neostriatal medium spiny neurons (MSNS).

Based on the results we believe that the Mn-inhalation model is optimal to reproduce PD because it is bilateral, noninvasive, chronic and progressive; with symptomatic behavior (hypokinesia and postutal instability) and emotional (anxiety), and induces great loss of dopaminergic neurons in the SNc (65.95%) and dendritic spines of the MSNS (82.2%). Treatment with 7-OH-DPAT and BDNF

preserve dopaminergic neurons in the SNc, recovers motor coordination evaluated with rotarod test, and retrieve the MSNS dendritic spines.

Cognitive and emotional impairment induced by chronic neuropathic orofacial pain

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Pain is a complex sensory modality and it is necessary for surviving. Nevertheless, chronic pain loses its protective function and it has emotional and cognitive implications that may lead to long-term changes in the central nervous system affecting welfare of those who suffer from it, above all, their quality of life. These disturbances involve a failure from the subject to adapt to the requirements demanded by his environment and depression. The aim of the present study is to assess experimentally cognitive and emotional impairment induced by chronic neuropathic orofacial pain in a rodent model, evaluating motivation, adaptation, self-regulation and persistence. 36 male Wistar rats (200-250g) were used in this study. Rats were randomly divided in two groups, (1) chronic constriction injury of the mental nerve, a branch of the trigeminal nerve and (2) sham surgery. In order to assess motivation, a method to measure motivation for sucrose (10%) consumption in a self-administration schedule was developed. During this test, rats were placed into a test chamber to press a lever in order to obtain a reward (sucrose 10%) in a fixed ratio schedule (FR), and then in a progressive ratio schedule (PR), where it became progressively more difficult to obtain each subsequent reward; break point was used as a motivational measure. In order to evaluate persistence and adaptation, it was used a go/no-go task followed by a persistence trial; during the go/no-go task, the animals had to press a lever four times to obtain a reward, but they only received it if they pressed the lever while a cue light was illuminated. This way, learning, memory and self-regulation abilities were evaluated. Persistence trial began immediately after this, in which the cue light was illuminated during ten minutes, but the rats had not a reward after pressing the lever, measuring the time that they were willing to persist to obtain a reward and also the number of lever presses. Time was used to evaluate adaptation, too. Thereby, a long time pressing the lever was taken as a sign of poor adaptation to environmental requests. In order to prove that mental nerve constriction induces pain, cold hyperalgesia test was made. It was found that chronic constriction of mental nerve induced hyperalgesia typical responses when the cold test was performed (KS- test, $P < 0.05$). For motivation test, the latency time for the first response was longer for the mental nerve injury group (mNI) compared to the sham group (t-test,$P<0.0001$), the break point was lower for the mNI group than for the sham group, which indicated lower motivation to obtain a reward as a sign of depression. It was observed that learning and memory processes were not affected by the chronic constriction injury (t-test,$p>0.05$) during the go/no-go task. For the persistence trial, two variables were compared in each group, the total amount of responses and the total time of persistence. It was found that, in the mNI group, the number of responses decreased significantly (KS-test, $p<0.05$), in a time lapse longer than 5 minutes, which indicates that the injury not only affected the expectation and motivation to obtain a reward, but the individual's ability to adapt was disturbed, too. These results support the hypothesis that persistent neuropathic orofacial pain causes cognitive and emotional impairment, and could lay the foundations for the search for different therapeutic alternatives and help us in our understanding of the physiopathology of orofacial pain.

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CONTRIBUTION OF STRIATAL CHOLINERGIC NEURONS TO CHANGES IN THE ACTION-OUTCOME CONTINGENCY

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The striatum (STR) is the integrator of inputs coming from the cortex, thalamus and midbrain. It is critically involved in motor skills, learning, habits and goal directed behaviors. It is composed predominantly by GABAergic projections neurons (95%) and a small proportion of interneurons of which 3% are cholinergic. The cholinergic interneurons (CINs) provide the main source of acetylcholine to the striatum. It has been reported that CINs are important for learning mediated by reinforces and response selection by pointing to the existence and motivational value of behaviorally relevant stimuli and recently they had been suggested to be controlling striatal flexibility mechanisms of behavior, an ability that allow us to adapt to changes in the contingency, crucial for survival of an organism.

The aim of this study is to evaluate the contribution of the striatal cholinergic interneurons to the adaptation in the change of contingency of an action-outcome relationship.

To evaluate the contribution of CINS to the change in contingency we trained animals in a goal directed task using operant boxes. Once they learned the task we specifically inhibit the activity of CINs during a change in contingency. To specifically inhibit the activity of the CINS during the change in contingency we inject AAV2.1 containing the sequence to express ArchT-eYFP into of the dorsomedial striatum (DMS) of Chat Cre mice.

Our preliminary results indicate that when we inhibit the CINs during the change in contingency the subjects are less sensible to identify the change in the contingency. These data may shed light on to mechanism controlling the adaptability in behavior, mechanism that are affected in many neurological syndromes.

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Contribution of the Indirect Pathway from Basal Ganglia to Switch Between Action Sequences.

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Neuronal activity of the basal ganglia has been implicated in action selection and switch between action sequences. Disorders in this subcortical nuclei includes a heterogeneous group of pathologies related with dysfunctions in motor control as in Parkinson's, Huntington's disease, Tourette's syndrome and obsessive compulsive disorders. The understanding of the physiology, pharmacology and anatomy of the basal ganglia, has allowed to propose a functional model for this system. This model of the basal ganglia comprise two parallel pathways originated by two distinct types of medium spiny projection neurons in the striatum, the direct and indirect pathway. In a classical view these pathways have an opposing effect, it is thought that the direct pathway facilitates movement while the indirect pathway inhibit it. However recent studies have shown that both pathways are active to promote movements.

The aim of this work is to probe if there is a correlation between indirect pathway activity and 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 probe our hypothesis, we used *in-vivo* extracellular recordings and photostimulation-assisted identification of neuronal populations (PINP) in vivo using A2a-Cre mice. First we designed a training program to teach the subjects to switch between action sequences [chain of fixed ratio sequences of lever presses FR4→FR4→Reward]. For the electrophysiological recordings we used electrodes arrays (4x4). To perform the PINP in vivo we expressed channelrhodopsin-2 (ChR2-eYFP) into the striatum of the A2A cre mice by the injection of AAV2.1 (DIO-Floxed system).

As preliminary data we had identified striatal activity correlated with the switch between action sequences (35%) – specifically we observed that 44% of the recorded units showed an increase and 56% a reduction in activity– at the time of the switch. A percentage of this units was photo-identified as indirect pathway's neurons by optogenetic stimulation. This findings support our hypothesis about the basal ganglia indirect pathway been implicated with the switch between action sequences.

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Dystrophic microglia and hyperphosphorylated tau are present in old common marmoset (*Callithrix jacchus*)

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The common marmoset (*Callithrix jacchus*) is a small New World non-human primate widely used for biomedical research. Marmosets have a high genetic homology to humans and a shorter life-span compared to Old World monkeys and apes. As the world population increases its life expectancy, there is a high incidence of neurodegenerative diseases. Therefore, there is a growing need for adequate aging models. In this context marmosets may be a good model for aging research.
Microglia are cells in central nervous system that participate in immune- and inflammatory reactions. Dystrophic microglia is found in Alzheimer´s disease (AD), suggesting a link between microglia dysfunction and neurodegeneration. Tau protein is part of the microtubule-associated protein family which main function is to facilitate microtubule assembly and stabilization. Phosphorylation of tau may occur under physiological and pathological processes. In aging brains, hyperphosphorylation of tau promotes its own aggregation and in AD this hyperphosphorylation impedes axonal transport causing neuronal dysfunction. Recently, some studies have shown the presence of phosphorylated tau in principal neurons, astroglia and oligodendrocites of old non-human primates. However, up to now there are no reports of phosphorylated tau in microglia cells from marmoset monkeys.
The objective of this research was to detect phenotypic changes in microglia cells accompanied by phosphorylation of tau in marmosets at different ages (young, adult and old subjects) by immunohistochemistry and immunofluorescence techniques.
Our results demonstrate the presence of hyperphosphorylation and aggregation of tau in hippocampus and cortical zones in young individuals, mainly in cytoplasm. Tau aggregation increases with age, were somato-dendritic staining was found in the form of fibrillary aggregation. Importantly, tau aggregation was present in microglia cells of aged subjects, specifically in dystrophic microglia. This type of microglia shows morphological alterations such as loss of fine cytoplasmic processes, spheroids processes, twisted and shortened dendrites and fragmentation of cytoplasm. Moreover, amyloid beta$_{1-42}$ plaques were identified in the cortices of aged marmosets.
We conclude that marmoset monkeys are good models for aging research as they present important hallmarks of aging and neurodegeneration. Further studies are needed to determine the impact of these morphological alternations in brain functioning.

Dystrophin Dp71 transcripts are differentially expressed in mouse brain and retina

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Abstract

The Duchenne muscular dystrophy (DMD) is an X-linked disease characterized by progressive muscle degeneration due to the mutation in DMD gene. Presence of cognitive impairment and retinal abnormalities in a subpopulation of DMD patients has been related with mutations within the region coding for Dp71 and Dp40 proteins. To date, several alternative splicing events of Dp71 mRNA into the exons 71, 71 to 74, 78 and intron 77 have been identified in rat, mouse and human, and in other models, resulting in the expression of multiple Dp71 isoforms. These Dp71 isoforms are grouped in Dp71d, Dp71f and Dp71e based on their C-terminal end. Dystrophin Dp71 is highly expressed in brain and retina, the two main central nervous system tissues; however, specific Dp71 isoforms present in these tissues have not been determined. To explore the Dp71 isoforms expressed in adult mouse brain and retina, RT-PCR assays were carried out and PCR products were ligated into the pGEM-T Easy vector. The ligation mixture was used to transform DH5α cells and Dp71 positive colonies were analyzed by PCR multiplex to determine the alternative splicing of exons 71 to 74 and exon 78 and intron 77. All Dp71 isoforms were further analyzed by DNA sequencing. We found the expression of Dp71 transcripts for Dp71, Dp71a, Dp71c, Dp71b, Dp71ab and $Dp71_{\Delta 110}$ and novel Dp71 isoforms spliced out in exon 74, exons 71 and 74 or 74 and 78, which we named $Dp71d_{\Delta 74}$, $Dp71d_{\Delta 71,74}$ and $Dp71f_{\Delta 74}$, respectively. Additionally, we show a differential expression of Dp71 isoforms, the Dp71d group was highly expressed in brain while Dp71f group was most abundant in retina. In conclusion, the mouse brain and retina expresses multiple Dp71 transcripts generated by several alternative splicing events and these Dp71 isoforms are differentially regulated. These results support a detailed regulation at post-transcriptional level and suggest that Dp71 isoforms may play different roles in brain and retina.

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EFFECT OF DOCOSAHEXAENOIC ACID ON MITOCHONDRIAL FUNCTION IN ROTENONE-TREATED RATS

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The causes or neuronal death in neurodegenerative diseases are currently unknown. However, it has been suggested that mitochondrial dysfunction can promote neuronal death and the symptoms of neurodegenerative diseases. Among the experimental models used, rotenone is one of the most widely used to inhibit respiratory chain's complex I, leading dopaminergic neuronal death resulting in a Parkinson disease-like. Furthermore, it has been reported that the essential polyunsaturated fatty acids (EPUFA) have important effects on the nervous system. It this regard, it has been shown that docosahexaenoic acid (DHA) has a neuroprotective affect on dopaminergic neurons. Also, in recienty years, it has been shown that the EPUFA has an effect on mitochondrial biogeneis. Aim. The aim of this study was to evaluate the potential neuroprotective effect of DHA on mitochondrial dysfunction induced by rotenone in rat striatum and midbrain. Methods. Eighty male Wistar rats were assigned under the following conditions: pretreatment of DHA (35 mg /kg / day) for 7 dyas + rotenone for 8 and 14 days to analyze the exploratory activity, tyrosine hydroxylase (TH) enzyme and, isolated mitochondria, complex I activity, respiratory control index, transmembrane potential and ATP synthesis capacity. Results: DHA did not attenuate the damage in the exploratory activity at 14 days of treatment with rotenone. In addition, at 8 days, DHA prevented damage of rotenone in the exploratory activity (40%), and attenuated both the histology damage and the decreased in the TH protein content induced by rotenone in striatum and midbrain. On the other hand, DHA was unable to attenuate the rotenone-induced mitochondrial dysfunction in striatum and midbrain. Conclusion: DHA administration has a neuroprotective effect after 8 days of rotenone treatment but this effects was not related to the attenuation of rotenone-induced mitochondrial alterations.

Área: Neuropatología

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“Effect of Dystrophins Dp71a and Dp71c overexpression on PC12 cells”

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Dp71 protein is coded by Duchenne Muscular Dystrophy (DMD) gene and is a member of the dystrophin family. This protein is the first *DMD* gene product detected in embryonic stem cells and the most abundant in adult brain and retina. Dp71 preserves Central Nervous System important functions [(1)]. Its absence has been associated with slower neural growth [(2)] and cognitive impairment in DMD patients [(3)]. Dp71 transcript undergoes alternative splicing for exons 71-74 and 78 and the resulting protein isoforms are grouped according to its unique C-terminal. It has been suggested that this region is responsible for the difference in localization and function. Most of the information obtained to date corresponds to Dp71d group, which has exon 78. This Dp71 group includes isoforms that differ in the splicing of exon 71 (Dp71a) or 71 to 74 (Dp71c) [(4)]. The C-terminal region of these proteins contains 13 hydrophilic amino acids present in all dystrophin family. It is unknown if these further splicing could confer each isoform different functions. A recent study shows that the absence of exons 78 and 79 in Dp71 increases PC12 cells proliferation compared to normal growth [(5)]. The aim of the present study was to analyze the proliferation of PC12 cells overexpressing Dp71a and Dp71c. For this, we transfected PC12 Tet-On cells (Clontech) with plasmid pTRE2pur-Myc/Dp71a or Dp71c construct. We obtained the stably transfected clones PC12 Tet-On/Dp71aC4 and PC12 Tet-On/Dp71cC1, which were induced with Doxycycline (DOX) and the Myc/Dp71a or Myc/Dp71c expression was evaluated by western blot using anti-Myc antibody and RT-PCR. Myc/Dp71a and Myc/Dp71c subcellular localization was analyzed by indirect immunofluorescence: Dp71a was localized in cytoplasm and periphery, while Dp71c was found mainly in cell periphery. Proliferation of PC12 Tet-On/Dp71aC4 was not affected in relation to control clone, while PC12 Tet-On/Dp71cC1 was significantly slower than both control and PC12 Tet-On/Dp71aC4 clones. These results suggest different functions for Dp71 isoforms.

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Effects of lipopolysaccharide-induced inflammation in adult hippocampal neurogenesis

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Neurogenesis refers to the generation of new neurons. This process continues in the adult mammalian brain in two main regions, one of which is the subgranular zone of the hippocampal dentate gyrus. Dysregulation of adult hippocampal neurogenesis has been shown to be a critical mechanism underlying cognitive impairment. Several events, such as acute inflammation, downregulates the neurogenic process. However, it is still unknown if: 1) in chronic inflammation conditions, which are common to pathological events, a downregulation of neurogenesis persists and, 2) at which cellular level is inflammation affecting the neurogenic process. Thus the aim of the study was to generate a model of chronic neuroinflammation in mice and evaluate the effects of this condition on neural proliferation, differentiation and survival. For this purpose, we used young adult (two month-old) male C57Bl/6 mice and administered 4 i.p. saline or LPS (1 mg/kg) injections separated by one week each. Physiological sickness responses were assessed by changes in body weight and open field test. The pro-inflammatory cytokine IL-6 levels in hippocampus were tested by western blot. The inflammatory milieu in the brain was assessed by the presence of morphologically activated microglial cells ($Iba1^+$) and neural proliferation was analyzed by immunohistochemical co-labeling of $BrdU^+DCX^+$ cells (neuroblasts and immature neurons). Cell counting was performed in images obtained from confocal microscopy coronal sections. Our results show that LPS treatment induced the loss of the animals' body weight 24h after each injection but within 7 days all animals recovered to control values. No locomotion deficits, suggesting sickness signs, were observed in any of the treated animals. Analysis of microglia showed that in saline-treated animals, these cells had ramified processes, typical of a resting state. After one LPS injection IL-6 levels increased and microglia changed from resting-state morphology to an activated morphology, characterized by an amoeboid shape. IL-6 increased levels and activated microglia persisted even after 4 LPS injections, suggesting that no LPS resistance was developed. Our results show a decreased hippocampal cell proliferation ($BrdU^+$) and a decrease in $BrdU^+/DCX^+$ cells, which could either reflect a downregulation in the neurogenic process or in the survival rate of newly born neurons. We propose long-term LPS i.p, injections as a model of chronic brain inflammation, a condition that maintains low levels of neurogenesis. Further experiments addressing proliferation decrease vs apoptosis will help dilucidate our present results and the effect of such process in cognitive tasks will be performed.

Evaluation of mitochondrial fusion/fission proteins and bioenergetics defects in synaptosomes from the 3xTg mouse model of Alzheimer´s Disease.

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Alzheimer´s Disease (AD) is the most common form of dementia in the elderly. Mitochondrial dysfunction, oxidative stress and β-amyloid (Aβ) accumulation are thought to cause neuronal and synaptic degeneration leading to cognitive decline in AD. Mitochondrial alterations could be derived from the loss of balance in proteins that regulate mitochondrial dynamics, particularly fusion (Mfn1, Mfn2 and OPA1) and fission proteins (Drp1 and Fis1). In AD patients the number and size of mitochondria is reduced (Hirai et al., 2001). Besides, proteins involved in fission are increased while proteins involved in fusion decrease in hippocampal and cortical neurons. These changes are often accompanied by energy deficits in mitochondria isolated from brain of mice exhibiting markers of AD (Yao et al., 2009). However, there is little evidence of mitochondrial alterations that may result in synaptic dysfunction, which is considered an early manifestation of AD. In this work we analyzed mitochondrial bioenergetics and the content of mitochondrial fission/fusion proteins (Drp1/pDrp1 and Mfn1) in isolated nerve endings from different brain regions (hippocampal, cortex and cerebellum) in the 3xTg-AD, over time. Our results show a significant decrease of metabolic activity measured by MTT reduction in the 9-11 months old female mice. In addition, preliminary results indicate a decrease in synaptic mitochondrial oxygen consumption at the same age in the three brain regions. In addition, we have observed a significant increase in the active form of Drp1 (phosphorylated-Drp1) in hippocampal synaptosomes. The fusion protein, Mfn1 did not show changes in any of the studied brain regions. At present, these results suggest that mitochondrial dynamics and activity are altered mainly in hippocampal synaptosomes from the 3xTg-AD along with disease progression that may contribute to the synaptic deficit in AD.

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Evaluation of the RVG peptide ability as a DNA carrier for gene therapy in neurodegenerative diseases.

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Introduction: The neurodegenerative diseases treatment is a huge challenge for today's neuroscience. Gene therapy opens a new field in its treatment as it can be specifically directed to the affected neurons. The RVG peptide is an amino acid sequence of the rabies virus glycoprotein, which confers tropism to neurons of the central nervous system. This RVG peptide has been used to transport different kinds of molecules but gene expression has not been demonstrated yet.

Objective: To evaluate the RVG peptide ability as a DNA carrier for gene therapy in neurodegenerative diseases.

Material and methods: The RVG peptide, a karyophilic peptide (PK) and a plasmid expressing a reporter gene (GFP) were bind by electrostatic charges to form the complex called RVG. The formation of the RVG complex with different concentrations of the components was analyzed by retention agarose gels to determine the optimum ratio. RVG complex capacity to transfect neuronal phenotype cells (SH-SY5Y) and HeLa cells was assessed by immunofluorescence. Subsequently, RVG complex (100 ng) was injected by stereotaxic surgery into coordinates of the cerebral cortex and hippocampus of C57BL/6 mice. The mice were sacrificed on days 4 and 20 post-surgery and their brains were prepared for histological analysis. In order to analyze the expression of the reporter gene in cerebral cortex and hippocampus, we performed immunohistochemistry and RT-PCR techniques.

Results: The optimum ratio, in which we obtained efficient transfection, was 1 microgram (µg) of RVG, 4 µg of PK and 2 µg of DNA. GFP expression was detected 48 h post-transfection in SH-SY5Y cells but not in HeLa cells. *In vivo* experiments demonstrated the GFP expression is detected 4 days post-transfection in the cerebral cortex and hippocampus and continues at 20 days post-transfection.

Conclusion: RVG complex has the ability to transfect neuronal phenotype cells. RVG complex has the ability to transfect cells in the regions of cerebral cortex and hippocampus. The expression of the RVG complex is stable at 20 days post-transfection in the region of the cortex.

FRECUENCY OF THE POLYMORPHISM 5-HTTLPR IN PATIENTS WITH PARKINSON´S DISEASE.

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Introduction: The depressive major syndrome is the principal neuropsychiatric and non motor symptom in Parkinson's disease (PD) and its treatment is not considered within the manage of this disease, affecting the quality of life of the patients. The codificant gene of the serotonine transporter (SLC6A4) has a polymorphic sequence that modulates its transcription and it is known as 5-HTTLPR. The presence of the short allele (S) of this gene SLC6A4 it's been associated to depressive disorders.

Objective: To analyze the frequency of the polymorphism 5HTTLPR of the gene SLC6A4 in patients with PD and controls.
Material and methods: Cases and controls study. We analyzed genomic DNA samples of 116 patients diagnosed with PD of early onset and late onset, also analyzing 116 controls peered by age and sex.
The polymorphic region 5-HTTLPR was amplified using PCR. The amplified products were analyzed by agarose gel electrophoresis at 2%. It was used descriptive statistics; it was established the association using the chi-squared test ($\chi 2$) and Odds Ratio. The tests were evaluated with a significance of 5% ($p<0.05$).

Results: The average age of the 116 patients with Parkinson´s disease was 64.9 years and the same average age for controls . To determine the risk of depressive major syndrome in PD Odds Ratio was 1.28 and $\chi 2$ 4.50 ($p<0.05$). Finding a higher numer of presence of the polimorfism in PD patients respect to controls.

Conclusion: The presence of 5HTTLRP for PD is higher (OR 1.28) respect to controls. This increases the risk of the depressive major syndrome in PD patients.

Generation of an inducible cell model system to the study of spinocerebellar ataxia type 7 (SCA7)

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Area: Neuropathology

Spinocerebellar ataxia type 7 (SCA7) is an autosomal dominant neurodegenerative disorder characterized by progressive ataxia and retinal degeneration. SCA7 is caused by a CAG/glutamine repeat expansion in the ataxin-7 gene/protein. SCA7 is the most common autosomal dominant spinocerebellar ataxia in the central region of Veracruz Mexico, where one of the largest series of cases worldwide has been reported. Progressive degeneration is caused by the neuronal loss of Purkinje cells in the cerebellum and photoreceptor loss in the retina. Detailed pathogenic mechanisms involved in the pathophysiology of SCA7 remain still unknown, however the importance of astrocytes in the disease progression has been recently demonstrated. In this work we have generated an experimental model for ataxin-7 expression based on the human Müller Stem cell line MIO-M1, as a first step into the study of the effects on the glial cells at retina level. Inducible pTRE3G system was used to clone ataxin-7 carrying the normal tract of CAG repeats. MIO-M1 cell model expressed ataxin-7 transcript and protein only under inducing conditions. With this model, we will be able to identify potential alterations in the MIO-M1 cells and subsequently the glial cell contribution into the retinal degeneration of SCA7.

Glycine receptors expression in the retina from streptozotocin- diabetic rats

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Abstract

Background. Diabetes mellitus is associated to a variety of complications including retinopathy. Diabetic retinopathy is generally associated to damage in blood vessels, but alterations in neural retina have been reported. Indeed, there is evidence which suggest alterations in retinal neurotransmission in diabetic patients. Glycine plays an important inhibitory action in retina neurotransmission and its action, restricted to the inner retina, is mediated through the strychnine-sensitive postsynaptic glycine receptor (GlyR). The GlyR are composed by two α (1-4) subunits and three β subunits. **Objective.** In this study we analyzed possible alterations on GlyR expression in the retina of diabetic rats. **Methods.** Long Evans rats were used and diabetes was induced by a single administration of streptozotocin (Sánchez-Chávez et al, 2008). GlyR expression was assessed by both, protein and mRNA, following western blot standard procedure and qPCR, respectively. **Results.** Western blot analysis revealed a considerably decrease in GlyR α1 subunit at early stages of diabetes induction, which corresponds with undectable levels of its mRNA at P7D and a 13-fold decrease at 20 D. Intriguingly, α1 subunit expression showed a ~4-fold and 50-fold increase at 45D compared to control and to 20D, respectively. **Conclusions.** These results provide evidence of alterations in retinal neurotransmission function after early diabetes induction, suggesting these as initial step of retinopathy.

