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Comunicaciones Orales

ALZHEIMER DISEASE-LIKE CELLULAR PHENOTYPE OF NEWBORN GRANULE NEURONS CAN BE REVERSED IN GSK-3β OVEREXPRESSING MICE

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AIMS: To determine the reversibility of granule neuron alterations caused by GSK-3β overexpression in a murine model of Alzheimer´s disease.

MATERIAL AND METHODS: We have used a conditional mouse model to study the involvement of GSK-3β overexpression in different aspects of adult hippocampal neurogenesis. By injecting GFP- and PSD95:GFP-expressing retroviruses we have been able to analyze alterations in both morphology and connectivity of newborn granule neurons. These alterations were compared with those present in Golgi-stained hippocampal samples obtained from Alzheimer´s disease patients. The reversibility of previously mentioned alterations was evaluated by switching off the transgenic system and also by using a physiological approach (environmental enrichment) to increase hippocampal plasticity.

RESULTS: Several neurodegenerative diseases such as Alzheimer disease are accompanied by memory deficits that could be related to alterations in AHN. We have determined that GSK-3β overexpression causes dramatic alterations in newborn neuron dendritic tree morphology and post-synaptic density number and volume. Alterations in previously damaged neurons were reverted by switching off the transgenic system and by environmental enrichment. Furthermore, comparative morphometric analysis of granule neurons from patients with Alzheimer disease and from GSK-3β overexpressing mice revealed shared morphological alterations.

CONCLUSIONS: Taken together, these data indicate that GSK-3β is crucial for hippocampal function, thereby supporting this kinase as a relevant target for the treatment of Alzheimer´s disease.

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1) Alzheimer disease-like cellular phenotype of newborn granule neurons can be reversed in GSK-3β-overexpressing mice. Llorens-Martín M, Fuster-Matanzo A, Teixeira CM, Jurado-Arjona J, Ulloa F, Defelipe J, Rábano A, Hernández F, Soriano E, Avila J. Mol Psychiatry. 2013 Apr;18(4):395.

2) GSK-3β overexpression causes reversible alterations on postsynaptic densities and dendritic morphology of hippocampal granule neurons in vivo. Llorens-Martín M, Fuster-Matanzo A, Teixeira CM, Jurado-Arjona J, Ulloa F, Defelipe J, Rábano A, Hernández F, Soriano E, Avila J. Mol Psychiatry. 2013 Apr;18(4):451-60.

Áreas Temáticas:

1ª: Desarrollo

2ª: Trastornos y reparación del sistema nervioso

ANALYSIS OF BRAIN ALTERATIONS IN A MOUSE MODEL OF LAFORA DISEASE

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Lafora Disease (LD) is an autosomal-recessive, neurodegenerative disorder, characterized by the presence in most tissues of intracellular polyglucosan deposits, called Lafora Bodies (LBs). LD manifests during adolescence with neural tonic-clonic seizures and rapidly leaves the patients in a vegetative state followed by death few years after disease onset. LD is caused by loss-of-function mutations in two genes: *EPM2A*, encoding laforin, a dual phosphatase with a carbohydrate binding domain; and *EPM2B*, encoding malin, an E3-ubiquitin-ligase. Laforin and malin have been implicated in the regulation of glycogen biosynthesis and dephosphorylation, in the modulation of the ubiquitin proteasome system and in macroautophagy. Although these functions may provide an explanation for LB formation, they do not fully account for the massive loss of brain functions observed in the patients. To explore the possibility that impairment of malin/laforin activity interferes with neuronal function well before the appearance of LBs, we undertook a systematic analysis of the brain phenotype of *Epm2b*$^{-/-}$ mice. These mice recapitulate most of LD pathological traits. Comparison of the morphology of hippocamapal neurons in P16 wt and *Epm2b*$^{-/-}$ animals revealed alterations in the dendritic arborization of mutant neurons, indicative of possible alterations in synaptic transmission and neuronal excitability. Supporting this possibility, we found by electrophysiological field-recordings that the basal hippocampal synaptic transmission of the mutants is higher than that of WT, despite a similar number of inhibitory interneurons in both genotypes. To determine the causes of this difference we are currently comparing the biochemical composition of synaptosome preparations from wt and mutant mice or from surface protein biotinylation of hippocampal cultures. We are also analyzing by ankyrinG immunostaining the length and disposition of the axonal initial segment as a read out of neuronal excitability state. We will illustrate the results of these experiments and discuss their relevance in the context of LD.

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1. Trastornos y reparación del sistema nervioso.
2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

CAJAL-RETZIUS CELLS INSTRUCT NEURONAL MIGRATION BY COINCIDENCE SIGNALING BETWEEN SECRETED AND CONTACT-DEPENDENT GUIDANCE CUES

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Cajal-Retzius (CR) cells are a transient cell population of the CNS that is critical for brain development. In the neocortex, CR cells secrete reelin to instruct the radial migration of projection neurons. It has remained unexplored, however, whether CR cells provide additional molecular cues important for brain development. Here, we show that CR cells express the immunoglobulin-like adhesion molecule nectin1, whereas neocortical projection neurons express its preferred binding partner, nectin3. We demonstrate that nectin1/3-mediated interactions between CR cells and the leading processes of migrating neurons are critical for radial migration. Furthermore, reelin signaling to Rap1 promotes Cdh2 function in neurons via nectin3 and afadin, thus directing the broadly expressed homophilic cell adhesion molecule Cdh2 towards mediating heterotypic cell-cell interactions between neurons and CR cells. Our findings identify nectins/afadin as components of the reelin signaling pathway and demonstrate that coincidence signaling between CR cell-derived secreted and short-range guidance-cues direct neuronal migration.

Áreas Temáticas:
1ª: Desarrollo
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

CALCIUM-INDUCED CALCIUM RELEASE MODULATES THE RECRUITMENT OF VESICLES IN HAIR-CELL RIBBON SYNAPSES

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Hair cells, the sensory receptors of hearing and balance, convert mechanical stimulation into neurotransmitter release through the exocytosis of vesicles at ribbon synapses. Unlike most neuronal synapses, hair cells can maintain exocytosis for long period, but the mechanism supporting the continuous recruitment of new vesicles for release is unknown. Using real-time dual-sine hair-cell capacitance measurements, we found that released vesicles seem to be substituted by vesicles from reserve pools after continuous exocytosis in a calcium-dependent manner. Swept field confocal microscopy (SFCM) showed a nonlinear increase in calcium levels that paralleled the superlinear capacitance component corresponding to vesicle recruitment, suggesting a role for calcium-induced calcium release (CICR) in synaptic release. Calcium substitution by barium, a cation that cannot be reuptaken by the ER, delayed the release of readily releasable pools and impaired vesicle recruitment. Pre- and postsynaptic recordings demonstrated that pharmacological modulation of calcium stores and ryanodine receptors impaired the recruitment of new vesicles under prolonged stimulation. These data suggest the potential role of CICR in the recruitment of vesicles in auditory hair-cell ribbon synapses.

Áreas temáticas: 1. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2. Nuevos métodos y tecnologías

CAMBIOS EN LA EXPRESIÓN DE PROTEÍNAS SINÁPTICAS ASOCIADOS A LOS PROCESOS COGNITIVOS

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El almacenamiento de la información adquirida requiere de cambios sinápticos duraderos que parecen depender de la modificación de la expresión génica. Tanto es así, que la inhibición farmacológica o la modificación de la expresión de elementos estructurales de las sinapsis modifican los procesos cognitivos.

El objetivo principal de este trabajo es identificar cambios sinápticos en las principales sinapsis del cerebro, glutamatérgicas y GABAérgicas, asociados a los procesos cognitivos. Para ello, 5 grupos de ratones fueron sacrificados entre 0.5 y 24 horas después de una sesión de exploración en un ambiente enriquecido. Tras el sacrificio, se comparó el número de espinas dendríticas así como la expresión de proteínas sinápticas de adhesión (neurexinas y neuroliginas), vesiculares (transportadores vesiculares de Glut y GABA) y de andamiaje (gefirina y PSD95), en hipocampo y corteza prefrontal de ratones expuestos a ambiente rico frente a ratones no expuestos.

Nuestros resultados muestran que la exposición a un ambiente rico produce un aumento en el número de espinas dendríticas en el hipocampo y en la corteza prefrontal de los animales que exploraron con respecto a animales controles. Además, se produce un aumento de los genes que codifican para proteínas sinápticas: a tiempos cercanos a la finalización de la exploración aumenta la expresión de genes codificantes de proteínas de adhesión; y posteriormente, se produce un incremento en la expresión de genes codificantes de proteínas vesiculares y de anclaje. Estos resultados, sugieren que tras la exploración de un ambiente enriquecido se produce un incremento en el número de sinapsis, tanto excitadoras como inhibidoras. La relevancia funcional de estos cambios aún está por determinar.

1ª: Neurociencia cognitiva y conductual.
2ª Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.

CANNABIDIOL PROVIDES LONG-LASTING PROTECTION AGAINST THE DELETERIOUS EFFECTS OF INFLAMMATION IN A VIRAL MODEL OF MULTIPLE SCLEROSIS: A ROLE FOR A_{2A} RECEPTORS

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Inflammation in the central nervous system (CNS) is a complex process that involves a multitude of molecules and effectors, and it requires the transmigration of blood leukocytes across the blood-brain barrier (BBB) and the activation of resident immune cells. Cannabidiol (CBD), a non-psychotropic cannabinoid constituent of C*annabis sativa*, has potent anti-inflammatory and immunosuppressive properties. Yet, how this compound modifies the deleterious effects of inflammation in TMEV-induced demyelinating disease (TMEV-IDD) remains unknown. Using this viral model of multiple sclerosis (MS), we demonstrate that CBD decreases the transmigration of blood leukocytes by downregulating the expression of vascular cell adhesion molecule-1 (VCAM-1), chemokines (CCL2 and CCL5) and the proinflammatory cytokine IL-1β, as well as by attenuating the activation of microglia. Moreover, CBD administration at the time of viral infection exerts long-lasting effects, ameliorating motor deficits in the chronic phase of the disease in conjunction with reduced microglial activation and pro-inflammatory cytokine production. Adenosine A_{2A} receptors participate in some of the anti-inflammatory effects of CBD, as the A_{2A} antagonist ZM241385 partially blocks the protective effects of CBD in the initial stages of inflammation. Together, our findings highlight the anti-inflammatory effects of CBD in this viral model of MS, and demonstrate the significant therapeutic potential of this compound for the treatment of pathologies with an inflammatory component.

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CB_1 AND GPR55 RECEPTORS FORM FUNCTIONAL HETEROMERS IN STRIATUM: A POTENTIAL THERAPEUTIC TARGET FOR NEURODEGENERATIVE DISEASES

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Aim: The endocannabinoid system has emerged as a major player in brain neuromodulation, participating in the control of neurotransmitter release and regulating processes such as motor activity, memory and learning and motivational responses. Accordingly, it is known that the cannabinoid CB_1 and CB_2 receptors form functional heteromers in brain that are considered as therapeutic targets for neurodegenerative diseases. GPR55 receptor was considered a member of the cannabinoid receptor family, but it is not activated by cannabinoids and its endogenous ligand is L-α-lysophosphatidylinositol. GPR55 receptor activation may explain physiological effects that are non-CB_1/CB_2 mediated. This receptor is expressed in the CNS, although its exact function remains unclear. Based on the similar signalling, localization and distribution displayed by CB_1 and GPR55 receptors, the aim of this work is the study whether GPR55 form heteromers with CB_1.

Methods: CB_1-GPR55 receptor heterodimerization was studied in mammalian cells cotransfected with the cDNA for human GPR55 and CB_1 by Bioluminescence Resonance Energy Transfer, Reporter gene and ERK phosphorylation assays. Furthermore, the presence of CB_1-GPR55 heteromer in the brain was determined in rat striatum slices by measuring MAPK signalling.

Results: CB_1 receptor can form heteromers with GPR55 receptor in transfected mammalian cells. Within CB_1-GPR55 receptor heteromer, CB_1 receptor antagonist, SR141716, can block the effect of GPR55 receptor agonist, CID1792197, in MAPK activation. This cross-antagonism phenomenon was used as a "biochemical fingerprint" of CB_1-GPR55 receptor heteromers, allowing their identification in the rat striatum.

Conclusion: Taken together, the data suggest the occurrence of CB_1-GPR55 receptor heteromers in the striatum and more give insight into the mechanism by which CB_1 receptor can negatively modulate GPR55 receptor function, opening new avenues for drug discovery and therapeutic targets for neurodegenerative diseases.

Áreas Temáticas:

1ª: Neurociencia de sistemas
2ª: Nuevos métodos y tecnologías

cGKII KNOCKDOWN IMPAIRS SYNAPTIC VESICLE RECYCLING AND CONTRIBUTES TO THE FORMATION OF ENDOSOMAL-LIKE STRUCTURES AFTER STRONG STIMULATION IN SYNAPTIC BOUTONS

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Nitric oxide and cGMP have been implicated in the development of neurons from neurogenesis and neuron migration to synaptogenesis. This variety of physiological functions can be mediated by different effectors, including cGMP-regulated phosphodiesterases (PDEs), cGMP-dependent serine /threonine kinases (cGKs I and II) and cGMP-gated ion channels (CNGC). We have demonstrated that cGMP influences neurite outgrowth and synaptic bouton function and maturation in cerebellar granule cells, although the downstream mechanisms involved still remain unknown. We have investigated whether the cGKII, which expression increases with cell development and shows a synaptic localization, affects to the functionality of synaptic boutons.

We have found that cGKII knockdown or inhibition leads to a decrease of functional boutons in rat cerebellar granule cells. By using FM1-43 dye to track vesicle recycling we have found that synaptic boutons recycle their vesicles with different efficiencies (Bartolomé-Martín et. al. 2012). The lack of cGKII or its long lasting inhibition with KT5823 increased tremendously the proportion of boutons that recycled their vesicles inefficiently, observed as a strong labeling in response to a first stimulation but a weak loss of dye in response to a second stimulation. Moreover, cGKII knockdown or inhibition leads to ultrastructural changes related to the number, distribution and morphology of the vesicles in the boutons after strong stimulation. Cells that have undergo stimulation show an increase in endosomal-like organelles, structures that cannot render releasable vesicles.

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DJ-1 REGULATES ADULT NEUROGENESIS BY PROTECTING MITOCHONDRIA FROM OXIDATIVE STRESS

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Life-long neurogenesis is supported by neural stem cells residing in the subventricular zone (SVZ) of the adult mammalian brain. SVZ neural stem cells continuously generate neuroblasts which migrate to the olfactory bulb (OB) and functionally integrate there as mature interneurons. Neural stem cells have been described to have high endogenous levels of reactive oxygen species and regulate their self-renewal during brain development in a PI3K/Akt-dependent manner. Here we show that lentiviral knockdown of DJ-1 in the SVZ reduced the number of new neurons in the olfactory bulb. The proliferation, self-renewal and differentiation rate of neural progenitors was also affected, demonstrating a key role of DJ-1 in the coordination of the neurogenic process. However, lack of DJ-1 did not influence dendritic development. Moreover, increased mitochondrial superoxide accumulation, reduced mitochondrial membrane potential and ATP production were found in adult neural progenitors after DJ-1 loss of function. We propose that DJ-1 is essential to maintain mitochondrial homeostasis in oxidative stress conditions produced by high endogenous levels of reactive oxygen species in adult neural progenitors.

Áreas Temáticas: Seleccione las **2** áreas temáticas que más se ajusten a su trabajo en orden de prioridad:
1ª: Transtornos y reparación del sistema nervioso
2ª: Desarrollo

DRR1 AS A NEW MOLECULAR LINK BETWEEN STRESS, SYNAPTIC PLASTICITY AND BEHAVIOR

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The tumor suppressor gene down-regulated in renal cell carcinoma 1 (DRR1) is a glucocorticoid-dependent stress-inducible gene in the brain and was recently pointed as a new molecular link between stress, synaptic efficacy and behavioral performance [Schmidt et al, 2011]. In the adult mouse brain, DRR1 mRNA expression is high in the cerebellum and in limbic areas such as the hippocampal CA3 region and the lateral septum. Particularly in the CA3 region, DRR1 mRNA expression is up-regulated in a GR-dependent manner by a strong acute stressor.

Overexpression of DRR1 in mouse CA3 -by means of an adeno-associated viral (AAV) vector- enhanced cognitive flexibility, decreases long-term potentiation (LTP) magnitude and reduces spine density. These effects rely on the ability of DRR1 to directly modulate actin dynamics.
We currently aim to further dissect the physiological role of DRR1 in distinct brain areas, particularly in the CA3 and the septum. We used gain-of-function (overexpression) and loss-of-function (knockdown) approaches by stereotaxically injecting an AAV construct containing either the DRR1 coding sequence or a shRNA against DRR1 in the CA3 region and septum of mice. In the hippocampus, knockdown of DRR1 in the CA3 region significantly impaired cognitive performance as measured in the novel and spatial object recognition test. Moreover, electrophysiological recordings showed increased LTP and decreased paired-pulse facilitation at CA3-CA1 synapses in slices. In the septum, DRR1 mRNA expression was also up-regulated by acute stress. Stereotaxic septal overexpression of DRR1 increased social behavior but did not significantly modulate anxiety and cognitive-related behavior.

In summary, our data suggest that the stress-induced increase in DRR1 expression might play an intriguing role in modulating different aspects of behavior in response to stressful experiences. Moreover, this molecular link between stress, structural plasticity, neuronal actin dynamics and the modulation of behavior is brain region specific.

Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Neurociencia cognitiva y conductual

EARLY ONSET OF SYNAPTIC AND COGNITIVE DEFICITS IN A PS1/APP MODEL OF ALZHEIMER'S DISEASE

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Cognitive and memory decline in Alzheimer's disease (AD) is highly likely caused by synaptic dysfunction followed by neuronal loss. We have previously reported an early neuronal loss in the hippocampus of PS1M146L/APP751SL model of this pathology. The aim of the present work was to investigate the early synaptic alterations, analyzing the effect of Abeta plaques on synaptic morphology (in both pre- and post- synaptic elements) and efficiency, along with the onset of memory deficits using a variety of tasks designed to identify early subtle changes in cognition. Hippocampal synapses/dystrophic neurites were investigated by optic and transmission electron microscopy. Dendritic spines density was assessed by Golgi-Cox staining, mRNA and protein levels were measured by RT-PCR and Western-blot, respectively. The density (number/surface) of synaptic vesicles in the active zone was quantified at those presynaptic terminals close to the Abeta deposits, and found to be decreased in 4.5 month-old PS1/APP mice in both the molecular layer of dentate gyrus and stratum oriens of hippocampus proper. Moreover, there was also a correlation between this loss and the distance to the Abeta deposits. Some of these elements were abnormally swollen and contained abundant autophagic vesicles. However, they were making synaptic contacts onto morphologically normal dendrites with postsynaptic density. Finally, these mice presented mild cognitive deficits using the Morris water maze task at the early ages examined that correlated with the observed initial synaptic and axonal pathology. In conclusion, this AD model displays some of the initial synaptic changes of the human pathology and may represent a valuable tool to assess novel future treatments against this disease during the earlier stages. *Supported by FIS-PI12/01431 (AG) and FIS-PI12/01439 (JV).*

5. Trastornos y reparación del sistema nervioso
2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

ECTOPIC C-FIBERS WITH SPONTANEOUS ACTIVITY PREVAIL IN PARTIALLY DAMAGED SAPHENOUS NERVES

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Spontaneous pain is frequently present in patients with peripheral neuropathies; accordingly aberrant spontaneously active C-fibers have been recently recorded from human patients. Current animal models of neuropathy evaluate the progress of hyperalgesia and allodinia, but are unsuitable for the study of spontaneous pain; besides, the low numbers of spontaneously active C-fibers reported in these models, pose a difficulty to perform pharmacological studies.

Here we describe an experimental model in which 1/2 of the fibers showed ectopic discharges, of which 50% are spontaneously active.

Nerve-end neuromas were generated in a peripheral branch of the saphenous nerve in adult mice. Four weeks later, the saphenous nerve in continuity with the neuroma at the axotomized branch and the skin were excised and maintained *in vitro* and extracellular recordings from identified single units were performed.

Most of the fibers examined (80/83) fired upon electrical or natural (mechanical or thermal) stimulation of the neuroma. Of those, 23 responded also to natural stimulation of the skin. Six units showed post-discharges to mechanical stimulation of their receptive fields and 2 units showed erratic responses to repetitive stimulation. Interestingly, 20/83 were spontaneously active with a mean frequency of discharge of 0.27 + 0.07 Hz Fifty % of those fibers lacked thermal responsiveness.

In conclusion, 24% of the fibers examined in partially damaged nerves, present ectopic spontaneous discharges (80% of which were C-units). Furthermore, as our model enables identifying the origin of the fiber (neuromatose/damaged vs peripheral skin/“presumed intact”), we could observe that 28 % of the units had inputs from both the neuroma and the peripheral skin.

Although we cannot infer the underlying mechanisms of the prevalence of fibers with ectopic discharges in a partially damaged peripheral nerve, communication between damaged and undamaged units by ephaptic contacts and/or collateral sprouting fibers seems to be a plausible mechanism.

EXPANSION OF Tbr2 INTERMEDIATE PROGENITORS IN THE DEVELOPING MOUSE CORTEX VIA CB_1 CANNABINOID RECEPTOR/mTORC1 SIGNALING

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The CB1 cannabinoid receptor regulates different aspects of cortical development and neural progenitor proliferation. Here we aimed at unravelling how this receptor drives the expansion of the intermediate cortical progenitor pool in the developing mouse brain. We achieved this by using a wide array of pharmacological and genetic gain and loss of function approaches, both in *in vitro* and *in vivo*. Pharmacological stimulation of CB1 receptor signaling in cortical slices and progenitor cell cultures increased Tbr2-expressing progenitor generation and mTORC1 pathway activity, assessed as the phosphorylation of its substrate the ribosomal protein S6, while acute CB1 receptor ablation exerted the opposite effects. Luciferase reporter assays based on Pax6-responsive elements and the Tbr2 promoter, together with ChIP analysis, showed that the CB1 receptor drives Tbr2 expression downstream of Pax6 induction in an mTORC1-dependent manner. Examination of CB1 receptor-deficient mouse embryos revealed premature cell cycle exit of cortical progenitors, decreased number of radial glial (Pax6+) and Intermediate progenitor (Tbr2+) cells, and reduced mTORC1 activation in the ventricular and subventricular zone. Likewise, CB1 receptor knockdown *in utero* reduced Tbr2-positive cell generation. Characterization of CB1 receptor expression in samples from patients of type IIb focal cortical dysplasia and tuberous sclerosis, two diseases characterized by developmental focal cortical malformations associated to overactivation of the mTORC1 pathway, revealed an enrichment of CB1 receptor expression in phospho-S6-positive cells. Altogether, our results demonstrate that the CB1 receptor exerts a crucial role in tuning dorsal telencephalic progenitor proliferation by inducing the Pax6/Tbr2 axis via the mTORC1 pathway.

EXTRACELLULAR CALCIUM CONTROLS THE EXPRESSION OF PHYSIOLOGICAL AND PATHOLOGICAL HIPPOCAMPAL RIPPLE OSCILLATIONS

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Physiological ripple oscillations (100-200 Hz) are recorded during non-theta states and are proposed to play a role in memory consolidation. In contrast, pathological fast ripples (200-600 Hz) originate from similar areas from the hippocampus in temporal lobe epilepsy and represent markers of epileptogenesis. Here, we examined the cellular and network dynamics underlying the expression of normal and pathological forms of ripple oscillations in vivo and in vitro. Using multi-site silicon probes, we recorded ripples and fast ripples from freely moving and urethane-anesthetized normal and epileptic rats. We found ripple and fast ripples share a common spectral structure, but differ in their dominant frequency component and local field potential signatures. We then asked whether physiological ripples in vivo could be transformed into fast ripples by locally manipulating extracellular ionic composition using a novel integrated tetrode-fluidic probe. We found that lowering Ca2+ in situ (0-1 mM) transformed the characteristic ripple positivity into a spikier component that slightly accelerated, giving a fast ripple like power spectrum. This effect ran in parallel with an impairment of paired-pulse inhibition and enhanced pyramidal cell excitability. Increasing Ca2+ up to 4 mM did not affect ripple expression and paired-pulse inhibition. We then looked at the effect of extracellular Ca2+ in the expression of different forms of ripple oscillations in vitro. Using 2.5-3 mM Ca2+ resulted in the emergence of ripple like oscillations with similar spectral features than in vivo ripples. In contrast, using 1-1.5 mM Ca2+ resulted in the emergence of larger and slightly accelerated cycles (>200 Hz) similar to in vivo fast ripples. Using paired recordings we found that lowering Ca2+ from 3 mM to 1 mM resulted in enhanced cell excitability and failure of GABA release that impairs disynaptic inhibition and promote excessive synchronization of pyramidal cell firing. We conclude that extracellular Ca2+ in a physiological range has major effects on neuronal excitability and dynamics in situ.

Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.

HIPPOCAMPAL LOCAL FIELD POTENTIAL SIGNATURE OF A NOVEL EXPERIENCE

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Novelty induces plastic processes at hippocampal synapses and facilitates the consolidation of memories encoded simultaneously with the novel experience (Moser et al. 1993; Wang and Morris 2010). In our group, we have previously shown that synaptic plasticity in the dentate gyrus drives the reorganization of functional connectivity in long-range hippocampal networks supporting memory (Canals et al. 2009; Álvarez-Salvado et al. 2013). Recently, we have further demonstrated that such phenomenon involves an inhibitory gating of activity propagation in the hilus. This mechanism is operated by synaptic plasticity (i.e. triggered by Long-Term Potentiation) and is hypothesized to facilitate hippocampal-cortical interactions. Therefore, we wondered whether a similar mechanism could be involved in the novelty-induced facilitation of memory consolidation. To address this question we performed multichannel electrophysiological recordings of spontaneous local field potentials (LFPs) as well as evoked activity in non-anesthetized freely moving animals exposed to novel environments. We implanted adult Long-Evans rats with array electrodes (Michigan probes) and platinum-iridium stimulating electrodes targeted to the dorsal hippocampus (across CA1 area and dentate gyrus) and perforant pathway, respectively. Analysis of the electrophysiological signals based on independent component analysis (ICA) combined with electrophysiological protocols of pair-pulse facilitation and pharmacological manipulations allow us to dissect the on-going activity of LFP generators including inhibitory networks. Interestingly, novelty significantly depressed the inhibitory tone in the hilus of the dentate gyrus. Concomitantly, we observed alterations in the temporal structure of the LFPs recorded in different hippocampal sub-areas. These results are in good agreement with previous findings from the lab and suggest that novelty facilitates activity propagation in hippocampal networks by modulating the hilar inhibitory tone.

Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Neurociencia cognitiva y conductual

IDENTIFICATION OF GENES INVOLVED IN GABAergic SYNAPTOGENESIS

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Synapse formation during postnatal development is one of the most determining processes in brain circuits wiring. Whereas numerous molecular players have been identified in glutamatergic synaptogenesis in the cortex, few are the ones identified specifically in GABAergic interneurons, mainly because of the difficulty of isolating those cells. We have now the tools to explore these neurons in detail, thus we search for genes particularly implicated in GABAergic synapse formation in the cortex with the aim to get more insight into the molecular mechanisms that mediate this process.

To choose the proper time point of the study, we first quantified the number of putative glutamatergic and GABAergic boutons in the developing cerebral cortex by using VGLUT-1 and GAD65 presynaptic markers. We observed that the number of both GABAergic and Glutamatergic synapses formed is increased along development from postnatal day 5 (P5) to P30. We found that the highest synaptogenic rate takes place at P10-P12 and therefore we defined this time point as the most suitable for identifying synaptogenic genes.

GABAergic interneurons and glutamatergic pyramidal cells were sorted by fluorescent activated cell sorting (FACS). Subsequently, mRNA was extracted from the isolated cells, amplified and hybridized to gene expression microarrays to assess which genes are up or down-regulated along this process. The *in silico* bioinformatic analysis of microarray data and later comparison between different groups generated several lists of genes differentially expressed during GABAergic and/or glutamatergic synaptogenesis. The validity of the experimental approach is confirmed by the presence of several genes known to play a role in synaptic function and formation including signaling molecules and receptors. This result suggests that many of the identified genes with unknown function might also participate in different steps of synapse development.

Áreas Temáticas:

1ª Desarrollo
2ª Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.

INCREASED BETA OSCILLATORY ACTIVITY IS NOT A NECCESARY PHYSIOLOGICAL FEATURE OF THE EARLY PARKINSONIAN STATE

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Enhanced beta oscillatory activity in the basal ganglia is generally accepted as a functional feature of the parkinsonian state as shown by recording in patients and in animal models. Here, we show that increased beta band appears when the nigro-striatal lesion is very severe but it is not a feature of the early parkinsonian state.

We used different degrees of 6-OHDA-induced lesion in the rat and recorded oscillatory activity with chronic implanted electrodes in the SNpr, comparing the effect of a complete (6 µg of 6-OHDA) and partial (3 µg of 6-OHDA) unilateral lesion in the MFB in order to ascertain how the severity of dopaminergic depletion influences activity in the beta range. We also recorded a more progressive dopaminergic lesion by intracerebroventricular (icv) 6-OHDA administration over 7 days (700 µg of 6-OHDA as final dose). Different behavioral tests were performed in order to evaluate the motor features. Local Field Potential (LFP) recordings in SNpr showed a clear peak of oscillatory activity in the high beta/low gamma frequency range (25-40Hz) was just observed after the complete unilateral 6-OHDA lesion (Tyrosine Hidroxilase (TH+) cell loss: 97%). A similar activity was not present in animals with partial 6-OHDA lesion through either MFB injections or icv 6-OHDA administration (TH+ cell loss: 72% for MFB-partial and 65% for icv-partial).

These results suggest that the basal ganglia-cortical network becomes hyper-synchronous in the beta range only when nigro-striatal DA depletion is severe, although initial motor deficits may appear before such physiological changes. Accordingly, the emergence of enhanced beta activity could be taken as an indicator of defective compensatory mechanisms and a target for new therapies in Parkinson's disease.

1. Áreas temáticas: Opción 6: Trastornos y reparación del sistema nervioso y
Opción 4: Neurociencia de sistemas

INCREASED LEVELS OF IGF-1 AND IL-1β WITHIN NEURONS – BUT NOT WITHIN GLIAL CELLS – AS A PLASTIC MECHANISM INVOLVED IN REPAIRING SYNAPTIC HOMEOSTASIS IN THE COCHLEAR NUCLEUS FOLLOWING COCHLEAR ABLATION

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Upregulation of activity-dependent molecules such as insulin-like growth factor 1 (IGF-1) and interleukin-1β (IL-1β) is one of the main mechanisms used by neurons and glial cells to promote repair following brain injury. IGF-1 is crucial for restoring synaptic transmission in auditory nuclei following hearing loss; however, whether IL-1β is also involved in this process remains unknown. In this study, we evaluated the expression of IGF-1 and IL-1β within neurons and glial cells of the ventral cochlear nucleus in adult rats at 1, 7, 15 and 30 days following bilateral cochlear ablation. Following deafferentation, significant increases in IGF-1 levels within cells of the cochlear nucleus were observed at 1, 7 and 15 days compared with control animals. The expression and distribution of IL-1β in the ventral cochlear nucleus of ablated animals was temporally and spatially correlated with IGF-1. We also observed a lack of colocalization between IGF-1 and IL-1β with either astrocytes or microglia at any of the time points following ablation. In conclusion, the present findings indicate that impairment of synaptic transmission by removal of cochlear inputs leads to a series of cellular events within the cochlear nucleus that activate both neurons and non-neuronal cells such as microglia and astrocytes. Interplay between these distinct activated cell types may lead to the induction of synaptic repair mechanisms.

Áreas Temáticas:
1. Neurociencia de sistemas.
2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.

GLIAL-NEURONAL INTERACTIONS IN ADULT RETINA CELL CULTURES

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Introduction: Following retinal disease or injury, for example in glaucoma or retinal ischemia, axonal degeneration and death of retinal ganglion cells (RGC) results in irreversible blindness. The retinal glial cells, astrocytes and Müller glia, provide structural and trophic support to RGCs in the healthy retina and may also have a function in promoting cell survival after injury.

Objective: We are interested in understanding the mechanisms of regeneration in adult RGCs and how glial plasticity may be influencing this process. In rat models of glaucoma and ischemia, retinal glia were found to adopt a branched morphology, suggesting that there exists a degree of cell plasticity, which may have functional significance.

Methods: In this study, we have investigated the relationship between adult rat glia and RGCs in the healthy and damaged retina *in vivo* and also how these cells interact *in vitro*. Using a cell culture system, we have investigated the interactions between retinal glia and RGCs using cells isolated from adult rat.

Results: The organisation of astrocytes in the retina of a rat model of glaucoma was found to be significantly different than in a healthy retina. These alterations in glial cell morphology may reflect changes in their relationship with RGCs. We found that cultured adult RGCs in close contact with adult Müller cells exhibit improved cell viability and significant neurite elongation. Müller cell conditioned media was also found to have a positive effect on RGC growth, however to a lesser extent.

Conclusion: These results suggest that Müller glia support RGC regeneration not only by direct interaction, but also releasing soluble trophic factors. Further understanding of the relationship between retinal glia and RGCs is important in order to identify potential therapeutic targets to encourage retinal neuroregeneration.

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Subject Areas:
1st: 5.Trastornos y reparación del sistema nervioso
2nd: 2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

LA DISFUNCIÓN SINÁPTICA INDUCIDA POR EL PÉPTIDO *β*-AMILOIDE EN LA SINAPSIS SEPTOHIPOCAMPAL FIMBRIA-CA3 IMPLICA A LOS CANALES GirK

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El sistema septohipocampal juega un papel fundamental en los procesos de aprendizaje y memoria. Diversos estudios han mostrado su implicación en los estadíos tempranos de la enfermedad de Alzheimer (EA), donde la alteración de la actividad de redes neurales subyace al deterioro cognitivo y los déficits de aprendizaje y memoria. El objetivo del presente trabajo ha sido investigar el mecanismo de acción del péptido *β*-Amiloide (A*β*), tratando de dilucidar cómo puede perturbar el equilibrio existente entre los sistemas de neurotransmisión excitador-inhibidor del sistema septohipocampal necesario para su correcto funcionamiento.

Para alcanzar los objetivos propuestos se realizó un abordaje multidisciplinar, incluyendo técnicas de electrofisiología clásica (registro intracelular con electrodos finos) combinadas con técnicas de biología molecular (RT-PCR cuantitativa). La preparación utilizada en ambos casos fueron rodajas que incluían el extremo septal de la fimbria y el hipocampo para estudiar los efectos del A*β* sobre la sinapsis septohipocampal fimbria-CA3.

Nuestros resultados muestran que en las células piramidales de la región CA3 del hipocampo, el péptido A*β* induce tres efectos principales: una despolarización de la membrana, un aumento en la resistencia de entrada y una disminución de la respuesta postsináptica GABAérgica metabotrópica. Estos efectos fueron mimetizados por bloqueo del efector del receptor GABAB, los canales de potasio rectificadores de entrada acoplados a proteína G, GirK. Además, al estudiar los cambios inducidos por A*β* en el patrón de expresión génica hipocampal encontramos alteraciones en los niveles de mRNA de distintas subunidades de canales, entre ellas en los canales GirK.

Nuestros resultados nos permiten proponer los cambios observados en la función del canal GirK de las neuronas piramidales de CA3 como un mecanismo potencial para la alteración inducida por el A*β* en la red septohipocampal.

Agradecimientos: MINECO (BFU2011-22740, SAF2010-14878, BFU2009-07341, RYC-2009-03827) y Fundación Eugenio Rodríguez Pascual.

1ª: Trastornos y reparación del sistema nervioso
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

LIPIDOME ANALISYS IN MULTIPLE SCLEROSIS REVEALS PROTEIN LIPOXIDATIVE DAMAGE AS A POTENCIAL PATHOGENIC MECHANISM

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Aim: The objective of this work was study the changes induced by MS in metabolome and lipidomic profile of cerebrospinal fluid (CFS).

Methods: We have applied Metabolomic and lipidomic analyses, both in targeted and untargeted approaches, to the study of cerebrospinal fluid (CSF) samples of multiple sclerosis (MS) patients (n=9), compared with samples of non-MS individuals (n=9) using mass-spectrometry. We have used western-blot and analyzed cell culture to confirm pathogenic pathways suggested by mass-spectrometric measurements.