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Área: Neuropatología

Impaired Autophagy is Associated with Neuronal Senescence *in vitro*

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Cellular senescence is a biological state characterized by an irreversible cell cycle arrest, senescence-associated β-galactosidase activity (SA-β-gal), senescence-associated heterochromatin foci, DNA damage foci and accumulation of lipofuscin. Senescent cells accumulate in various tissues and organs with ageing where they express a complex senescence-associated secretory phenotype (SASP). The SASP includes inflammatory cytokines, chemokines, growth factors and proteases and is proposed to underline age-related diseases. Cellular senescence has been described in mitotic cells, and it has been assumed that post-mitotic cells are incapable of entering into a senescent state. Nevertheless, neurons with several senescent features have been observed in old mouse brain. Therefore, we developed an *in vitro* model of senescence of primary cultures of rat cortex to study the mechanisms of both neuronal and glial senescence establishment and compare similarities with senescent neurons and glia from old rat brains. We found that during neuronal and glial senescence autophagy flux is impaired. Autophagy is a catabolic process that, through lysosomes, degrades intracellular components into basic biomolecules. Our findings demonstrate that long-term cultures of cortical cells can be useful for study the cellular and molecular mechanisms of neuronal and glial senescence and suggest that defective autophagy would contribute to neuronal senescence.

Key words: Cortex; Neurons; Glia; Autophagy; Aging; Senescence; SASP.

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Improvement in learning and memory after treatment with a bifunctional tetrapeptide in triple transgenic mice for Alzheimer's disease

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The interaction of amyloid-β (Aβ) with metal ions such as copper (Cu) has been implicated in the pathological events of Alzheimer's disease (AD), including memory deficits. In this study, we evaluated the effect of a bifunctional tetrapeptide (TP), which is capable of modulating Aβ aggregation and chelating Cu ions[1], on neuronal histopathology and cognitive dysfunction in a triple transgenic mouse model (3xTg-AD) that develops many of the features observed in AD. At the end of the treatments, 3xTg-AD subjects infused with TP (Tg-TP) performed similar to non-transgenic control animals (NoTg-CTRL) and better to transgenic mice treated with saline as vehicle (Tg-Vh) in a spatial reference learning and memory task, the Morris water maze. Interestingly, this cognitive improvement in Tg-TP was accompanied by a different kind of Aβ aggregation compared to Tg-Vh. The amount of large Aβ oligomers increased in the Tg-TP group. Furthermore, we found no differences in the number of GFAP positive cells in hippocampus regions of Tg-TP animals in contrast to Tg-Vh. Our results suggest that a change in the size of Aβ aggregates may affect their toxic properties, reflected on neuroanatomical and behavioral levels. Therefore, the design of chemical compounds may not only offer a therapeutic approach but it also helps understanding the underlying mechanisms of AD pathology.

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MicroRNAs 1303, 130a and let-7g* expression levels in blood serum of patients with Pediatric Astrocytoma

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Abstract

Background. Astrocytoma, the leading cause of death associated with cancer, are the most common primary central nervous system tumors in childhood. A microRNA (miRNA) array –performed from pediatric tumors- showed expression changes of different miRNAs, such as **miR-1303**, **miR-130a**, **let-7-3p, and miR-16**. Secretion of microvesicular miRNAs (circulating miRNAs) is a characteristic of different types of cancer which allowed the identification of many miRNAs as diagnostic and/or prognostic biomarkers. **Objective:** To determine the expression changes of miRs 1303, 130a, let-7-3p, and 16, in blood serum of pediatric patients with astrocytic tumors. **Methods.** RNA extraction –from blood serum- was performed with the total RNA purification Plus kit (Norgen Biotek Corp) and changes on miRNA expression were tested by qPCR using miScript Primer Assays of QIAGEN. **Results.** Preliminary data showed a higher expression of miRNA 130a in diffuse astrocytoma (7 fold) and in GBM (24 fold) than pilocytic tumors. **Conclusions:** These results suggest that miR-130a might be used as biomarker of progression and/or malignancy in pediatric astrocytoma.

Modifications in white matter tracts in MPTP-lesioned non-human primate model of Parkinson's disease

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The Parkinson´s disease (PD) is a progressive disorder characterized by motor and non motor disturbances. The motor disturbances are mainly tremor, rigidity and bradykinesia, while the non-motor disturbances involve cognitive, emotional and peripheral alterations. Currently, PD has been studied through imaging tools as magnetic resonance diffusion imaging to provide information about anatomical connectivity in the brain, by measuring the anisotropic diffusion of water in white matter tracts (WMT). One of the measures most commonly is fractional anisotropy (FA), which quantifies how strongly directional the local tract structure, in order to localize brain changes related to neurodegeneration. Up to now, there are a few reports where evaluated changes in WMT in PD at basal condition, before any dopaminergic treatment. To evaluate this condition, the animal models are very important, mainly nonhuman primate (NHP) models of PD, because they play an essential role in the understanding of PD pathophysiology and the assessment of PD therapies. In this work we evaluated the WMT of 6 nonhuman primates (*Cercopithecus aethiops*), 3 control subjects and 3 MPTP subjects. Images were acquired using a 3 Tesla GE Discovery MR750 (General Electric, Milwaukee, WI) in the Instituto de Neurobiología of Universidad Nacional Autónoma de México, Juriquilla, Querétaro. The DTI sequences consisted of *Single Shot Echo Planar Imaging sequences*, acquiring 65 volumes of 70 axial slices (2 mm slice thickness and no separation), one for each one of the 60 independent directions of diffusion with b= 2000 s/mm^2 and one corresponding to b=0 s/mm^2, TR/TE=6500/100 ms, FOV 256 × 256 mm^2 and an acquisition and reconstruction matrix of 182×182, resulting in an isometric resolution of 1×1×1 mm. We evaluated the WMT changes with *Tract-Based Spatial Statistics* FSL's tool on FA maps from the whole brain, brainstem and cerebellum to compare anisotropy of water diffusion. A decrement was found in the subjects' FA ($p<0.05$, corrected) in the cerebellum's white matter, *corpus callosum*, anterior and posterior internal capsule, external/extreme capsule, temporal fasciculus, occipital fasciculus, and frontal fasciculus white matter. These findings are in accordance with histopathology results where the most important degeneration occurs in the BG and its connections with the related cortical areas, thus the white matter changes support the idea that the PD is a connectivity disorder between the BG and cortex that

have a close correlation with the motor and non motor symptomatology as occurs in the PD patients.

Field: Neuropatología

Neuronal correlates in cortex with the switch between action sequences.

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The study of switch between actions sequences is important to understand syndromes as the obsessive compulsive disorder (OCD). We hypothesized that in OCD there is loss of the capacity to switch between action sequences. The neuronal activity in the cortex and striatum has been correlated with the control to appropriately initiate/perform/stop an action sequence both in primates and in rodents.

It's has been suggested that basal ganglia (BG), specifically the striatum and the several parts of the cortex contain neuronal activity signaling when to initiate movement sequences. The striatum is highly innervated by several cortexes. This nuclei contain the neurons who axons are the main output of BG which had been classified in two systems: the indirect-pathway and direct-pathway, and specific functions had been suggested for these pathways but little is known about how the different cortexes modulate the striatal activity to appropriately switch between action sequences. To address this question we developed a rodent two sequences task to study the switch between action sequences. Then once the training had been stabilized we recorded the neuronal activity from M2 and m-PFC while animals perform the switch between action sequences in vivo. We also implemented optogenetic manipulations to identify if the units we record project to the striatum.

As preliminary data, after signals analysis process (Offline Sorter™ and MATLAB ®) we had found that neural activity in the cortex is correlated with the moment of transition between action sequences presenting differential patterns of activity. Currently we are developing loss of function experiments (using Arch3.0) to test whether this activity is contributing to the switch.

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Quercetin protects against spinal motor neuron degeneration induced by chronic excitotoxicity *in vivo* through a Sirt1-dependent mechanism

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Excitotoxicity results when there is an excessive activation of glutamate receptors, and it may result in neuronal death. This is a mechanism of paramount importance in most neurodegenerative diseases, including motor neuron (MN) disorders (MND) (*Expert Opin Ther Targets* 11:1415-1428, 2007). We designed an *in vivo* model of chronic spinal excitotoxicity in rats, by infusing AMPA directly in the spinal cord, that permits to assess the potential protective action of drugs (*J Neuropathol Exp Neurol* 66:913-922, 2007). Polyphenols, such as quercetin (QCT) and resveratrol (RSV), are plant-derived compounds that have emerged as a promising therapeutic approach for several neurodegenerative disorders (*Antioxid Redox Signal* 18:1818-1892, 2013). The positive effects of polyphenols have been attributed to the allosteric activation of sirtuin 1 (SIRT1), a NAD+-dependent histone deacetylase, that is being extensively studied as a potential therapeutic target for neurodegenerative disorders (*Front Aging Neurosci 5:53, 2013*). Thus, SIRT1 activators, such as RSV and QCT, have been shown to be beneficial in an *in vitro* transgenic model of ALS (*EMBO J* 26:3169-3179, 2007). In this work, we report the effects of RSV and QCT infusion in our *in vivo* model of AMPA-induced chronic spinal neurodegeneration. AMPA [1 mM] infusion caused a progressing paralysis during 10 days, initiating in the ipsilateral hindlimb and manifested as an increasingly reduced time to fall from the Rotarod. At this time, the number of MNs was reduced by ~90% in the ipsilateral side and by ~50% in the contralateral side. RSV and QCT infusion per se, at a dose of 1 nmole/day, did not alter the motor behavior or the number of MNs. Both RSV and QCT, when co-infused with AMPA, significantly increased the time to fall from Rotarod, but only QCT significantly decreased MN loss, and overall showed a better protective effect. We also tested the effects of EX527, an inhibitor of SIRT1, at a 1 nm/d dose. EX527 alone did not cause any effect, and when co-infused with AMPA protected similarly to RSV in both the Rotarod performance and MN number. When we co-infused either RSV or QCT with EX527+AMPA, only the beneficial effects of QCT were suppressed. In addition, we confirmed the selective expression of SIRT1 protein in MNs by immunofluorescence. Thus, we conclude that QCT prevents MN loss against chronic excitotoxicity *in vivo* through the activation of SIRT1, and that this opens new therapeutic strategies for ALS. However, other mechanisms cannot be ruled out. The contradictory effect of EX527 may be due to the fact that this inhibitor is selective for SIRT1 and therefore the inhibition could result in reductions in NAD+ consumption, which under excitotoxic conditions may stimulate other protective NAD+-dependent mechanisms in the stressed MNs.

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Presynaptic terminals afferent to spinal motoneurons are modified during the degeneration process induced by chronic excitotoxicity in vivo.

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In previous work we developed a model of degeneration of lumbar spinal motoneurons (MN) in vivo by excitotoxicity, through the chronic intraspinal infusion of AMPA and the consequent overactivation of the receptors. MN death is bilateral and produces paralysis of the rearlimbs. (J. Neuropathol. Exp. Neurol. 66:913, 2007). These alterations are similar to those observed during MN degeneration in amyotrophic lateral sclerosis (ALS), and due to the clinical and experimental evidence of alterations in the neuronal circuits during the progress of the disease, in this work we studied whether the MN degeneration caused by AMPA resulted in alterations of the synaptic inputs onto MN. For this purpose we analyzed by synaptophysin immunofluorescence, specific marker of synaptic terminals, the changes in the number and location of those terminals in close contact with the membrane of the MNs (identified by SMI32), at one and three days after the beginning of AMPA infusion. We analyzed 15-17 MNs in 4-5 sections for each rat (N=6 per group). Only MNs that were >25 um in size in the microscope image and with visible nucleus were considered, to ensure that the terminals were afferent to the MN. Control vehicle-treated or intact rats showed an average of 16 terminals on each MN, and this value increased about two-fold one day after the beginning of AMPA infusion; at this time the MN somas showed no signs of damage. In contrast, at day 3 the MN cytoplasm showed heterogeneous SMI32 staining, suggesting cell damage, and the number of terminals was about half the control value, while numerous synaptophysin-stained structures resembling terminals appeared in the surrounding neuropil. At this time animals showed complete paralysis of the rearlimbs. These findings suggest that at the early stages of the excitotoxic MN degeneration induced by AMPA there is an increased synaptic input resembling sprouting, whereas at more advanced degeneration stage this input decreases. These data also suggest that the functional state of the spinal MN may modify the synaptic input they receive, and therefore offer a novel strategy to better understand the mechanisms of neuronal degeneration and possible therapeutic measures in ALS. This work was supported by Consejo Nacional de Ciencia y Tecnología de México (CONACYT, project 128229) and Dirección General de Asuntos del Personal Académico, UNAM (IN201013). UNRJ is recipient of a scholarship from CONACYT.

Protective effect of D-β-Hydroxybutyrate against excitotoxic neuronal death and its relationship to autophagy.

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Abstract-During cerebral ischemia oxygen and glucose supply to the brain is interrupted, affecting vital cellular processes such asthe release and uptake of the excitatory amino acids neurotransmitters, glutamate and aspartate, leading to excitotoxic damage due to the prolonged stimulation of NMDA and Non-NMDA glutamate postsynaptic receptors. Excitotoxic neuronal damage involves a series of molecular processes including oxidative stress and energy deficiency, which contribute to neuronal death. Autophagy is a catabolic process triggered during nutrient deprivation, which leads to the degradation of damaged organelles and proteins for the maintenance of cellular homeostasis. This process is characterized by the formation of double membrane structures known as autophagosomes, which engulf damaged cellular components and subsequently fuse with lysosmes forming autophagolysosomes, where the autophagosomal content is degraded. Excessive autophagy degradation can induce cell death. Autophagy is activated during excitotoxicity induced by ischemia but is role in cell survival or cell death is still controversial. In previous in vivo and in vitro studies we have shown that the ketone body, D-β-hydroxybutyrate (BHB) has neuroprotective effects against glucose deprivation- and hypoglycemia-induced damage through the decrease in reactive oxygen species (ROS) production and the increase in energy levels. In addition, we have observed that BHB stimulates autophagic degradation favoring cell survival in cultured cortical neurons exposed to glucose deprivation. The aim of this study is to investigate whether BHB can protect against excitotoxic neuronal death induced by the administration of NMDA in the rat striatum, and whether this effect is related to the modulation of autophagy. Results show that BHB reduces the size of the excitotoxic lesion induced by NMDA administration as well as the number of damaged cells. In addition, results show that the levels of three autophagic markers: Beclin 1, a protein involved in autophagic initiation; LC3-II a protein involved in the maturation of autophagosomes and p62, involved in the recruitment of damaged proteins to the autophagosome, increase at different times after NMDA administration. The effect of BHB on the levels of the autophagy markers is under study.

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Protective effects of S-allyl cysteine on behavioral, morphological and biochemical alterations in the brains of rats subjected to chronic restraint stress

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Antioxidants exhibiting a broad range of neuroprotective actions constitute attractive tools to design therapeutic alternatives to different neurological and psychiatric alterations. In this work we evaluated the effects of S-allyl cysteine (SAC) on behavioral, biochemical and morphological indicators of toxicity and brain damage induced by chronic restraint stress to rats. Restraint stress sessions were performed every day for 6 h during 21 consecutive days. SAC (100 mg/kg, i.p.) was administered daily 30 min before every single stress session. Behavioral tests comprised three different cognitive, social and locomotor tasks (open-field, T plus-maze and forced swimming devices), whereas biochemical (lipid peroxidation, protein content and activities of glutathione peroxidase (GPx) and glutathione S-transferase (GST)), as well as morphological (Haematoxylin & eosin and immunohistochemistry for glial fibrillary acidic protein (GFAP)) endpoints were estimated in three brain regions: striatum (S), hippocampus (Hp) and cortex (Cx). When compared to control (unstressed) rats, the stressed animals exhibited enhanced motor activity in open-field and increased number of visits to close spaces in the plus-maze, as well as decreased struggling in the swimming device. Stressed animals also produced morphological alterations, stimulating reactive gliosis in the brain regions studied. These changes were accompanied by increased oxidative damage and compensatory activation and expression of antioxidant enzymes. SAC prevented the aberrant behavioral patterns elicited by stress in rats, and significantly reduced their morphological and biochemical alterations in brain regions. Our findings support the concept that the neuroprotective properties exerted by SAC in this and other toxic paradigms are mostly related with its broad range of antioxidant properties.

Recurrent moderate hypoglycemia exacerbates neuronal death and induces cognitive dysfunction after the hypoglycemic coma

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The most common consequence of insulin treatment in diabetic patients is the presence of moderate and even severe hypoglycemia. Recurrent hypoglycemia (RH) induces a defective hormonal glucose counterregulatory response increasing the risk to severe hypoglycemia (SH), which might result in the hypoglycemic coma. This is associated with neuronal death in vulnerable brain regions such as the cortex, the hippocampus and the striatum. Although moderate hypoglycemia does not represent an immediate threat for the life of patients, if recurrent, it may lead to several clinical sequelaes. Previous studies have shown that RH produces only scarce death in the cerebral cortex, while the presence of synaptic dysfunction was observed in the hippocampus in absence of neuronal death. The effects of moderate RH in cognitive function and neuronal death induced by the hypoglycemic coma, are still unknown.Therefore the aim of the present study was to investigate whether (RH) previous to the induction of the hypoglycemic coma (SH) induces oxidative damage in different brain regions, and whether it correlates to the vulnerability to neuronal death and a decline in cognitive functions. Male Wistar rats were subjected to repeated episodes of (RH) (55-35 mg/dl) previous to the induction of (SH) (< 20 mg/dl) by insulin administration. Rats were rescued with glucose 10 minutes after the presence of coma (8-10 min), as monitored by EEG recording. 24 h after insulin injection, rats were sacrificed and oxidative damage was evaluated monitoring the levels of reduced glutathione (GSH), 4-Hydroxy-2-nonenal (4-HNE), and 3-nitrotyrosines (NT) by immunostaining, while neuronal death was evaluated 24 h and 15 day after the coma, using Fluoro Jade B and the Tunel Assay. Cognitive function was assesed by water maze 24 h and 15 days after coma induction. Results indicate that animals subjected to HR/HS had a significative increase in the presence of FJB (+) cells in parietal cortex, striatum and hippocampus relative to the HS group. These data are consistent with the presence of positive cells to TUNEL, 4-HNE and NT in the parietal cortex and the hippocampus and with a decrease in the GSH in the hippocampus. In agreement with these observations, cognitive impairment after HR/HS was more severe than that observed in animals only exposed to SH, 15 days after the induction of the hypoglycemic coma. The present data suggest that an increase in oxidative stress

and a reduced antioxidant defense, contribute to the vulnerability of HR/HS treated rats to neuronal death and cognitive decline.

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RESVERATROL REDUCES BRAIN EDEMA IN CEREBRAL ISCHEMIA

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Abstract: Brain vascular endothelial cells (BVEC) is a component of the neurovascular unit (NVU) which is integrated also by neurons and glial cells. Among other functions, BVEC regulates the flow of ions and molecules between the blood stream and brain tissue, thereby maintaining homeostasis within the NVU. During cerebral ischemia, decreased blood flow due to an embolus or thrombus formed in a major cerebral artery, leads to an immediate reduction in the concentrations of oxygen and glucose that reach the tissue. This situation causes mitochondrial damaged and an increased production of reactive oxygen species (ROS) that quickly alter the function of BVEC. Accordingly, ionic flow is changed, causing the passage of water from the vessel into the brain parenchyma (ionic edema). This provoke that microvessels gradually lose their structural integrity permitting the formation of vasogenic edema wherein the crossing of proteins through the blood brain barrier (BBB) is observed. If vascular deterioration continues, the extravasation of cells from the bloodstream and progression to hemorrhage is allowed. It is possible that antioxidants prevent the formation of edema and decrease damage to the BBB through regulating the level of ROS during cerebral ischemia. Therefore, we evaluated the effect of RSV on the prevention of edema in an *in-vivo* model of cerebral ischemia. **Methods.** Male Wistar rats (280 to 350 g) were subjected to 2 h of middle cerebral artery occlusion (MCAO) followed by 24 h of reperfusion. RSV was administered (1 mg/kg; i.v; diluted in 50% ethanol) at the onset of reperfusion. Cerebral edema formation was evaluated by measured the brain water content and the Evans blue extravasation. Damage to the brain tissue was evaluated by the technique of 2,3,5-Triphenyltetrazolium chloride (TTC) dye. The protective effect of RSV on survival was quantified 24 h after reperfusión. **Results.** MCAO/reperfusion increased the 4.1 % of water in the brain. Similar results were found in this condition when protein extravasation was evaluated with the Evans blue stain and infarct area with TTC showing a 13.42 and 27.7 % increase, respectively. Administration of RSV to rats subjected to MCAO/reperfusion reduced the percentage of brain water to 2.9 %, the extravasation of Evans Blue to 5.20 % and the infarct area to 20.9 %. MCAO/reperfusion reduced the survival rate to 61.9 % and administration of RSV prevented death and increased survival to 90.47 %. **Discussion.** Our results indicated that RSV prevents formation of edema and reduces the damage caused by ischemia and reperfusion. The mechanisms is unknown, but

the effect could be right through the regulation of proteins such as the matrix metallo-proteinases which are activated by ROS and their function is to degrade the junction proteins cell-cell.

Role of autophagy on severe hypoglycemic neuronal death and its possible modulation by beta-hydroxybutyrate.

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Hypoglycemia occurs as a consequence of insulin treatment in diabetic patients, leading to brain glucose deprivation and neuronal damage when the hypoglycemic coma is attained. Animals exposed to the hypoglycemia coma (glucose <20 mg/dl) show neuronal death in vulnerable brain areas such as the cortex, the hippocampus and the striatum. However, the molecular mechanisms involved in hypoglycemic neuronal death are still unclear. Previous studies from our group have suggested that autophagy, a lisosomal-dependent degradation process activated during energy failure, participates in neuronal death induced by glucose deprivation in cultured cortical neurons, and that the ketone body (KB), D-beta-hydroxybutyrate (D-BHB), stimulates the autophagic flux and prevents neuronal death. Is well know that KB can substitute for glucose under certain conditions, such as prolonged fasting, and we have previously shown that D-BHB can prevent neuronal death after insulin-induced hypoglycemia in in vivo. In the present study we aimed to investigate whether autophagy is activated during hypoglycemia and glucose reperfusion in vivo and whether the neuroprotective effect of (D-BHB), is related to the regulation of autophagy. Male wistar rats were exposed to the hypoglycemic coma and recovered with glucose reperfusion in presence or the absence of D-BHB. Samples were obtained after 24 h. Other rats were euthanized after 2 h of severe hypoglycemia (SH). The levels of different autophagy markers were determined by inmunoblot (Beclin-1, LC3I-II and p62) in the cortex, the hippocampus and the striatum. Results show that after 2 h of SH there is no change the levels of Beclin-1, a protein involved in the initiation of autophagy, while the conversion of LC3-I to LC3-II, an index of autophagosome formation, increases in all regions studied. The levels of p62, a protein involved in autophagic degradation show no change. When autophagy markers were analyzed 24 h after the hypoglycemic coma, the transformation of LC3-1 to LC3-II partially increased in all regions studied. Preliminary results suggests that in rats treated with D-BHB, no increase in LC3-I transformation was observed, while apparently no changes in Beclin 1 and p62 levels are observed in rats treated or not with D-BHB. Results suggest that autophagosomes are formed during the hypoglycemic episode and 24 h after glucose reperfusion and that D-BHB might prevent late autophagosome accumulation.

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Synergistic interaction in the antinociceptive and anti-inflammatory effect of DHA-diclofenac combination

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Diclofenac is one of the most used traditional nonsteroidal antiinflammatory drug (NSAID). Furthermore, this drug exhibits antipyretic, antiinflammatory and analgesic effect. Diclofenac is amply used for the treatment of acute and chronic inflammatory pain; is one of the most common treatments of pain in chronic degenerative diseases. The apparition of gastritis, gastric and duodenal ulcers in patients treated with diclofenac is the principal adverse effect.. In the other hand, there have been reports of experimental therapies for the treatment of inflammatory pain like docosahexaenoic acid (DHA) that has another metabolic way to counter the pain, this compound belongs to the family of polyunsaturated fatty acids (PUFAs), and there are several reports demonstrating the anti-inflammatory, antinociceptive and gastric safety properties. It is known that, combination of drugs with other molecules that possessing similar pharmacological effects could improve their therapeutic effects and reduce the adverse effects. Then, the aim of this work was to evaluate the synergistic interaction of anti-inflammatory and anti-nociceptive effect of the combination of DHA and diclofenac. The anti-inflammatory effect was evaluated using the paw edema induced by carrageenan test and anti-nociceptive effect was evaluated using the formalin test, for these test female Wistar rats were used (200-220 g). For this, we evaluated the anti-inflammatory effect of diclofenac and DHA to determine the effective dose 30 (ED_{30}) of each compound and then evaluate the oral combination in different fixed ratios: 1:1 1:3 and 3:1. The isobolographic analyses showed that these combinations exhibited a super additive effect, as it is shown below with the values of ED_{30} theoretical and experimental: For combination of 1:1, the theoretical DE_{30} (ED_{30t}) value ED_{30} was 67.94±4.3 mg/kg and the experimental (ED_{30e}) was 6.97±2.4 mg/kg, the same manner, combination 1:3 was 35.37±2.2 mg/kg and 1.1±0.5 mg/kg respectively and combination 3:1 was 100.51±6.4 mg/kg and 11.34±1.59 mg/kg respectively, showing super additive effect in the three combinations. To evaluate the analgesic effect, first we assessed the anti-nociceptive effect of diclofenac and DHA to determine the ED_{30} of each compound and then we evaluated the most potent combination 1:3. The isobolographic analysis shows that the combination exhibited a super additive effect, since the ED_{30t} was 15.92±0.5 mg/kg and the ED_{30e} was 1.25±0.5 mg/kg. This combination also showed gastric safety, because when we carry out the dissection of the stomach after 3 hours of the administration, no gastric damage was found in this group. In summary, these data suggest that co-administration of DHA and diclofenac presented a super additive action in the anti-nociceptive and anti-inflammatory effect with gastric safety.

Thalamic contribution to activation of the basal ganglia during the initiation and executions of action sequences

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Currently it is thought that the neuronal circuits responsible for the selection of actions (initiation of action sequences) are the loop cortex-basal ganglia-thalamus-cortex. In this study we are currently investigating the interaction between the thalamus and basal ganglia with the aim of answering the following question: Which is the thalamic contribution to the initiation of an action sequence? To address this question we performed three experiments 1) we recorded the activity of different thalamic nuclei, 2) we inhibited the activity of the neurons in the thalamus or 3) its projections to the striatum while an animal initiated a self paced action sequence.

To study the contribution of the thalamus to action initiation we trained animals to initiate and perform sequences of lever press in an operant task (fix ratio 8). To record the activity of the thalamus we performed electrophysiological recordings using electrodes arrays (4x4) implanting them into the thalamus. To inhibit the neuronal activity of the thalamus or their projections to the striatum we use the VGLUT2-Cre mice, which express the Cre recombinase under the promoter VGluT2 (vesicular glutamate transporter), which gives the specificity to express opsins into the thalamic neurons. Then by expressing the opsin Archeorhodopsin-eYFP (proton pump which when illuminated with green light can inhibit the neuronal firing) we specifically inhibit the cell bodies of thalamic neurons or their axons in the striatum depending on the positioning of a fiber optics to achieve the ArchT illumination in vivo while animals initiate/perform an action sequence.

Our preliminary results shown neuronal activity in the thalamus correlated with the initiation of action sequences, some recorded units showed an increase in their activity before the sequence of lever press initiated. When investigating if the activity of the thalamus is contributing to the initiation of action sequences by inhibiting the thalamic activity just before action sequence initiation we observed that decreasing the activity of the thalamus (inhibiting the cell bodies) or when inhibiting their thalamic axons into the striatum we observed a delay in the initiation of the action sequence. These results are implicated in the understanding of the thalamic subcircuits contributing to action sequence initiation, which we hypothesized it is important to understand the symptoms of neuropathologies as Parkinson´s and Huntington disease and syndromes as the obsessive compulsive disorders.

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The tetanus toxin C-fragment protects spinal motor neurons against degeneration by chronic excitotoxicity in vivo

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Topic: Neuropathology

In our laboratory we have shown that the chronic infusion of AMPA in the rat lumbar spinal cord leads to progressive motor neuron (MN) death mediated by excitotoxicity and induces consequent paralysis of the rear limbs (J. Neuropathol. Exp. Neurol. 66:913, 2007). Thus our model, which excludes genetic alterations, resembles the pathology and motor alteration present in sporadic amyotrophic lateral sclerosis (ALS). The tetanus toxin C-fragment (TTC) does not have the toxic effects of the native toxin, but maintains the capacity to be retrogradely transported from peripheral axons into the central nervous system (Proc. Natl. Acad. Sci. 94:9400, 1997), with preferential localization in MNs (Neurodegener. Dis. 1:101, 2004). In addition, the TTC has shown neuroprotective properties in vitro, involving the activation of PLCγ-1, PKC y ERK-1/2 signaling cascades through TrkA receptors (J. Biochem. 356:97, 2001; J. Neurochem. 373:613, 2003; J. Neurochem. 90:1227, 2004), and against neurodegeneration in animal models of Parkinson's disease (Neurosci. Res. 74:156, 2012; Neurosci. Res. 84:1, 2014) and familial ALS (Orphanet. J. Rare. Dis. 21:6, 2011).

In this work we evaluated the possible protective effect of TTC in our in vivo model of chronic spinal neurodegeneration. We found that the i.m. injection of the fragment is more efficient than the intraspinal infusion to protect against MN death and thus reduced the motor deficits induced by AMPA infusion. Furthermore, the protection exerted by TTC is more intense when administered two hours after the beginning of AMPA infusion as compared with the co-treatment. The i.m. administration of GW-441756, a potent inhibitor of TrkA receptor, significantly decreased the protection exerted by TTC. We conclude that the protection exerted by TTC against excitotoxic MN death is mediated by the activation of TrkA receptors located in the neuromuscular junction and the further retroaxonal transport of the fragment to spinal MNs. Because of this tropism for MNs, the efficacy of their effects, the lack of toxicity at low concentrations and the facility of the route of administration, we consider that TTC could be used as a therapeutic option for neurodegenerative diseases as ALS. This work was supported by CONACyT, Mexico (project 240817) and DGAPA, UNAM (IN201013). C.N. is recipient of a scholarship from CONACyT.

Protective effects of *Ilex paraguariensis* extracts in oxidative stress parameters in mice brain with Diabetes mellitus induced

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Background: Diabetes mellitus is a metabolic disorder that has increased its incidence in several countries. In Mexico, this disease is the leading cause of death, generating high spending on public health. For these reasons, the search for natural and/or synthetic compounds that can minimize the deleterious effects generated by diabetes and its complications has gained attention worldwide. *Ilex paraguariensis* is used in the preparation of stimulant drinks well consumed by the population of South America. Extracts of this plant are known to have a wide variety of compounds, such as polyphenols, methylxantines, and caffeine, which can exert beneficial health effects. It is know that the brain needs continuous supply of glucose to keep active, but chronic hyperglycemia triggers a series of complications related to oxidative stress in Diabetes mellitus. Thus, the aim of this study was to evaluate the effect of yerba mate extracts in a mice model of type 1 diabetes induced by streptozotocin, on oxidative stress parameters. **Methods:** We evaluated glucose levels, serum transaminases, and fructosamine in blood. We also evaluated the levels of lipid peroxidation, nonproteinthiols (NPSH), and the activity of the enzymes δ-aminolevulinate dehydratase (δ-ALA-D), and catalase (CAT) in the brain. Finally, we analyzed the thermal sensitivity as an indicator of diabetic neuropathy. **Results:** Our results showed that mice with STZ-induced diabetes exhibited high levels of glycemia, transaminases and fructosamine in blood, as well as an increase in lipid peroxidation. We also found a decrease in the levels of NPSH and in CAT and δ-ALA-D activities. The treatment with *Ilex paraguariensis* extracts reversed hyperglycemia caused by STZ and normalized oxidative stress parameters, while reversed peripheral neuropathy assessed by thermal sensitivity. **Conclusions:** Excess glucose can stimulate the accelerated secretion of the neurotransmitter glutamate, which increases brain oxidative stress, generating free radicals that can be damaging for neurons. Peripheral neuropathy and oxidative stress are important mechanisms for triggering damage during chronic hyperglycemia caused by STZ. Our extract was able to reverse this damage and this effect can be attributed to its potent antioxidant capacity combined with a potential hypoglycemic effect thereof, becoming targets of research as potential components reducing the deleterious effects of DMI at the brain level.

Short term effect of early overnutrition in the transcriptome of Wistar rat hypothalamus (Rattus norvegicus).

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Introduction. The hypercaloric nutrition is highly associated with metabolic syndrome, diabetes mellitus type 2 and cardiovascular diseases. The postnatal overnutrition (PON) influences the generation of metabolic disorders such as obesity and dyslipidemia. Recent reports in animal models with hypercaloric diet show changes in the concentration of peptides that regulate the activation or inhibition of neurons that induced behaviors of appetite and satiety, resulting in hyperfagia after treatment.

Objetives. Analyze the short-term effect of the PON in the hypothalamus transcriptome. Particularly analyze metabolic pathways related to the regulation of the processes of hunger, satiety and inflammation.

Materials and methods. Overnutrition model in Wistar rats by overlactation from day 3 to day 21 of life by litter size reduction method using pup male was generated; control (n = 5) and overnutrition (n = 5). Transcritome was analyzed by microarrays of expression technology (Ratgene ST 2.2, Affymetrix®), with a coverage of 28.407 transcripts. The pathway analysis was performed with the Ingenuity Pathway Analysis software (IPA). Bioinformatic analysis was performed on the R statistical software, statistical analysis for phenotypic and biochemical parameters were analyzed in Graphpad Prism 6® software was applied Student t with $p < 0.05$.

Results. The data obtained shows overweight in treatment group, also high concentration of triglycerides and glucose serum, but cholesterol levels unchanged between. Transcriptome analysis showed 226 deregulated transcripts of which 98 are under-expressed transcripts overexpressed and 128 with a cutoff of fold change (Log fold change) of 1.5 were found. Differentially expressed transcripts belong to genes encoding proteins of different types: ion transporters, adhesion molecules and receptors.

Conclusion. Early postnatal overnutrition generates insulin resistance and dyslipidemia. We show for the first time the effects of early postnatal overnutrition in the hypothalamus transcriptome to 21 days. Some of differentially expressed transcripts controls neuronal circuits involving the hypothalamus control circadian rhythms (Per, Timeless, Bmal, ITGB4, Cdc6, COL3A1, etc.) and endocrine regulation, this is an evidence of metabolic disruption in hypothalamus.

A dietary portfolio modulates SIRT1 expression in astrocytes and reduces brain inflammation while improving working memory in a transgenic mice model of Alzheimer disease.

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Alzheimer's disease (AD) is the most common form of dementia that involves neurodegenerative processes affecting synaptic function and memory formation. Recent investigations have described brain metabolic alteration in early stages of AD, such as a hypometabolism of glucose, brain insulin resistance and reduced mitochondrial bioenergetics. Astrocytes function as key components on brain metabolism and can protect against oxidative stress; however, in AD there is an over activation of astrocytes that leads to inflammatory reactions and neuronal damage. Sirtuin 1 (SIRT1) has emerged as a key metabolic sensor in various cellular processes. SIRT1 mediates mitochondrial biogenesis, while suppresses the expression of pro-inflammatory cytokines by astrocytes. Functional foods contain bioactive components that can modulate expression and function of proteins and enzymes related to energy metabolism. A dietary portfolio (PD) containing dried nopal, chía seed, and soy was able to restore metabolic disturbances in obese subjects (Guevara-cruz et al., 2012), while nopal reduced neuroinflammation, APP levels, oxidative stress, and increased synaptic contacts in obese rats (Leonhardt et al., 2014). Moreover, food may have epigenetic effects and may accelerate or delay the progression of disease (Martin et al., 2013, Martin et al., 2014). Therefore, we aim to evaluate in 3xTgAD mice (AD transgenic animal model) whether ingestion of a PD over two generations may increase SIRT1 expression in astrocytes and principal neurons. We hypothesize that 3xTgAD mice fed with a PD will present higher levels of SIRT1 in astrocytes, reduced neuroinflammation, and improved cognitive abilities compared to transgenic mice fed with control chow diet.

3xTgAD and age matched wild type female mice were fed with either control diet (AIN-93) or PD over two subsequent generations. Cognitive performance was asses at 9 months-old by the T-maze and water maze before sacrifice. Whole cell and synaptic extracts were used to asses SIRT1, PGC-1, PPAR γ, BDNF, Irisin, ARC and PSD-95 by Western Blot. Double immunofluorescence was used to confirm the presence of SIRT on GFAP possitive cells (astrocytes) and dendritic processes were analyzed under laser confocal microscopy (Leica SP-8).

Our results showed that 9 months old 3XTgAD female mice fed with PD had an improved spacial and working memory compared to AIN-93 fed 3xTgAD mice. This was accompained by decrease inflamation in hippocampus and altered regional distribution of SIRT1 in astrocytic processes. In addition, synaptic proteins were enhanced in mice 3xTgAD mice fed with PD.

Guevara-cruz et al. (2012). J Nutr. *142*, 64–69; Leonhardt Avalos et al., (2014) 44th Society of Neuroscience Meeting program # 144.15/V27 Martin et al. (2014). Plos one. 9(6): Martin et al. (2013) Curr Med Chem. 20(32): 4050–4059.

A dietary portfolio restores SIRT1 levels and reverses dendritic spine loss in prefrontal cortex´s neurons of obese rats

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Mild-life obesity has been associated with alterations in gene expression, decreased metabolic expenditure and learning and memory deficits. Sirtuin 1 (SIRT1) is a NAD- dependent class III deacetylase that function as cellular energy sensor. Recent investigations shows the permissive role of SIRT1 in memory consolidation and dendritic out-growth. Diet-induce obesity (DIO) reduces SIRT1 expression in brain and liver, while SIRT1 activation reduces body weight and increases life-span. Moreover, resveratrol treatment recovers memory deficits in obese mice. Functional food also enhances the expression of enzymes and proteins related to energetic metabolism.

The objective of study was to evaluate the effects of a dietary portfolio (PD) containing nopal, chia seed, soy and curcumin in dendritic spine density and SIRT1 levels in prefrontal cortex of obese rats.

Male Wistar rats weighing 250 ± 20 g were divided in two groups during 4 months: Control group (standard diet AIN-93, n=6) and High-fat-diet group (HFD plus 5% sucrose in water, n=6). Once animals in HFD-S had reached metabolic alterations (i.e. overweight, high plasma levels of triglycerides, cholesterol, LDL and glucose intolerance) they were asses for behavioral performance (T-maze, novel object recognition and open field) before a PD was added into the diet (5% dehydrated cactus, 20% soybean oil, 3% chia and 1% turmeric) during 2 months to half of the animals in each group. Cognitive assessment was done before sacrifice at 9.5months of age. Immunofluorescence for SIRT1 and diolistic labelling for spine quantification was performed in the right prefrontal cortex.

Our results shows that obesity decreased the number of spines in prefrontal cortex, accompanied by a decrease in SIRT1 protein. Addition of PD into the diet was able to recover the levels of SIRT1 and modify the morphology of spines in same region.

Thus, brain alterations induced by high-fat-diet affecting prefrontal cortex and behavior can be abated by ingestion of a PD.

A High Calorie Diet Causes Memory Loss, Metabolic Syndrome And Oxidative Stress Into Hippocampus And Temporal Cortex Of Rats

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A High Calorie Intake Can Induce The Appearance Of The Metabolic Syndrome (Ms), Which Is A Serious Public Health Problem Because It Affects Glucose Levels And Triglycerides In The Blood. Recently, It Has Been Suggested That Ms Can Cause Complications In The Brain, Since Chronic Hyperglycemia And Insulin Resistance Are Risk Factors For Triggering Neuronal Death By Inducing A State Of Oxidative Stress And Inflammatory Response That Affect Cognitive Processes. This Process, However, Is Not Clear. In This Study, We Evaluated The Effect Of The Consumption Of A High-Calorie Diet (HCD) On Both Neurodegeneration And Spatial Memory Impairment In Rats. Our Results Demonstrated That HCD (90 Day Consumption) Induces An Alteration Of The Main Energy Metabolism Markers, Indicating The Development Of Ms In Rats. Moreover, An Impairment Of Spatial Memory Was Observed. Subsequently, The Brains Of These Animals Showed Activation Of An Inflammatory Response (Increase In Reactive Astrocytes And Interleukin1-B As Well As Tumor Necrosis Factor-A) And Oxidative Stress (Reactive Oxygen Species And Lipid Peroxidation), Causing A Reduction In The Number Of Neurons In The Temporal Cortex And Hippocampus. Altogether, These Results Suggest That A HCD Promotes The Development Of Ms And Contributes To The Development Of A Neurodegenerative Process And Cognitive Failure. In This Regard, It Is Important To Understand The Relationship Between Ms And Neuronal Damage In Order To Prevent The Onset Of Neurodegenerative Disorders. Funding For This Study Was Provided By Grants From VIEP-BUAP Grant Difa-Nat15-I And From The PRODEP DSA/103.5/15/7449; BUAP-PTC-395.

Area of study: Metabolism

Exhibition in poster

B_2 and B_6 Vitamins Restore Dopamine Levels in Rat Brain Regions Altered by 3-Nitropropionic Acid.

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Abstract

3-Nitropropionic Acid (3-NPA), a complex II inhibitor of the electron transport chain, causes Huntington disease-like symptoms after administration into animals. However, primary mechanisms of cell death and free radicals are not clearly understood. This study tested the hypothesis that riboflavin (B_2) and pyridoxine (B_6) vitamins leads to the protection of brain against free radicals as consequence of 3-NPA single administration, measuring the levels of dopamine and select oxidative stress markers.

Methods. Male Fisher rats (weight 250g) received the next treatments as follow: group A, control (NaCl 0.9%); group B, 3-NPA (20mg/kg); group C, B_2 (10mg); group D, B_2 (10mg) + 3-NPA (20mg/kg); group E, B_6 (10mg); group F, B_6 (10mg) + 3-NPA (20mg/kg). all administered intraperitoneally daily for 3 days and 3-NPA in single doses, in the noticed doses. At the moment of sacrifice the brain was obtained to measure dopamine, reduced glutathione (GSH), lipid peroxidation, Ca^{2+}, Mg^{2+} ATPase and H_2O_2 concentrations through spectrophotometry and fluorescence standardized methods.

Results. Dopamine levels increased significantly ($p<0.05$), in cortex, hemispheres and cerebellum/medulla oblongata regions of animals that received 3-NPA alone. GSH decreased significantly ($p < 0.05$) in cortex regions of animals that received 3-NPA alone or combined with B_2 or B_6 vitamins, and this biomarker increased significantly ($p<0.05$) in cerebellum/medulla oblongata regions in the same groups. Lipoperoxidation levels increased significantly ($p<0.05$), in cortex, hemispheres and cerebellum/medulla oblongata regions of animals treated with B_2 vitamin alone. ATPase dependent of Ca^{+2}, Mg^{+2} and H_2O_2 increased significantly ($p<0.05$), in all regions of animals that received 3-NPA alone.

Conclusion. The results suggest that B_2 or B_6 vitamins restored dopamine levels in rat brain, and showed the capacity of 3-NPA to generate free radicals.

Does the dorsomedial nucleus-lateral hypothalamus thyrotropin-releasing hormone projection sense energy balance alterations?

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Key words: thyrotrophin-releasing hormone; dorsomedial nucleus, lateral hypothalamus; feeding

In mammals, multiple brain circuits contribute to the maintenance of energy balance (Schneeberger et al. 2014). Central administration of thyrotropin-releasing hormone (TRH) or TRH agonists reduces food intake in normal rodents and hungry rats. These effects may involve hypothalamic targets, since local injection of TRH into medial and lateral hypothalamus (LH) reduces feeding behaviour in rats (Suzuki et al. 1982). Apart from the hypophysiotropic TRH neurons of the paraventricular nucleus of the hypothalamus, various additional groups of TRH neurons are present in the hypothalamus, and TRH receptors are expressed in multiple hypothalamic nuclei. However, the circuits in which these TRH neurons are involved are poorly understood. The dorsomedial nucleus of the hypothalamus (DMH) has an important role in energy homeostasis (Schneeberger et al. 2014). In this region, a significant population of TRH neurons receives afferents from the subparaventricular zone, an output region of the suprachiasmatic nucleus (Hokfelt et al. 1989, Horjales-Araujo et al. 2014). The DMH sends glutamate-TRH projections to the LH area (Chou et al. 2003), which expresses both TRH receptors, predominantly TRH-R1 (Heuer et al. 2000). TRH exerts an indirect inhibition of the firing rate of melanin-concentrating hormone (MCH) neurons of LH through the activation of GABA neurons (Hara et al. 2009). This result is consistent with the detection of TRH axons terminating on or near LH GABA neurons (Zhang & van den Pol 2012). Since MCH neurons send orexigenic projections, the TRHergic DMH-LH projection may transmit anorexic signals through the GABAergic neurons. The purpose of this project is to determine whether the TRHergic DMH-LH projection senses energy balance changes. We used RT-PCR, immunocytochemistry and *in situ* hybridization to analyze the functional state of the projection. In male rats, fasting enhanced TRH-R1 expression in the LH. In female rats fasting decreased TRH expression in the DMH. Experiments are in progress to analyze in adult mice expressing GFP in TRH neurons the response of the projection to fasting and refeeding Thus, preliminary data suggest that the TRHergic DMH-LH projection senses energy balance.

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Hipoglycemic coma induces overexpression of endoplasmic reticulum stress markers and caspase activation: B-hydroxybutyrate as an alternative energy source.

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The brain consumes 20% of the total body energy and therefore it requires a continuous supply of glucose. Glucose is the main energy source in the brain and when blood levels drop below 54 mg/dl, some symptoms arise such as irritability, dizziness, blurred vision, confusion and tachycardia. If this condition is not corrected and blood glucose decays to less than 20 mg/dl, the hypoglycemic coma might occur leading to neuronal death. It has been observed that under conditions of severe fasting and some neuronal diseases such as Parkinson and Alzheimer, which show a decrease in glucose metabolism, other energy substrates, such as the ketone body, D-B-hydroxybutyrate (BHB) can be used as energy source, as it is converted to acetyl coenzyme A (acetyl-CoA). In previous studies we have observed that rats subjected to severe hypoglycemia and treated with D-BHB, show decreased cell death in the cerebral cortex and cortical cultures exposed to glucose deprivation in the presence of D-BHB, preserve ATP levels and show increased survival, suggesting that D-BHB can be used as an alternative energy source in these conditions (Julio-Amilpas et al. 2015. J.Cereb. Blood Flow Metab 35:851-60). One of the cellular processes than demands more energy, is protein synthesis and the organelle sensing this process is the endoplasmic reticulum (ER). The decrease in ATP levels cause protein misfolding, and their accumulation in the ER induces cellular stress. This stress triggers the unfolded protein response (UPR). The UPR acts through the activation of three sensors able of detect the accumulation of misfolded proteins. One of these sensors is the PERK (Protein Kinase RNA-like Endoplasmic Reticulum Kinase) pathway. This kinase is inhibited by its interaction with the chaperone GRP78; under ER stress, GRP78 dissociates from PERK and triggers its dimerization and activation. PERK phosphorylates eIF2a (Eukaryotic Translation Initiator Factor 2), which inhibits global protein synthesis, alleviating ER stress. However, if ER stress persists, eIF2a promotes the translation of the mRNA of the transcription factor ATF4, which up-regulates chop gene expression. CHOP protein promotes the mitochondrial apoptosis pathway by the down regulation of Bcl-2. We show that rats subjected to the hypoglycemic coma, show an increase in the protein content of GRP78, ATF4 and CHOP, as well as an increase in eIF2a phosphorylation and in the proteolytic activation of caspase-12. These effects are attenuated when rats are treated with BHB. Results suggest that the hypoglycemic coma induces ER stress, which is possibly involved in apoptotic neuronal death and that ER stress is abated by D-BHB treatment.

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Involvement of energy metabolism and Sirtuin 1 inhibition in the expression of Alzheimer's disease markers

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Metabolic alterations associated with the consumption of high fat diets (HFD) are considered as risk factors for neurodegenerative diseases, including Alzheimer's disease (AD). Some of the deleterious effects of HFD depend on the high content of saturated fat acids such as palmitic acid (PA). It is presumed that PA might be altering the cellular metabolism by modulating the NAD+/NADH ratio. A group of Histone Desacetylaces (Class III – Sirtuins) might be modulated by cellular metabolism due to the utilization of NAD+ as a cofactor. Sirtuin 1 (Sirt1) is responsible for the deacetylation of several cytoplasmic proteins, transcription factors and residues in histone tails, leading to activation or repression of genes. Nevertheless, the mechanisms that relate the intake of HFD, the alteration of cellular metabolism and the expression of cellular markers of AD remains to be elucidated. Thus, we have analyzed the effects of neuronal exposure to PA on Sirt1 function and its correlation with changes in tau and NF-κB acetylation and with the expression of the limiting enzymes of the amyloidogenic APP processing, Bace1 and Adam10. Primary hippocampal neuronal cultures were exposed to different concentrations of PA or EX527 (Sirt1 inhibitor), followed by protein and RNA extraction for Western Blot and RT-qPCR analyses. Quantification of NAD+/NADH ratio was measured with a colorimetric kit. Our results show that neuronal exposure to PA at non-neurotoxic doses diminished the NAD+/NADH ratio. Furthermore, several cytoplasmic targets of Sirt1, tau and NF-κB increased their acetylated residues, while total protein content of Adam10 decreased and Bace1 increased after the treatment with PA or EX527. At present our results suggest that an alteration in cellular energy metabolism might be implicated in reduced Sirt1 function which in turn may have an impact in the expression of biochemical markers of AD such as Aβ, hyperacetylation of Tau and activation of NF-κB.