Results: The results of the untargeted approach of metabolomics and lipidomics suggest the existence of several metabolites and lipids discriminating both populations. Applying targeted lipidomic analyses focused to a pathogenic pathway in MS, oxidative stress, reveal that the lipid peroxidation marker 8-isoprostaglandin F2α is increased in CSF from MS patients. Furthermore, as lipid peroxidation exerts its pathogenical effects through protein modification, we studied the incidence of protein lipoxidation, revealing specific increases in carboxymethylated, neuroketal and malondialdehyde-mediated protein modifications in proteins of CSF from MS patients, despite the absence of their precursors glyoxal and methylglyoxal. Finally, we report that the level of neuroketal-modified proteins correlated with a hitherto unknown increased amount of autoantibodies against lipid peroxidation-modified proteins in CSF, without compensation by signaling induced by lipid peroxidation via peroxisome proliferator-activated receptor γ (PPARγ).

Conclusion: The results, despite the limitation of being obtained in a small population, strongly suggest that autoimmunity against in situ produced epitopes derived from lipid peroxidation can be a relevant pathogenic factor in MS.

Thematic Areas: 1. Nuevos métodos y tecnologías.
2. Trastornos y reparación del sistema nervioso

METHAMPHETAMINE CAUSES DEGENERATION OF DOPAMINE CELL BODIES AND FIBERS OF THE NIGROSTRIATAL PATHWAY EVIDENCED BY SILVER STAINING

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Background: Methamphetamine is a widely consumed illicit drug with high abuse potential. Recent epidemiological studies showed that methamphetamine abuse increases the risk for developing Parkinson's disease. Studies in animals have shown that this drug produces dopaminergic neurotoxicity in the nigrostriatal pathway. We examined the effect of repeated low and medium doses versus single high dose of methamphetamine on degeneration of the dopaminergic system, including terminals in the striatum and cell bodies in the SNpc.
Methods: Mice were given methamphetamine using one of three protocols: three i.p. injections of 5 or 10 mg/kg at 3 hour intervals or a single 30 mg/kg injection. The integrity of dopaminergic fibres and cell bodies were assessed 1 and 3 days after methamphetamine by TH immunohistochemistry and silver staining.
Results: All three administration protocols induced significant hyperthermia and loss of striatal dopaminergic fibers. The 3x10 protocol yielded the highest effect, followed by the 3x5 and 1x30. Some degenerating axons could be followed from the striatum to the SNpc. All three protocols induced similar significant degeneration of dopaminergic neurons in the SNpc, evidenced by the presence of amino-cupric-silver stained dopaminergic neurons. Both necrosis and apoptosis were apparent in degenerating neurons. Silver staining was also observed in striatal neurons after methamphetamine. By using D1-Tmt/D2-GFP BAC transgenic mice, we observed that striatal degenerating neurons were equally distributed between direct and indirect projection pathway neurons and interneurons.
Conclusions: These data provide direct evidence that methamphetamine administration to mice causes destruction of striatal neurons and dopaminergic fibers, along with significant irreversible degeneration of cell bodies in the SNpc.

Áreas Temáticas
Trastornos y reparación del sistema nervioso

MODULATING INCREASED HIGH FREQUENCY OSCILLATIONS IN THE PREFRONTAL CORTEX IN AN *IN VITRO* MODEL OF SCHIZOPHRENIA

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Objetives: Hypofunction of NMDA receptor (NMDAr) has been widely used as a model of schizophrenia. Underactivation of these receptors has been related with aberrant increase in gamma synchronization and disrupted excitation-inhibition balance on prefrontal cortex (PFC). Our objective was to develop an *in vitro* model of schizophrenia for a better understanding of PFC dynamics in these conditions. Additionally, antipsychotic drugs and modulation of the network with electric fields were tested in order to modulate NMDAr hypofunction effects.

Material and Methods: Extracellular recordings were obtained from prefrontal cortical slices from adult ferrets. Slices were placed in an interface style recording chamber and bathed in different ACSF solutions. These cortical slices *in vitro* generated slow (≤1Hz) oscillations showing synchronized fast rhythms (10-100 Hz) during Up states. We explored the effect of progressively increasing the concentration of NMDAr antagonist, dizocilpine maleate (MK-801) as an in vitro model of schizophrenia. The effect of antipsychotic drugs clozapine and haloperidol and of a uniform electric field were explored**.**

Results: The average power of high frequencies was significantly increased during Up states with MK-801. Besides, this slow oscillation pattern suffered gradual changes with NMDAr blockade: oscillation frequency and Up state duration decreased while Down state duration increased. Clozapine application could prevent NMDAr hypofunction effect, while haloperidol had no significant effect. Electric fields of the appropriate orientation were powerful modulators of the altered slow and fast rhythms.

Conclusions: PFC network *in vitro* can be used as an *in vitro* model of schizophrenia. Results obtained with the antipsychotic drugs suggested that acting on the serotonergic system with clozapine is one way of preventing electrophysiological effects depending on NMDAr hypofunction. Further, proper electrical stimulation can achieve highly specific spatiotemporal control of the aberrant activity and it remains to be explore if this is extensive to some of the symptoms.

Áreas Temáticas:
1ª: Neurociencia de sistemas.
2ª: Trastornos y reparación del sistema nervioso

PROPIEDADES DE LA ADAPTACIÓN E UN ESTÍMULO TÁCTIL REPETITIVO EN LA CORTEZA DE BARRILES DE LA RATA

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La adaptación es una forma de aprendizaje no asociativo, entendida como la disminución de la frecuencia de descarga de las neuronas ante una estimulación repetitiva. Hemos realizado registros unitarios en la corteza de barriles en ratas adultas, anestesiadas con uretano y a lo largo de la vía somestésica. Se estimuló con un tren de 40 estímulos en una única vibrisa que consistían en un soplo de aire de 20 ms y 1-2 atm. La frecuencia de los estímulos en el tren fue de 1-8 Hz. El tren de estímulos se dividió en 4 periodos de 10 estímulos y comparamos el porcentaje de respuesta considerando el primer periodo como 100%. En la capa 2/3, encontramos que la respuesta disminuyó rápidamente a todas las frecuencias de estimulación, llegando a un porcentaje de adaptación del 71% a 5 Hz. En la capa 5/6 la adaptación fue similar, alcanzando un porcentaje de adaptación del 76%. La aplicación de acetilcolina en la corteza de barriles disminuyó la adaptación, alcanzando valores de un 38% en capa 2/3 y 56% en capa 5/6 respecto al primer periodo de estimulación. La inhibición provocada por interneuronas de la corteza de barriles no fue responsable de la adaptación ya que la aplicación de muscimol, agonista de receptores $GABA_A$, no aumentó la adaptación. Para determinar si la adaptación tiene su origen en la corteza cerebral, realizamos registros unitarios en el núcleo del trigémino principal e interpolar y en núcleo ventral posteromedial del tálamo. La adaptación en el núcleo principal del trigémino fue de un 76% mientras que en el tálamo alcanzó el 55%. Estos resultados indican que la adaptación se produce a lo largo de la vía somestésica y es mayor a nivel de la corteza somestésica, probablemente por cambios en las propiedades electrofisiológicas de estas neuronas.

PTEN OVEREXPRESSION ALTERS SYNAPTIC DENSITY, SOCIAL BEHAVIOR AND FEAR MEMORY FORMATION

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PTEN (phosphatase and tensin homologue deleted on chromosome 10) is a negative regulator of the PIP_3 (phosphatidylinositol-(3,4,5)-triphosphate) signaling pathway, that modulates growth, proliferation and cell survival. PTEN is highly expressed in the brain, where it controls neuronal growth, synaptogenesis and synaptic plasticity (Jurado *et al*, 2010). There is also evidence that the PIP_3 pathway, and PTEN in particular, control social behavior and cognitive function, as evidenced from the association between some forms of autism and PTEN deficiency (Kwon *et al*, 2006; Zhou & Parada, 2012). To further explore the importance of PTEN to synaptic organization and cognitive function, we have employed a new transgenic mouse presenting moderate overexpression of PTEN ($Pten^{tg}$ mice, Ortega-Molina *et al*, 2012). At the structural level, $Pten^{tg}$ mice display decreased synapse density in both hippocampal CA1 and lateral amygdala. In addition, synapses are smaller in the lateral amygdala but normal in the CA1 region. At the behavioral level, $Pten^{tg}$ mice display lower anxiety, higher sociability, and impaired amygdala-dependent fear memory, as determined with elevated plus-maze, social recognition test and cued-fear conditioning, respectively. In contrast, hippocampal dependent learning and memory were normal in $PTEN^{tg}$ mice. To conclude, this study strengthens the role of PTEN in synapse organization and cognitive function.

Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

RESPONSE TO COMPLEX PATTERNS OF REGULARITY IN THE INFERIOR COLLICULUS OF THE ANESTHETIZED RAT

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Single neurons in the inferior colliculus (IC) of the rat show a specific decrement of response to simple repetitive stimuli, or stimulus specific adaptation (SSA).

This ability to encode the complex auditory past in multiple time scales would enable the auditory system to generate expectations about the incoming stimuli.

So far, SSA has been studied in animal models using the classical oddball paradigm, yet it remains to be determined whether violations of more complex regularities can also be detected at the single neuron level in the form of expectation suppression, as they are in humans at the population level through EEG and MEG.

We performed experiments on female Long-Evans rats anesthetized with urethane (1.5 g/kg, i.p.) to make extracellular recordings of well-isolated single units in the IC. Each stimulus was presented as “expected” (embedded in a regular pattern) or “unexpected” (breaking a regularity) and their responses were compared to a corresponding control “random” condition having the same stimulation context. Thus, the only difference between experimental and control conditions was the degree of expectancy or “predictability” of the stimulus.

We have recorded data from both strongly adapting (high SSA) and non-adapting (low SSA) neurons and we have found signs of pattern violation sensitivity in some neurons from both classes, as a difference between the response to a stimulus under the expected or unexpected conditions and their respective control conditions. Furthermore, for two out of the three patterns used, we found a positive correlation between the pattern recognition index (PRI) and common SSA index (CSI) at the population level.

These preliminary results suggest that there is a subpopulation of IC neurons sensitive to pattern regularity breaks, and that the neurons that show deviancy detection are more likely to have the ability to code for complex patterns of regularity.

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1. 1ª: Neurociencia de sistemas

2. 2ª: Neurociencia cognitiva y conductual

ROBO1 SIGNALLING IS MODULATED BY FLRT3, A NOVEL INTERACTING PROTEIN

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The correct projection of the growing axons during nervous system development is controlled by axon guidance cues that act through specific receptors located at the growth cones. How this restricted number of molecules can organize the entire nervous system connectivity is, however, poorly understood. Here we show one example of how one of these receptors, Robo1, can be modulated to fine tune its response to its cognate ligand Slit1.

The Fibronectin Leucine Rich Transmembrane proteins (FLRT1-3) have been previously shown to be important during nervous system development[1]. In order to elucidate the intracellular signalling mechanisms of FLRTs we performed a yeast two-hybrid screening using the intracellular domain of FLRT3 as a bait and a fetal human brain cDNA library as a prey. Interestingly, we found a clone coding for Robo1 among the possible interactors. We confirmed this interaction in HEK2937T cells by immunoprecipitation and immunofluorescence assays. Deletion of the intracellular domains of FLRT3 or Robo1 impaired the co-immunoprecipitation, suggesting that the interaction happens in *cis*, when the two proteins are expressed in the same cell. We also constructed single deletion mutants of the CC1, CC2 and CC3 domains of Robo1 and found that any of them was necessary for the interaction. FLRT3 expression is high in the rostral thalamus where Robo1 regulates the attraction of these axons to the anterior cortex[2]. We were able to detect Robo1-FLRT3 interaction in dissociated cultures of rostral thalamic neurons suggesting that FLRT3 could modulate Robo1 signalling in this context. Indeed, we provide evidence that FLRT3 regulates Slit1-Robo1 signalling at the level of the RhoA-ROCK pathway and we suggest that this is important for the attraction of rostral thalamic axons to the cortex.

To summarize, FLRT3 is a new regulating partner for Robo1 signalling in axon guidance.

[1]Yamagishi et al., 2011.
[2]Bielle et al., 2011.

Áreas Temáticas:
1ª: Desarrollo

SCWAHNN CELLS TRANSFECTED WITH A LENTIVIRAL VECTOR OVEREXPRESSING FGF-2: IN VITRO AND IN VIVO EFFECTS TO PREFFERENTIALLY PROMOTE MOTORNEURON REGENERATION

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Fibroblast growth factor 2 (FGF-2) is a trophic factor expressed by glial cells and different neuronal populations. Addition of FGF-2 to spinal cord and dorsal root ganglia explants increases specifically neurite outgrowth of motor neurons. With the aim to further explore the potential capability of FGF-2 to selectively promote motor regeneration in vivo, we have produced a lentiviral vector (LV) that overexpresses FGF-2. Addition of cultured Schwann cells infected with FGF-2 into a collagen matrix embedding spinal cord or dorsal root ganglion (DRG) explants significantly increased motor neurite outgrowth but not sensory neurite outgrowth when compared to co-cultures transduced with LV-GFP, thus demonstrating that the LV construct was as effective as direct addition of the trophic factor to promote motor axon growth. Schwann cells transduced to overexpress FGF-2 were grafted in a silicone tube to repair a 6 mm defect in the rat sciatic nerve. In LV-FGF-2 group, all animals show regenerative cables with myelinated fibers at one month. Number of myelinated fibers was slightly higher in LV-FGF-2 group compared to GFP-LV, with a tendency to have a higher proportion of motor regenerating axons. Onset of musucular reinnervation, evaluated by electrophysiology tests, was earlier in LV-FGF2 animals.

Áreas temáticas.

1. Trastornos y reparación del sistema nervioso.
2. Neurociencia de sistemas.

STATISTICAL WIRING OF THALAMIC RECEPTIVE FIELDS OPTIMIZES SPATIAL SAMPLING OF THE RETINAL IMAGE

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It has been widely assumed that retinal mosaics set the limit on visual resolution. However, cat visual acuity is higher than would be expected from the density of On or Off X- retinal ganglion cells (RGCs) alone, suggesting that other factors improve the resolution visual processing. Retinal output reaches the primary visual cortex (V1) through relay cells in the dorsal lateral geniculate nucleus (LGN) of the thalamus which often receive convergent retinal inputs. Thus visual space is rewired, transforming the retinal message sent to V1.

In cat, LGN relay cells outnumber RGCs. Retinothalamic convergence, combined with this increase in cell number from stage to stage could provide an interpolated map of visual space. If thalamic RFs were interpolated versions of their retinal precursors, then the array of RFs in the thalamus would have a greater discrimination capacity than the retinal mosaic, an improved coverage of visual space and a higher signal-to-noise ratio (SNR).

To test these and associated functional consequences of retinothalamic resampling we constructed a statistical connectivity model based on the synaptic structure of retinal and thalamic RFs. The scheme of retinothalamic convergence that the results of our analysis predict explains the empirically determined level of visual acuity in cat (9 cycles/degree). The benefits of interpolation, increased spatial resolution, SNR and coverage, come at a cost, however. Interpolation blurs the image, reducing local contrast to degrade edge perception. We have found experimental evidence for a solution to this problem. Our results show that the anatomical organization of relay cells and interneurons in the LGN produce physiological arrangements of excitation and inhibition within the RF-centers that effectively boost contrast borders, thereby increasing the dynamic range of the visual message through the LGN. Thus, the circuit design we describe operates like most local contrast enhancement techniques used in digital image processing.

Áreas Temáticas:
1ª: Neurociencia de Sistemas
2ª: Neurociencia cognitiva y conductual

THE E3 UBIQUITIN LIGASE APC/C-CDH1 REGULATES NEUROGENESIS DURING BRAIN DEVELOPMENT

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Objective

The morphology of the adult brain is the result of a delicate balance between neural progenitor proliferation and the initiation of neurogenesis in the embryonic period. Although the length of the G1-phase of the cell cycle in progenitor cells is known to be a key factor, the specific molecular events controlling neurogenesis are yet undefined. Using an embryo-restricted Cdh1 knockout mouse model, we assessed whether the anaphase-promoting complex/cyclosome (APC/C) cofactor, Cdh1, which regulates mitosis exit and G1-phase length in dividing cells, regulates neurogenesis *in vivo*.

Material and Methods

Brains from embryos E14 were extracted from wt or Cdh1 knockout (-/-) mice and fixed with 4% paraformaldehyde. The sagittal plane sections (5 or 20 μm) were processed for immunohistochemistry analysis using Nestin (neuronal progenitor), Tuj1 (early-) and Map2 (mature-differentiated neurons), BrdU incorporation and Ki67 (proliferation), active-Caspase3 and TUNEL (apoptotic death), pH2AX and p53 (DNA damage, replicative stress) and pH3 (mitotic marker).

Results

Genetic ablation of Cdh1 increased proliferation of neuronal progenitor cells in the ventricular/subventricular zones (VZ/SVZ) of the cerebral cortex at the expense of a reduction of newly differentiated neurons. This caused a significant reduction in cerebral cortex length. In addition, loss of Cdh1 triggered replicative stress, mitotic cell acccumulation and apoptotic cell death in the VZ/SVZ. Thus, loss of Cdh1 causes aberrant cell cycle progression in progenitor cells and delays their exit from the cell cycle to generate postmitotic neurons. In turn, Cdh1 ablation causes genomic damage triggering p53-dependent apoptotic death in VZ/SVZ neural progenitors of the cerebral cortex.

Conclusions

Our results demonstrate that APC/C-Cdh1 coordinates cortical neurogenesis and size, thus posing Cdh1 in the molecular pathogenesis of congenital neurodevelopmental disorders, such as microcephaly.

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THE GFP-NEURAL CELL TRANSPLANTATION AND INTRAMEDULLAR INJECTION OF SYNTHETIC COMPOUND INDUCES A RECOVERY IN RATS WITH SPINAL CORD INJURY.

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The aldaynoglia, a subpopulation of glia cells, promotes myelination and axonal regeneration, and these are found in different locations in the central nervous system (CNS) such as olfactory bulb, hypothalamus, pituitary, spinal cord, and so on. Our group has described how to obtain large amounts of aldaynoglia cells from neural stem cells (NSCs) performing neurospheres and this procedure has been increased their availability. Here we studied the role of synthetic glycolipid in the propagation and differentiation of NSCs into aldynoglía and the effect of these new compounds on growth, regeneration and myelination of axons in vitro. We combined the study of NSCs transplantation therapies and the injection of synthetic molecule in spinal cord contusion model. The repairing of CNS lesions was evaluated by: cytoskeletal markers (GFAP, neurofilament, etc.), cell migration, neuritic growth and myelination of axons promoted by the activity of the synthetic agent and / or aldaynoglia. The functional recovery of animals was followed by standardized behavioral test, BBB. Also we have found evidence of a therapeutic route that it is specific for glial and microglial cells, and which did not interfere with the functionality of neurons and oligodendrocytes. We conclude that synthetic compound has a high activity as an inhibitor of astrocyte growth and microglia, and supported the axonal growing promoted by aldaynoglia cells.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

TRESK AND TREK CHANNELS IN SENSORY AND MOTOR GANGLIA. A MOLECULAR AND FUNCTIONAL STUDY.