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Telomerase activity and cell cycle length are associated to the transition from neurogenesis to gliogenesis in the mouse embryonic spinal cord

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Neural stem cells (NSC) shift their differentiation potential over time during development of the mammalian central nervous system. At early stages, embryonic NSC in the neuroepithelium divide symmetrically to self-renew and then divide asymmetrically to generate a differentiated progeny; neurons are generated first, then glial cells emerge. The timing mechanisms that regulate the transition from one phase to the next one depend on extracellular signals as well as on an interplay between neurogenic and gliogenic transcription factors. Nevertheless, the mechanisms that restrict the ability of older NSC to generate neurons after the gliogenic phase is established remain unclear. Recently, it has been shown that signaling cascades that regulate replicative senescence contribute to embryonic development without any pathological implication. This opens the possibility that mechanisms regulating the duration of the cell cycle also could be involved in restricting stem cells differentiation potential. Therefore, we addressed whether the onset of gliogenesis and the loss of the neurogenic potential are associated with the lengthening of the NSC cycle and the activity of telomerase enzyme. Accordingly, we show that telomerase expression and activity are down regulated during transition from neurogenesis to gliogenesis in the mouse embryo spinal cord. We also observe that NSC displaying a predominant neurogenic differentiation potential divide faster than the gliogenic ones. As expected, these changes are consistent with an accumulation of some cell cycle inhibitors. Therefore, our data suggest that the loss of neurogenic potential in the murine spinal cord could result from a process of replicative senescence.

Glycine receptors expression during rat retina development

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Abstract

Background. Glycine receptors (GlyR) have a pentameric structure, composed of two α (1-4) and three β subunits, which form the chloride channel gated by glycine. The physiological role of these subunits is unknown. In the spinal cord, the α2 is expressed in immature stages and it switches to α1 expression in adult neurons, where it establishes most of the synaptic GlyR. In the adult retina, all the α subunits are present. **Objective.** Therefore, we analyzed the expression of α1 and α2 during the postnatal development of the rat retina. **Methods.** The retina of Long Evans rats were processed for western blot proteins expression, using specific commercial antibodies, following standard procedures. In addition, mRNA expression was assessed by means of qPCR. **Results.** qPCR studies showed that the α2 isoform of GlyR exhibited its highest levels at P0 (newborn), decrease around two-fold at 7P, 15P, and adult rat retina. Meanwhile, the mRNA for the α1 GlyR subunit increased progressively along postnatal development, reaching expression values ten-fold higher in the adult retina, compared to P0-P15. However, the α2 protein expression was not significantly modified at any development period studied during development. **Conclusions.** The α2 subunit of GlyR showed relative high expression levels in the immature and in the mature retina. Although the expression of α1 subunit increased during development, it did not appear to be associated with the decrease of the α2 subunit expression, as in spinal cord. These results, in addition to support a role of α2 in development, suggest a particular function of α2 GlyRs in the adult retina.

Área: Desarrollo

CYTOMEGALOVIRUS INFECTION AFFECTS THE DIFFERENTIATION OF NEURAL STEM CELLS

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Human cytomegalovirus (CMVh) is the most important infectious agent cause of birth defects affecting the central nervous system (CNS) as a consequence of intrauterine infection. The CMVh is a specific virus species but presents a great similarity with the murine (CMVm), because of the similarity of the genome structure and biological characteristic between CMVh and CMVm, many studies of congenital infection have been performed in the mouse model. Using this model, in immunostained brain slice cultures, the virus-susceptible cells were found in the subventricular zone and cortical marginal regions, the site where neural stem cells are located. In our laboratory we have the human neural stem cell line (hNS1) is a clonal line, its origin is of human fetal tissue 10.5 weeks gestation which the region of the diencephalon and telencephalon was dissected and were later immortalized conditionally and in the absence of growth factors (EGF and FGF) they differ giving rise to astrocytes, oligodendrocytes and neurons.Taking this into account, hNS1 can be a good model to explore the effects of CMVh infection in the CNS. We studied apoptosis caused by CMVh infection in differentiated cells hNS1 and evaluate specific markers of neural differentiation. As for the assessment of cell viability it was found that (17.8% ± 9.54%, n = 4) infected cells differentiated and have a higher incidence of apoptosis that differentiated and uninfected cells (0.3% ± 2.2%, n = 4), including the incidence of apoptosis in infected cells differentiated and, is higher compared to cells irradiated by UV (12.8% ± 7.1%, n = 4), which they were used as control of apoptosis. By analyzing the differentiation of cells using neuronal markers (MAP-2) and glial (GFAP), was observed in infected cells is a decrease in the percentage of astrocytes compared to uninfected crops. So far it has been found that CMVh infection produces death in glial cells

whereas the number of neurons remains constant, it is therefore necessary to explore in future experiments physiological effects produced by the presence of virus in neuronal function in infected cells.

Transcriptome comparative analysis of mouse and chick telencephalon during embryonic development

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Morphological differences between the brains of the members of the different classes of vertebrates are the result of differential regulation of genetic networks, that regulation occurs at the level of transcription and translation at the posttranscriptional and posttranslational level. Has been described that transcription factors as Pax6 lead early events regionalization telencephalon, changes in the expression or function of genetic networks involving participation signaling proteins such as reelin are responsible for morphological differences in the brain of the various classes of vertebrates. Here we analyze the early development of the forebrain of mice and chickens. Morphological divergence between the telencephalon of mammals and birds. We sequenced the transcriptome telencephalon embryonic stages using two equivalents HiSeq Illumina platform by the method of paired readings. Using bioinformatic analysis of RNA-seq, two sets of samples mapped against the reference genome mouse and chicken, we determine the abundance of each transcript differentially expressed in the mouse. We identified 84 transcripts increased in both species, we distinguish genes related to processes regulating the development of the cerebral cortex, differentiation forebrain neurons, pallium development and development of the olfactory bulb like Cdk5r2, Csr, Dlx1, Lhx6, Lhx8, Neurod1, Neurod1, Uncx, Eomes and Tbr1. Later we compare the transcripts increased only in mice (400) against increased only in chicken (177) and we determine that in mice Cdk5r1, CDH5 and chicken Fat4, Gal, grin1, Msx2 regulate differentially process relate to maturation of anatomical structures and in the same manner we identify gene networks in mouse involving Bcl11b, Bhlhe, Cdk5r1, CX3CR1, Dlx2, Gsx2, NRP1, Slit1, Trp73 while chicken Fat4 involved, Grin1, Neurod6, NRP2, Plxna4, Reln, Robo2 and Sema3A of telencephalon developmental regulation during this stage of early development.

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ON THE PRESENCE OF SYNAPTIC PROTEINS IN MELANOPSIN CONTAINING GANGLION CELLS OF RAT RETINA.

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The retina of vertebrates is one of the best characterized phototransducer tissues among animals. Their classic photoreceptors rods and cones, transduce light into a nerve impulse conveyed by retinal ganglion cells to the encephalon. A subset of retinal ganglion cells expresses the photopigment melanopsin which allows them to function also as photoreceptors. These so-called intrinsically photosensitive retinal ganglion cells (ipRGCs) display a very wide spectroscopic range, do not habituate and thus work as luminance detectors. The ipRGCs and classic photoreceptors work together conveying the light stimuli information into central nervous system, including that related to biorythms. Nonetheless, only the ipRGCs form the retinohypothalamic tract projecting directly to the suprachiasmatic nuclei. Hence the ipRGCs function as the sensory/projecting neurons in charge of the photoentrainment of the circadian clock and other actions which do not lead to image formation and are collectively known as non-visual functions of the retina. Because of this the ipRGCs have become a main character in sensory physiology.

In this work we aimed to characterize the presence in ipRGCs of three proteins known by their location to regular ganglion cells: medium chain neurofilaments (NFm), the cytomatrix protein Bassoon (bass) and the cell surface protein Thy1. Retinae isolated from rat at different ages were used both in vertical sections and whole mount preparations for applying the immunofluorescence detection method. Tissue was analysed by epifluorescence microscopy, ipRGCs were identified by melanopsin detection and colocation with proteins was accounted.

Melanopsin was immunodected at the earliest ages (P2) compared to NFm, bass and Thy1. The amount of positive cells was increasing with age for all the proteins assayed. The location of NFm and Thy1 did not match that of melanopsin, accounting less than 10% of co-labelling at all ages assayed, whereas most of the axons reaching the optic nerve were either NFm- or Thy1-positive. The location of bass was detected as punctae distributed all over the synaptic layer of interneurons and ganglion cells (inner plexiform layer, IPL), we detected most of these punctae (70%) occurring at the axons or dendrites of the ipRGCS compared to the location at cell soma. Most of the ipRGCs co-labeled with bass were of the M1 subtype (80%), even though we could detect ipRGCs of the M2 and M3 subtypes. These results add to the evidences that ipRGCs show features distinctive to regular ganglion cells aside the presence of melanopsin and thus make worth analyzing the set of neurotransmitters, receptors and synaptic proteins expressed by these cells.

Comparison of synaptic proteins distribution within the retinae of Wild animals

Óscar Iván Sánchez-Estrada, Diana Martínez-López, Jorge Alberto Pérez-León

The first steps of vision occur within the retina. In vertebrates this tissue shows a highly ordered organization in cell and synaptic layers, with the same pattern in all classes of the Phylum. There are several proteins and molecules used as retinal cell markers, the location of these by means of histological methods has allowed to infere the functional organization of retinal circuits overmost in typical lab animals as fishes, amphibians, rodents, and domestic cat. The output of this kind of studies led to interesting conclusions in species from which is only possible to analyse *postmortem* tissue, as primates including humans. On the other hand, the analysis of wild animals has also provided insight into particular organization divergent from lab animals. The present study was developed to evaluate the organization of the retina from wild animals: the rodents *Ammospermophilus interpres, Peromyscus spp.,* and *Chaetodipus spp.,* and the bat *Anthrozous pallidus* (Chiroptera). Specimens were collected for ecodistribution studies and prepared for taxidermia, eyeballs were dissected and the retinae prepared for cryosectioning. Vertical sections were used for immunohistofluorescence with antibodies detecting proteins involved in the synapses of the retina. The sections were analysed with epifluorescence microscopy and compared with rat retina.

We observed that the labelling was obtained in a very similar way among the different specimens and in comparison to rat, however, some remarkable differences were noted: the density and location of parvalbumin positive cells were higher and widespread in wild life rodents compared to rat; calretinin was located to horizontal cells compared to the labelling of amacrine cells in rat; PKC immunoreactivity, mainly located to rod bipolar cells in rat and mouse, was extended to amacrine cells in our samples. Another striking differences were the absence of mGluR6 from bat retina and the immunoreactivity of recoverin restricted to photoreceptor somata in wild life rodents

Even when the studies realized on the retina along the years have determined that the structural organization is conserved among vertebrate species, this kind of work shows that some proteins location may vary according to the species and thus point to physiologic traits worth of analyzing.

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K_V and K_{IR} channels identified at presynaptic terminals of the corticostriatal synapses in rat

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In the last few years, it has been increasingly clear that the activity of voltage-dependent (K_V) as well as the inward rectifier (K_{IR}) potassium channels influence neurotransmitter release. The subcellular localization and composition of K^+ channels is crucial to understand the extent of its function and its influence in controlling neurotransmitter release. In this study we investigated the role of K_V and K_{IR} channels in presynaptic modulation of corticostriatal synapses. Extracellular field recording of population-spike and pharmacological blockade with specific and non-specific blockers were used to identify several families of K_V channels. Exposure to non-specific K^+-channel blockers Ba^{2+}, Cs^+, and tetraethylammonium (TEA) increased the amplitude of the orthodromic population-spike, decreasing, therefore, the value of paired pulse ratio (PPR). Exposure to A-type current blocker 4-aminopyridine (4-AP) also reduced PPR in a extent similar to that observed with TEA. In both cases the effects were dose dependent. We tested several K^+-channel blockers specific for families K_V1, K_V3 and K_V4. We observed decrement in PPR with several K_V1- and K_V3-channel blockers, but not with K_V4-blockers phryxotoxin and heteropodatoxin. These results suggest that type A-current is most probably produced by $K_V1.4$ or K_V3 channels, but not the K_V4 channels. Heterologous combinations of K_V1 family channels K_V 1.1, K_V 1.3, K_V 1.4 and K_V 1.6 isoforms can be expected to be at presynaptic action sites since blockage with margatoxin, tytiustoxin, hongotoxin, agitoxin and dendrotoxin toxins, all render a reduction in PPR. Further pharmacological characterization with tertiapin-Q, a specific $K_{IR}3$ channel family blocker; also induce a reduction of PPR, suggesting that $K_{IR}3$ channels are present at corticostriatal terminals. In contrast, exposure to Lq2, a specific K_{IR} 1.1 blocker, did not induce any change in PPR, indicating the absence of these channels in the presynaptic corticostriatal terminals.

Neurotransmitter release from vesicles last only few milliseconds, due in part, to the balance between the activation of voltage-gated Ca^{2+} and K^+ currents. Up-regulation of K_V-channels activity presumable will lead to a stronger and faster repolarization, reducing the time window for calcium influx trough voltage-dependent Ca^{2+} channels, with the consequent reduction in the neurotransmitter release. Altogether our data indicates the presence of corticostriatal presynaptic K_V channels with a complex stoichiometry. Such complexity may be relevant to the mechanisms of modulation of neurotransmitter release, since the variety of tetrameric composition of these channels widens the range for regulation of channels activity. On the other hand, the presence of $K_{IR}3$ channels points to additional mechanisms to modify synaptic strength. Since $K_{IR}3$-channels activity can be modified by G-protein gamma-beta subunits, indirect regulation can be expected in response to a vast

repertoire of G-protein coupled receptors. The variety of K^+channels at presynaptic sites provides additional pathways to fine-tuning synaptic activity.

ON THE PRESENCE OF NEUROTRANSMITTER MOLECULES IN THE *Eurythoe complanata*

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The Neurophylogeny has advanced in the field of Evolution in order to explain the origin of the nervous system making comparisons among distinct phyla of animals using various experimental approaches. In this project we are using the annelid worm *Eurythoe complanata* to detect neuroactive molecules shared with vertebrate organisms. Using the immunofluorescence technique we detected neurotransmitter molecules and neuroactive proteins throughout the body of this worm. The molecules PKC, glycine, glutamine synthase (GlnS), GABA and FMRFamide were detected by epifluorescence and the immunopositive cells morphology and location through the body axis were recorded. The "nearest neighbour" technique was perform to measure the density and average distance for cell somata distribution. We also searched in databases for the amino acid and nucleic acids sequences of trying to find homology among the proteins detected in our specimen and other metazoans. The results show the identification of different cell types positive for each of the molecules PKC, glycine, GlnS, GABA and FMRFamide; these cells display different densities and distances, supporting the notion of different cell types. GABA and FMRFamide immunopositive cells had very similar characteristics indicating a possible co-expression of two molecules in a single cell type. The description of these cell types would lead to the proposal of the existence of a neurotransmission system with resemblance to that of the vertebrates and

thus to feasible homologies explaining common mechanisms for the action of toxins of wide-spectrum.

Melatonin-induced dendritogenesis in hippocampal hilar neurons: study of the signaling pathway.

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Área: Transducción de señales

Melatonin, the main product of the pineal gland, modulates neurodevelopment in adult brain. This indoleamine induces both axonogenesis and dendritogenesis in hippocampal neurons (Liu et al, 2015; Domínguez-Alonso et al, 2012). In particular, dendrites' number as well as their length, thickness and complexity are stimulated by melatonin. These events are triggered by the binding of Ca^{2+}-calmodulin (CaM) to CaM kinase II (CaMKII), which induces its activation. Previously, melatonin was proven to induce CaM synthesis and translocation to different subcellular compartments in epithelial cells, so these mechanisms could account for stimulating CaMKII-mediated pathways. In this study we explored the participation of CaM, CaMKII and melatonin receptors in the mechanism by which melatonin stimulates dendritogenesis. Results indicated that CaM was increased in the soluble (cytoplasmic) fraction of hippocampal organotypic slices incubated with melatonin. Also, phospho-CaMKII (T286) and phospho-PKC (S660), the autophosphorylated active forms of these kinases, were significatively augmented after melatonin treatment. We also found increased phospho-ERK1/2, which may be activated downstream CaMKII in the signaling pathway. Furthermore, pharmacological inhibition of CaMKII and PKC as well as the antagonism of MT1/MT2 membrane receptors abolished melatonin-induced dendritogenesis, which confirms their involvement in this pathway. Our results indicate that melatonin stimulates dendrite formation in adult rat hippocampus through the binding to its specific membrane receptors, the translocation of CaM to the cytoplasmic compartment and the further activation of CaMKII. Dendritogenesis elicited by melatonin also involved PKC and ERK1/2 activation. Data strongly suggest that melatonin could repair the loss of hippocampal dendrites that occur in neuropsychiatric disorders by increasing the availability of CaM and the subsequent activation of CaMKII. Future studies will elucidate the specific protein targets of CaMKII, PKC and ERK1/2 in this signaling pathway.

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Role of Wnt signaling pathway on hippocampal reorganization after entorhinal cortex lesion

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Wnt signaling plays a fundamental role during early and postnatal development regulating cell differentiation, proliferation and plasticity. In recent years, it has been shown that components of canonical or non-canonical Wnt pathways are also expressed in adult brain. There is evidence that emphasizes the involvement of Wnt ligands, particularly Wnt5a and Wnt7a, in the modulation of neuronal circuit assembly at pre- and post-synaptic levels. However, it remains unknown if Wnt pathways are activated in response to synaptic loss and participate in the synaptic reorganization after damage. In this work, we have evaluated changes in the expression and function of Wnt agonists (Wnt5a, Wnt7a) and antagonists (Dkk1 and Sfrp1) in a model of hippocampal deafferentation. Male Wistar rats of 250-300 g were used throughout the study. Unilateral injection of kainic acid (2 µmoles) was applied to the right medial entorhinal cortex (EC) (A=27.64, L= 25.4 and V= 25.6). Animals were sacrificed by decapitation 6, 24, 72h and 7 days after lesion and the right hippocampus was dissected. Control animals received 1µL of 10 mM phosphate buffer. Total RNA and protein were obtained using TRizol. Expression analysis of *Wnt5a*, *Wnt7a*, *Sfrp1* and *Dkk1* were carried out by qRT-PCR. Protein levels of Wnt5a, Wnt7a, Sfrp1, Dkk1, CycD1, c-Myc and ABC were analyzed by Western Blot. We have found that *Wnt5a,* a Wnt non-canonical ligand, is upregulated in the hippocampus 6h after EC lesion and remains elevated until 72h. Interestingly, *Wnt7a,* which acts as a Wnt canonical ligand reached significant higher levels after 7 days of hippocampal deafferentation. Similar to *Wnt7a*, the antagonist *Sfrp1* increased after 7 days, while the antagonist, Dkk1 protein levels show a sustained elevation as early as 6h after the injury. Cyclin D1, a downstream component of the canonical Wnt signaling, increased at all times tested suggesting the activation of the Wnt canonical signaling cascade in the hippocampus in response to EC damage. We can conclude that the Wnt components we have analyzed show a differential expression pattern in time that may participate in different processes of synaptic reorganization through the

modulation of canonical and non-canonical Wnt signaling in response to hippocampal deafferentation.

This work was partially supported by CONACYT scholarship 393073.

Twin, a Component of the CCR4-NOT Complex, is involved in Thermal Nociception in *Drosophila*

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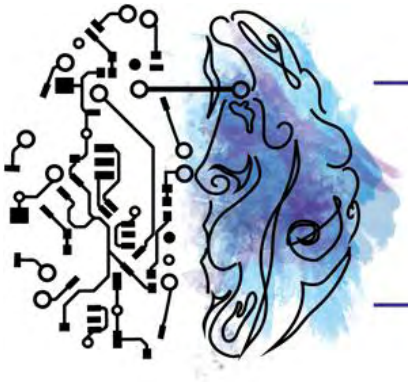
I Congreso de la Rama de Neurobiología

Área: Transducción de señales

Abstract:

The animal capacity for the detection of potentially damaging stimulus is called nociception. If the system is to be capable of integrating sensory information such as nociception and elicit a correct behavioral response, an extremely complex network of cellular and molecular machinery must work in exquisite coordination. *Drosophila melanogaster* has proven to be an excellent model for the identification and characterization of genes involved in sensory perception and nociception (Tracey, Wilson, Laurent, & Benzer, 2003) (Neely et al., 2010).

In this work we demonstrate that *twin* loss of function is sufficient to reduce thermal nociception and cause synaptic hypertrophy. This gene codes for a deadenylase and is part of the CCR4-NOT complex that is involved in translational control of gene expression (Morris, Hong, Lilly, & Lehmann, 2005). Furthermore, using an interactome analysis approach we propose that the Twin and the CCR4-NOT complex interact with transcripts of other genes also involved in nociception via the "AU rich elements" (AREs) most likely regulating their translational levels by deadenylation (Temme, Simonelig, & Wahle, 2014). Finally we propose an integrative model for *twin's* regulatory function in nociception.



Sociedad Mexicana
de Bioquímica
Neurobiología

Posters Session II
Tuesday April 5. 17:30 – 19:30

Drug Abuse
Electrophysiology
Stress
GLIA
Neuroendocrinology
Neuropharmacology
Plasticity and Cognition

Prenatal ethanol exposure during late gestation facilitates drug intake in offspring and selectively alters Met-enkephalin content in brain reward areas

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Ethanol-induced activation of the endogenous opioid system is involved in alcohol reinforcement and reward. Ethanol modifies opioidergic transmission at different levels in adult rats. Selective changes in opioid synthesis and release, as well as in ligand binding to opioid receptors have been reported in distinct brain areas in response to ethanol administration. However, the effects of ethanol exposure to low or moderate doses during early ontogeny have been barely explored. The aim of this work was to investigate the effect of prenatal ethanol exposure on alcohol intake and Met-enkephalin (Met-enk) content in rat offspring. Pregnant rats were exposed to ethanol (2 g/kg) or water during GDs 17-20. At PDs 14 and 15, preweanlings were evaluated in an intake test (5% and 10% ethanol, or water). Met-enk content in infants (PD15) was assessed by radioimmunoassay in the following brain areas: ventral tegmental area (VTA), nucleus accumbens (NAcc), prefrontal cortex (PFC), amygdala, substantia nigra (SN), caudate-putamen (CP), hypothalamus and hippocampus. Ethanol consumption in infants was facilitated by prenatal experience with the drug, particularly in females. Prenatal ethanol exposure increased Met-enk content in the PFC and NAcc, but decreased peptide concentration in the VTA. Prenatal ethanol treatment also increased peptide levels in the medial-posterior zone of the CP, and strongly augmented Met-enk content in the hippocampus and hypothalamus. These findings show that prenatal ethanol exposure stimulates consumption of the drug in infant rats, and induces selective changes in Met-enk levels in regions of the mesocorticolimbic and nigrostriatal systems, the hypothalamus and hippocampus. Our results support the role of mesocorticolimbic enkephalins in ethanol reinforcement in offspring, as has been reported in adults. This work was supported by the Consejo Nacional de Ciencia y Tecnología

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Association between promoter variants of the dopamine and serotonin transporter genes in undergraduate Mexican students with alcohol abuse or dependence

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Area: 5. Drogas de abuso/Drugs of abuse

Introduction

The identification of factors that are associated with alcohol abuse and dependence in different populations is of great significance. This may be the basis for designing new drug therapies that could contribute to meet the specific treatment needs of each patient and/or the basis for preventive measures.

Aim: To determine if there is an association between alcohol dependence/abuse/self-esteem and genetic variants of the dopamine and/or serotonin transporter.

Methods and results

We analyzed DNA samples of 212 undergraduate students of a public university in Mexico City that were assessed with the Composite international Diagnostic Interview (CIDI 3.0) and the Coopersmith Self-Esteem Inventory. We genotyped the -67 A/T polymorphism of the SLC6A3 and the 5-HTTLPR of the SLC6A4. We found a statistically significant association between diagnosis and the -67 A/T polymorphism (X^2=13.78, 4 df, p= 0.008). We also identified an association between self-esteem at home and the 5-HTTLPR polymorphism X^2=20.084, 8 df, p= 0.010).

Discussion and Conclusion

The SLC6A3 and SLC6A4 transporters are key regulators of the dopaminergic and serotonergic system respectively. The identified associations had not been evaluated in Mexican population and may help recognize important avenues of research.

Energy Drink Administration In Combination With Alcohol Cause An Inflammatory Response And Oxidative Stress In The Hippocampus And Temporal Cortex Of Rats

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Energy drinks (ED) are often consumed in combination with alcohol because they reduce the depressant effects of alcohol. However, different research suggest that chronic use of these psychoactive substances in combination with alcohol can trigger an oxidative and inflammatory response. These processes are regulated by both a reactive astrogliosis and an increase of pro-inflammatory cytokines such as IL-1β, TNF-α and iNOS, causing cell death (apoptosis) at the central and peripheral nervous system. Currently, mechanisms of toxicity caused by mixing alcohol and ED in the brain are not well known. In this study, we evaluated the effect of chronic alcohol consumption in combination with ED on inflammatory response and oxidative stress in the temporal cortex (TCx) and hippocampus (Hp) of adult rats (90 days old). Our results demonstrated that consuming a mixture of alcohol and ED for 60 days induced an increase in reactive gliosis, IL-1β, TNF-α, iNOS, reactive oxygen species, lipid peroxidation and nitric oxide, in the TCx and Hp. We also found immunoreactivity to caspase-3 and a decrease of synaptophysin in the same brain regions. The results suggested that chronic consumption of alcohol in combination with ED cause an inflammatory response

and oxidative stress, which induced cell death via apoptosis in the TCx and Hp of the adult rats. Funding For This Study Was Provided By Grants From VIEP-BUAP Grant Difa-Nat15-I And From The PRODEP DSA/103.5/15/7449; BUAP-PTC-395.

Area of study: Metabolism

Exhibition in poster

Ethanol induces selective effects on opioid peptide mRNA expression in the rat brain.

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The endogenous opioid system (enkephalins, endorphins and dynorphins) is associated with a variety of functions, including alcohol reward and reinforcement. Ethanol reinforcement and high alcohol drinking behaviour are partially mediated by the ethanol-induced activation of the endogenous opioid system. Ethanol and opiates share numerous pharmacological properties and exhibit similar behavioural effects in animals and humans. Low doses produce psychomotor activation and euphoria, whereas high doses reduce locomotor activity and induce sedation. Ethanol may alter opioidergic transmission at different levels, including the synthesis, processing, release and/or ligand binding to opioid receptors. The aim of this work was to investigate the effects of different doses of ethanol on Pro-enkephalin (Pro-enk) and Pro-opiomelanocortin (POMC) mRNA expression in distinct brain areas, particularly those associated with the reward circuit. Male Wistar rats were administered with saline or different doses of ethanol (0.5, 0.75, 1.0, 1.5, 2.0, 3.0 g/kg i.p.) and 30 min later brain regions were dissected: prefrontal cortex (PFC), nucleus accumbens (NAcc), amygdala, anterior-medial (amCP) and medial-posterior (mpCP) regions of the caudate-putamen (CP), hypothalamus and hippocampus. Pro-enk and POMC mRNA levels were quantitated by real time PCR. Pro-enk mRNA expression was increased by high ethanol doses in the amygdala, hypothalamus and hippocampus, but was decreased in the amCP. In other brain areas, ethanol induced biphasic effects on Pro-enk mRNA expression. In the NAcc and mpCP, low to moderate doses (0.5 - 2.0 g/kg) increased, but high doses (3.0 g/kg) decreased mRNA levels. In the PFC, the opposite effects were found. POMC mRNA levels in the PFC, NAcc, amygdala, CP and hippocampus were increased by low to high ethanol doses, whereas mRNA expression in the hypothalamus was significantly decreased. These results indicate that ethanol-induced changes in Pro-enk and POMC mRNA expression are dose-dependent and region-specific, and suggest a selective responsiveness of enkephalin- and endorphin-containing neurons to ethanol exposure. The observed effects on opioid biosynthesis could be involved in the locomotor and/or sedative actions of the drug. This work was supported by the Instituto de Ciencia y Tecnología del DF

(ICyTDF) (PICD 08-13) and the Consejo Nacional de Ciencia y Tecnología (CONACyT) (82728).

Nicotine effect in a *Drosophila melanogaster* model of Parkinson's disease induced by human α-Synuclein and Synphilin expression

Area: Drug Abuse
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Parkinson's disease (PD) is the second most common neurodegenerative disease only after Alzheimer's disease. There are genetic and environmental factors in the development of this disease being the aging the principal cause. One of the main characteristics in PD is the death of dopaminergic neurons in a brain region called *substantia nigra pars compacta*. PD continues to increase as one of the main health problems in terms of disability and mortality in worldwide (*de Lau, 2006*).

There is evidence that nicotine has neuroprotective properties and that the risk of PD in smokers is slightly lower than in general population. Nicotine is an antioxidant that could slow the effects of neuronal degradation and the loss of function of the dopaminergic neurons (*Quik, 2000*).

We evaluated the effect of nicotine in a PD model induced by two human proteins, α-Synuclein and Synphilin, which are expressed in *Drosophila melanogaster* dopaminergic neurons under control of UAS-GAL4 system. In a PD Drosophila model the expression of α-Synuclein or Synphilin induced a decrease lifespan associated with the progressive loss of dopaminergic neurons (*Hernández-Vargas, 2011*). Therefore survival curves were performed to determine if different nicotine concentrations suppress neurotoxicity. Additionally we evaluated the flies motor deficits performed climbing assays.

Interestingly on the one hand we have shown decrease lifespan and increase in a motor deficits in control flies but on the other hand we have shown increase in lifespan and decrease in motor deficits in the 2.4 μM nicotine concentration in α-Synuclein and Synphilin flies. We concluded that nicotine only causes a beneficious effect in parkinsonian flies being toxic for control flies.

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Gap junction inhibitor, carbenoxolone, alters glucose-induced electrical activity in mouse pancreatic beta cells

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ABSTRACT

Glucose induced insulin release depends critically on the electrical activity of pancreatic islet beta cells. In the mouse, the electrical activity consists of slow membrane potential oscillations (< 0.5 Hz) between hyperpolarized silent (-55 mV) and depolarized active phases (-45 mV); in the last one bursts of action potentials are generated by activation of voltage dependent Ca^{2+} and K^{+} currents. Moreover, since most islet beta cells are electrically coupled, this electrical activity occurs synchronously and in phase in all beta cells from a single islet. The duration of the active phases increases with higher glucose concentration. There are evidences that support that the burst pattern depends on gap junctions. To further explore this hypothesis, we tested carbenoxolone (CBX), a gap junction inhibitor, on the electrical activity of beta cells from microdissected pancreatic islets using intracellular membrane potential recordings during perfusion of CBX at concentrations previously reported to inhibit gap junction intercellular communication. We demonstrate that CBX increases -in a dose dependent manner- the active phase of electrical activity induced by glucose with activatory concentration 50% (AC=50) of 114.3 µM, and a total inhibition of the silent phase at concentrations above 300 µM. These results support the hypothesis that gap junctions may be involved in the generation of the burst pattern of electrical activity and in islet beta cell glucose sensing. Proyecto DGAPA IN IN224514

Recording of Native Ionic Currents from Primary Cultured Chromaffin Cells by Automated Patch-Clamp Electrophysiology.

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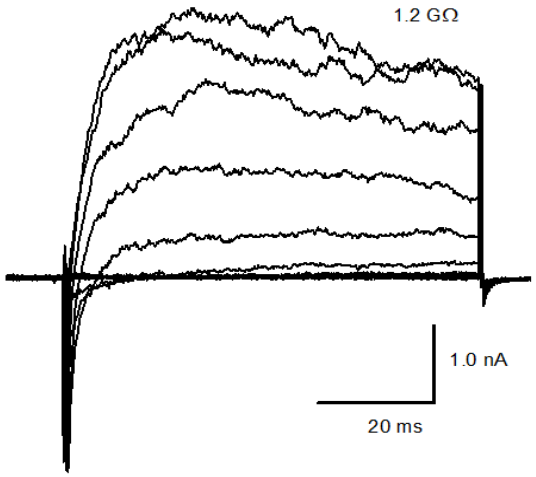
The patch-clamp technique is well established as the gold standard for the study of all kind of ion channels. The complexity of the analysis of ion channel activity, requiring highly specialized experimental operations and time-consuming methodologies, has historically determined the notoriously slow production of experimental data, particularly when the goal is to test a large number of chemical compounds trying to identify those exerting a pharmacologic effect with therapeutic purposes. The introduction of automated patch-clamp (APC) instrumentation has revolutionized the strategies to approach the discovery and development of new drugs targeting ion channel function. Of critical relevance for APC technology is to increase data production in at least one order of magnitude maintaining the high quality of ion current recordings under voltage clamp and Giga-seal (GΩ) conditions. Since its inception there has been a mounting interest in extending the success of APC technology to the study of ion channels endogenously expressed in native and primary cells. The objective of the present work is to optimize the recording of native ion currents from neuroendocrine chromaffin cells utilizing an APC recording system (QPatch 16X, Sophion-Biolin Scientific, Denmark).

The figure shows a representative example of whole-cell total membrane currents recorded by the QPatch 16X from disaggregated chromaffin cells obtained by enzymatic digestion of bovine adrenal glands, after 2-3 days culture (37 ˚C, 5% CO_2) and at room temperature. Symbols at the right upper corner indicate seal resistance while recording. At least two outward delayed currents (only one shown in the figure) and one inward early current were observed. Their characteristic kinetics, voltage dependence and pharmacology strongly suggest they correspond to K^+ and Na^+ permeable channels, respectively. In all aspects they were identical to the equivalent currents recorded by classic manual patch-clamp in chromaffin cells from the same preparation and on the same day.



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Mechanisms underlying the ischemic damage in area CA3 of the hippocampus

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There is consensus that the hippocampus is differentially affected by ischemia. Whereas area CA1 is highly vulnerable to its deleterious effects, area CA3 exhibits a degree of resistance. Nevertheless, the cellular mechanisms underlying ischemic damage on area CA3 are not well understood. Here, we explored the participation of several mechanisms that contribute to the recovery of synaptic responses and population spikes following a brief period (10 min) of oxygen glucose deprivation (OGD) in vitro. Simultaneously acquired mossy fiber-mediated excitatory postsynaptic potentials (MF fEPSPs) and antidromic-elicited population spikes (PS) were totally suppressed after a 10 min exposure to OGD without apparent recovery up to 90 min after the insult. In another series of experiments, the blockade of AMPARs/KARs and NMDARs receptors with kynurenic acid did not prevent the effects of OGD on the MF fEPSPs (12.8±1.5%) but was effective to revert the PS responses (69±2.4%). Then, slices were preincubated with BAPTA-AM. Under these conditions, PS but not fEPSPs exhibited a marked recovery. When the external Na^+ was substituted with NMDG, the responses exhibited a larger degree of recovery (MF-fEPSP 86±5%; PS-CA3 107±2%; n=8). Blockade of hemichannels with carbenoxolone did not prevent the OGD effects. Lastly, we investigated the contribution of TRPV's. Slices preincubated with capsazepine showed a strong recovery following the OGD insult (MF-fEPSP 66±1%; PS-CA3 94±1%; n=7).Our data suggest that ischemic damage involves Na^+ and Ca^{2+} influx but is independent of ionotropic glutamatergic receptors activity. These results also suggest that TRPV's can play a critical role in the ischemic damage on area CA3.

A type of dendrotoxin inhibits the ASIC current in dorsal root ganglion neurons from rat

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Dendrotoxins are a group of peptide toxins purified from the venom of several mamba snakes. α-dendrotoxin (α-DTx, from the Eastern green mamba *Dendroaspis angusticeps*) is a canonical blocker of voltage-gated K^+ channels and specifically of Kv1.1, Kv1.2 and Kv1.6. In this work we show that α-DTx inhibited the ASIC currents in DRG neurons (IC_{50} = 0.8 μM) when continuously perfused during 25 s (including a 5 s pulse to pH 6.1) but not when co-applied with the pH drop. α-DTx was found to reversibly inhibit the peak of ASIC currents from rat DRG neurons in a concentration-dependent manner without affecting the time course of desensitization nor the sustained component of the current. Additionally, we show that α-DTx abolished a transient component of the outward current that, in some experiments, appeared immediately after the end of the acid pulse. Dendrotoxins are basic proteins with a net positive charge at neutral pH; Arg and Lys residues conforming a cationic domain could, in principle, bind to anionic regions in the pore of potassium channels, in the case of ASICs is possible that α-DTx binds in specific amino acids in the lateral fenestrations in ASICs. Our data indicate that α-DTx inhibits ASICs and Kv with IC_{50} in the nM range. Furthermore, our data indicate that many results already in the literature should be re-evaluated taking into account this new finding. The α-DTx could be the basis for the development of more specific tools for the study of the ASIC which in the future could help to better

understand their function and provide molecular targets for drug design with probable clinical use skeletons.

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ÁREA: Electrofisiología

Characterization of electrophysiological alterations in the hippocampal CA3 region a model of prenatal infection.

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Area: Electrofisiología

Maternal infections during pregnancy have been identified as a risk factor for the development of neurological and psychiatric disorders in the progeny; a condition that is mimicked by prenatal exposure of rats to lipopolysaccharide (LPS). In this model, the resultant offspring will experience impaired learning and psychotic-like behaviors. Thus, we explored the changes in the synaptic strength of glutamatergic inputs converging on area CA3 of the hippocampus as well as the interneuron-mediated inhibition of animals exposed to prenatal infection (LPS administration in gestational days 15 and 16, 100 μg/Kg). A histological analysis revealed a structural disorganization of the dentate granule cell layer and pyramidal cell layer of the hippocampus of animals prenatally exposed to LPS. The strength of the glutamatergic inputs assessed by mean of input-output curves (I-O curves) indicates an increase in the excitability ratio of the perforant path to dentate granule cells (PP-DG) synapse, the PP-CA3 pyramidal cells (PP-CA3) synapse. The increased excitability was also found at the mossy fiber-CA3 synapse (MF-CA3). Paired-pulse protocols were used to unmask changes in the interneuron-mediated inhibition and short-term synaptic enhancement of the synapses converging on area CA3. Compared to control, the PP-DG, PP-CA3, and MF-CA3 synapses exhibited a decreased index of depression. Conversely, the three synaptic inputs exhibited an increased index of facilitation. Together, these electrophysiological alterations indicate an imbalance in the inhibition/excitation ratio of the CA3 network on animals prenatally exposed to an infection with LPS.

The synaptic communication between dentate gyrus and area CA3 take part in the transfer of the somatosensorial information arriving from the entorhinal cortex, a cortical region highly susceptible to damage in several psychiatric disorders including schizophrenia. Thus, the synaptic imbalance observed in this study, may represent the mechanistic basis underlying the cognitive impairment triggered by prenatal infections.

Dye coupling between granule cells and their target neurons as evidence of intercellular communication.

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Area: Plasticity and cognition

Gap junctions are present in the mossy fiber terminals of granule cells making synapses with the apical dendrites of pyramidal cells of CA3. They contribute to mixed chemical-electrical synaptic transmission. The mixed synapses have only rarely been described and has been found low incidence. Here, we used co-cultures of hippocampus cells and by immunocytochemistry we identify cells of the three neurons types of hippocampus with connexin36. The connexin enables the granule cells form gap junctions with their synaptic targets. Dye coupling has been accepted like evidence of electrical transmission between cells. To corroborate the connectivity by dye coupling, we conducted recording of granule cells and neurobiotin injection. This study demonstrated that the dye passes to other cells, suggesting the presence of gap junctions. We found that only in approximately 17.8% of coupling in control condition. We expose the cultures to trimethylamine (TMA) and ammonium chloride (NH_4Cl), these drugs has been reported to open gap junctions as a result of intracellular alkalinization. The cultures exposed to NH_4Cl exhibited similar coupling to control cultures (11.1%). No coupling was found in cultures with extracellular TMA. We added TMA to internal solution to compare the effect in the coupling. We found 75% of dye coupling in these conditions. Additionally the spontaneous activity revealed an increase of electrical synaptic events.

Functional role of low-voltage activated Ca^{2+} current (LVA) in vestibular afferent neurons in culture

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Resting discharge pattern of Vestibular-afferent neurons (VANs) may vary from regular to irregular, spanning a continuum of variation coefficients. The specific firing properties of each neuron are correlated with the soma size and the synaptic input, however, firing patterns of these neurons cannot be explained completely only by means of these characteristics. It has been speculated that intrinsic properties (variable expression and density of the ion channels) help to establish the firing pattern observed in VANs. Based on previous results it has been suggested that heterogeneous distribution of the LVA currents contribute to differentiate the discharge pattern of VANs.

Here, we study the functional role of LVA current on VANs-discharge. Using whole-cell patch-clamp recording, we observed a blocking effect of Nickel (300 µM) and Mibefradil (2 µM) -typical LVA blockers- , on LVA currents generated with a double pulse voltage clamp protocol. Additionally, in calcium imaging recordings using the intracellular dye Fluo 4-AM, Ni^{2+} and Mibefradil significantly reduced the calcium responses generated by depolarization induced by perfusion of high potassium solution (20 mM), 60% and 43%, respectively. Current-clamp recordings showed that Ni^{2+} decreased the amplitude and increased the duration of the action potentials (AP), and Mibefradil decreased the amplitude and threshold (T) of the AP. Moreover, the frequency of the repetitive firing generated by long depolarizing pulses (500 ms) decreased after applying both Ni^{2+} and Mibefradil. Sinusoidal current injection shown a group of cells where the AP number increased and another group of cells where the AP number decreased after the use of both mibefradil and Ni^{2+}. Our results shown that VANs possess a LVA current and that its heterogeneous expression in neurons contribute to explain some of the differences in the action potential discharge patterns observed in VANs, demonstrating that LVA calcium current has significant role in the sensory coding of vestibular afferent information.

Title: **Pallidoreticular pathway control the electrical activity of reticular thalamic nuclei.**

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Area: Electrophysiology

The reticular thalamic nucleus (RTn) coordinates the overall traffic information of thalamic nucleus to the cerebral cortex. RTn receives collateral axons from cortico-thalamic and thalamic-cortical fibers where it modulates the exchange of information between thalamus and the cerebral cortex. There are two spiking modalities of RTn neurons: electrical burst activity (synchronized mode) and electrical tonic activity (desynchronized mode). The electrical activity of RTn depends of their afferent fibers from other nuclei and the membrane properties of reticular neurons. In addition to cortical and thalamic projections, the RTn receives information from different brainstem nuclei and the globus pallidus (GP).

We study the spontaneous electrical activity of RTn neurons recorded in vivo in anesthetized rats and under the chemical activation or inhibition of the GP. To determine if the pallidoreticular pathway can modify the spontaneous RTn neurons discharge activity, we either activate it or block it by ipsilateral intrapallidal infusion of different concentrations of glutamate or GABA respectively.

We found that activation of pallidal neurons modify the spontaneous firing rate of RTn neurons. In a dose-dependent manner, glutamate decreases the spontaneous spiking rate of RTn neurons relative to basal values (30 PMol decreased the spiking rate by 35.13 ± 5.86%,n=11; 300 pMol by 45.82 ± 6.07%, n=10 and 3 nMol by 56.2 ± 6.81, n=13), without significance change in either the bursting index.
Additionally, intrapallidal GABA application increases RTn neurons spiking rate in eight neurons tested per concentration (30 pMol increased the spiking rate by 25.96 ± 6.08%, 300 pMol by 64.3 ± 19.57%, 3 nMol by 77.16 ± 19.89%). Burst index remained without changes.

Our results suggests that the GP exerts tight control over RTn activity.

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Serotonin modulates ionic currents in vestibular afferent neurons.

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Recent findings in vestibular afferent neurons have identified the expression of both metabotropic (5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F}) and ionotropic (5-HT_{3A} and 5-HT_{3B}) serotonin receptors. However, the physiological role of serotonin in the vestibular system is unclear. Recently, drugs such as serotonin re-uptake inhibitors have been implemented in the clinic for the treatment of vestibular disorders (e.g. vertigo), suggesting that serotonin may play an important role in vestibular activity.

Here, we study the functional role of serotonin on electrical activity in cultured vestibular afferent neurons of rat. Using whole-cell patch-clamp recording, we study the effect of serotonin (30 µM) on total currents generated with a protocol of square pulses from -120 mV to +40 mV, with 400 ms duration and steps of 10 mV; the holding voltage (V_h) was -60 mV. Application of serotonin (30 µM) significantly decreased the amplitude of the inward current (attributable to Na^+ current) by 53% measured at +20 mV and the steady-state outward current by 27% measured at +30 mV. Additionally, in calcium imaging recordings using the intracellular dye Fluo 4-AM, serotonin (100 µM) significantly reduced the calcium responses generated by depolarization induced by perfusion of high potassium solution (20 mM).

Our results indicate that metabotropic serotonin receptors are functionally expressed on vestibular afferent neurons and their activation modulate the inward currents of these neurons.

Design software acquisition and analysis techniques nonlinear dynamics signals obtained through a Holter monitor prototype

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Electrofisiology

Techniques nonlinear dynamics have been used successfully for analyzing electrocardiographic signals and cardiac series.

Considering this, the design of a Holter monitor is performed with a sampling frequency of at least 500 Hz the results conjoined traditional diagnostic techniques and the nonlinear dynamics. Software capable of obtaining signals stored in the memory of the monitor, generate tachograms, make separation for hours and separate series in the stages of sleep and wakefulness was developed.It has the ability to assess the main techniques of nonlinear dynamics that are used to analyze time series , such as multifractal analysis , detrended fluctuation analysis , calculation of the fractal dimension of Higuchi , etc. , and apply traditional analysis to assess heart rate variability . Based on the results of such analysis it was added a pre - diagnosis in which deviations from normal values are evaluated for the parameters calculated whether or not the nonlinear dynamics. In addition, the software has the ability to generate an electronic medical record for each patient.

Key words – Electrocardiography, nonlinear dynamics, beat cardiac, Holter monitor, multifractal, tachogram.

Voltage-activated calcium channels as functional markers of mature neurons in human olfactory neuroepithelial cells: implications for the study of neurodevelopment in neuropsychiatric disorders

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ABSTRACT

In adulthood, differentiation of precursor cells into neurons remains in several brain structures as well as in the olfactory neuroepithelium. Isolated precursors allow the study of the neurodevelopmental process *in vitro*. The aim of this work was to determine whether the expression of functional Voltage-Activated Ca^{2+} Channels (VACC) is dependent on the neurodevelopmental stage in neuronal cells obtained from the human olfactory epithelium. The expression of channel-forming proteins associated specifically to Olfactory Sensory Neurons (OSN) was analyzed by immunofluorescence, and evaluation of VACC functioning was performed by microfluorometry and patch-clamp technique. Alpha subunits of VACC were immunodetected only in OSN. These mature neurons responded to forskolin with a five-fold increase in free Ca^{2+} with regard to precursor cells. The involvement of VACC in the precursor's response was discarded for the absence of transmembrane inward Ca^{2+} movement evoked by step depolarizations. These data suggest differential expression of VACC in neuronal cells depending on their developmental stage and also that the expression of these channels might be acquired by OSN during maturation, to enable specialized functions such as ion movement triggered by membrane depolarization. Evidence supports that VACC in OSN might constitute a functional marker to study the neurodevelopment in neuropsychiatric disorders.

KEYWORDS

Calcium flux; differentiation process; human precursors; neuronal excitability; voltage-gated Ca^{2+} channels; neuropsychiatric disorders

Chronic levodopa treatment induces nitrosative stress, astrogliosis and spatial working memory impairment in rats with intra-nigral 6-OHDA lesion

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The Parkinson's disease (PD) is a disorder, which the patients present motor disturbances, as tremor, rigidity and bradykinesia, however, during the early stage there are deficits in both learning and memory task. PD is characterized by the death of dopaminergic neurons in the substantia *nigra pars compacta* (SNpc) with the consequent decrease of dopamine (DA) in the striatum and prefrontal cortex (PFC). The nigro-striatal-cortical pathway is affected in this pathology, with this neuronal circuit involved in cognitive processes such as spatial working memory (SWM). However, cognitive dysfunction appears even when the patients are receiving L-DOPA treatment used to improve motor skills. There is evidence that DA metabolism formed by L-DOPA generates free radicals such as nitric oxide (NO•), which may cause damage through the nitrosative stress (NS). Besides the neurodegeneration other changes occur during pathology as the inflammatory process characterized by activation of glia. However, currently the impact of the L-DOPA treatment on NS, SWM and astrogliosis in PD animal model is unknown. The aim of this study was to evaluate both the effects of chronic L-DOPA administration on SWM and the production of NS in rats using an intra-nigral lesion caused by 6-hydroxydopamine (6-OHDA). Post-lesion, the animals were administered orally with L-DOPA/Carbidopa (100 mg/kg) for 20 days. An SWM task in a Morris water maze was conducted post-treatment. Nitrite levels and immunoreactivity of 3-Nitrotyrosine (3-NT), Inducible Nitric Oxide Synthase (iNOS), Glial Fibrillary Acidic Protein (GFAP), and Tyrosine Hydroxylase (TH) were evaluated in the SNpc, the dorsal striatum and the PFC. Our results show that chronic L-DOPA administration in rats with intra-nigral 6-OHDA-lesion caused significant increases in SWM deficit, nitrite levels and the immunoreactivity of 3-NT, iNOS and GFAP in the nigro-striatal–cortical pathway. These facts suggest that as L-DOPA can induce NS and astrogliosis in rats with dopaminergic intra-nigral lesion, it could play a key role in the impairment of the SWM, and thus can be considered as a toxic mechanism that induces cognitive deficit in PD patients.
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Field: Estrés

Effects of Extremely Low Frequency Stimulation in the Oxidant System of Stressed Wistar Rats.

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Area. Stress

The World Health Organization has been interested in health effects of the extremely low frequency electromagnetic fields (ELF-EMF). This interest is due to the existence of domestic apparatuses in our daily life. There are several investigations referring this ELF-EMF as probably oncogenic. There are also beneficial effects as in articulation regeneration and recovery from depression. Oxidative stress is one of the most important mechanisms of action proposed of ELF-EMF effects in rat´s brain. Stress also causes oxidative stress in the rat´s brain. In previous work in our laboratory we have found that restraint stress causes changes in lipid peroxidation in rats that were stimulated with ELF-EMF during 2 hours. The effects induced by ELF-EMF stimulation depends on several parameters of stimulation: frequency, amplitude and duration of exposition.