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Objectives: TREK and TRESK channels are subfamilies of two pore domain potassium channels (K2P) implicated in the control of resting membrane potential and excitability. They are expressed in peripheral ganglia (sensorial and motor) and they exert a powerful effect on those membrane properties.
The main goal of this study is to determine which members of those subfamilies are present in the superior cervical (SCG) and nodose ganglia (NG) from mice and study their electrophysiological properties from a single channel perspective.

Material and Methods: mRNA levels for the TRESK and TREK subfamilies were quantified by qRT-PCR using TRESK as calibrator. Protein expression was studied by immunocytochemistry from cultured SCG and NG neurons and functional expression and electrophysiological properties were characterized by single-channel recording.

Results: TRESK and all members of the TREK subfamily (TREK-1, TREK-2 and TRAAK) are present in neurons from both ganglia. mRNA levels were TRESK>TREK-2>TREK-1>TRAAK for SCG and TRESK>TREK-1>TREK-2>TRAAK for NG. Single channel recording show mainly TRESK and TREK-2 channel activity in SCG while in NG TRESK and TREK-1 are the main active channels. P_O, dwell time, amplitude and voltage dependency were also determined for each subunit. TRESK, TREK-1 and TREK-2 show a clear increase of P_O with the depolarization in both ganglia.

Conclusions: All TRESK and TREK channels are expressed in SCG and NG ganglia. TRESK mRNA is the most expressed in both NG and SCG, followed by TREK-2 in SCG and TREK-1 in NG. From a functional point of view the majority of the cells show TRESK and TREK-2 single-channel activity in SCG, while in NG TRESK and TREK-1 are the most active subunits.

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Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

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Tema 1

Desarrollo

Posters

IN VITRO EVIDENCE THAT SERUM NITRO-ALPHA-SYNUCLEIN COULD PARTICIPATE ON PARKINSONIAN NEURODEGENERATION

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Objectives. The role of cerebrospinal fluid (CSF) and serum in Parkinson disease (PD) is not well known. In vitro studies with CSF have given contradictory results. We have recently detected alterations in nitrosylated tyrosines (Tyr) of serum alpha-synuclein in PD patients. The objectives of this study were to discern: 1) if CSF from PD patients causes morphological injuries in dopamine substantia nigra neurons, and 2) the role of PD serum and nitrosylated serum alpha-synuclein in cytotoxicity and aggregation.

Methods. CSF and serum from PD patients (n=40) and control subjects (n=30) was used. Culturing cell techniques, LDH assay, immunofluorescenee and western blotting were employed. Patients were classified according to the Hoehn-Yahr scale.

Results. The findings indicated that Parkinsonian CSF was cytotoxic to substantia nigra cells only in Hoehn-Yahr-stage-4 patients, whose CSF induced significant retraction of neurites of cultured dopamine neurons. Regarding serum, 60K-filtrated serum (not non-filtrated serum) was cytotoxic at every stage, as measured with the LDH assay. Serum-induced cytoxicity was fully abolished after blockade of the nitrosylation at the amine-terminus Tyr39 residue of the molecule. 60K-filtrated serum from PD patients was also pro-aggregative at every stage, inducing proteinaceous aggregates inside substantia nigra neurons, expressing nitro-alpha-synuclein. Interestingly, serum-induced aggregations were reliably diminished after blockade of nitrosylated Tyr125/136 residues, located at the carboxyl terminus.

Conclusions. Stage-4 CSF and serum at all stages are cytotoxic for substantia nigra neurons, and serum nitro-alpha-synuclein and its nitrosylated tyrosine residues at carboxyl and amine terminus could participate on PD neurodegeneration.

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1. Trastornos y reparación del sistema nervioso
2. Nuevos métodos y tecnologías

A PROTOMAP OF CEREBRAL CORTEX FOLDING DEFINED BY STEP-WISE PATTERNS OF GENE EXPRESSION IN GERMINAL LAYERS

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The cerebral cortex is a modular structure organized in distinct cytoarchitectonic and functional areas, which are limited by sharp borders and distributed along the rostro-caudal and latero-medial axes. In mammals with a folded cortex this modularity also involves the three-dimensional accommodation of the neuronal layers to form folds and fissures along the cortical mantle. All these different modules of cortical neurons emerge during development from embryonic neural stem and progenitor cells arranged in germinal layers.

Objectives- Given the tight genetic regulation of the cell cycle and proliferation of germinal cells during cortical development, we proposed that proliferation differences between prospective folds and fissures might result from differences in gene expression along cortical germinal layers during embryonic development.

Material y methods- We performed a gene expression analysis of germinal layers in the developing cerebral cortex of the gyrencephalic ferret.

Results- We found thousands of genes differentially expressed (DEGs) that defined unique transcriptional fingerprints for each cortical area and germinal zone analyzed. Significantly, we found the existence of mosaics of gene expression along each of the germinal layers correlating with gyrus/sulcus formation. Expression patterns were frequently characterized by sharp, step-wise changes in expression levels for genes which are otherwise expressed in shallow gradients across the mouse cortex. Our findings are consistent with the existence of a transcriptional protomap of cortical folding in germinal layers.

Conclusions- The fact that DEGs between gyrus and sulcus had a 7-fold bias towards genes mutated in human cortical malformations, validates our strategy and indicates that many other DEGs may be involved in patterning cortical folds. These findings also indicate that our list of DEGs is a valid entry point to search for genes mutated in human cortical malformations but for which specific genetic mutations have not yet been identified.

Áreas Temáticas:

1ª: Desarrollo
2ª: Nuevos métodos y tecnologías

PATTERN OF PAX7 GENE EXPRESSION IN THE BRAIN OF *Xenopus laevis* THROUGHOUT DEVELOPMENT

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Pax7 gene is a member of the highly conserved Pax family that is expressed in restricted regions during development of the CNS, where is involved in early establishment and maintenance of the regional identity. Using immunohistochemitry and in situ hybridization techniques, the expression of Pax7 in combination with other ancillary markers, was studied in the brain of *Xenopus laevis* throughout development and in adults. Pax7 appeared early in three main domains that correlate with the neuromeric organization of the CNS. In early embryos Pax7 cells were found in the basal plate of p3, roof and alar plate of p1 and mesencephalon, and the alar plate of r1. As the development proceeds Pax7 cells were observed in the hypothalamus, close to the catecholaminergic cell population of the mammillary region. In the diencephalon, Pax7 was expressed in a portion of the basal plate of p3, in the roof plate of p2 and scattered superficial cells of the thalamus and throughout the roof of p1 and the commissural and juxtacommissural domains of the pretectum. In the mesencephalon, Pax7 cells were localized in the optic tectum and in the torus semicircularis. The rostral portion of the alar part of r1, including the ventricular layer of the cerebellum expressed Pax7. Some of these cells then populated ventrally the interpeduncular nucleus and rostrally the ventral portion of r0. Additionally, Pax7 positive cells were found in the ventricular zone of the ventral part of the alar plate along the rhombencephalon and the spinal cord. These results show that Pax7 is maintained in the brain of adult amphibians with a well-conserved pattern among vertebrates. The persistence in adult specimens, in contrast to other tetrapods could reflect a functional role in the maintenance of territorial and cell identity in the plastic amphibian brain.
Supported by Grant by: BFU2012-31687.

Áreas Temáticas:
1ª: Desarrollo
2ª: Neurociencia de sistemas

PHENOTYPING THE CENTRAL NERVOUS SYSTEM OF THE EMBRYONIC MOUSE BY MAGNETIC RESONANCE MICROSCOPY

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Genetic mouse models of neurodevelopmental disorders are being massively generated, but technologies for their high-throughput phenotyping are missing. The potential of high-resolution magnetic resonance imaging(MRI)for structural phenotyping has been demonstrated before. However, application to the embryonic mouse central nervous system has been limited by the insufficient anatomical detail. Here we present a method that combines MR microscopy and staining of live embryos with a contrast agent to provide unprecedented anatomical detail at relevant embryonic stages. By using this method we have phenotyped the embryonic forebrain of Robo1/2$^{-/-}$ double mutant mice enabling us to identify on a single subject-basis most of the well-known anatomical defects in these mutants, as well as novel more subtle alterations. We thus demonstrate the potential of this methodology for a fast and reliable screening of subtle structural abnormalities in the developing mouse brain, as those associated to defects in disease-susceptibility genes of psychiatric relevance.

Áreas Temáticas:
1ª: Desarrollo
2ª: Nuevos métodos y tecnologías

STUDY OF MOLECULAR MICRODOMAINS IN MOUSE CORTEX AND CEREBELLUM USING THE ALLEN DEVELOPING MOUSE BRAIN ATLAS

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Cortex and cerebellum are interesting brain regions due to its sophisticated cellular structure and circuitry, high degree of functional modifiability combined with unique operational mechanisms, and their multiple roles in animal motor, cognitive and sensory behavior. Both cerebellum and cortex are composed of horizontal layers with different neuronal and connectivity patterns. Each layer contains glutamatergic excitatory neurons and GABAergic inhibitory interneurons. Developmental genes have been shown to play important roles regulating the principal processes in cortical development; such are neurogenesis and regional patterning. Here we have studied gene expression patterns at E13.5, E15.5 and E18.5 and postnatal (P4, P14, P28) stages using the Allen Developing Mouse Brain Atlas (www.brain-map.org/). First we analyzed genes expressed in the neuroepithelium at developmental and postnatal stages and have followed these expression patterns during brain development to identify presumptive microdomains for different neocortical or cerebellar layers. This study allowed us: 1) establish molecular references to detect morphogenetic field limits; 2) analyze maintenance of the ventricular molecular labeling in cellular migratory streams; 3) describe specific molecular markers for neuroepithelial presumptive regions and 4) identify potential regulatory genes underlying neural differentiation in cerebral and cerebellar cortex. The comparison of these molecular microdomains during cortical and cerebellar development suggested differences in the molecular and cellular mechanisms underlying layering and functional regionalization.

1ª: Desarrollo
2ª: Nuevos métodos y tecnologías

STUDY OF THE BASAL FOREBRAIN IN LIS1 MUTANT MOUSE

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LIS1 is one of principal genes related with Type I lissencephaly, a severe human brain malformation characterized by an abnormal neuronal migration in the cortex and underlaying predisposition to develop mental disorders. The role of this gene has been studied using the *Lis1/sLis1* murine model, which has deleted the first coding exon from the *Lis1* gene. Homozygous mice are not viable but heterozygous have shown abnormal neuronal morphology and cortical dysplasia, hippocampal abnormalities and enhanced excitability. *Lis1/sLis1* embryos also exhibited a delay of cortical innervation by the thalamocortical fibers. Moreover, it has been suggested that the maturation of cortical neurons plays an important function in the incursion of thalamocortical axons in the developing cortex. Basal forebrain cholinergic neurons migrate from pallium to subpallium and represent the main cholinergic input to the cerebral cortex. Interestingly, this projection plays a crucial role in modulating cortical activity and facilitating processes of attention, learning, and memory; and has not been studied in *Lis1/sLis* mice. We hypothesized that disorganized cortex and hippocampus could affect cholinergic projections from the basal forebrain and septum, respectively, in *Lis1/sLis1* mouse. Here we first studied the basal forebrain in *Lis1/sLis1* mice during development and observed significant structural and hodological differences between wild-type and *Lis1/sLis1* embryos. In addition, septo-hippocampal projections showed a delayed development in mutant embryos. This retard may be related with the cell disorganization of the dentate gyrus in the same way that cortical plate neurons are related with the invasion of thalamocortical axons. Basal forebrain abnormalities could contribute to explain the enhanced brain excitability and deficits in hippocampal-dependent learning behaviors in *Lis1/sLis1* mutant mice and comorbidity of cognitive symptoms associated to mental diseases.

1. Desarrollo
2. : Trastornos y reparación del sistema nervioso

REGULATION OF *TRKA* EXPRESSION BY BEX3

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TrkA protein levels are tightly regulated during development to ensure the survival of NGF-dependent neurons. Using a yeast two-hybrid screening, we have found an interaction of TrkA with Bex3 (Brain Expressed X-linked gene), a protein that has been previously described to interact with p75NTR. Bex3 protein contains a leucine-rich nuclear export sequence, two boxes for ubiquitination and a CaaX box. The association between TrkA and Bex3 occurs through the juxtamembrane region of TrkA and the C-terminus of Bex3. Bex3 is expressed in the same DRG neurons that express TrkA but prior to it. Interestingly, Bex3 depletion led to a decrease of protein and mRNA TrkA levels in sensory neurons but not of TrkB levels in hippocampal neurons. Using reporter and ChIP assays we have observed that Bex3 modulates and binds to *trkA* gene promoter. In addition, Bex3 translocates from cytoplasm to nucleus and we noticed that in response to NGF, Bex3 dimerizes and accumulates in nucleus. Finally, we have observed that Bex3 levels regulate the survival of NGF-dependent sensory neurons. These findings indicate that Bex3 protein modulates TrkA levels through the regulation of the gene´s promoter.

Áreas Temáticas:
1ª: Desarrollo.
2ª: Trastornos y reparación del sistema nervioso.

POTENTIAL INTERACTIONS BETWEEN MULTIPLE NEURON POPULATIONS AND PROGENITOR CELLS IN THE DEVELOPING FERRET CEREBRAL CORTEX

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Three main germinal layers have been described in the developing cerebral cortex of gyrencephalic mammals: Ventricular Zone, Inner and Outer Subventricular zone. These layers are populated by three main kinds of progenitor cells: Apical Radial Glia (aRG), Basal Radial Glia (bRG) and/or Intermediate Progenitors (IP). Both aRG and bRG are characterized by having a long basal process spanning through the cortical thickness and contacting the pial surface, but only aRG possess an apical process contacting the ventricle. These different progenitors are regulated by intrinsic and extrinsic factors. Thalamo-cortical afferents have been shown to modulate cortical progenitor cell proliferation *in vitro*. In addition, migrating interneurons are likely to also influence DNA synthesis of progenitors.

Here we have investigated additional extrinsic elements that may also modulate cortical progenitor proliferation.

We have analyzed the spatio-temporal distribution of a wide variety of neuronal markers during ferret cortical development. The ferrets were perfused and the brain was cutted in cryotome. Then, we performed simple and double inmunohistochemistry of several neuronal markers together with progenitor markers.

We have found that several populations of neurons are intermixed with the different populations of progenitors. Some neuron markers like Tuj1 were expressed through the entire cortical thickness, while others like Calretinin were present in subsets of cortical germinal layers. Remarkably, many of the markers examined labeled axonal processes but not cell somas, and were expressed from very early in embryonic development.

These findings strongly support a model in which early-born cortical neurons could influence the proliferative kinetics and cell fate potential of progenitors. Given that both aRG and bRG extend a long basal process up to the pial surface, we propose a model in which cell-extrinsic influences act onto cortical progenitor cells not only on cell soma of but also through all their basal process.

Áreas Temáticas:
1ª: Desarrollo
2ª: Neurociencia de sistemas

ERK1/2 ARE THE INTRACELLULAR PATHWAYS ACTIVATED BY ANOSMIN-1 AND FGF2 IN FGFR1-MEDIATED OPC MIGRATION

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Signalling through fibroblast growth factor receptors (FGFRs) is essential for many cellular processes including proliferation and migration, as well as differentiation events such as myelination. Anosmin-1 is an extracelullar matrix glycoprotein that interacts with the fibroblast growth factor receptor 1 (FGFR1) to exert its biological actions through this receptor. This protein is defective in the X-linked form of Kallmann syndrome (KS) and has a prominent role in the migration of the GnRH neurons. We have also shown that anosmin-1 exerts a chemotactic effect via FGFR1 on neuronal precursors from the SVZ and the essential role of the ERK1/2 signalling in this effect. We report the positive chemotactic effect of FGF2 and anosmin-1 on rat and mouse postnatal OPCs via FGFR1. The same effect was observed with the truncated N-terminal region of anosmin-1 (A1Nt). The introduction in anosmin-1 of the missense mutation F517L found in patients suffering from KS annulled the chemottactic activity; however, the mutant form of anosmin-1 carrying the disease-causing mutation E514K also found in KS patients, behaved as the wild type protein. The chemoattraction exhibited by FGF2 and anosmin-1 on OPCs was blocked by the mitogen-activated protein kinase (MAPK) inhibitor U0126, suggesting that the activation of the ERK1/2 MAPK signalling pathway following interaction with the FGFR1 is necessary for FGF2 and anosmin-1 to exert their chemotactic effect. In fact, both proteins were able to induce the phosphorylation of the ERK1/2 kinases after activation of the FGFR1 receptor.

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Áreas Temáticas: Seleccione las **2** áreas temáticas que más se ajusten a su trabajo en orden de prioridad:
1ª: 1
2ª: 2

THE PAX-HD FAMILY: PERSPECTIVE FROM EVOLUTIONARY DEVELOPING GENOARCHITECTONIC ANALYSIS

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In the last years the interest in evolutionary/developmental biology has increased notably. Specially there are many groups of evolutionary biologist whose research is focused to understand how changes in regulatory and coding regions of genes contribute to species evolution and adaptation. In this evolutionary scenario the novel notion of genoarchitectonics refers to the neural expression of genes coding for proteins activated or repressed in spatially restricted patterns regulated by genomic regulatory regions. This is the case of the Pax gene family, a gene family with highly important functions that have arisen from a single ancestral gene and/or chromosome duplication during early metazoan history. The developing expression patterns of Pax6 and Pax7 have been analyzed by means of immunofluorescence in the developing brain of representative groups of vertebrates, such the lungfish *Neoceratorus fosterii* as anamniote no tetrapod, the amphibians *Xenopus laevis* and *Pleuordeles waltl*, representatives of anamniote tetrapods, in the saurpsida group, the turtle *Pseudemys scripta* and the bird *Gallus gallus*, as no mammalian amniotes and finally in the mammalian amniote *Mus musculus*. The degree of conservation of the region recognized by the antibodies has been assessed by the antigen sequence analysis by BLAST, showing high degree of homology. Our results about the expression patterns show that the Pax genes studied are largely evolutionary conserved and, therefore, could unequivocally be used to identify subdivisions in the vertebrate brain that are not clearly discernible with classical techniques. In addition, the spatiotemporal sequences of expression provide indirect evidences of putative migratory routes across neuromeric limits and the alar-basal boundary. Thus the results show the high interest of the Pax gene family, suited for evolutionary and comparative analysis of the brain, since in the same study is possible to compare the topological organization and also the specific cell groups produced in each brain subdivision.

DYRK1A OVEREXPRESSION LEADS TO ALTERATIONS IN EARLY CORTICAL DEVELOPMENT THAT RESEMBLE THOSE DESCRIBE IN DOWN SYNDROME

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Alterations in the cerebral cortex circuitry likely contribute to the intellectual disability in Down syndrome (DS). Early cortical neurogenesis is severely reduced in the trisomic Ts65Dn mouse, a model for DS. *DYRK1A*, lying on chromosome-21, is one of the best candidates for DS intellectual disability and several evidences indicate that kinase DYRK1A plays a fundamental role in brain growth and development. However, the contribution of the extra copy of *DYRK1A* in cortical development has not been assessed. Here we used a mouse – the BACtg*Dyrk1a* mouse – that carries an extra copy of *Dyrk1a* and studied the embryonic phase of cortical development. Excitatory cortical neurons are generated in the dorsal pallium from an asymmetric neurogenic division of a radial glial (RG) cell, or from a symmetric division of an intermediate progenitor (IP). Here we show that neuron production in BACtg*Dyrk1a* embryos was diminished since the onset of neurogenesis to E13.5, while the opposite occurred for the IPs. As we did not observe changes in the number of dividing RG cells nor in the size of the RG cell pool during this period, our results indicate that the number of asymmetric proliferative divisions in the BACtg*Dyrk1a* embryos is increased at the expenses of the asymmetric neurogenic divisions. Preliminary results indicate that alterations in the cell cycle of RG progenitors could be the underlying cause of this phenotype. The advanced production of IPs leads to an advanced fate of the neurons generated at E13.5. During late corticogenesis, the number of IPs in mutant embryos decreased, indicating an advanced exhaustion of the IP pool. In accordance, the numbers of cells expressing layer VI or layer II-III neuronal markers were decreased in postnatal BACtg*Dyrk1a* mice. This work strongly suggests that trisomy of *Dyrk1a* is contributing to the cortical defects previously described in the Ts65Dn model.

1ª: Desarrollo
2ª: Trastornos y reparación del sistema nervioso

SEQUENTIAL ESTABLISHMENT OF CEREBRAL CORTEX LAYERING

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During cortical development, excitatory neurons are generated from progenitors sited in the ventricular zone of the cortical neuroepithelium at mice embryonic (E) day 13. Those cells migrate in a radial way to form the cortical plate in an apparently controlled temporal order, following an inside-out pattern of cortical lamination.
To unveil the specific time of generation of the different layers of the neocortex, in utero electroporation was performed at different cortical developmental stages using a plasmid encoding GFP. The birthdates and sequential waves of proliferating cell generation were determined by combining BrdU, CldU and IdU. Brains were analyzed at birth and postnatal stage P15. Finally, we characterized the fate of transfected progenitors by immunostaining using specific markers. At P0, GFP+ neurons transfected at E13 were mostly located in layers 5 and 6 (approximately 60%) with a small percentage (about 4%) populated the dense cortical plate (layers 2 to 4). However, 40% of GFP+ cells transfected at E14 and E15 settled the dense cortical plate with a small population in layers 5 and 6 (about 8%). E16 transfected cells were mainly sited at P0, in both the germinative ventricular zone and in layer 2. E17 transfected cells occupied only the ventricular zone, likely corresponding to progenitor cells.
In summary, the cortical projecting cells are generated between E13 and E17 and follow an inside-out gradient of generation.

Área Temática: Desarrollo

EFFECTS OF MORPHINE ON MIRNA 212 EXPRESSION LEVELS

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Morphine effects on central nervous system (CNS) cell proliferation have been studied during the last years. This drug can alter proliferative cell expression patterns, as is shown through phosphohistone 3 immunohistochemistry. In this work, we study the relationship between the effects of morphine and the expression of different important molecules in zebrafish CNS development. With this purpose we try to elucidate the role of neurotrophins and the regulation they exert through miRNA 212. The expression levels of the miRNA 212, measured by qPCR and *in situ* hybridization, changed after morphine exposure (10 nM and 10 μM) in zebrafish embryos at 24 and 48 hours post fertilization. Besides, when we used morpholino to knock down mu opioid receptor, we observed that miRNA 212 levels were similar between the morpholino control group and morphine treated embryos, as indicated by qPCR and *in situ* hybridization. These results show that morphine is triggering its proliferative effects through a different pathway than the one triggered by the mu receptor. We have also studied the levels of BDNF at the mentioned stages in morphine treated embryos, as a previous way to understand its possible bond to opioid effects. These results may lead to a better understanding of the influence of morphine in the mechanisms of the pain and addiction processes.

DCC RECEPTOR FUNCTIONS AS AN ACCELERATOR FOR THALAMOCORTICAL AXONAL EXTENSION

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Developing axons must control their growth-rate in order to follow the appropriate pathways, although the regulatory mechanisms involved remain elusive. We recently found that the calcium spontaneous activity of thalamic neurons governs axon growth and extension through the cortex *in vivo* (Mire, Mezzera et al., Nat Neurosci, 2012). This activity is developmentally regulated in thalamic neurons and modulates axon growth by regulating the transcription of Robo1 through an NF-kB binding site in its promoter. We found that Robo1 receptor acts as a brake for thalamic axonal extension being upregulated at the time thalamic growth cones approximate to target regions. However, deceleration of axonal extension may not be the only mechanism that control axonal extension. Interestingly, we found that DCC transcription is also controlled by spontaneous thalamic activity in an opposite manner to Robo1 suggesting that it may play a role on thalamic axon growth. Silencing thalamic spontaneous activity decreases both DCC mRNA and protein levels. Moreover, opposite to Robo1, DCC is downregulated during embryonic development. By combining dissociated cell cultures *in vitro* with gene manipulation of thalamic neurons *in vivo*, we found that DCC receptor modulates thalamocortical axon growth by acting as an accelerator. When DCC levels are increased both in dissociated thalamic cells and in thalamic neurons in vivo, thalamic axon length is significantly augmented. Accordingly, thalamic axons lacking DCC show a delay in their cortical extension. In sum, our results demonstrate that spontaneous activity modulate axonal extension of developing axons by controlling the levels of Robo1 and Dcc that play antagonistic roles this process.

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Áreas Temáticas:
1ª: Desarrollo
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

CUX1 IS DETERMINANT FOR THE CORPUS CALLOSUM CONNECTIVITY THROUGH THE EFFECTIVENESS OF ACTION POTENTIALS

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The development of precise corpus callosum (CC) connections is essential for the functions of the cerebral cortex. Agenesis of the corpus callosum is the only clinical feature that univocally correlates with autisms and autism spectrum disorders (ASD). Autism is also proposed as related to defects in long range connectivity as opposed to better preserved local circuitry. We focus our study on how *Cux1* transcription factor determine the inter-hemispheric connectivity of callosal projecting neurons of cortical layer II-III. We show that *Cux1* is essential both for the development and maintenance of the corpus callosum contralateral connections, but not for midline crossing or ipsilateral synapses. This defect correlates with reduced intrinsic excitability in *Cux1* loss of function cells as well as abnormal development of the axonal initial segment (AIS). In vivo experiments demonstrate that defects in contralateral innervation are not due to the decreased action potential firing, but rather, to abnormal formation of the AIS, axonal excitability and to propagation of synaptic efficacy by ionic currents. These results indicate that *Cux1* plays a determinant role on the innervation of homotypic contralateral areas. Through these functions, *Cux1* might regulate the formation of the functional cerebral areas having main implications on autism and ASD.

Áreas Temáticas:
1ª: Desarrollo
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

FLRT3 IS A ROBO1-INTERACTING PROTEIN THAT DETERMINES NETRIN-1 ATTRACTION IN DEVELOPING AXONS

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Thalamocortical axons (TCA) form a precise topographical projection that conveys the majority of sensory and motor information to the cerebral cortex, where integration of this information leads to perception and the organization of appropriate responses to internal and external stimuli. The establishment of these connections depends on the precise navigation of axons, which is controlled by attractive and repulsive guidance cues. This guidance molecules are normally presented to cells in an overlapping fashion, however little is known about how their signals are integrated to control the formation of neural circuits. In this study, we are investigating how distinct guidance cues interact with each other to set specific axonal behaviours during brain development using the thalamocortical projection as a model system. In particular, we are studying the interaction between Slit1 and Netrin-1 guidance cues, which are known to modulate TCA behaviour. Here, we show that the attractive response to the guidance cue Netrin-1 is controlled by Slit/Robo1 signalling and by FLRT3, a novel co-receptor for Robo1. While axons lacking FLRT3 are insensitive to Netrin-1, axons containing FLRT3 can modulate their Netrin-1 responsiveness in a context-dependent manner. In the presence of Slit1, convergent activation of Robo1 and FLRT3 receptors induces Netrin-1 attraction by the upregulation of surface DCC through the activation of PKA. Finally, the absence of FLRT3 produces defects in axon guidance *in vivo*. In sum, these results highlight a novel mechanism by which interactions between limited numbers of axon guidance cues can multiply the responses in developing axons, as required for proper axonal tract formation in the mammalian brain. Beyond its relevance to thalamocortical guidance, our study has important implications for understanding the complex and delicate balance between attractive and repulsive signalling pathways that is required for the wiring of neural circuits.

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Áreas Temáticas:
1ª: Desarrollo
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

CORTICAL NEUROPLASTICITY INDUCED BY EARLY SENSORY LOSS IS INTRINSICALLY REGULATED IN THE THALAMUS

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The establishment of connections between specific thalamic nuclei and primary cortical areas depends both on intrinsic and sensory-driven mechanisms. The specific mechanisms by which thalamic axons influence and specify the identity of different sensory areas of neocortex remain unclear. Moreover, it has been previously shown that sensory deprivation in one modality provokes neuroplasticity in spared cortical areas (known as cross-modal plasticity) such as mice enucleated at birth display an expansion of the barrel-field in the primary somatosensory area (S1) in the adult (Bronchti et al., 1992). Sensory-driven experience is thought to drive such changes, however the mechanisms involved in these processes remain still unknown. Our aim is to determine the role of thalamic neurons in the processes of neuroplasticity upon sensory deprivation. To this end, we performed embryonic bilateral enucleations to provoke significant neuroplastic changes in spared sensory systems using the mouse as a model. We found that cortical changes appear before than previously though and in an experience independent fashion. As the thalamus is the first structure in the CNS were sensory input converges, we performed a microarray screening to look for changes in the expression profile in distinct principal thalamic nuclei comparing enucleated mice with their control littermates at early postnatal stages. We found candidate genes which significant differential expression between deprived and normal conditions which function we are currently testing through gain- and loss-of-function experiments.

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Áreas Temáticas:

1. Desarrollo
2. Neurociencia de sistemas

EXPRESIÓN DEL GEN *AHR* DURANTE EL DESARROLLO EMBRIONARIO DE AVES

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El sistema nervioso central (SNC) y el oído interno de vertebrados son estructuras complejas, las cuales se originan de diferentes porciones del ectodermo, la placa neural y la placoda ótica, respectivamente. El receptor de compuestos aril-hidrocarbonados o receptor de dioxina (AHR) es un factor de transcripción activado por unión a ligando que media los efectos tóxicos y carcinogénicos de una amplia variedad de xenobióticos. Estudios cada vez más detallados de *Ahr* implican al receptor en rutas de señalización celular que controlan proliferación y diferenciación celular, migración, desarrollo, apoptosis, ciclo celular, homeostasis hepática y desarrollo tumoral. Diferentes estudios muestran que *Ahr* tiene un papel importante en el desarrollo embrionario, tanto de invertebrado como de vertebrados. Además, la exposición de dioxina se asociaba con defectos en desarrollo del SNC, así como a alteraciones cognitivas y del comportamiento. Por todo ello, hemos realizado un análisis detallado mediante hibridaciones *in situ* de la expresión de *Ahr* en el desarrollo del SNC y el oído interno de aves, *Gallus gallus*. Los resultados obtenidos muestran que la expresión del gen *Ahr* se localiza en regiones muy definidas del mesencéfalo, diencéfalo y epitelio ótico. Además, ciertas poblaciones de neuroblastos, que se desprenden del tubo neural o el territorio presuntivo de los elementos sensoriales del oído interno en desarrollo, muestran una clara expresión del gen *Ahr*. En particular, la mácula del utrículo y el sáculo corresponden a los elementos sensoriales donde se desprenden un mayor número de precursores neuronales. La combinatoria de diferentes marcadores celulares permitiría identificar subpoblaciones de neuroblastos que formarán el ganglio acústico-vestibular. Nuestros resultados sugieren una implicación del gen *Ahr* en la diferenciación de determinadas porciones del tubo neural y del epitelio ótico, así como en la transición epitelio mesénquima de determinadas poblaciones de neuroblastos.

Áreas Temáticas:
1ª: Desarrollo
2ª: Neurociencia de sistemas

Este trabajo ha sido financiado por el proyecto de investigación del Ministerio de Ciencia e Innovación, BFU2010-19461.

EXPRESIÓN DEL GEN *EPHA5* DURANTE EL DESARROLLO EMBRIONARIO DE AVES

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El oído interno es una compleja estructura sensorial con funciones auditivas y del equilibrio. Durante el desarrollo embrionario, el esbozo ótico sufre importantes cambios morfogenéticos y una determinante especificación celular que conducen a la formación de las subdivisiones del laberinto membranoso del oído interno adulto y a la generación de los diferentes elementos sensoriales en una correcta posición espacial. Las neuronas del ganglio acústico-vestibular, las cuales se originan a partir de neuroblastos desprendidos de la porción más ventro-medial del esbozo ótico, se encargan de la inervación de los diferentes elementos sensoriales que constituyen el laberinto membranoso. El sistema Eph-ephrinas es el principal complejo molecular que guía el crecimiento axonal en la formación de mapas de conexiones neuronales. Estudios descriptivos y experimentales han mostrado la existencia de señales repelentes desde el epitelio ótico en desarrollo. Entre ellas, cabe destacar el sistema Eph/ephrinas. En este trabajo, se presentan los patrones de expresión espacial y temporal de *EphA5* en embriones de pollo de 3-8 días (E3-8), mediante la técnica de hibridación *in situ* sobre secciones de criostato. La expresión del gen *EphA5* se observó en una porción del epitelio ótico correspondiente a la mácula del sáculo y la papila basilar. El resto de las máculas (la mácula del utrículo, mácula neglecta y mácula de la lagena) no fueron marcadas por la expresión del gen *EphA5*. Cabe destacar una expresión débil del gen *EphA5* en un pequeño área localizada entre la mácula del utrículo y la cresta lateral. Todos estos resultados muestran que el sistema Eph-ephrinas podría estar implicado en el control de la guía axonal implicada en la inervación específica de algunos elementos sensoriales durante el desarrollo del oído interno de ave.

Áreas Temáticas:
1ª: Desarrollo
2ª: Neurociencia de sistemas

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EXPRESIÓN DEL GEN *IRX3* DURANTE EL DESARROLLO DEL OÍDO INTERNO DE AVES

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Los genes *Iroquois (Iro/Irx)* pertenecen a una familia de homeoproteínas conservada en todo el reino animal, las cuales poseen un homeodominio altamente conservado de la superclase TALE (*Three Aminoacid Loop Extention*). Desde un punto de vista molecular, los seis genes *Irx* de vertebrados están organizados en dos grupos (*IrxA* y *IrxB*), cada uno de ellos constituido por tres componentes. Estos genes homeobox juegan un papel importante durante el desarrollo embrionario, tales como la determinación de los patrones antero-posterior y dorso-ventral, así como de regiones específicas del sistema nervioso central y de los elementos sensoriales. Recientemente se ha mostrado que el patrón de expresión de algunos genes *Irx* delimita los dominios sensoriales del oído interno de aves. Nuestro interés se ha centrado en el estudio del posible papel del gen *Irx3*, pertenece al grupo *IrxB*, en la especificación del epitelio del oído interno, un complejo elemento sensorial derivado de una porción del ectodermo cefálico, la denominada placoda ótica. Mediante la técnica de hibridación *in situ* sobre secciones de criostato, hemos analizado la expresión del gen *Irx3* durante el desarrollo embrionario del oído interno de embriones de aves (E3-E8). Nuestros resultados ponen de manifiesto que el gen *Irx3* se expresa en la porción posterior del epitelio ótico, en un territorio que incluye a parte de la papila basilar, la cresta posterior y la mácula neglecta, el resto de los elementos sensoriales no están marcados por la expresión de este gen. Estos datos sugieren un posible papel de *Irx3* en la especificación de la porción posterior del esbozo ótico.

Áreas Temáticas:
1ª: Desarrollo
2ª: Neurociencia de sistemas

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CONTROL OF METALLOPROTEINASE ACTIVITY: AN IMPORTANT STEP FOR BOTH CORTICAL DEVELOPMENT AND NEURODEGENERATION

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The A Disintegrin And Metalloproteases (ADAMs) are a family of membrane proteases that cleave the extracellular domains of a variety of membrane bound proteins, releasing soluble peptides that modulate cell-to-cell communication or priming the protein for further cleavage, indispensable to generate intracellular signalling. Prominent examples of ADAM substrates are the Notch receptor, N-cadherin, L1, several cytokines, or the amyloid precursor protein (APP). All these proteins control different events in CNS development and homeostasis, suggesting that ADAM activity must be tightly regulated to allow proper brain development and function. However, the identification of such regulators is just at the beginning. A recent example is Secreted Frizzled Related Protein1 (Sfrp1), which we showed binds to and negatively regulates ADAM10 proteinase activity. As a consequence, genetic inactivation of *Sfrp1* in mice increases Notch, N-Cadherin, L1 and Amyloid Precursor Protein (APP) processing. Here, we will show that this abnormal processing has several important consequences during cortical development, including an increase in early precursors proliferation and a premature differentiation of early-born projection neurons. In contrast, crossing *Sfrp1*$^{-/-}$ mice with a mouse model for Alzheimer Disease (AD), result in the generation of a mouse line that hardly develop the otherwise characteristic histopathological and cognitive defects of the disease, opening the interesting possibility that *Sfrp1* may represent a useful therapeutic target for AD.