The aim of the study was evaluate the effect of ELF-EMF stimulation on the brain's oxidant system of stressed rats versus unstressed.

Chronical unpredictable mild stress (CUMS) model was used. Fifty six male Wistar rats were divided in 2 groups: with or without ELF-EMF stimulation. These groups where subdivided in control (unstressed), and with CUMS during 21 days or 14 days. The ELF-MF groups were stimulated with a pair of Helmholtz coils in the last 7 days (60Hz, 2 hours/day). The cerebrum was immediately obtained and cerebellum, cortex and subcortical areas were separated. The lipid peroxidation in the brain was measuring with the Tiobarbituric Acid Reactive Species (TBARS) method. We also measured the antioxidant enzymes catalase and superoxide-dismutase. We found that neither ELF-EMF stimulation nor stress cause more lipid peroxidation than controls in cerebellum. In contrast they do cause more lipid peroxidation than controls in cortex. In subcortical tissue ELF-EMF significantly reduces lipid peroxidation caused by chronical variable stress. In conclusion the ELF-EMF stimulation induces changes in the oxidant balance differentially on the brain areas studied of stressed Wistar rats.

$CoCl_2$-induced chemical hypoxia provokes differential responses at transcript and protein levels as well as changes at subcellular localization of hemeoxygenase isoforms.

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The heme oxygenase system (HO) is integrated by two microsomal oxidoreductase enzymes (HO-1 and HO-2). Both isoenzymes transform free heme to biliverdin (BV), carbon monoxide (CO) and Fe^{2+}. They are considered cytoprotective enzymes against different cellular stress events since they are responsible of detoxification of free heme levels and the production of metabolites such as BV and CO with antioxidant, anti-inflammatory and anti-apoptotic actions. HO-1 is regulated by several transcription factors and increases significantly in response to different stimulus. HO-2 has been considered a constitutive isoenzyme and it plays an important role in different tissues. Its basal expression is higher than HO-1 and is one of the primary sources of defense against reactive oxygen and nitrogen species. Several studies have shown that both proteins have responses in both *in vivo* and *in vitro* models of hypoxia. Nowadays, there are not enough studies to completely understand the behavior of the HO-1 and HO-2 in hypoxia or in environments that mimic hypoxic conditions through stabilization of Hypoxia Inducible Factor-1 (HIF-1). In this work we evaluated the response of HO system at transcript level by real-time PCR assay, protein levels and cellular distribution by Western blot, ELISA and immunofluorescence using a $CoCl_2$-induced chemical hypoxia model in PC12 cells. This cell line reproduces the behavior of carotid body cells, the main cells that physiologically detect oxygen levels.

Cells were incubated with $CoCl_2$ (0.5 or 1.0 mM) for 24 or 48 h. We have previously reported that HIF-1 alfa is stabilized and translocated to the nucleus at these conditions. mRNA and protein levels of HO-1 increased significantly in cytoplasm as well as in nucleus and mitochondria in response to 0.5 and 1.0 mM $CoCl_2$ at 24 and 48 h. $CoCl_2$ did not induced changes in HO-2 mRNA levels, however, protein levels decreased in cytoplasm at 24 and 48 h and both $CoCl_2$ concentrations. Surprisingly, HO-2 was detected in the nucleus under physiological conditions and protein levels decreased in response to $CoCl_2$ 1.0 mM during 24 h as well as in both concentrations of $CoCl_2$ at 48 h. HO-2 was also detected in mitochondria in physiological conditions and protein levels increased in this organelle with $CoCl_2$ 0.5 and 1.0 mM at 48 h.

These results suggest particular behaviors of transcript and protein of heme oxygenase 1 and 2 under $CoCl_2$ induced-chemical hypoxia in PC12 cells, suggesting that both isoenzymes may have dependent-and- independent enzymatic functions in different cellular compartments such as cytoplasm, nucleus and mitochondria. We also observed that HO-2 is not strictly a constitutive isoform, and the intracellular location of both isoenzymes are not

exclusive of the endoplasmic reticulum and could be localized in the nucleus and mitochondria as an important response to survival of PC12 cells in a hypoxic stress.

Corticosterone into the dorsal striatum impairs memory retrieval of an inhibitory avoidance task

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There is evidence that the striatum is involved in learning and memory. It is also known that stress plays an important role in our ability to recall past experiences, and the nature of its effects on memory depends crucially on the time of exposure to stress. Glucocorticoid hormones are released by stressful situations, and their involvement in the process of memory consolidation has been thoroughly studied. However, there are still unanswered questions about the involvement of glucocorticoid hormones and about the structures involved in the process of retrieval. It has been reported that stress induced before a retention test has mainly negative effects on memory recall. The aim of this study was to determine the involvement of striatal glucocorticoids in memory retrieval. To address this issue, five different groups of male Wistar rats were implanted bilaterally with cannulae into the dorsal striatum. An intact group of rats was also studied. After a week of recovery from surgery, rats were trained in an inhibitory avoidance task (IA), using a foot-shock of 1.0 mA. Forty-eight hours later, their retention latencies were measured, but 30 min before this test the animals were infused with vehicle or 3, 5, 10, or 20 ng of corticosterone (CORT). Our results showed that the group treated with 5 ng of CORT produced a retriveal impairment. These findings suggest the involvement of glucocorticoids in the dorsal striatum in the retrieval of an IA.

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Effect of delayed and abbreviated environmental enrichment on hippocampal neurogenesis after traumatic brain injury.

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Comentario [NLA1]: No revisaste los requisitos del congreso: piden la dirección de correo electrónico, el area tematica y otros datos.

Area: Estrés

Traumatic brain injury is one of the main death causes at the global level; patients that survive present long term motor, behavioral and cognitive disabilities tha can be mitigated by rehabilitation. Environmental Enrichment (EE) in rodents is a model of clinical rehabilitation that increases the size of the cerebral cortex, increases dendritic ramifications, hippocampal neurogenesis and improves cognitive performance in a variety of tasks. However, the traditional models of EE do not replicate to clinical rehabilitation practice since patients have a short period of exposure to physiotherapy, and the beginning of the rehabilitation is not immediate after the injury. Previous results from our group have shown that the delayed (3 days) and abbreviated (6 hours/day) EE promotes motor and cognitive recovery in a similar way to continuous EE. Therefore, the aim of the study was to evaluate the effect of delayed and abbreviated EE on hippocampal neurogenesis in a model of traumatic brain injury. For which we used Sprague-Dawley male rats that suffered controlled cortical impact (CCI 2.8 mm tissue deformation at a speed of 4 m/s) and controls (sham). Rats were assigned to continuous EE, delayed and abbreviated EE or standard animal facility conditions (STD) for 21 days. Animals were anesthetized and intracardially perfused, brains were dissected, 40μm slices were obtained with a cryostat and we performed an immunostaining against the marker of neuroblasts doublecourtin (DCX). We estimated the volume of the subgranular layer (SGL) of the dentate gyrus and the number and density of DCX+ cells by stereology. Our results showed that under STD conditions CCI causes an increase in SGL volume when compared to SHAM animals. In addition, we observed that continuous EE causes an increase of DCX+ cell number and density in CCI animals but not in the SHAM group. We did not observe effect of delayed and abbreviated EE on any of the parameters studied. In conclusion our results suggest that the motor and cognitive recovery observed in animals subjected to delayed and abbreviated EE is not directly related to an increase in hippocampal neurogenesis.

Effect of early life stress on hippocampal neurogenesis and its relationship with metabolic risk on mature rats

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Área 8: Estrés

Depression and metabolic syndrome (MetS) are important health problems due to their increased incidence in the adult population. Early life stress (ELS) decreases hippocampal neurogenesis and causes a deregulation of the hypothalamic-pituitary-adrenal (HPA) axis that could increase both metabolic risk and depression vulnerability. Yet, the relationship between MetS and hippocampal neurogenesis is poorly understood. Periodic maternal separation (MS) is a widely used rodent model of ELS that consists of separating the pups from their mothers for periods of 3 h a day during the first weeks of life. MS males show increased basal corticosterone (CORT) levels, a passive coping strategy on the forced swimming test (FST), decreased hippocampal neurogenesis and increased metabolic risk in juveniles; however, the effect of MS has not been studied in mature animals. Therefore the aim of the study was to evaluate the effect of MS on metabolic risk and its relationship to hippocampal neurogenesis in mature rats. To test this, Sprague Dawley male rats were subjected to MS. We evaluated depressive-like behavior FST and anxiety-like behavior in the elevated plus maze (EPM) at 10 months of age. Animals were catheterized in the jugular vein and let to recover for one week. We evaluated glucose tolerance and determined the concentration of glucose, triglycerides, cholesterol, CORT and insulin. Rats were intracardialy perfused, brains were dissected and we performed an immunostaining against the immature neurons marker doublecortin (DCX). We estimated the number and density of labeled cells by stereology. MS animals showed a passive coping strategy in the FST without affecting anxiety-like behavior in the EPM. The glucose tolerance test showed that both MS and CONT animals have impaired glucose regulation. We observed no significant differences when comparing other metabolic parameters. However, MS decreased the density (cel No/mm^3) and number of DCX+ cells in

the hippocampal neurogenic niche, without causing differences in the volume. In conclusion, our results suggest that metabolic alterations induced by age did not correlate with the effects of MS on hippocampal neurogenesis.

Effect of stress on gut mucopolysaccharide content from BALB/c mice

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Introduction. Gut *mucus* secreted by globet cells forms a sticky layer composed by mucopolysaccharides (Mucopol) that displays a pivotal anti-inflammatory role by preventing the direct contact of epithelial surface with microbiota. Small intestine is covered by a single loose (unattached) *mucus* layer whereas the colon is covered by an inner *mucus* layer firmly attached to the epithelial surface and upon it, an outer loose *mucus* layer. Normal levels of Mucopol are disturbed by stress as evidenced in the whole length of the intestinal tract **Aim.** To assess the influence of stress on Mucopol content in terms of intestinal regionalization. **Methods.** Groups (n=12) of 8 week-old male BALB/c mice either unstressed or stressed by board immobilization 2 h during 4 consecutive days were euthanized by an isoflurane overdose and ex-sanguinated by cardiac puncture. Intestinal tract was collected and rinsed with isotonic saline solution for full removing of feces and divided in three regions: proximal (next to stomach), distal (next to cecum) and colon (next to rectum). Sections of 1 cm of each region were collected, weighed and stained with alcian blue solution. Mucopol quantitation was accomplished with a standard curve of known concentrations of chondroitin 4 sulfate stained with alcian blue *versus* the corresponding absorbance at λ=620 nm. Mucopol content expressed in mg/g from both mice groups was compared with Student´s *t* test and the statistical differences were regarded at $p<0.05$ **Results.** By comparison with the unstressed mice, stressed mice had lower Mucopol content in all intestinal regions ($p<0.05$). **Discussion.** Stress induced a Mucopol decrease presumably associated with globet cell depletion by prostaglandings released during the degranulation of mast cells; the latter is induced by corticotropin releasing factor (CFR) produced by hypothalamus and/or intestine in response to stress. Decrease of mucus layer may enabled inflammatory responses by favoring adherence and penetration of microbiota on epithelial surface. **Perspectives.** Mucopol estimation may evidence neuroendocrine mechanisms by means the stress contributes to inflammatory diseases and impact on the pharmacological strategies for prevention or control of gut inflammation.

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Effect of stress on the expression of claudins in the ileum of BALB/c

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Introduction. Stress is an alarm signal that evokes the release of neuroimmune factors that affects the expression of transmembrane proteins at the paracellular surface of the epithelial cell layer such as claudins (1,2). Alterations on claudin expression in ileum may contribute to the development of inflammation i.e. ileitis (3). **Aim.** To analyze the effect of stress on the expression of claudin-2, -4 and -7 in ileum of BALB / c mice. **Methods.** Group of 6 BALB/c female mice (8 weeks old) were stressed by immobilization for 2 h/day during 4 days and a mice group without stress was included as control. Mice were euthanized with isoflurane, distal small intestine (next to cecum) was dissected and rinsed with saline solution; the luminal surface was scraped with a glass slide for sampling whole mucosa to assess the relative protein expression or mRNA expression by Western blot and RT-QPCR, respectively. Data were normalized to β-actin housekeeping gene in both techniques. Results were compared with Student's *t*-test and the significant differences were regarded at P values <0.05. **Results.** By comparison with control mice, in stressed mice expression of claudin-2 was lower at protein and mRNA levels (P <0.05) but in the case of claudin-4 and -7 only at mRNA level. **Discussion.** Decreased expression of claudin-2 may result from the release of pro-inflammatory interleukins (IL) such as IL-6, which promote intestinal inflammation. **Conclusion.** Reduced expression of claudin-2 by stress may contribute alter permeability favoring inflammation associated with disorders such as ileitis **Perspectives.** The study of stress impact on claudins could contribute to the understanding of factors involved in inflammation in ileum and the development of immunological and pharmacological strategies for prevention and/or control.

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Epigenetic effects of maternal separation on thyrotropin-releasing hormone .

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Epigenetic modifications, (DNA methylation, histone modification) represent the way by which the environment influences gene expression. Critical periods like embryonic development or early life are particularly sensitive to modify these patterns. Early life stress causes epigenetics modificationson the expression of glucocorticoid receptor (GR) in the hippocampus, and in variables that affect the stress response of the adrenal axis. However, the effects on the thyroid axis have not been reported despite their importance on the energy balance regulation and its susceptibitlity to stress.

In this work, we investigate the effects of early life stress on DNA methylation patterns of two genes related to thyroid axis, in Wistar male and female rats under maternal separation (MS) paradigm during lactation. The offspring was separated from their mother daily,from post-natal day 2(PND2) to PND21, between 10hrs and 13hrs and euthanized at PND60.We analyzed the thyrotropin-releasing hormone (TRH) and thyroid hormone receptor β2 (TRβ2) in the paraventricular nucleus of the hypothalamus (PVH). DNA methylation analysis was performed by bisulfite genomic sequencing. mRNA levels in PVH of male and female were determined by *in situ* hybridization (ISH) at various ages. We found that TRH mRNA was unchanged in MS males in all ages but their response to threats as fasting are higher than in females (Jaimes-Hoy this congress). We analyzed the proximal promoter sites of Trh gene and no differences were observe in female samples from ELS or from normal rearing conditions. ELS males showed differences in a CpG site next to the initiation site. More work is needed to define if this difference diminishes the response to metabolic threats that alter the concentrations of TH. We conclude that changes in TRH expression in PVH after early life stress are not due to changes in DNA methylation at promoter sites that correspond to consensus sequences for example to phosphorylated CREB or, to GR. Differential responses between ELS and controls to threats that alter HPT activity may be due tochanges in histone modifications, transcription factors and co-regulators or, to the differential stress response that affects that of the HPT as we have previously demonstrated (Sotelo-Rivera, this congress).

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Restraint Stress increases TRH and TRH-R2 expression in thalamic nuclei.

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The thalamus is the brain´s sensory relay station. Several thalamic nuclei are regulated during stress, one of these, the posterior paraventricular thalamic nucleus (pPVT), communicates behaviourally relevant information from PVN to amygdala, hippocampus and cortex. Thyrotropin releasing hormone (TRH) is a tripeptide with neuroendocrine and neuromodulatory roles; expressed in the PVN it controls the activity of the thyroid axis; in other brain areas as amygdala it has anxiolytic functions[1, 2]. Other areas contain TRH neurons or its receptors but its function is still unknown for example in the thalamus where high levels of TRH mRNA are found in the reticular thalamic nucleus and thalamic nuclei express the highest concentrations of its receptor TRH-R2. Since the pPVT has been postulated to be involved in habituation to chronic stress we evaluated the elements involved in TRH transmission in thalamic areas of Wistar rats submitted to acute and chronic stress by restraint.

Restraint of movement (Res) is a widely used model of psychogenic stress. We have previously demonstrated that TRH mRNA levels in PVN decrease after acute restraint stress[3] but return to baseline in the chronic condition. In amygdala, TRH content increased and decreased mRNA levels suggesting inhibition of TRHergic neurons[2].

Animals were introduced for 30 min in a polycarbonate tube with adjustable length, with slots along the sides and an opening at the end to let the rats´ tail free. Rats were sacrificed 45 min after a single restraint episode (acute condition) or after 14 consecutive daily 30 min episodes (chronic condition). Systemic blood was collected and brains carefully removed and frozen (-70ºC) until processed.

Rats habituated to the homotypic stress since corticosterone serum concentration increased only after acute RES. Acute or chronic RES did not modify the activity of the thyroid axis. Chronically stressed animals gained less weight than naïve. In situ hybridizations were performed in ventral slices containing thalamic nuclei (Bregma -7.6 to -4.28) and PVN (Bregma -8.1 to -7.6). TRH-R2 mRNA increased in the pPVT and ventral posterolateral thalamic nucleus (VPL) specific to chronic restraint; in response to both acute and chronic restraint in: anterior paraventricular thalamic nucleus (aPVT), centromedial (CM), reuniens nucleus (Re), anteroventral and anterodorsal nuclei (AV and AD). Aditionally, proTRH mRNA levels increased in reticular (Rt) nucleus only in the chronic condition. These results provide evidence of the involvement of thalamic TRH in psychological stress and suggest that its signalling occur through TRH-R2. Our results broaden the knowledge of the molecules that participate in the circuits of stress response by restraint. A direct effect of TRH on habituation remains to be tested.

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Role of ROS produced by mitochodria and NOX (NADPH-Oxidase) in apoptotic death of cerebellar granule neurons.

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It has been described that reactive oxygen species (ROS) play a role in multiple processes during physiological and pathological conditions. There are several sources that produce ROS in the cell, including xanthine oxidase, CYP450, lipoperoxidase, the mitochondria and NADPH-oxidase (NOX). Recent lines of evidence show that exist an interplay between different sources, suggesting that ROS produced by the mitochondria induce the ROS production by NOX. In cultured neurons, it has been shown that ROS act as early signals during the process of apoptotic cell death, and that one of the ROS sources implicated are NOX. However, it is unknown if there is a crosstalk between ROS produced by NOX and those produced by the mitochondria. In cerebellar granule neurons (CGN) treated with staurosporine (ST) or potassium deprivation (K5), ROS production occurs at different times along of the death process, which is critical for apoptotic cell death of CGN. In the present study, we evaluated the mitochondrial ROS generation induced by two apoptotic conditions (ST and K5) and their possible role in the NOX-mediated production of ROS. For that, we used cultured cerebellar granule neurons maintained in a medium with 25mM potassium (K25) during 7 days in vitro (DIV) and treated with ST (0.5 μM) or K5. Under these conditions, we measured ROS production by using dihidroethidium and we confirmed that ST or K5 treatment induced a significant increase of ROS levels after 5 hours. Interestingly, we also found an early ROS production (0-30 min), which was less extensive than that observed at 5 hours. Both, the cell death and the early ROS increase produced by treatment with ST were markedly reduced by the mitochondrial antioxidant MitoTEMPO. Preliminary studies also showed that the treatment with ST induces the loss of mitochondrial membrane potential at short times. These results suggest that the mitochondrial ROS may be necessary for the process of neuronal death mediated by ST and that these ROS could be required for the NOX-mediated ROS production.

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El papel de las NADPH oxidasas durante el daño excitotóxico en el núcleo estriado del ratón.

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The excitotoxic damage is a common phenomenon in various pathologies of the central nervous system (CNS). The mechanism of such damage depends on several factors, including the increase in the intracellular concentration of calcium (Ca ++) and production of reactive oxygen species and nitrogen, phenomena which contribute to neuronal death and subsequent loss of function. Recently found evidence pointing to the NADPH oxidases (NOX), particularly NOX-2 counterpart, as the main source of reactive oxygen species (ROS) responsible for oxidative stress during this process both in vitro and in vivo models. The NOX enzymes are transmembrane whose only known function is the production of reactive oxygen species (H2O2 and O2 •); this production, in small quantities involved in redox regulation processes during physiological conditions, while the rise in activity has been observed in inflammatory phenomena, necrosis and apoptosis. These features postulated to NOX as an excellent therapeutic target in the control and prevention of excitotoxic damage. In an in vivo excitotoxic damage model produced by intracerebral injection of glutamate striatum of C57-BL6 mice, we have been characterized based on surrogate markers the time course of the activity of the NOX and by means of a mouse NOX2 KO - / - the specific gravity of the counterpart in particular, observing a significant increase in activity with biphasic (1hr to 12 hrs), which is not observed in animals deficient enzyme. This increased activity correlated with the application cylinder test, since it was observed that those animals deficient NOX-2 outperform glutamate after administration compared to wild animals. Protein levels determined by Western blot analysis indicated that the increased expression regulate to the second peak of activity, however the first mechanism is independent of the control. Likewise the data obtained so far suggest that deficient animals NOX-2 have increased expression of NOX-4 as a compensatory mechanism, a phenomenon that has been observed in other models. On the other hand in vitro model treatment with glutamate causes 80% cell death in primary cultures of granular neurons in the first 24 hours, this effect is counteracted by the administration of MK-801 an inhibitor of NMDA receptors. Administration of granule neurons to glutamate produced neuronal death, which depends on activation of NMDA receptors. The biphasic behavior increased activity of NOX depends on the coordination of the different cell types present in the mixed cultures.

Analysis of the thyrotropin-releasing hormone-degrading ectoenzyme, pyroglutamyl peptidase II, in tanycyte primary cultures

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Tanycytes are a specialized glial cell population which surrounds third ventricle walls, in the medial basal hypothalamus. These cells have been implicated in barrier functions, as they limit the entrance and outflow of signals to the systemic circulation; generation of new hypothalamic neurons in the adult rat, based on their similarity with radial glia; and the regulation of the hypothalamus-pituitary-thyroid (HPT) axis, which sets thyroid hormone levels in serum. In this axis, thyrotropin-releasing hormone (TRH) neurons of the paraventricular nucleus of the hypothalamus project to the median eminence, where TRH is released into portal vessels connected with the anterior pituitary. Pyroglutamil peptidase II (PPII), the TRH-degrading ectoenzyme, is broadly distributed along the brain, but can be found in high levels in the tanycytes of the median eminence, including β2 tanycytes. Signals that decrease HPT axis activity, as systemic administration of thyroid hormones (TH) [1] or fasting [2], increase the expression of PPII in the median eminence, suggesting that tanycyte PPII controls the HPT axis, limiting the amount of TRH that reaches the anterior pituitary. This suggestion has been substantiated experimentally, since PPII inhibition can increase serum thyrotropin concentration [1].

Since median eminence is a blood brain barrier-free area, we intent to identify the local or peripheral signals that regulate PPII expression and activity, as they can possibly affect HPT axis activity. To accomplish this aim, we tested PPII expression and activity in tanycyte primary cultures obtained from the median eminence of 10 day old rats; cultures were established in serum supplemented medium. Tanycyte primary cultures were characterized by immunofluorescence, showing the presence of nestin, vimentin and DARPP-32, tanycyte specific markers. Cultures expressed PPII mRNA; however, we couldn't detect PPII activity in basal conditions, nor in cultures treated with TH, or with a low-glucose medium exposure, to mimic fasting. We postulated that the absence of PPII activity was related to the presence of a truncated isoform of PPII, which was previously found to exert a dominant negative activity over the complete isoform [3]. We found a higher expression of the truncated isoform of PPII with respect to the complete isoform in tanycyte culture extracts, which is consistent with the hypothesis. On the contrary, an analysis of the development of PPII expression and activity in the median eminence *in vivo* indicated that the truncated PPII was a minor component. Thus, standard culture conditions switch the balance between both PPII isoforms, and favor the expression of the truncated isoform. The factors that modify the ratio of truncated/complete PPII isoforms are unknown.

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APP metabolism is modulated by cholesterol in cultured astrocytes

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Cholesterol is essential for maintaining lipid raft integrity and has been regarded as a crucial regulatory factor for amyloidogenesis in Alzheimer's disease (AD). The vast majority of studies on amyloid precursor protein (APP) metabolism and amyloid β-protein (Aβ) production have focused on neurons. The role of astrocytes remains largely unexplored, despite the presence of activated astrocytes in the brains of most patients with AD and in transgenic models of the disease. The role of cholesterol in Aβ production has been thoroughly studied in neurons and attributed to the participation of lipid rafts in APP metabolism. Thus, in the present study, we analyzed the effect of cholesterol loading in astrocytes and analyzed the expression and processing of APP. We found that cholesterol exposure induced astrocyte activation, increased APP content, and enhanced the interaction of APP with BACE-1. These effects were associated with an enrichment of ganglioside GM1-cholesterol patches in the astrocyte membrane and with increased ROS production. Also, we found that cholesterol increased the formation of amyloidogenic fragments C-99 and sAPPβ, and decreased the non-amyloidogenic fragment sAPPα.