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Áreas Temáticas:
1ª: Desarrollo
2ª: Trastornos y reparación del sistema nervioso

GENE PROFILING OF IDENTIFIED NUCLEI SPECIFIC THALAMIC NEURONS

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The thalamocortical system is remarkable with topographically ordered long-range axonal projections that convey sensory information to the cortex. Although a number of transcription factors and molecular guidance cues have been identified that play a role in thalamocortical guidance and topography, the complete expression profile for each principal thalamic nucleus during development remains to be determined. We developed a mouse model, in which neurons from principal thalamic nuclei are specifically labelled in colours. By using fluorescence-activated cell sorting (FACS) we isolated these nuclei specific thalamic neurons and reveal their gene expression profiles. Moreover, as we have recently demonstrated that spontaneous activity plays an essential role in thalamocortical development, we have designed an strategy to determine thalamic genes that are regulated by electrical activity and study the consequences of perturbing this activity in distinct steps of thalamocortical wiring. By learning the complete expression profiles of the principal thalamic nuclei and also the genes that are changed with perturbation of activity we will gain insights into the underlying mechanisms that determine the nuclei-specific formation of thalamocortical connectivity.

Áreas Temáticas: Seleccione las **2** áreas temáticas que más se ajusten a su trabajo en orden de prioridad:
1ª: Desarrollo
2ª: Nuevos métodos y tecnologías

SEX DIFFERENCES AND ESTRADIOL REGULATION OF NEURITOGENESIS IN DEVELOPING HIPPOCAMPAL NEURONS

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Previous studies have shown that estradiol promotes neuritogenesis in developing hippocampal neurons by a mechanism involving the upregulation of neurogenin 3, a Notch-regulated transcription factor (1, 2). In this study we have explored whether G-protein coupled estrogen receptor 1 (GPER) participates in this hormonal action. In mouse primary hippocampal neuronal cultures, in which cells from male and female embryos were cultivated together, GPER agonists (17β-estradiol, G1, ICI 182,780) increased neurogenin 3 expression and neuritogenesis and this effect was blocked by the GPER antagonist G15 and by a siRNA for GPER. In addition, GPER agonists increased Akt phosphorylation in ser473, which is indicative of the activation of phosphoinositide-3-kinase (PI3K). However, GPER agonists did not significantly affect the phosphorylation of Akt in the presence of G15. Furthermore, the PI3K inhibitor wortmannin prevented the effect of G1 and estradiol on neurogenin 3 expression and the effect of estradiol on neuritogenesis. In mouse primary hippocampal neuronal cultures, in which cells from male and female embryos were cultivated separately, the basal mRNA levels of neurogenin 3 were higher in female compared to male neurons. Furthermore, estradiol treatment increased the levels of expression of neurogenin 3 in males but not in females. In addition, female hippocampal neurons showed an enhanced neuritogenesis compared to male neurons. These findings suggest that estradiol participates in the control of hippocampal neuritogenesis by a mechanism involving GPER, the activation of PI3K signaling and the expression of neurogenin 3. Furthermore our findings have revealed sex differences in neuritogenesis. Future studies should determine whether GPER/PI3K/neurogenin 3 signaling is involved in the generation of sex differences in neuritogenesis of hippocampal neurons.

Áreas temáticas:
1. Desarrollo
2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

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ORIGEN DE LOS DIFERENTES ELEMENTOS DEL OIDO INTERNO DE AVES: PROPUESTA DE UN MODELO EVOLUTIVO

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El oído interno es un complejo elemento sensorial formado por una serie de cavidades interconectadas entre sí, las cuales constituyen el laberinto membranoso. Este laberinto está dividido, según su morfología y función, en dos sistemas: el vestibular y el auditivo. A pesar de los numerosos estudios sobre el desarrollo del oído interno, aún se conoce relativamente poco acerca del origen de cada una de las estructuras sensoriales/no sensoriales del oído interno adulto en la propia placoda. Una aproximación la proporcionan los estudios de especificación mediada por la expresión asimétrica de determinados genes reguladores. No obstante, la mejor manera para entender la diferenciación del epitelio ótico, e incluso la evolución del oído interno, es mediante la realización de un mapa de destino. Con la intención de establecer la correcta posición de las diferentes estructuras que componen el oído interno de aves en la placoda ótica, hemos realizado un mapa de destino usando la técnica de embriones quimeras pollo/codorniz. Para ello, hemos realizado trasplantes homotópicos en estadio de placoda ótica (10 somitas) y analizándolos en estadio HH29 (E6). Los resultados obtenidos nos han mostrado la relación espacial del origen de cada uno de los componentes del laberinto membranoso del oído interno de aves, todo ello en un contexto de cambios morfogenéticos. Además, nuestros estudios sugieren la existencia de un territorio placodal posterior que incluiría al territorio presuntivo de la placoda ótica y las placodas epibranquiales. La posible acción de señales difusibles desde los tejidos adyacentes, que proporcionarían información posicional, dividiría de manera secuencial a este territorio original placodal en dominios longitudinales (dorso-ventrales) y transversales (antero-posteriores). Estos resultados plantean un nuevo modelo de desarrollo embrionario y filogenético del oído interno de vertebrados.

Áreas Temáticas:
1ª: Desarrollo
2ª: Neurociencia de sistemas

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NEUROGENESIS EN EL OÍDO INTERNO DE AVES

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El oído interno es un complejo órgano sensorial constituido por una serie de cavidades y canales interconectados entre sí, que forman el laberinto membranoso. El oído interno se origina de la placoda ótica, que es un engrosamiento del ectodermo cefálico, localizada entre el rombómero 4 al 7 (estadio HH10). A continuación, los bordes de la placoda ótica se elevan dorsalmente (estadio HH12) y se acercan entre sí hasta contactar (estadio HH16). Posteriormente, el esbozo ótico se separa del ectodermo cefálico, dando lugar a una estructura casi esférica que se denomina otocisto o vesícula ótica (OT o VO; estadio HH17). Recientemente, se ha propuesto que los neuroblastos, el primer tipo celular que se diferencia en el epitelio ótico y que darán lugar a las neuronas del ganglio acústico-vestibular, se desprenden del territorio antero-medial de la placoda/copa ótica, el denominado dominio pro-neural, situado en la pared antero-medial de la vesícula ótica. A pesar del interés suscitado por el lugar de origen de los precursores neuronales y la posterior inervación de las neuronas sensoriales, aun no ha sido establecido un mapa detallado del origen de las diferentes subpoblaciones de los neuroblastos dentro del epitelio ótico. Por ello, hemos realizado un análisis minucioso mediante hibridaciones *in situ* e inmunohistoquimicas de los marcadores de neuroblastos conocidos hasta el momento. Durante este trabajo, hemos utilizado embriones de pollo, *Gallus gallus*, desde el estadio HH12 (E2) al HH34 (E4). Los resultados muestran que los neuroblastos se desprenden del territorio presuntivo de todos los elementos sensoriales del oído interno de aves en desarrollo, siendo la mácula del utrículo y el sáculo los elementos sensoriales donde se observo un mayor desprendimiento de neuroblastos. La combinatoria de diferentes marcadores celulares permitiría identificar subpoblaciones de neuroblastos que formarán el ganglio acústico-vestibular.

Áreas Temáticas:
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FoxP2 AND FoxP4 ROLE IN MOUSE STRIATAL DEVELOPMENT

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The lateral ganglionic eminence (LGE) is the proliferative region of the striatal domain in the basal ganglia of mammals. This region gives rise to striatal projecting neurons as well as interneurons, which migrate along the rostral migratory stream to the olfactory bulb. FoxP2 and Foxp4 are two repressor transcription factors express in the LGE at embryonic stages. Both genes participate in neurogenesis process in other regions of the central nervous system: FoxP2 and FoxP4 promote detachment of differentiating neurons from the proliferative region in the spinal cord; in the cortex, Foxp2 downregulation inhibits neurogenesis in cortical precursors by impairing the intermediate progenitor cell production. However, the role of FoxP2 and FoxP4 in LGE development has not been solved. In the present work, we analyzed the function of both genes during striatal neurogenesis. By double and triple immunofluorescence we studied the relation of FoxP2 and FoxP4 with proliferative markers and with molecules important for striatal development like Pax6 or Islet1. To test whether FoxP2 and FoxP4 elevation or downregulation control striatal neurons production we used *in utero* electroporation of pCIG expression vectors containing an IRES-nuclear-EGFP reporter or RNA (shRNA) vectors respectively. We observed an implication of FoxP2 and FoxP4 genes in striatal patterning at intermediate developmental stages and in striatal projecting neurons production in our experiments.

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Areas temáticas
1ª. Desarrollo
2ª. Trastornos y reparación del sistema nervioso

THE PATTERNS OF CELL DEATH AND OF LECTIN-POSITIVE MACROPHAGES/MICROGLIAL CELLS IN THE DEVELOPING VISUAL SYSTEM OF THE SMALL-SPOTTED CATSHARK *(Scyliorhinus canicula)*

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The main aim of the present study is to analyze the possible relationship between ontogenetic cell death and macrophage/microglial cells during fish visual system development.

We have studied the spatiotemporal patterns of cell death and phagocytes during development of the *Scyliorhinus canicula* visual system in embryos, juveniles and adults of this shark species. Cryosections were treated according to the TUNEL and lectin histochemical techniques.

There are four areas of cell degeneration observed during early shark visual system development. (I) An area of cell degeneration in the dorsal part of the optic cup is present through St21 to St23. Concerning to the optic pathways, (II) a large number of cells die in the presumptive optic chiasm during optic cup stages (St21-23), and (III) abundant dead neuroepithelial cells are found along the distal half of the optic stalk between St27 and St29. Finally, (IV) between St23 and St26, an area of cell death is apparent in the anterior wall of the developing lens. Cell death in the shark developing retina during stages where the emergence of the retinal layers takes place (St30 to St34) follows a central-to-peripheral and vitreal-to-scleral gradients. This sequence of histogenetic cell death recapitulates the sequence of maturation of the various layers and cell types.

Lectin-positive cells apparently enter the retina by the optic nerve head when the retinal layering is almost complete. As development proceeds, these labeled cells migrate parallel to the axon fascicles of the optic fiber layer and then reach more external layers by radial migration.

Our findings support the view that, during visual system development, there are differences in the topographic pattern of cell death in vertebrates. Furthermore, no evident correlation is found between the chronotopographical pattern of distribution of TUNEL-positive bodies and the pattern of distribution of lectin-labeled macrophages/microglial cells during the shark´s visual system ontogeny.

Área temática: Desarrollo
Área temática: Neurociencia de Sistemas

MIDBRAIN AUDITORY GENOARCHITECTURE COMPARED BETWEEN CHICKEN AND MOUSE

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The avian torus semicircularis is homologous to the inferior colliculus in mammals and is intercalated rostrocaudally between the optic tectum and the preisthmic area. The toral complex consists of an intercollicular area and the toral nucleus proper, which largely occupies a periventricular position; there exist also some superficial toral components, which are less massive (Puelles et al., 1994). The inferior colliculus (IC) of mammals is the major component of the auditory midbrain and contains three major subdivisions: a central nucleus, a lateral nucleus, and a dorsal cortex. These differ in various aspects, including gene expression patterns, though this issue has received little comparative attention. The first goal of our study was to characterize genoarchitectonically the chicken toral subdivisions, testing the model proposed by Puelles et al. (1994) on the basis of immunochemical mappings. The next step was to establish possible one-to-one homologies with the mouse IC parts. To achieve this, we performed ISH on postnatal (stages P3-P43) and embryonic chicken brain sections (stages HH30-HH45), as well as in postnatal and embryonic mouse brain sections (stages E16.5; E18.5; P8-P36), identifying the expression of homologous gene markers relative to the postulated subdivisions in chicken and mouse. We studied the expression of various transcription factors (Meis1, Meis2, Six3, Tcf712, Tfap2B), neurotransmitters or enzymes that produce them (Gad67, Npy, Tac1, Vglut2, Penk, nNOS), and other molecules with different functions (Enc1, Foxp1, ParvB, Cadps2). The study was complemented by immunohistochemical analysis of PAX3, PAX7 and LIM1. We fully corroborated the earlier subdivision model postulated in chicken, and obtained a consistent combinatorial molecular code for each component of the inferior colliculus and the toral complex. In both species, the central core nucleus is marked by Tcf7l2, Six3, Vglut2 and ParvB, while the external nucleus in both species expresses Tcf7l2, Penk1,Calb2, Vglut2, Npy, Tfap2B and Gad67. The superficial chicken toral derivatives and the mouse dorsal cortex massively express Meis1, Tcf7l2, Calb2, Penk1, Npy and Enc1.

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Áreas Temáticas
prioridad:
1ª: Desarrollo
2ª: Neurociencia de sistemas

GENE EXPRESSION AND ANALYSIS OF THALAMO-CORTICAL PROJECTIONS IN Fgf8$^{null/neo}$ MUTANT MOUSE FOREBRAIN

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Thalamocortical axons form a precise topographical projection that conveys the majority of sensory and motor information to the cerebral cortex. The formation of neural connections is dependent on extracellular attractant and repellent signals for axons and migrating cells. In previous studies we analyzed the expression pattern of *Mus musculus immunoglobin superfamily, member 21 (Igsf21), Phosphodiesterase 10A (Pde10a)* and *BTB (POZ) domain containing 3 (Btbd3)*, finding differences between wild-type and Fgf8$^{null/neo}$ mutant mouse forebrain during development. The expression of these genes in the Fgf8 hypomorphic mouse showed that the specific molecular pattern of thalamic ventral and lateral nuclear complexes was altered. In addition important alterations in the cortex were detected in the mutant mouse, principally in structure (from holoprosencephaly to microcephaly) and layering of remaining cortical regions. We hypothesized that thalamus defects together with a disorganized cortex in the Fgf8$^{null/neo}$ mouse could be related with thalamocortical connectivity impairment. In the present work, we investigated the molecular and cellular processes involved in the guidance of thalamocortical axons growing towards the cortex in the Fgf8$^{null/neo}$ mutant mouse. We performed a developmental analysis of the forebrain by immunohistochemistry and in situ hybridization technique, comparing the expression of different genes in wild-type and Fgf8$^{null/neo}$ mutant mice. For axonal tracing experiments, we placed single crystals of DiI and DiA in the thalamus and in the cortex subsequently analyzing the projections in 100 µm vibratome sections. We observed important differences in thalamocortical projections between wild-type and hypomorphic mice. Current results suggest that signaling molecules (Fgf8) code positional information involved in specification of thalamic and telencephalic structures. This thalamic disorganization could modify molecular cues to guide axonal projections to adequate targets.

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1º: Desarrollo
2º: Trastornos y reparación del sistema nervioso

WNT1 HAS A KEY ROLE IN THE ESTABLISHMENT OF POSITIONAL INFORMATION IN THE DIENCEPHALON

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The diencephalon is a complex brain area which derives from the caudal region of the prosencephalon. It is located in the middle of the brain, between the secondary prosencephalon (anterior forebrain) and the mesencephalon (midbrain). This structure is divided in transverse segments (prosomeres) and longitudinal zones (columnar domains), which constitute segregated domains of cells with similar properties (morphogenetic fields). The complexity of diencephalic anatomy makes difficult the full elucidation of the mechanisms underlying its formation during development. Nevertheless, recent experiments along the last years allowed to progress in our knowledge on the molecular regionalization and to propose mechanistic models for the generation of regional diversity and histogenesis of this neural territory.

Signaling molecules act as morphogenes to induce specific neural fate to the neighbouring epithelium. These signaling molecules belong to four different families: Shh, Bmps, Fgfs and Wnts. Although most of them have been implicated in different mechanisms of the ventro-dorsal and antero-posterior regionalization of the neural plate and tube, very little is known about their role in diencephalic regionalization; with the exception of Shh. In this study, we focus our investigation in Wnt1, a member of the Wnt family. Wnt1 is expressed in restricted regions of the central nervous system, including the dorsal midline of the diencephalon and mesencephalon. We have analyzed morphologically and transcriptomicallly Wnt1 mutant mice by means of in situ hybridization, inmunohistochemical and classical structural staining techniques. Moreover we have studied Wnt1 gain of function phenotype in the diencephalon of chick embryos by in ovo electroporation experiments. Our results show significant modification of shape and size of diencephalic columnar domains after Wnt1 loss and gain of function, suggesting a key role of Wnt signaling in the molecular regulatory code modulating diencephalic ventro-dorsal regionalization.

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1º. Desarrollo
2º. Trastornos y reparación del sistema nervioso

CREB'S POTENTIAL ROLE IN INTERHEMISPHERIC CONNECTIONS

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The human cerebral cortex represents the highest evolved structure of the nervous system and as such, the precise connectivity of cortical neurons is responsible for complex behaviour and high associative tasks. Callosal projection neurons play a key role in these tasks. They are interhemispheric neurons whose myelinated axons make up the corpus callosum, the largest white matter tract in the placental mammalian brain. The molecular mechanisms involved in its formation are not fully understood. Spontaneous neuronal activity and guidance molecules have shown to play critical roles but how the regulation of these aspects is coordinated is unknown.

CREB is a transcription factor known for mediating the consolidation of synaptic plasticity. It modulates intrinsic activity and induces the transcription of several genes known to mediate axonal response. We used in utero electroporation-mediated gene transfer method to explore its possible role in interhemispheric axon development. Using A-CREB as a tool to suppress CREB activity, we found that this protein plays a crucial role in callosal axon connectivity.
We also overexpressed CREB (VP16-CREB) finding suggesting evidences that, activation of CREB functions could be triggering exit of axonal projections from the main bundle of the corpus callosum and their turning and ascension through the cortical plate to reach the superficial layers.

Our preliminary studies suggest that CREB plays an important role in certain stages of interhemispheric axon development. Understanding corpus callosum formation is critical, as defects in this commissure have been implicated in cognitive syndromes presenting high-level associative dysfunction, such as autism spectrum disorders.

Áreas Temáticas:
1ª: Desarrollo
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

CHALLENGING THALAMOCORTICAL PLASTICITY: ROLE OF THALAMIC AXONS IN CORTICAL DEVELOPMENT AND CONNECTIVITY

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Thalamocortical axons (TCA) form a precise topographical projection that conveys the majority of sensory and motor information to the cerebral cortex. This connectivity is formed prenatally, and thus TCA may influence several aspects of cortical development such as neurogenesis, area specification and cortical wiring, although these possibilities remain either not fully demonstrated or controversial. We will test the capacity of thalamocortical axons to compete for neocortical space and to impose a sensory-input in place when the thalamus has been challenged by an insult. Therefore, we will also determine to what extend the input by nucleus-specific TCA is necessary for the formation of both reciprocal corticothalamic (CTA) connectivity and area specification. To this end, we have developed a genetic approach to selectively ablate specific thalamic nuclei genetically so that mice will develop be normally, receiving sensory input but lacking a specific thalamic relay station. Our preliminary results show that when a particular thalamic nucleus is ablated, reciprocal corticothalamic connections are affected, suggesting that CTA from specific sensory modalities require their correspondent thalamocortical inputs. Moreover, we will test whether thalamocortical input is required for a normal cortical neurogenesis and proper development and function of cortical sensory areas. Beyond its relevance to thalamocortical projection, our study has important implications for understanding the brain plasticity through thalamic manipulation and learn about its capacity to rewire and restore cortical function when an insult has occurred.