Aquaporin 1 is localized in the Schmidt-Lanterman incisures and at the paranodes of the nodes of Ranvier in the rat sciatic nerve

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Aquaporins (AQPs) are a family of small, integral membrane water-transporting proteins, found in prokaryotes and eukaryotes implicated in mediating bidirectional movement of water across cell membranes in response to osmotic gradients. There are at least 13 different members of the AQP protein family described in mammals. In the nervous system, most of the work has been focused in the central nervous system, but very little in the peripheral nervous system (PNS). In the PNS, AQP1, AQP2 and AQP4 have been reported in both peripheral neurons and glial cells. In this work we studied the expression of four AQPs (AQP1, AQP2, AQP4 and AQP9) by reverse transcription polymerase chain reaction (RT-PCR), showing that only AQP1 is present in the sciatic nerve. AQP1 is also observed at the protein level by Western blot analysis. We also studied the localization of AQP1 in the sciatic nerve by immunohistochemistry. The results show that AQP1 is present in both myelinating and non-myelinating Schwann cells. The expression of AQP1 in non-myelinating Schwann cells supports the involvement of AQP1 in pain perception. In myelin internodes AQP1 is enriched in the Schmidt-Lanterman incisures and in some internodes it is also present in the abaxonal membrane. AQP1 is also present in the paranodal regions of the nodes of Ranvier, which co-localizes with actin. Therefore, AQP1 might play an important role in myelin homeostasis maintaining the thermodynamic equilibrium across the plasma membrane in myelinated axons during electrical activity

CHARACTERIZATION OF Na+ DEPENDENT AMINO ACID TRANSPORTERS IN RAT ASTROCYTOMA C6 CELLS

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The importance of glia cells in the central nervous system (CNS) can be explained for the diversity of cell types and their variability of functions. Three different types of macroglia are present in de nervous system; astroglia, ependymoglia and myelinating glia. Astrocytes are the most abundant glia cells in CNS, their number and complexity correlate with their important role in brain development and function. Usually, astrocytes are associated with metabolic support of neurons. Nevertheless, their critical involvement in neurotransmission, trafficking of ions and molecules needed for brain homeostasis is supported by recent publications. Moreover, glia cells coordinate synaptic signals and modulate the activity of surrounding neurons. Glutamine shuttle between astrocytes and neurons is compulsory for the proper recycling and synthesis of glutamate and GABA. The amino acid efflux across the astrocytes and uptake into neurons is mediated mostly by the amino acid transport systems; A, L, N and ASCT. Particularly systems A and N, belonging to the Sodium neutral amino acid transport family (SNAT) have been associated with malignant gliomas. Functional characterization of these transporters is essential to understand their role in malignancy and proliferation in gliomas. However, the complexity of glial primary culture models calls for the use glioma stable cell lines such as rodent C6 cells to reveal their basic functional mechanisms. To this end, we characterized the expression of the different amino acid transporters in these cells with the final aim to develop a suitable cellular model in which the functional characterization of these transporter proteins under different stimulus could be easily determined .

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Conjugated linoleic acid induces selective inhibition of the astrocytoma cell survival through a PPAR-gamma independent mechanism

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Glioblastoma multiforme (GBM) is a fast-growing glioma derived from astrocytes and the most aggressive brain tumor in adults. Current chemotherapeutic agents are known to have a low therapeutic index in brain tumors due to their inability to reach the brain tissue, low selectivity towards cancer cells and generate side-effects. Therefore, new therapeutic approaches are needed for the treatment of GBM. Agonists of peroxisome proliferator-activated receptor-gamma (PPARγ) have shown decrease the viability of malignant astrocytoma cells without affecting the viability of primary astrocytes through a PPARγ independent pathway. Conjugated linoleic acid (CLA) is a natural PPARγ-agonist which crosses the blood-brain barrier and induces anti-proliferative activity on glioblastoma cells; however, neither the mechanisms of action involved in the anti-glioma effect of CLA nor has its toxicity on primary astrocytes been elucidated. The aim of this work was to determine the selectivity of the cytotoxic effects of CLA (bioactive isomers i.e. cis9,trans11-CLA and trans10,cis12-CLA) towards GBM and their possible mechanism of action. Our findings showed that bioactive isomers of CLA decreases GBM cell viability, but not modify the primary astrocytes effects. In contrast, linoleic acid (LA) and trans,trans-CLA did not modify the cell viability of glioma. PPARy antagonists did not block the anti-glioma effect induced by CLA (bioactive isomers), suggesting that the receptor is not involved in the mechanism of action. The necrosis and nitric oxide production were not involved in toxic CLA effects while oxidative stress pathways and the disruption of mitochondrial electron transport chain were activated. These data provide the first evidence that CLA does not induces toxicity on primary astrocytes and suggest that the toxicity on glioma cells depends only from bioactive CLA isomers and not from other CLA isomers. Furthermore it was shown that reactive oxygen species production and apoptosis induction are involved in the mechanism of action, while PPARy receptor does not. CLA may represent a novel adjuvant therapeutic alternative

in the treatment of GBM without causing adverse side effects. However, additional experiments are needed to determine the mechanism of action involved.

Mitochondrial complexes in astrocytes isolated from newborn and adult rats

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BACKGROUND. Astrocytes are the most abundant and diverse brain cells and play a central role in maintaining central nervous system homeostasis. Mitochondria provide ATP to astrocytes to perform their functions and mitochondrial complexes are the main target of several neurotoxic compounds. However, the study related to the metabolism and mitochondrial dynamics in astrocytes is still limited.

OBJECTIVE. Studying the expression of one subunit of each respiratory complex and the activity of complex I and II in astrocytes isolated from cortex, striatum and hippocampus from neonatal and adult rats.

METHODOLOGY.

I. Primary cultures of astrocytes were isolated from newborn (P0-P1) and adult rats (three months, 250-280 g).
II. GFAP was determined by immunofluorescence.
III. One subunit of each respiratory complex was determined by Western blot: NDUFB8 (complex I, 20KD), SDHB (complex II, 30 kD), Core protein 2 (complex III, 48 kD), MTCO1 (complex IV, 40kD) and ATPSA (complex V, 55 kD).
IV. Complex I and II enzymatic activities were determined.

RESULTS. The selected subunit of complexes I, II, III and V was detected by western blot in neonatal rat astrocytes; strong signal of complex III and V and weak signal for complex I was detected in adult rat astrocytes. In agreement, the activity of complex I was about 25-fold higher in neonatal than in adult astrocytes. In our experimental conditions, the activity of complex II was not detected in astrocytes isolated from newborn nor adult rats. The same result was found for all brain regions studied.

CONCLUSIONS. The activity measured for complex I agrees with the expression detected by western blot, showing difference depending on the age of the rats

used to isolate astrocytes. A thorough study of the complex II in astrocytes in vitro and in vivo is required.

Microglial and cytokine changes in the hippocampus and peripheral cytokine secretion in response to an immune – stress challenge in rat pups.

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Introduction: The stress response involves a range of adaptive alterations in multiple hormones and neurotransmitter systems. Early life stress permanently affects the development of the central nervous system. Adult patients who experienced stress during childhood may develop psychological disorders as anxiety and depression in adulthood. These diseases are accompanied by inflammatory processes in the brain. Microglial cells are brain´s counterpart to macrophages, they are the primary cells of the central nervous system´s innate immune response. These are distributed throughout the brain and can be activated by infection, inflammation or stress and are capable of secreting chemical messengers or cytokines. Early life stress increases the secretion of proinflammatory cytokines, however the response is not known against a second challenge. **Objective:** To demonstrate that early life stress leads to an immediate activation increase of microglial cells in the hippocampus and to a high circulating cytokine release in response to an immune challenge with LPS. **Material and methods:** Four groups of Male Sprague Dawley rat pups (n = 8 for each group) were used with the following treatments: 1) saline control group, 2) control + LPS group, 3) maternal separation (MS), 3h/day from postnatal day (PD) 1 to 14. 4) saline group and MS + LPS group. LPS was administered (1mg/Kg body weight) to animals only on PD14. One hour later the open field test was carried out to evaluate locomotion, and the animals were sacrificed on PD15. Brains and blood samples were collected from animals. Immunohistochemistry was used with antibodies specific for microglial cells, and concentrations of proinflammatory cytokines (IL-6-IL-1β, TNF-α) and plasma corticosterone were determined using ELISA commercial kits. **Results:** LPS treated animals presented a reduced locomotion in the open field. We observed a higher activation, but a decrease in the total number of microglial cells in both SM and LPS groups in CA3 and the hilus areas from the hippocampus. The highest activation and lowest number of microglial cells was observed in the SM + LPS group. On the other hand, LPS produces an increased secretion of plasmatic IL-1β, TNFα, IL-6 and corticosterone. SM only increases the secretion of TNFα in absence the LPS, but interestingly SM attenuates the secretion of the cytokines in response to LPS. **Conclusion:** Early life stress sensitizes microglial cells, inducing a higher activation in response to a second challenge with LPS, but on the contrary, the peripheral cytokine response to LPS is attenuated.

$P2Y_1$ RECEPTORS EXPRESSION IN MYELINATING OLIGODENDROCYTES OF RAT OPTIC NERVE

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Neuron-neuroglia communication directly or indirectly regulates all nervous system functions. One of these functions is myelination, the wrapping of neuronal axons with myelin membrane that in the central nervous system is made by glial cells known as oligodendrocytes (OLG). The ATP is one of the many signals that OLG and neurons use for their dialogue. This molecule and its metabolites stimulate membrane receptors named Purinergic Receptors (P1 mainly sensitive to adenosine, and P2 sensitive to ATP). P2 family is divided in ionotropic (P2X) and metabotropic (P2Y) receptors. In this study, we have characterized functionally the P2 receptors expressed in cultured myelinating OLG from rat optic nerve (P12). First, we evaluated the MBP, MAG and MOG expression in order to determinate their maturation stage during the culture. We found that >90% of the cells corresponded to myelinating OLG at 1 DIV to 3 DIV. To evaluated the function of the purinergic receptors we applied ATP, and others purinergic agonists, and estimated the intracellular calcium-free concentration ($[Ca^{2+}]_i$) by fluorometry. ATP stimulation evoked a robust $[Ca^{2+}]_i$ increase (EC_{50} of 1.2±0.25 μM) in the presence of normal extracellular Ca2+ (2.2 mM), response that was not affected when Ca2+ was removed. According to the potency displayed by different purinergic agonists (2meSADP=2meADP>ADP>ATP) the response was elicited mainly by P2Y1 receptor activation. This was supported by the effect of MRS2179 (100 μM), a specific P2Y1 antagonist that potently inhibited the ADP-induced response; also we have detected P2Y1 transcripts in the OLG myelinating stage by RT-PCR. It is proposed that ATP signalling through P2Y1 receptors might be important during myelin formation and to maintain neuron-OLG interactions.

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Possible role of NF-*k*B in Cytochrome P450 epoxygenases down-regulation during an inflammatory process in astrocytes

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Introduction: The development and progression of several neurodegenerative and neuropsychiatric illnesses have been related to chronic neuroinflammatory processes. The cytochrome P450 (CYP) epoxygenases and its metabolic products, the epoxyeicosatrienoic acids (EET) have been proposed as important therapeutic targets in CNS inflammation, due to its potent anti-inflammatory activity. However, CYP enzymes expression can be modified itself by diverse pro-inflammatory cytokines, in response to NF-kB binding to the promoter region of its genes. Until now, there is no evidence regarding CYP epoxygenases regulation during inflammation in the CNS, which would suggest its role in the homeostasis of this process. Our goal is to elucidate whether an inflammatory process developed in astrocytes is able to down-regulate CYP epoxygenases expression and if transcription factor NF-KB is involved in this regulation.

Materials and methods: Primary astroglial cultures were obtained from newborn rat's cortex. Identification of astrocytes was performed by immunofluorescence. Cultures were treated with LPS, TNF-α, LPS+IMD-0354 (selective NF-KB inhibitor) or TNF-α+IMD-0354. CYPs mRNA expression was determined by qRT-PCR, protein expression by immunofluorescence and enzyme activity by the ELISA determination of EETs. NF-κB binding site predictions in *cyp2j3* and *cyp2c11* promoter regions were calculated through AliBaba 2.1 free software.

Results: LPS-induced inflammation caused a decrease in CYP epoxygenases mRNA and protein expression, which was reflected in a decrease in EETs levels. The LPS-mediated down-regulation of CYP expression and activity was not observed when the NF-KB pathway was inhibited by IMD-0354. TNF-α addition reproduced the LPS effects, but NF-KB inhibition could not totally prevent it. Additionally, five possible NF-KB binding sites were found in the promoter region of *cyp2j3* and *cyp2c11* genes.

Conclusions: Astrocytes CYP epoxygenases expression and activity are decreased in a pro-inflammatory environment, due in part to the production of cytokines like TNF-α, since this cytokine is able to trigger this effect by itself. Transcription factor NF-kB may be involved in this CYP regulation, probably by its specific binding to CYP genes promoter. In concordance, when NF-kB pathway is inhibited, inflammation-mediated CYP regulation is not observed.

Searching for negative regulators of microglia activation: an alternative for Alzheimer's disease treatment?

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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 triggering the amplification of the pathology. Thus, the blockage of microglia activation has been proposed as a potential therapeutic strategy in neuropathologies. Therefore, the present study was focused on the anti-inflammatory effect of a hydroalcoholic extract (HE) of *Malva parviflora* (*M. parviflora*) using primary cultures of mouse microglia. Primary microglial cells were isolated from wild-type CD1 and from 5XFAD mice used as an AD model. We demonstrated that the HE of *M. parviflora* possesses immunomodulatory properties as it significantly decreased the activation of NF-kB and AP-1 in macrophages stimulated with LPS. We also observed an anti-inflammatory effect of *M. parviflora* HE in neonatal mice microglia as it reversed the amoeboid phenotype (associated with activated microglia) of these cells when treated with LPS. 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. It is known that TREM2 (triggering receptor expressed on myeloid cells 2) is highly expressed by microglia and mediates phagocytic clearance following injury or insult. Here we demonstrated that *M. parviflora* HE induces TREM2 expression resulting in

increased phagocytosis of βA peptide by microglia. This work was partially supported by grants from CONACYT (155290 and 154542) and DAGPA/UNAM (IN209212 and IN209513).

Keywords: Microglial cells, inflammation, Alzheimer’s disease, *Malva parviflora*, TREM2.

Differential recruitment of transcription factors and epigenetic changes on progesterone receptor isoform promoters in a mouse embryonic hypothalamic cell line

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Progesterone Receptor (PR) belongs to the nuclear receptor superfamily and regulates several reproductive and non-reproductive functions. PR gene codifies for two main isoforms (PR-A, and PR-B) that are regulated by two specific promoters and are transcribed from alternative transcriptional start sites. Differential regulation of PR isoforms in rodent hypothalamus has been related with the epigenetic status of their promoters. However, the molecular mechanisms involved in the regulation of PR isoforms expression in embryonic hypothalamic cells are poorly understood. In this study, the estradiol regulation of PR isoforms in a mouse embryonic hypothalamic cell line (mHypoE-N42), as well as the transcriptional status of their promoters was assessed. PR-B expression was specifically and transiently induced by estradiol at 6 h of treatment, via estrogen receptor alpha (ERα). This induction was associated with the recruitment of ERα to PR-B promoter. Interestingly, a downregulation of this PR isoform at 12 h of estradiol treatment was observed. This downregulation was associated with a decrease of specific protein 1, H3K4me3 and RNA pol II occupancy on PR-B promoter. On the other hand, there were no estradiol dependent changes in PR-A expression that could be related with the histone marks or the transcription factors assessed in the present study. In conclusion, PR isoforms are differentially regulated by estradiol in mouse embryonic hypothalamic cells and this differential regulation is associated to specific transcription factors interactions and epigenetic changes in PR-B promoter.

Characterization of energy balance in mice KO for the thyrotropin releasing hormone degrading enzyme

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Thyrotropin releasing hormone (TRH) is key in metabolic homeostasis. Paraventricular nucleus (PVN) TRH neurons send axonal projections into the median eminence (ME) of the hypothalamus. In the external layer, TRH enters the hypothalamus-pituitary portal capillaries, and regulates thyrotropin (TSH) secretion from the pituitary. Pyroglutamyl peptidase II, the TRH-degrading enzyme (TRH-DE) is detected mainly in brain; lower levels are present in pituitary, liver and serum. This ectoenzyme is expressed in tanycytes of the ME, where it degrades TRH in the extracellular space, switching off the hypothalamus-pituitary-thyroid (HPT) axis. In this compartment, its activity is regulated by thyroid hormones, and fasting, suggesting that it controls the activity of the HPT axis in response to energy homeostasis clues. To better understand the role of PPII in energy homeostasis, we made use of a TRH-DE knockout mouse line (Tang et al, 2010). We compared the phenotype of the KO mice (in both male and female animals) with that of heterozygous or wild type mice on a mixed background. In KO mice, the activity of PPII was strongly reduced in the brain, and TRH half-life increased in serum. The body weight of male KO mice was slightly reduced. KO males ate less than WT and heterozygotes. This was not associated with changes in the expression of melanin concentrating hormone in the lateral hypothalamus. The basal locomotor activity was slightly augmented in KO mice males compared to wild type mice. No differences were observed in females. In basal conditions, markers of HPT axis activity (PVN TRH mRNA levels or serum concentration of TSH or thyroid hormones) were independent of genotype. However, the drop of serum TSH concentration that is induced by fasting was attenuated in KO animals, in particular in male animals. In conclusion, the elimination of PPII alters energy intake and impairs the response of HPT axis to energy deficit; these effects are gender-dependent. Acknowledgements: CONACYT CB154931, DGAPA IN206712, DGAPA 206416.

Glucocorticoids curtail cAMP-induced TRH transcription by a protein:protein interaction between glucocorticoid receptor and catalytic PKA.

Sotelo-Rivera I, Uribe RM, Charli J-L, and Joseph-Bravo P.

Cold exposure activates the hypothalamic-pituitary-thyroid (HPT) axis, increasing the expression of thyrotrophin releasing hormone (TRH) in hypophysiotropic neurons of the paraventricular nuclei of the hypothalamus (PVN), release into the portal blood, as well as enhanced thyrotrophin (TSH) and thyroid hormone serum concentrations. Stress inhibits the activity of the HPT axis and we have recently shown that a previous stress exposure as well as a corticosterone injection interferes with the response of the HPT axis to acute cold (1).

We have also previously characterized the consensus sequences of the TRH gene promoter that bind to cAMP-induced phosphorylated CREB and to dexamethasone activated glucocorticoid receptor (GR), and demonstrated, in primary cultures of hypothalamic cells or in transfected heterologous system that dexamethasone intereferes with the stimulatory effect of cAMP on TRH mRNA levels, on TRH transcription and on pCREB binding to CRE or GR binding to GRE.

We searched for the mechanism responsible for this interference in primary cultures of rat hypothalamic cells that impedes GR and pCREB binding to TRH promoter is due to chromatin remodeling by recruitment of deacetylases as occurs when cells are incubated with T3. Chromatin immunoprecipitation assays demonstrated HDAC3 bound to TRH promoter of cells incubated with T3 but not with those incubated with db-cAMP+dexamethasone that did show diminished binding of polymerase. Results support an interaction between elements of GR and cAMP transduction pathways that impedes binding of the transcription factor. Protein-protein interaction between activated GR and the catalytic subunit of PKA (PKAc) or pCREB have been reported. We searched this possibility by immunocytochemistry of stimulated cells from primary cultures or, the neuroblastoma cell line SY-SY5Y incubated with forskolin, dex or forskolin+dex. As a consequence of this interaction, the proper translocation of GR or of PKAc to the nucleus was prevented. Coimmunoprecipitation analyses confirmed protein:protein interaction between GR:PKAc but not with pCREB. The amount of pCREB was increased by forskolin but not if coincubated with dex. These results confirmed that the interaction of GR with PKAc inhibited CREB phosphorylation and its binding to CRE but also, GR binding to GRE.

We are currently evaluating if corticosterone diminishes in vivo CREB phosphorylation by analyzing brain slices of rats injected with corticosterone and exposed 1h to cold (as in 1), measuring the total amount of cells expressing proTRH mRNA (detected by in situ hybridization) and the amount of these that express pCREB measured by immunocytochemistry. CONACYT 180009, DGAPA IN-204316.

(1) I. Sotelo-Rivera, *et al.*, 2014. *Journal of Neuroendocrinology,* 26, 861-869.

Maternal separation modifies the expression of regulatory elements of the TRH system in response to food deprivation or a palatable diet, in a sex-dependent manner.

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Maternal separation (MS) alters stress responses through tissue-specific effects on gene expression that are preserved until adulthood, predisposing to metabolic alterations. As the activity of the Hypothalamic-Pituitary-thyroid (HPT) axis is susceptible to various forms of stress, we studied if MS during the lactation period altered HPT axis function and its response to a metabolic stressor such as food deprivation or a hypercaloric diet in the adult rat. Wistar male and female pups were separated from their mothers from postnatal day 2-21 (PND) 3h/day (MS), or left undisturbed (non-handled [NH]). Behavioural tests were performed at juvenile and adult stages. At PND 60, half of NH and MS rats were fasted 48 h. Other NH or MS groups were fed 60 days with chow or, chow+peanuts+chocolate-cookies (palatable diet [pd]) and restrained for 60 min prior to sacrifice.

In the open-field test juvenile MS males, showed increased locomotion; this effect was no longer observed in adults. In the elevated-plus maze, MS decreased anxiety in males tested during puberty, but this effect was lost in adulthood. However, adult MS females showed increased anxiety. MS increased *Trh* mRNA levels in the paraventricular hypothalamic nucleus (PVN) of female rats but not males; basal serum concentration of TSH and T3 were reduced in males, while T4 serum levels were increased in females. Fasting reduced insulin serum concentration in both genders independently of rearing conditions, while corticosterone increased in MS males and in NH or MS females. PVN-*Trh* expression diminished by fasting more in females than in males of the NH group and the extent of fasting-induced inhibition was reduced by MS in males. Fasting decreased T4 and T3 serum levels of NH-females, less strongly than NH-males, but similar to MS-females; only MS-fasted females had decreased TSH serum concentrations. In response to a pd, corticosterone release after restraint was lower than in NH-chow but not in MS-pd compared to MS-chow. Food efficiency,

adipose tissue mass, body weight, insulin, leptin and T3 serum levels increased and, PVN-*Trh* expression decreased in MS-pd more than in NH-pd.

MS alters the response of the HPT axis at adulthood after 48h of food deprivation or 60 days ingestion of a palatable diet, in a gender specific manner.

Reproductive experience modifies GFAP-immunoreactivity in paravaginal ganglia of female rabbits

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The pelvic ganglia provide most of the autonomic innervation of the urogenital organs. We have previously reported that the reproductive experience influences on the morphometry of paravaginal ganglia adjoined to the pelvic vagina. Such a finding has been partially modeled in ovariectomized rabbits and we have proposed the involvement of endocrine estrogen actions linked to the expression of GDNF in satellite glial cells (SGC). The present work aimed to determine whether the activation of SGC of paravaginal ganglia changes in pregnant and primiparous rabbits. The immunoreactivity anti-GFAP was semiquantitatively evaluated as an indirect indicator of the SGC activation. Data showed that GFAP-immunoreactivity in SGC surrounding paravaginal neurons in virgin, pregnant at term (G30) and primiparous (P3) rabbits. The GFAP immunoreactivity was increased in G30 and P3 groups as compared to virgin rabbits. The increased GFAP immunolocalization in SGC in pregnant and primiparous rabbits suggest the relevance of these cells in the morphometric plasticity of paravaginal neurons.

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Association between a CLOCK gene polymorphism and circadian phenotypes in patients with bipolar disorder

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Area: 5. Neuroendocrinología/neuroendocrinology

Introduction

Many human behaviors and physiological activities have a circadian rhythm, which maintains homeostasis. State-independent circadian abnormalities have been identified in patients with bipolar disorder, during affective episodes and in euthymia. Circadian genes have been associated with bipolar disorder (BD).

Aim: To determine if there is an association between a polymorphism of the CLOCK gene and circadian phenotypes in patients with bipolar disorder.

Methods and results

We evaluated 122 Mexican Mestizo patients with BD, who were assessed with SCID-I, the Horne and Östberg questionnaire, and the Pittsburg Sleep Quality Index. DNA was obtained from blood samples and PCR was performed for the T3111C CLOCK polymorphism. Differences in fatigue at night ($p=0.045$), difficulty in final awakening ($p=0.001$), and self-rated quality of sleep ($p=0.007$) were explained by CLOCK genotypes.

Discussion and Conclusion

Specific phenotypes related with sleep may be associated with the T3111C CLOCK polymorphism in Mexican patients with BD. Circadian activity may be affected by CLOCK alleles. Disruption of sleep is associated with BD, but also with diabetes and other metabolic phenotypes including obesity, which are frequently observed in patients with BD.

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Effect of *Ibervillea sonorae* extracts in GLUT1 and GLUT3 expression in cells Bergmann Glia and neural granullar cells.