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Áreas Temáticas:
1ª: Desarrollo
2ª: Neurociencia de sistemas

LACKING TAU PROTEIN CONFERS CERTAIN NEUROPROTECTION TO NEWBORN GRANULE NEURONS

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Aim: alterations in tau protein metabolism are found in a subset of neurodegenerative disorders known as tauopathies, the most common of which is Alzheimer´s disease. As several tauopathies are accompanied by memory deficits, and adult hippocampal neurogenesis is crucial for learning and memory, tau protein could be involved in this process. So, the aim of this work is to study the effect of lacking tau protein in adult hippocampal neurogenesis.

Material and Methods: we have used tau knockout mice (Dawson et al., 2001) at two different ages (3 months and 12 months) to study different aspects of adult hippocampal neurogenesis in the dentate gyrus. We have used two approaches to analyze the maturation and integration of newborn neurons into the circuit: thymidine analogs and specific antibodies to study different states of differentiation, and PSD95:GFP expressing retroviruses to analyze newborn granule neuron morphology and postsynaptic cluster number and volume.

Results: we have demonstrated that both young and old tau knockout mice show an increased volume of their dentate gyrus, accompanied by a larger number of mature granule neurons. Surprisingly, proliferation and early survival were not affected in these mice as compared to wild type. On the contrary, a selective increase in survival was found at late survival times (4 weeks). However, in spite of being more numerous, mature granule neurons showed a reduced number and volume of postsynaptic PSD95-GFP+ clusters, which suggest worse afferent connectivity compared to wild type granule neurons.

Conclusions: lacking tau protein seems to confer certain neuroprotection at late survival times during adult hippocampal neurogenesis, although functional consequences of tau deletion in these neurons should be further investigated.

Áreas temáticas:

1ª: Desarrollo
2ª: Trastornos y reparación del sistema nervioso

THE ROLE OF FOCAL ADHESION KINASE IN AXONAL DEVELOPMENT

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During the last phases of brain development, once neurons reach their final destination, they extend axons that arborize through the extension of collaterals that bifurcate again to form terminal fields. Neurons typically produce more collaterals and axon terminals that will eventually endure in the adult brain, since many of them are pruned. It has been described two different types of pruning: large-scale pruning with an elimination of long collaterals, and small-scale pruning with a local refinement of axons within the termination zone. This remodeling of the axonal arbor allows the refinement of the network and generates a precise adult connectivity.

Over the past few years our group has explored the function of Focal Adhesion Kinase (FAK) in neural circuits development. We have described that FAK controls axonal arborization of hippocampal neurons *in vitro*. Mutant neurons form bigger axonal arbors due to both: increase in branch formation and reduction in branch retraction.

Here we have combined mouse genetics with a set of different experimental approaches to show that FAK is required for the proper remodeling of axonal arbors *in vivo*. The absence of FAK causes specifically an impaired small-scale remodeling but does not affect the long-scale pruning. In particular, FAK-deletion in pyramidal cells leads to an abnormal refinement of the axons in the terminal field.

Remodeling of the axons could be mediated by local and transcriptional changes. To understand the molecular mechanisms by which the kinase commands this process *in vivo*, we focused in a possible implication in pathways that promote transcriptional changes. FAK has been shown to be upstream of ERK-signaling cascades. Also, FAK can physically associate to regulatory regions of the targeting genes, regulating gene expression. Therefore, to test its role in transcription we have carried out a high throughput screening of genes that are transcriptionally regulated by FAK, using microarray analysis.

Áreas Temáticas:
1ª: Desarrollo
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

EXPRESSION PATTERN OF TRANSMEMBRANE PROTEINS WITH EXTRACELLULAR LEUCINE RICH REPEATS (eLRRs) IN THE DEVELOPING MOUSE BRAIN

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Objectives: Leucine Rich Repeats (LRR) is a consensus sequence domain involved in protein-protein interaction characterized by the conserved sequence of 11 aliphatic amino acids including leucine. Transmembrane proteins with extracellular LRRs (eLRRs) have recently emerged as key regulators of several processes during the establishment of neuronal circuits: myelination (LINGO1), axon extension (Islr2/Linx), synaptic function (Trk, LRRTM, and SALM) and neuron migration (FLRTs[1]). Their essential role is further emphasized by the fact that expression of genes coding for transmembrane proteins with eLRRs is highly enriched in the nervous system and that several of them have been linked to human neurological and psychiatric disorders. We therefore performed a systematic analysis of the expression map of several families of this type of genes aiming for the identification of new molecular mechanisms of neuronal connectivity.

Materials and Methods: Mouse embryonic brains were collected at different developmental stages and cryosectioned. In situ hybridization was performed using standard protocols with digoxigenin labelled riboprobes specific for each gene.

Results and Conclusion: We studied the expression pattern of genes coding for transmembrane proteins with eLRRs with special focus on the cortex, thalamus and hippocampus in different stages during development. Most of them showed discrete expression maps. Among all the genes analyzed, Islr2, NLRR3 and members of the SALM family showed very suggestive expression patterns in regions of the brain where many axon guidance and neuron migration decisions are taken. This study provides important clues about the genes encoding transmembrane proteins with eLRRs regarding their possible role in neural connectivity, in particular, in radial and tangential migration of cortical neurons.

[1]Yamagishi et al., 2011

Thematic Areas:
1st: Development
2nd: System Neuroscience

FLRT PROTEINS ARE CHEMOREPELLENT CUES FOR MIGRATING INTERNEURONS

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The migration of neocortical interneurons (INs) during brain development is a precisely regulated process, critical for brain function. Indeed, defects in IN migration are associated with psychiatric disorders including schizophrenia. Most of cortical INs originate in the medial ganglionic eminence (MGE) of the subpallium and use long stereotyped tangential routes to reach the cerebral cortex. Here, INs disperse in order to reach their final position in the appropriate cortical layer. Many of the molecular signals and the cellular mechanisms coordinating IN migration remain to be elucidated. The ectodomains (ECD) of the fibronectin and leucine-rich transmembrane proteins (FLRTs) were recently described as novel chemorepellent cues for the migration of a subset of excitatory neurons in the developing cortex[1]. Considering that FLRTs are also expressed in regions coincident with the routes of IN migration we tested if they could determine the appropriate migration paths of these neurons. We observed that FLRT2 and FLRT3 ECDs bind to calbindin-positive INs in dissociated cultures of MGE. This binding induced a repulsive response since interneurons derived from MGE explants avoided the secreted forms of FLRT2 or FLRT3 ECDs in confrontation assays with transfected HEK293T cells. At E14.5, calbindin positive INs migrate into the cortex by three main tangential streams in the marginal zone, subplate and lower intermediate zone (IZ). At this stage FLRT3 is expressed in the IZ suggesting that it may be an important signal in maintaining these three streams of migrating INs. Preliminary analysis of brain cortexes where FLRT3 was conditionally deleted from the nervous system revealed a different distribution of INs compared to controls. In particular we observed an increase of calbindin-positive neurons in the IZ of the mutant brains. These results suggest a possible function of FLRT3 controlling IN migration within the developing mammalian neocortex.

[1]Yamagishi et al., 2011

Áreas Temáticas:
1ª: Desarrollo
2ª: Desarrollo

TECTAL EPHA3 GUIDES NASAL RETINAL GANGLION CELLS AXONS DURING RETINOTECTAL MAPPING BY COMPETING WITH AXONAL EPHA4 FOR AXONAL EPHRINS-AS

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Eph and ephrins are expressed in complementary gradients in both, the retina and the tectum and guide retinotectal projections by producing bidirectional signaling. Previously we demonstrated that tectal EphA3, acting like a second mapping force, stimulates axon growth of nasal retinal ganglion cells (RGC) toward the caudal tectum preventing them from branching in the rostral tectum (Ortalli et.al. 2012). The aim of this work was to study the molecular pathway which mediates the EphA3 action. We postulated that activation of axonal EphA4 decreases axon growth and that tectal EphA3 increases axon growth by reducing EphA4 activation throughout competing with axonal EphA4 for axonal ephrin-As binding.

We used cultures of retinal explants from chicken embryos. Some of them were treated with EphA3-Fc or Fc, PIPLC (sheds ephrin-As), Lithocholic acid or KYL (inhibits ephrin-A-mediated EphA4 activation) and others were electroporated with EphA4, KiEphA4 (kinase-inactive dominant negative EphA4) or GFP pMES expression vectors. We performed immunocytochemistry, immunoprecipitation and Western blot against Eph/ephrins-As.

We showed that: a) Nasal RGC axons present higher levels of ephrin-As, colocalization of ephrin-A2 and EphA4, and tyrosine-phosphorylated EphA4 than temporal RGC axons. b) Axonal response to EphA3 ectodomain is associated to ephrin-A expression and EphA4 tyrosine phosphorylation. c) The EphA3 ectodomain and ephrin-A shedding both decrease the degree of EphA4 Tyr-602 phosphorylation. d) Removal of axonal ephrin-As and inhibition of ephrin-A-mediated-EphA4 signaling recapitulate the effects of EphA3 ectodomain on RGC axon growth and branching. e) Overexpression of EphA4 produces neurons with shorter axons than the neurons which express GFP or KiEphA4. Neurons expressing KiEphA4 have longer axons than the control. These results support the idea of a novel molecular mechanism whereby tectal EphA3 increases axon growth toward the caudal tectum and collaborate to inhibit axon branching in the rostral tectum by decreasing ephrin-A-mediated-EphA4 forward signaling.
Supported by CONICET-UBA

Áreas Temáticas:
1ª:Desarrollo
2ª: Neurociencia de sistemas

STUDY OF CELL DIFFERENTIATION PROMOTED BY VARIUS NEUROTROPHIC FACTORS IN A CELLULAR MODEL OF DOWN SYNDROME

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Down syndrome (DS) is a genetic defect that causes a delay in brain development.

Individuals with DS have different features such as cognitive impairment, causing intellectual disability. Some authors suggest that the absence of certain neurotrophic factors is responsible for cognitive impairment.

Previous studies in the laboratory have shown that oleic acid is a neurotrophic factor that promotes neuronal differentiation. For this reason, in this study we aimed to investigate the effect of different neurotrophic factors in our DS cell model and elucidate the possible synergistic effect of these factors with oleic acid. Therefore, both normal and trisomic cells were incubated in the absence or presence of oleic acid together with different factors: NGF, BDNF, GDNF and BMP-9 separately. After 24 hours of incubation, photographs were taken using an inverted microscope and cells with two or more dendrites were quantified. The results show that oleic acid promotes a significant increase in neuronal differentiation in normal cells. In addition, when oleic acid is incubated with neurotrophic factors, it results in a rate of differentiation, suggesting a synergistic effect with the factors. However, none of the studied compounds is able to promote higher differentiation in trisomic cells, suggesting that the characteristic gene dosage in DS increase prevents the differentiating effect of these effects. In this regard, inhibiting overexpression of DRK1A (double activity tyrosine kinase I), which is involved in the cortical region of DS, neurotrophic factors induce differentiation of DS cells. This effect is higher when cells were incubated with oleic acid with the studied neurotrophic factors. These results suggest that in order to achieve higher cell differentiation in trisomic cells, it is necessary to incubate oleic acid together with another neurotrophic factor.

Áreas Temáticas:

1. Desarrollo
2. Trastornos y reparación del sistema nervioso

PHOSPHATIDYLCHOLINE METABOLISM IS ALTERED IN CELLULAR MODELS OF DOWN SYNDROME (DS)

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Recent studies in our laboratory have shown that oleic acid induced choline acetyltransferase (ChAT) expression in euploid cells, but this effect was lower in trisomic cells. This suggests that synthesis of neurotransmitter acetylcholine in response to oleic acid could be affected in DS patients. The oleic acid is mainly incorporated into phosphatidylcholine (PC) of plasmatic membrane in cultured neurons. We hypothesize that oleic acid fails to induce ChAT expression in trisomic cells due to an altered phospholipids metabolism. Interestingly, we have observed that the amount of PC into plasmatic membrane is reduced in trisomic in comparison to normal cells. Moreover, we have analyzed the expression levels of different enzymes involved in PC synthesis by polymerase chain reaction real time, and we have obtained that choline kinase alpha (Chka) and phosphate cytidylyltransferase 1 choline alpha (Pcyt1a) have lower expression in trisomic than in normal cells. The dual-specificity tyrosine (Y) phosphorylation-regulated kinase 1A (DYRK1A) gene is located on human chromosome 21 and encodes a proline-directed protein kinase. DYRK1A plays an important role in several biological functions, leading to mental retardation in DS patients. Here, we report that overexpression of DYRK1A could be involved in the impairment of PC metabolism in trisomic cells.

Áreas Temáticas:

1- Desarrollo
2- Trastornos y reparación del sistema nervioso

ARMS/KIDINS220 AND SYNEMBRYN-B MODULATE NGF-MEDIATEDDIFFERENTIATION AND REGULATED SECRETION IN PC12 CELLS

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Neurotrophins are growth factors essential for nervous system development, but currently gaining strength data implicate neurotrophins in synaptic activity regulation in adult brain. Nerve Growth Factor (NGF) has been previously linked to differentiation and regulated secretion in PC12 cells. Neurotrophin receptors use scafold proteins to activate signaling pathways that regulate different events such as differentiation and secretion. ARMS/Kidins220 (Ankyrin-Repeat Rich Membrane Spanning / Kinase D-interacting substrate 220 kDa) is a specific scafold protein for Trk neurotrophin receptors that has been implicated in differentiation and synaptic activity modulation. In this study, we have observed that ARMS/Kidins220 levels affect NGF regulated secretion. To identify potential partners of ARMS/Kidins220 that may be involved in secretion, we performed a yeast two-hybrid assay. This trial identified Synembryn-B, an homologue of Ric8 protein (resistance to inhibitors of cholinesterase 8) originally described in *Caenorhabditis elegans* mutants affected in neurosecretion, as an interactor of ARMS/Kidins220. We have further demonstrated that this interaction plays a crucial role in NGF-regulated secretion through Rho family small GTPases Rac1 and RhoA. Moreover, ARMS/Kidins220 and Synembryn-B also affect NGF-mediated neurite outgrowth in PC12 cells. We conclude this two proteins cooperate to regulate NGF-mediated differentiation and regulated secretion in PC12 cells.

Áreas Temáticas:

1ª: Desarrollo
2ª: Sistemas homeostáticos y neuroendocrino

INSULIN-LIKE GROWTH FACTOR-I (IGF-I) REGULATES THE GENERATION OF PROGENITOR CELLS AND THE POSITIONING AND MATURATION OF HIPPOCAMPAL GRANULE NEURONS

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In the rodent and human brain, the great majority of neurons are formed during embryonic development. Nevertheless, neurogenesis is maintained in the adult subventricular zone-olfactory bulb (OB) and the dentate gyrus (DG) of the hippocampus. Neural stem cells located in these areas give rise to more restricted progenitors that readily differentiate into OB and DG neurons. Our previous studies indicated that the extracellular factor IGF-I promotes the incorporation of newly formed neurons in the adult OB. We then decided to study the role of IGF-I during DG neurogenesis. First, we detected the expression of IGF-I in the postnatal/adult DG, being more abundant in parvalbumin$^+$-neurons. Additionally, some Prox1$^+$-cells expressed both IGF-I and IGF-IR. To study the influence of IGF-I on the different stages of neuronal generation and maturation, we analyzed sections from postnatal/adult IGF-I knockout (KO) and wild-type mice previously immunostained with specific antibodies. The KO mice had an accumulation of Ki67$^+$-cycling cells and Tbr2$^+$-intermediate progenitor cells, which were localized in the outer area of the granule cell layer (CCG) and, even, in the molecular layer (ectopic position for these cells). Moreover, the morphology of doublecortin (DCX)$^+$-cells was more immature and the CCG was disorganized in the KO, having more Prox1$^+$-cells out of the layer. The labelling of proliferative cells with retroviral particles in P21 mice and their analysis 21 days postinjection allowed us to determine that the majority of the newly formed cells were Prox1$^+$-neurons in the mice of both genotypes. However, the neurons in the KO mice showed a less complex dendritic tree. Our findings indicate that IGF-I is a critical factor during adult DG neurogenesis, regulating the number of progenitors as well as the positioning and maturation of newly formed granule neurons.

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Áreas Temáticas:
1ª: Desarrollo

CORTICAL DEVELOPMENT ALTERATIONS INDUCED BY EMBRYONIC Δ^9-TETRAHYDROCANNABINOL ADMINISTRATION ALTER SKILLED MOTOR ACTIVITY AND SEIZURE SUSCEPTIBILITY

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Developmental cannabinoid exposure has been shown to induce long-lasting behavioral alterations in adult mice. However, the impact of embryonic administration of plant-derived Δ^9-tetrahydrocannabinol (THC) and its neurobiological mechanism of action remain obscure. Here we investigated the developmental and functional consequences of embryonic THC exposure (i.p. administration to pregnant female mice from gestational day 12.5 to 16.5, 3mg/Kg) in wild-type (WT) and CB_1-deficient littermates. Analysis of motor activity revealed that embryonic THC exposure impaired the skilled motor function (assessed by the skilled reaching- and staircase pellet reaching-tests), but did not affect unskilled activity and general motor activity. Immunofluorescence characterization of cortical excitatory populations revealed that embryonic cannabinoid administration induced deep-layer projection neuron alterations. In addition, the use of transgenic Thy1-YFP mice, that label layer V pyramidal neurons, confirmed the existence of THC-induced alterations in subcortical projection neurons, likely responsible of the deficits observed in skilled motor activity. Ongoing analysis after vital retrograde tracing of corticospinal motor neurons labelled in the cervical spinal cord of THC- and vehicle-treated WT and $CB_1^{-/-}$ mice will define the contribution of the CB_1 cannabinoid receptor in THC-induced alterations. In addition, the latency to the GABA antagonist pentylenetetrazol-induced seizures was decreased in THC-exposed WT but not $CB_1^{-/-}$ mice. Subsequent analysis of the inhibitory neuronal lineage revealed a shift in the distribution of GABAergic interneurons of THC-treated animals. Finally, gene expression analysis in the cortex and hippocampus of THC-administered mice pointed to altered differentiation of the excitatory and inhibitory neuronal populations and indicated a pro-epileptic transcriptional signature. These findings demonstrate that prenatal cannabinoid exposure exerts functional alterations in the adult brain, owing to the role of CB_1 cannabinoid receptors in the differentiation of subcortical long-range projection neurons and the appropriate coordination of excitatory and inhibitory neuronal specification.