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ÁREA: Neurofarmacología

ABSTRACT

Mexico heads the first places with major number of persons sick with diabetes, according to the International Federation of Diabetes (FID), it occupies sixth place of incident worldwide. National survey of Health and Nutrition (ENSANUT) in 2012 reported that 20 % of the diagnosed cases do not have access to any treatment, and that the patients 85.6 % is treated by oral agents, whereas 6.2 % do not use pharmacological therapy and that 13 % uses insulin treatment or in combination. In spite of the advances in medical treatments, there are many patients who are using alternative therapies as complement to the pre-written medication or as the unique treatment. The traditional remedies with plants exist from ancient times and are widely used in spite of the whole controversy related to his efficiency or security. In Mexico, about 5000 species of plants have medicinal attributes. Nevertheless, it thinks that the chemical, pharmacological and biomedical validation of the active principles that they contain has been carried out only in 5 % of these species. At least 306 species of 235 kinds and 93 families have been reported for the treatment of the Diabetes. There are only 30 species have been studied some scientific study of pharmacological activity, like the cucurbitacea *Ibervillea sonorae*. It is species of the semiarid region to the north of Mexico. It is a plant dioica everlasting that has been used for several centuries for the treatment of sufferings as diabetes, arthritis, rheumatism and some cardiac diseases. Diabetes is a glucose metabolism disorder. The glucose to the being the principal source of energy in eukarionts, supports a strong dependence in the activity neuronal since it needs near 80 % for the development of activities in the Nervous Central System. This work, it is centered on the primary system of transport of glucose realized by (GLUT's), on specific GLUT 1 and GLUT 3 (that are present in glial cells and neurons respectively), due to the fact that its expression, regulation and activity play a basic role in the homeostasis neuronal. One sought to determine the activity of watery and ethanol extracts of *Ibervillea sonorae* in the expression of the above mentioned carriers in an in vitro system using cells gliales of Bergmann and neuronal granullar cells, for it technologies were used of western blot in solid phase

and captures of glucose in cultures and hereby to evaluate the expression to level membranal.

Bioavailability of a bifunctional molecule in a blood-brain barrier model.

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The amyloid β-peptide (Aβ) plays a central role in the pathogenesis of Alzheimer's disease (AD). Metal ions, such as Cu(II), are involved in the aggregation of Aβ and its neurotoxic effects [1]. The peptide *MDᴅWAib* (TP) is a bi-functional molecule that competes with Aβ for Cu(II) and modulates Aβ aggregation [2], thus, having therapeutic potential against AD. However, drugs aimed to reach the central nervous system via systemic administration have a limited action into the brain due to the blood brain barrier (BBB). Peptide drugs can be degraded by enzymes and have low permeability through the BBB. Given its molecular weight and polarity, it is likely that TP can not cross the BBB.

The aim of this study was to assess the permeability and susceptibility to proteolytic degradation of TP in a BBB *in vitro* model. The BBB model was based on rat brain microvascular endothelial cells (RBMECs) cultured on Millicell-inserts and bathed on the basolateral side with conditioned media from astrocytes. We added TP at the apical side and collected samples from both, apical and basolateral side. TP was quantified by high-performance liquid chromatography (HPLC).

Our results show that TP can not cross the BBB and it is degraded rapidly. As a strategy to prevent the bio-degradation of TP, we designed two new peptides with different stereochemistry of the amino acids: TP' and TP''. These peptides retain their bifunctional characteristics and have lower degradation rates than TP. We conclude that TP' and TP'' may have higher therapeutic potential to treat AD after systemic administration.

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Choice behavior guided by learned taste aversion requires the orbitofrontal cortex

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The ability to select an appropriate behavioral response guided by previous emotional experiences is critical for survival (e.g. avoid a stimulus previously associated with danger). While much is known about brain mechanisms that underlie emotional associations, comparatively little is known about brain mechanisms required to use these stored emotional associations to guide choice behavior. To address this, we performed local pharmacological inactivations of several cortical regions before the retrieval of an aversive memory in choice-based vs. no-choice based conditioned taste aversion (CTA) tasks. Unexpectedly, we found that only inactivation of the orbitofrontal cortex (OFC), but not dorsal or ventral medial prefrontal cortices, blocked the retrieval of choice-CTA. Yet OFC inactivation left retrieval of no-choice CTA intact, suggesting its role in choice but not in retrieval of CTA. Further, we show that OFC inactivation effect on choice behavior was not due to impairment in taste detection, taste aversive learning, and motivation to drink or cognitive demands of the task. Notably, OFC inactivation did not affect choice behavior when it was guided by innate taste aversion. Consistent with the involvement of anterior insular cortex (AIC) in storing taste memories, we found that AIC inactivation impaired retrieval of both choice and no-choice CTA. Together, our results suggest that OFC may be required to compare the relative value of each of the taste memories stored in AIC and use this calculation as incentive to guide choice behavior. Thus, this study provides novel evidence for the role of OFC in guiding choice-behavior, dissociable from AIC-dependent taste aversion memory.

Effect of oral levodopa and reboxetine on motor recovery and total striatal catecholamine levels in a model of subarachnoid hemorrhage in rats

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Introduction: Cerebrovascular diseases (CVD) are the main cause of disability in adults worldwide. It is estimated that over 30% of these patients develop severe sequelae and up to 50% of them persist with severe and permanent sequelae despite receiving adequate rehabilitation therapy. Therefore, it is very important to develop new strategies to improve the results of conventional rehabilitation. In this sense, the pharmacological stimulation has been proposed as adjunctive therapy to rehabilitation for several decades. There is experimental evidence on the positive effects of noradrenergic and dopaminergic stimulation on motor recovery, however, is unknown which of the two systems take part predominantly, and there are no studies that have compared the two types of treatment, and even fewer in models of cerebral hemorrhage.
Objective: Demonstrate the effects of oral administration of levodopa (L-DOPA) or reboxetine (RBX) on motor recovery and striatal level of catecholamines in a model of subarachnoid hemorrhage in rats.
Methods: Male Wistar rats (280-300g) were used, and were divided into four groups (n=7-10 each): 1) Control group, without brain lesion; 2) experimental group injured with subarachnoid administration of FeCl2 + vehicle, 3) FeCl2 + L-DOPA 50mg/kg/day by 10 days; and 4) FeCl2 + RBX 10mg/Kg/day by 10 days. FeCl2 administration was performed by stereotaxic surgery placing a cannula in the subarachnoid space over the right sensorimotor cortex. The behavioral performances were assessed at 1, 5 and 10 days after brain lesion through four behavioral tests: cylinder test, adjustment steps test, footprint test and balance beam, finally the total striatal catecholamine levels were measured by HPLC.
Results: Both treatments the L-DOPA and RBX significantly improve the motor function in some of behavioral the tests (steps adjustment, balance beam and footprint test). The positive effect was earlier (Day 5) and significant in the RBX group. Likewise, a significant increase in total striatal catecholamines and its metabolites (DOPAC and HVA) was observed with L-DOPA administration, especially in the contralateral striatum.
Conclusion: L-DOPA and RBX improves motor recovery, however, noradrenergic stimulation RBX shows more precocious and significant effects.

Effects of clonidine and oxytocin in the modulation of anxiety in rat.

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Anxiety, is an adaptative response that prepares an individual to contend a potential threat, when this response is disproportionate to the stimulus that is causing it or appears without apparent cause, then this response is consider to become pathological. The amygdala is a key structure for the modulation of anxiety and within it, both alpha-2 adrenoceptors and oxitocinergic receptors, play an important role in the modulation of anxiety. Because these receptors can be found close from each other in the central amygdala, it is possible that an interaction between both receptors exists and this interaction may cause an increase anxiolytic effects.

The main aim of this study is search the existence of the interaction between oxitocinergic receptors and alpha 2 adrenoreceptors within the amygdala in rat. So, through bilateral microinjections within the central amygdala of clonidine (adrenergic agonist α-2) and oxytocin (OT), we define effective dose and sub-threshold doses of both agonists, these doses was test in the elevated plus-maze (EPM), this test is employ to evaluate anxiety. Our results show that 1.2 µg clonidine increased the time spent in the open arms in elevated plus-maze. While,10 ng OT showed tendency to increase the time spent in the open arms in EPM. Alpha-2 adrenoreceptor activation has anxiolytic effects in elevated plus-maze, nevertheless, oxcitocinergic receptor activation seems elicit anxiolytic effects. In accordance with our results, we will apply jointly sub-threshold doses of both agonists to examine the possible existence of an interaction between oxitocinergic receptors and α-2 adrenoreceptors, and this interaction is involving to increase the anxiolytic effects both receptors have.

Effects of oxytocin and quinpirole in the amygdaloid modulation of anxiety

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Anxiety is an adaptative response which prepares an individual against danger. Amygdala is one of the most important structures in anxiety modulation. Oxytocin plays a prominent role in traffic information in the amygdala reducing the activity of the central amygdala (CeA), site in where anxiety-like responses are implemented. Previous results of our laboratory showed that the administration of 25ng/side of oxytocin in CeA evoked an anxiolytic-like effect in shock probe burying test; as reflect of decrease of the total time of burying compared with the control group treated with saline solution. Likewise, the administration of 3μg/side of quinpirole, which is a D2 receptor agonist, in CeA evoked an anxiolytic-like effect, decreasing the total amount of burying time compared with the control group treated with saline solution in the same test of anxiety. Given that oxytocin receptors and dopamine D2 receptors are co-expressed in CeA it is possible they to interact each other and that interaction evokes an increase of anxiolytic effects. This work is aimed to pharmacologically evaluate the existence of interaction between oxytocinergic receptors and dopaminergic d2 receptors in CeA and its role in the amygdaloid modulation of anxiety. The existence of the oxytocinergic and dopaminergic receptors interaction is studied employing four groups 1) sub-threshold dose of oxytocin, 2) sub-threshold dose of quinpirole, 3) co-administration sub-threshold doses of quinpirole + oxytocin at the same time, 4) saline solution as control group. All drugs are administrated in CeA. Anxiety behavior is assessed using shock probe burying test. Results show that the group treated with co-administration of oxytocin and quinpirole has a tendency to decrease the total time of burying compared with control group, which is an anxiolytic effect. Whereas the other groups treated with only oxytocin and only quinpirole are not different to control group. Under these conditions the presence of anxiolytic-like effects provoked by quinpirole and oxytocin co-administration compared with groups only treated with quinpirole or oxytocin would point out the

existence of interactions between oxytocinergic and dopaminergic transmission systems in amygdaloid modulation of anxiety.

Área Neurofarmacología.

Role of arginine vasopressin on the amygdaloid modulation of fear and anxiety in the rat

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The amygdala plays a central role in fear and anxiety. The arginine vasopressin is a neuropepide synthetized in supraoptic and paraventricular nucleus of hypothalamus. Vasopressinergic fibers project from the hypothalamus and strongly innervate medial amygdala (MeA) and bed nucleus of the stria terminalis (BNST). Behaviorally, the intra-amygdaloid infusion of arginine vasopressin agonists and antagonists elicits anxiogenic and anxiolytic effects respectively on conditioned models of anxiety. Three different receptors have been so far identified for vasopressin effects (V1a, V1b and V2 receptors). V1a and V1b receptors has been identified within the central amygdaloid nucleus and its participation in the amygdaloid modulation of anxiety has been suggested. However, is less known the role of V1b receptor in unconditioned anxiety. The aim of this study was to evaluate the behavior of rats in the elevated plus-maze, the dark light box and the shock-probe burying test following vasopressin administration (1 ng/side) and the simultaneous administration of vasopressin and SSR149415 (1 and 10 ng/side), a specific V1b vasopressin receptor antagonist, within the central amygdaloid nucleus. The results showed that the bilateral microinjection of arginine vasopressin had no effects in anxiety during the elevated plus maze or dark light box. The infusion of arginine vasopressin (1ng/side) significantly increased the burying behavior in the shock-probe burying test as compared with their saline-treated controls. Interestingly, this last behavior was not observed when SSR149415 was administered simultaneously with vasopressin. Our results suggest, that the bilateral administration of arginine vasopressin is dependent effects of the test used to assess anxiety and seems to have effects on higher aversion paradigms while those that are less aversive its after effects seem to be low or absence of such.

Validation of a behavioral task to study the brain mechanisms of active suppression of fear

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Fear is a defense response to threat that enables the individual to avoid danger. However, when the goal is to obtain a reward the optimal adaptive response is to actively overcome fear. The brain mechanisms that underlie the active suppression of fear to get a reward are practically unknown. To address this, we developed a behavioral task in which hungry rats are trained to suppress their fear to a dangerous zone (electrified grid) to obtain a reward (food) in a safe zone (not electrified floor). The training involves that individuals learn to discriminate between safe and dangerous trials. Safe trials involve crossing a un-electrified grid, signaled by a light that predicts the availability of food on the opposite side of the box. Dangerous trials involve crossing to obtain food signaled by the light despite the electrified grid zone signaled by a conditioned sound. The active fear suppression test in well-trained rats involves the simultaneous presentation of light that signals reward and sound that signals fear. To validate our task we evaluated the effect of separately devaluating the aversive (fear to the dangerous zone) and appetitive (food in the safe zone) motivations. To evaluate the devaluation of the aversive motivation, well-trained rats were injected with an anxiolytic drug (diazepam 1mg/kg, s.c). Then, we devalue the appetitive motivation with hunger satiation (free access to food for one day). We found that devaluation of the aversive motivation with diazepam significantly facilitated the active suppression of fear, as indicated by short latencies to cross during dangerous trials compared to saline injected rats. Additionally, the devaluation of the appetitive motivation delayed active suppression of fear as indicated by long latencies to cross in both safe and dangerous trials as compared to hungry rats. Together our results validate our task and indicate that the expression of active fear suppression depends of the optimal balance between aversive and appetitive motivations. Ongoing experiments in the lab will determine which brain structures are necessary for the interaction of the

opposing motivations (avoid vs approach) that allow active fear suppression to occur.

Effect of chronic stress and streptozotocin-induced diabetes on medial prefrontal cortex`s neuronal plasticity and behavior in adolescent rats

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Chronic stress triggers maladaptive changes altering learning and memory functions. During adolescence, some parts of the brain are still under development and exposure to stress events may cause plastic changes in the central nervous system with enduring effects in adulthood. Furthermore, metabolic diseases such as diabetes can also trigger cognitive decline and may increase the risk of developing anxiety and depression. Despite the importance of those two factors involved in cognitive functions, there are no reports regarding the impact of chronic stress during adolescent, and the development of diabetes on behavioral and neural plasticity in adulthood. Diabetes can be experimentally induced by streptozotocin (STZ) administration, with damaging effects on brain and behavior.
We hypothesized that STZ-administration in adult rats previously submitted to chronic restraint stress (CRS) during adolescence will show alterations in medial prefrontal cortex (mPFC) neurons and magnocellular nucleus basalis (NBM), accompanied by behavioral changes.
Twenty-one days old rats were submitted to CRS protocol or handled over three weeks, followed by 2 weeks of rest. Half of rats received a second treat with a STZ-administration. Three weeks later animals were evaluated in the elevated plus maze, the T-maze and force-swimming-test to explore behavioral alterations. Soon later, animals were intra-cardiac perfused and brain samples were used for immunohistochemistry (post-synaptic protein 95, PSD-95; choline acetyltransferase, ChAT). mPFC`s neurons were diolistically labeled to analyze number and morphology of dendritic spines. Peripheral energetic metabolism was also evaluated in plasma (corticosterone, insulin, glucose, cholesterol and triglycerides). Our results showed that rats that received CRS during adolescence followed by STZ-induced diabetes, have higher triglyceride plasma levels, and increased ChAT and PSD-95 immunoreactivity in mPFC. Furthermore, stress-diabetic rats have a decreased number of mushroom spines, while thin spines were increased in mPFC`s neurons. These effect can be related with an increased in anxiety and depression like-behavior in CRS-diabetic rats.
These data indicate that adolescence is a sensitive period that results in a higher vulnerability to further threats. Future studies may focus in the mechanisms involved in brain plasticity during adolescence and the development of therapeutic

strategies to manage the incidence of stress`s related pathologies commonly found in young people.

Aftereffects of high-sucrose diet on cognitive function in a rat model of metabolic syndrome

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Abstract

In the last two decades several studies have reported different mechanisms that could link type two diabetes mellitus (T2DM) and dementia. Most of the Alzheimer's disease patients show alterations in the glucose metabolism and insulin signal transduction. It is thought that people develop clinical manifestations of dementia many years after the onset of brain deterioration, hindering the establishment of the disease temporality. Due to this fact, we decided to evaluate the cognitive performance in an earlier stage of metabolic dysfunction, i.e. metabolic syndrome (MS). Since MS is a multifactorial complex of signs that increases the probability to develop several types of health problems, cardiovascular processes, diabetes, certain types of cancer and even cognitive deficit among them. We treated young adult Wistar rats during a period of six months with sucrose 20% in drinking water. After the treatment, we performed three different memory tasks (Morris-Water Maze, Object Recognition Memory and Object Location Memory) and several metabolic parameters were monitored (blood glucose levels, insulin levels, body mass index and oral glucose tolerance test). We observed that MS rats showed a selective hippocampus-dependent cognitive impairment, since cortical function seems to be spared at this stage. In addition, we observed decreased levels in the synaptophysin vesicle protein as well as an impaired hippocampal long-term potentiation and increased levels of the Glial Fibrillary Acidic Protein (GFAP). We concluded that the MS rats present a mild hippocampal dysfunction due to the decreased levels of the synapses and the cognitive function performance in the memory tasks.

Brain plasticity induced by sexual experience in female mice.

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In the case of the female mammals sexual behavior is regulated by sex hormones like estradiol benzoate (EB) and progesterone (P) . In the absence of E and P females don't display receptivity behaviors. An unreceptive female doesn't present lordosis reflex (spine curvature while elevating the head and the tail to facilitate the male intromission) to any of the male attempts to copulate. Previous studies have demonstrated that ovariectomized female mice don't display lordosis in their first sexual experience even if induced receptivity with exogenous E and P. The aim of this study is to determine if sex experience induce brain plasticity changes such as axonal growth or an increase in the number of astrocytes.

In order to evaluate our hypothesis we used 20 female mice, ovariectomized and hormonally supplemented with the injection of E (50**µg**) and P (300**µg**) 48 and 4 hours respectively; mice were divided in four groups as following: (A) females who didn't copulate, (B) females with one sexual experience group (C) females with three sexual experiences (D) females with six sexual experiences.

All animals were sacrificed 24 hours after the last behavioral test and brains were recollected, sliced in 30**µm** and the accessory olfactory bulb was recovered. Then, we perform an immunofluorescence staining to analyze the markers GAP-43 (Growth Associated Protein) to mark axonal growth and GFAP (Glial fibrillary acidic protein) to mark astrocytes. We quantified the expression of GFAP positive cells and a densitometric analyze for the GAP-43 expression in the layers of the BOA (mitral, granular and glomerular). For the statistical analyses a Kruskal-Wallis was performed (astrocytes count) and an ANOVA (for the GAP-43).

We demonstrated no change in GAP-43 after the first sexual experience; by contrast after the sixth test we observed a significant difference in the mitral layer of the BOA. The astrocytes count showed significant difference in the glomerular layer after one and six trials. Our results demonstrate that sexual experience increases the expression of GAP-43 in the mitral layer of the BOA after six weeks of sexual experience as well as a fluctuating difference in the GFAP positive cells localized in the glomerular layer during those weeks.

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Chronic exposure to Wnt ligands modulates the expression of synaptic plasticity markers in the hippocampus *in vivo*

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Wnt signaling regulates important aspects of neuronal structure and function during early and late developmental stages. However, it is still unknown if Wnt signaling pathways regulate neuronal morphology and synaptic structure in the adult brain and whether these changes have an impact in cognitive processes such as learning and memory. In this work we studied some functional and structural changes in the adult hippocampus caused by the activation and inactivation of the canonical Wnt pathway in vivo. Bilateral cannulation into CA1 region connected to an Alzet® osmotic mini-pump was performed in adult male Wistar rats. We analyzed the effects of chronic infusion (7-11 days) of the agonist, Wnt7a and the antagonist, Dkk-1 on pre and post-synaptic remodeling and analyzed their impact on the object place recognition memory task. After the treatments, the memory task was performed and subsequently brains were extracted for biochemical and histological analysis. Although no significant differences in the object preference index was observed in the first 3 min an increment in this preference index was sustained by Wnt7a after 10 min of the test. Histological analysis showed no modification in the distribution of cytoskeleton-associated protein Tau. However, chronic treatments with Wnt7a induced changes in the distribution and contents of the synaptic proteins PSD-95 and synaptophysin. Moreover, Wnt pathway activation significantly increased the density of doublecortin positive neurons in the dentate gyrus associated with more elongated processes, suggesting an increase in the number and maturity of new neurons. Furthermore, electron microscopy analysis revealed that chronic infusion of Wnt7a significantly increased the number of excitatory perforated synapses by two fold without changing the total number or synapses. On the other hand Dkk-1 blocked the retrieval of the memory task and diminished the number of doublecortin positive cells. Taken together these results suggest that activation of Wnt signaling induces neurogenesis and promotes synaptic plasticity markers in the adult hippocampus.

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Modifications in Tau protein content in frontal cortex and hippocampus of mice of the strain C58/J with autism phenotype

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Introduction

Some of the core symptoms of autism spectrum disorder (ASD) are communication disturbance, abnormal social interactions and repetitive patterns or stereotyped behavior. It has been reported that the murine strain C58/J, has a high level of motor stereotypes, low social skills and hyperactivity, therefore it has been used as an animal model of autism. The differences in the behavior of the C58/J mice, could be related to changes in neuronal plasticity, and thus, with modifications in the dynamics of the neuronal cytoskeleton. Two of the most important cytoskeletal components are Tau and tubulin proteins. Microtubules are composed predominantly of α and β-tubulin heterodimers, and Tau is a microtubule-associated protein which phosphorylated form is active.

The objective of this research was to analyze changes in the expression of cytoskeletal proteins Tau and tubulin in frontal cortex and hippocampus of an autistic murine model corresponding to the C58/J strain.

Methodology.

Frontal cortex and hippocampus from C58/J and C57 BL/6 (wild type) mice were dissected. Samples were processed by Western Blot technique. To identify the cytoskeletal proteins we used anti-Tau, anti-PhosphoTau (serine 396) and anti-tubulin antibodies. GAPDH was the protein loading control.

Results.

We observed 3 abundant Tau isoforms in frontal cortex and hippocampus of both strains: 30, 60 and 80 kDa Tau isoforms. The 80 kDa Tau isoform expression in frontal cortex and hippocampus of autistic mice (C58/J) was significantly lower compared to the WT strain. The level of 60 kDa isoform showed a significant decrease only in cortex of autistic mice compared to WT. While 30 kDa isoform appears to show no change in both structures. Moreover, it was also observed that Tau protein content in WT phenotype differ between the two brain regions analyzed, but not on the autistic phenotype.

No significant difference was found in PhosphoTau/Tau ratio corresponding to 80 and 60 kDa isoforms in both brain areas, neither in WT strain nor in autistic animals, suggesting modifications in the content of the Tau protein, but not in its activity. However, 30 kDa isoform PhosphoTau/Tau ratio showed a tendency to decrease in autistic frontal cortex compared to the WT strain, suggesting a possible disturbance in Tau activity.

Tubulin protein content showed no change, neither in frontal cortex nor in hippocampus, which could mean no alteration in this cytoskeletal protein.

Conclusions

Our work showed important changes in expression of Tau isoforms and their phosphorylation state in frontal cortex and hippocampus between wild type and autistic animals. These differences could be associated with disturbances in neuronal cytoskeleton dynamics of ASD.

Structural and synaptic plasticity in SHANK3 mutant mice as autism genetic model

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Dendritic spines are highly dynamic structures whose morphology and lifespan are modified as a response to synaptic efficacy changes. Structural modifications in response to different forms of activity may underlie the long term encoding of information through remodeling of neural circuits. Long lasting changes in synaptic efficacy require new protein synthesis, and lead to long lasting growth or shrinkage of spines. Misregulation of these processes, such as through aberrant receptor signaling or altered protein synthesis, may lead to cognitive impairments.

It is known that a correlation exists between different neurological disorders and morphological alterations of dendritic spines. Evidence indicates that several of the genes that are mutated in patients diagnosed with autism spectrum disorders are genes involved in the expression of activity-dependent signaling pathways that regulates the synaptic structure and function. For instance, Phelan-McDermid syndrome caused by *Shank3* gene deletion in one allele in human, is thought to be the cause of core neurodevelopmental and neurobehavioral deficits in this syndrome. SHANK3 is a scaffold protein in the postsynaptic density and regulates NMDA receptors as well as mGluRs. **For this reason, it is important to understand how dysregulation in this protein can affect synaptic, structural and molecular response in neurons.** In order to develop this research, we use a genetic mouse model of Phelan-McDermid syndrome that bears the same deletion detected in human patients (heterozygous mouse SHANK3$^{-/+}$). These features make it a unique model for understanding the mechanisms of synaptic and structural plasticity mediated by NMDA and mGluR receptors, and thus determine the role of these receptors in the autism.

In order to investigate this issue, we will use two-photon imaging to determine if SHANK3 haploinsufficiency promotes any structural alterations in dendritic spines, also we evaluate the synaptic plasticity in response to long-term depression mediated by mGluR by two-photon calcium imaging with GCaMP6f, the expression of NMDAR and mGluR in CA1 neurons of organotypic slice cultures from SHANK3$^{-/+}$ and SHANK3$^{+/+}$ mice transfected with different plasmids using a Gene gun system.

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