Áreas Temáticas:
1ª: Desarrollo
2ª: Neurociencia cognitiva y conductual

Reelin$^+$ AND p73$^+$ NEURONS IN THE DEVELOPING LATERAL OLFACTORY TRACT OF MICE

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The axons of principal cells in the olfactory bulb form the lateral olfactory tract (LOT) that develops within a preformed LOT territory, located over the lateral pallium since E10.5 in mice. The LOT territory contains diverse types of early-generated neurons whose functions during development are not fully understood. These include, first, Lhx5$^+$ cells that migrate from the thalamic eminence (TE) to the caudal tier of the accessory olfactory bulb, where they differentiate as principal cells (Huilgol et al., 2013). Second, mGluR1$^+$ cells that have been considered guidepost cells in this system (Sato et al., 1998) and, according to our ongoing studies, also derive from the TE. To gain insight into the functional significance of LOT-associated neurons during development, we are analyzing these cell populations in genetically modified mice using immunohistochemistry and in situ hybridization. Here, we focus on two major populations of Reelin expressing cells in the LOT. First are the typical Cajal-Retzius cells in this region, which were Reelin$^+$/p73$^+$/Tbr2$^-$ and located subpially over the LOT. We will show evidence that at least part of the p73$^+$ Cajal-Retzius cells in the LOT derive from p73$^+$/Tbr2$^+$ secondary progenitors in the telencephalic tier of the TE subventricular zone. Notably, these Cajal-Retzius cells may migrate rostrally to reach the olfactory bulb. The second population is composed of Reelin$^+$/p73$^-$ cells, which were found inside the LOT and expressed Tbr2, in contrast to Cajal-Retzius cells that are Tbr2$^-$. Current evidence (Huilgol et al., 2013; our work in progress) seems to indicate that these Reelin$^+$ cells may express Lhx5 and/or mGluR1.

Áreas Temáticas:
1ª: Desarrollo
2ª: Neurociencia de sistemas

$p27^{kip1}$ CAN PREVENT EXTRA ROUNDS OF ENDOREDUPLICATION IN TETRAPLOID RETINAL GANGLION CELLS

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Objective

Endoreduplication (i.e. multiple rounds of DNA synthesis in the absence of cell division) is a common mechanism leading to somatic polyploidy in multiple organisms. In the developing chick retina, a population of nascent retinal ganglion cells (RGCs) becomes tetraploid after undergoing one single round of DNA synthesis followed by G2/M transition arrest while they migrate to the ganglion cell layer (Morillo et al. *PNAS* 107:109-14, 2010; Ovejero-Benito and Frade, *PLoS ONE* 8: e64890, 2013). The aim of this work was to explore the mechanism that prevents extra rounds of genome duplication in these RGCs, thus maintaining them in a tetraploid state.

Materials & Methods

Chicken embryos were staged according to Hamburger and Hamilton, *J.Morphol.*88:49-92, 1951. Cell cultures were made as described previously (Frade and Rodríguez-Tébar, *Methods.Mol.Biol.*139:257–264, 2000). Enrichement of differentiated RGCs was performed as described by De Curtis et al, *J.Cell.Biol.* 113:405-16, 1991. RNA interference was carried out as described previously (Das et al., *Dev.Biol*.294:554-63, 2006). Lipofection was performed as described by García-Domínguez et al. *Mol.Biol.Cell*. 22:1227-39, 2011. Immunostaining was carried out as described previously (Morillo et al. *PNAS* 107:109-14, 2010).

Results

$p27^{Kip1}$ can be detected in layered RGCs that express $p75^{NTR}$ and TrkB, and lack retinoblastoma expression, a neuronal population previously shown to be tetraploid. To study the role of $p27^{Kip1}$ in these neurons we designed two specific siRNA constructs able to strongly downregulate $p27^{Kip1}$ expression. By using these constructs we found that knocking-down of $p27^{Kip1}$ results in a significant increase of BrdU incorporation in RGC-enriched cultures. This finding is consistent with the observed incorporation of BrdU in RGCs from $p27^{Kip1}$ knock-out mice (Cunningham et al. *Mol.Cell.Neurosci*. 19: 359-374, 2002).

Conclusions

We propose that $p27^{Kip1}$ prevents extra rounds of genome duplication in tetraploid RGCs. This mechanism may also keep diploid RGCs with normal DNA levels.

RELATION BETWEEN CELL POSITIONING AND MÜLLER GLIA IN THE DEVELOPING SHARK RETINA: A DOUBLECORTIN IMMUNOCYTOCHEMICAL STUDY

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Development of the nervous tissue is a coordinated process of progenitor cell proliferation, differentiation and migration to correct locations. The retina of sharks has been exploited as a model to investigate neurogenesis. As in all vertebrate species studied, the retina is composed of six major types of neurons and Müller glia, arranged in different layers that result from adequate cell positioning and migration. As in other fishes, it contains stem cells within the ciliary marginal zone that gives rise to new cells throughout life. This fact, together with the slow development of embryos, makes them appropriated for detailed analysis of neurogenesis. While patterns of proliferation and cell differentiation in sharks are currently well known[1-3], the molecular cues that control retinal cell positioning are not well-understood. Cell-to-cell interactions as well as Müller cells have been reported to be involved in this process. However, in sharks, the maturation of Müller cells occur largely after mature neurons can be identified in its respective layers[4]. To investigate the relation between cell positioning and Müller glia in the developing retina, we have analyzed by the immunohistochemical pattern of expression of doublecortin (DCX, a microtubule-associated protein related with neurogenesis and migration of immature neurons) and glial fibrillary acidic protein (GFAP, a glial cell marker typically expressed in Müller cells). Our results reveal high DCX-immunoreactivity in the soma and processes of different cell types in the immature (not laminated) retina, before the radial glia can be identified by means of anti-GFAP. Interestingly, some cell bodies and fibers in the mature retina show DCX-immunoreactivity long after proliferation and lamination have been completed. Our results suggest that DCX can be involved in cell positioning in early stages of retinogenesis and highlight the importance of DCX in roles other than neurogenesis and cell migration in the mature retina.

Supported by Xunta de Galicia (10PXIB200051PR), Ministerio de Ciencia e Innovación (BFU2010-15816) and European Community-Research Infrastructure Action under the FP7 "Capacities" Specific Programme (ASSEMBLE 227799). References: (**1**) Ferreiro-Galve et al. (2010) **J.Chem.Neuroanat.** 39:1-14; (**2**) Ferreiro-Galve et al. (2010) **Exp. Eye Res**. 91:378-86; (**3**) Ferreiro-Galve et al. (2011) **J.Exp.Zool**. 318:91-108; (**4**) Bejarano-Escobar et al. (2012) **J.Anat.** 220:318-35.

1ª: Desarrollo

ACK1 EN PROCESOS DE RAMIFICACIÓN AXONAL Y DENDRÍTICA EN NEURONAS EN CULTIVO

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Ack1 es una tirosina quinasa altamente expresada en sistema nervioso central y capaz de interaccionar con numerosas proteínas intracelulares involucradas en señalización como Grb2, Cdc42 o Nck. Con el objetivo de conocer las funciones de esta molécula en el sistema nervioso, hemos comprobado que el tratamiento con diferentes neurotrofinas (NGF, BDNF, NT-3), conduce a una activación de Ack1 y también hemos detectado capacidad de coinmunoprecipitación entre receptores Trk como TrkA, B y C con la propia Ack1. Además, hemos llevado a cabo abordajes de alteración de los niveles de expresión mediante transfección con el cDNA y siRNA en neuronas en cultivo y en células PC12. Hemos observado un aumento significativo de las ramificaciones axonales y dendríticas como consecuencia de la sobreexpresión de esta molécula. De forma consistente con lso resultados anteriores, hemos comprobado que la disminución de la expresión de esta quinasa provoca una reducción estadísticamente significativa del grado de arborización dendrítica y axonal. Resultados similares han sido observados en el modelo de diferenciación celular representado por las células PC12.

Áreas Temáticas:

1ª: Desarrollo
2ª: Neurociencia de Sistemas

BMP AND WNT SIGNALLING REGULATE NEURONAL DIFFERENTIATION OF ADULT HIPPOCAMPAL STEM CELLS

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New neurons are born in defined brain regions throughout the whole life of an individual due to the existence of neural stem cells (NSCs). Neurogenesis in the adult brain occurs in two different locations: the subventricular zone (SVZ) of the lateral ventricles, and the subgranular zone (SGZ) of the hippocampal dentate gyrus. Local signals, such as Wnt ligands, regulate NSC proliferation and differentiation. Other signals, such as Bone Morphogenetic Proteins (BMPs), are involved in NSC maintenance and quiescence. However, most of the downstream mechanisms involved in these processes are still unknown. Here we show that adult hippocampal stem/progenitors express BMP receptors, and signalling through these receptors regulates NSC differentiation. We found that BMP2/4 signalling is sufficient to increase hippocampal neurogenesis *in vitro*, and that this signalling determines the neuronal fate within a 24 hour time window. We also observed that BMP signalling increases Lef1 expression, a member of the Wnt canonical pathway, suggesting a synergic effect between both, BMP2/4 and Wnt3a. Indeed, our results demostrate that BMP2/4 signalling enhances Wnt3a-induced neuronal differentiation. These findings point to a crosstalk between the BMP and Wnt pathways during adult hippocampal neurogenesis. We anticipate our assays to be the starting point for future *in vitro* or *in vivo* experiments aimed at understanding the molecular mechanisms of adult neurogenesis.

Áreas Temáticas:
1ª: Desarrollo.
2ª: Trastornos y reparación del sistema nervioso.

NKX6.2 POSITIVE TANGENTIAL MIGRATION IN TO THE BASAL INTERSTITIAL COLUMN.

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In a previous work we proved the localization of Nkx6.2 positive neurons along the interstitial column located in the basal diencephalon and mesencephalon. The fact that Nkx6.2 ventricular expression is restricted to the alar plate prompted us to analyse a possible tangential migration of this cells into the basal plate. This hypothesis was also supported by fate map analysis that proved the non basal origin of this population. We have used a transgenic line with the cre endonuclease under the promoter of Nkx6.2 inducible by tamoxifen (Dr. Fisher, NYSUM). We crossed this line with a tdTomato reporter line (The Jackson Lab, #007905) in order to label the Nkx6.2 positive neuroblasts and follow them through development. We have developed an organotypic neural tube culture, dissecting the area of the neural tube of our interested, opening it as a book through the roof plate and culturing it with the ventricular surface upwards. Neural tubes labelled at E9.5 and cultured at E10.5 displayed the ventricular domain and no cells were observed in the mantle layer a t0. One day later, the first labelled cells could be observed in the mantle layer. The migrating stream of neurons positive for Nkx6.2 become, two days later, stronger and the cells reached their final destination in the area of the interstitial column. The results obtained have proven that Nkx6.2 positive neurons migrate tangentially, they move from a caudodorsal position into their rostroventral final destination.

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Áreas Temáticas: Seleccione las **2** áreas temáticas que más se ajusten a su trabajo en orden de prioridad:
1ª: Desarrollo
2ª: Desarrollo

IMPLICATION OF NPAS1 IN THE DIFFERENTIATION GENETIC PATHWAY OF THE RED NUCLEUS.

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The identity of the basal midbrain is specified by the signals emitted mainly from the isthmus and the floor plate. The Shh gradient is translated in the trigger of the different genetic specification programs and these in the generation of the neuronal nuclei. Npas1 is a member of bHLH-PAS family of transcription factors. It is related with the neuronal differentiation. It is expressed in two populations of the basal midbrain, the red nucleus and the oculomotor complex. Our aim is to unveil the implication of Npas1 in the genetic pathway that directs the differentiation of the red nucleus. This study has been realized in two mouse strains, En1cre/+; Shhflox/flox and Pou4f1TauLacz/+ through immunohistochemistry and in situ hybridization.
The expression of Npas1 in the basal midbrain is first detected at E13.5. We confirmed the location of the Npas1 using double staining with Islet1 (oculomotor nucleus) and Pou4f1 (red nucleus). In order to elucidate a direct relation between Npas1 and Shh, we analysed a conditional mutant for Shh in the territory of En1. The expression of Npas1 is lost in the oculomotor complex but not in the red nucleus. This phenotype is due to the disappearance of the neurons of this nucleus. Pou4f1 is a transcription factor related with the specification of the red nucleus. We analysed Npas1 in a Pou4f1 null mouse, it is specifically lost in the red nucleus and maintained in the oculomotor complex. The lost of Npas1 could be responsible of the Pou4f1 null described phenotype, the red nucleus neurons are not able to develop a correct innervation of their target in the spinal cord.
This work was supported by the grant MICINN BFU2010-16538 to Puelles, E. BFU2011-27326 and GVA-Prometeo 2009/028 to Martinez, S. Moreno-Bravo, JA is supported by the JAE-Predoc-ESF.

Áreas Temáticas: Seleccione las **2** áreas temáticas que más se ajusten a su trabajo en orden de prioridad:
1ª: Desarrollo
2ª: Desarrollo

ROLE OF CONNEXIN43 DURING THE INITIAL STEPS OF FGF8 PLANAR INDUCTION ARISING FROM THE VERTEBRATE'S ISTHMIC ORGANIZER

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Gap junctions (GJs), composed of proteins from the connexin family allow for intercellular communication between cells in essentially all tissues by the transport of different ions and small molecules of less than 1000 Daltons through them. In brain, Connexin 43 (Cx43) is the dominant GJ expressed. At neural tube stages Cx43 and fibroblast growth factor 8 (FGF8) expressions co-localize in morphogenetic signaling centers. In one of them, the isthmic organizer (IsO) FGF8 is found as the key molecule for patterning the mesencephalon and rhombencephalon. FGF8 is secreted to the extracellular space but rapidly binds to Heparan Sulfate Proteoglycans (HSPGs) on the cell surface and in the extracellular matrix. Thus, release from HSPG and binding to another HSPGs creates, within time, a long-range morphogen gradient through the neuroepithelium. We observed though, that the initiation of the signaling triggered by FGF8 seems to travel much faster long distances than the supposed hindered diffusion protein, suggesting that other mechanisms may require for this long-range FGF8 planar induction activity. In the present work we have initiated embryological experiments in embryonic mouse brain tissue to assess the relationship between gap junctions (Cx43) and the FGF8 morphogenetic signal activity. This work was supported by the *MICINN FONDOS FEDER (BFU2011-27326).*

Áreas Temáticas:
1ª: Desarrollo
2ª: Desarrollo

FGF15 REGULATES PROLIFERATION AND NEUROGENESIS IN THE DEVELOPING DIENCEPHALON

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The establishment of anatomical structures of the mammalian brain requires a balanced proliferation and cell cycle exit of neural progenitors. In that sense, a precisely orchestrated interplay between extrinsic and intrinsic signals is required. However, little is known about the signals regulating these processes, particularly in the diencephalon. The diencephalon is a complex region located in a central area of the vertebrate brain that receives afferent sensory information, relaying it on to the cerebral cortex. Within this structure, gene expression characterizes the molecular regionalization, by regulating main histogenetic processes such as proliferation, migration, differentiation and establishment of neuronal connections. Among these genes, members of the fibroblast growth factor (Fgf8), hedgehog (Shh), bone morphogenetic proteins (Bmps2,4,6 and 7) and wingless (Wnts2b, 3a, 5a, 7a and 8b) family act as morphogenetic regulatory genes. Fibroblast growth factor 15, the mouse ortholog of human, chick and zebrafish Fgf19, is expressed in the ventricular layer of the diencephalon. The aim of this work is to determine the role of Fgf15 in diencephalic development. To assess this issue we used Fgf15-/- mutant embryos and analyzed the molecular and cellular phenotype by using immunohistochemistry and *in situ* hybridization. We studied proliferation by detecting Phosphohistone-H3 (PH3), as well as analyzed markers for postmitotic neurons, neural stem and progenitor cells such as Doublecortin (Dcx), neuronal class III beta-Tubulin (Tuj1) and Nestin among others, in each region of the diencephalon during development. Therefore, we observed that despite the increased numbers of cycling progenitors, these cells did not generate the proper amount of postmitotic progeny. To conclude, we provide evidence that Fgf15 controls cells cycle exit and differentiation in the mouse diencephalon.
This work was supported by the following Grants: European Consortium: EUCOMMTOOLS (Contract 261492), the Spanish Ministry of Science and Innovation: FEDER (BFU-2010-27326) and CONSOLIDER (CSD2007-00023).

1ª: Desarrollo
2ª: Desarrollo

CONTRIBUTION OF SIX3 TO SUBSTANTIA NIGRA PARS RETICULATA DEVELOPMENT.

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In the adult basal midbrain, there are gabaergic neurons in three main populations. The reticular formation, the periaqueductal grey and the susbtantia nigra pars reticulata. Recently, it has been proved that these gabaergic neurons display a complex origin. Fate map analysis has proven that these neurons are not originated from the basal plate. Six3 is a member of the sine oculis related homeobox gene. It presents a complex expression pattern in the midbrain, the ventricular expression is restricted to the alar plate however later on it is highly expressed in the susbtantia nigra pars reticulata. The aim of our work is to prove the contribution of Six3 to the generation of the gabaergic neurons of this population. We have developed our analysis in different mouse models using immunohistochemistry and in situ hybridization. We have labelled the alar neuroblasts at E10.5 and follow them in development. They colonize by tangential migration the basal prospective territory of the susbstantia nigra pars reticulata. Our E10.5 labelled neurons colocalized with Gad1 in the localization of the nucleus at E18.5. Therefore, our results have demonstrated that Six3 positive neuroblasts contribute to the heterogeneous gabaergic population of the substantia nigra pars reticulata.
This work was supported by the grant MICINN BFU2010-16538 to Puelles, E. BFU2011-27326 and GVA-Prometeo 2009/028 to Martinez, S. Moreno-Bravo, JA is supported by the JAE-Predoc program co-financed by the European Social Fund.
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Áreas Temáticas: Seleccione las **2** áreas temáticas que más se ajusten a su trabajo en orden de prioridad:
1ª: Desarrollo
2ª: Desarrollo

STUDY OF STRIATAL GENE EXPRESSION PROFILE DURING DEVELOPMENT

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In recent years cell and gene therapies have been suggested as encouraging treatments for neurological disorders such as Huntington's disease (HD). However, little is known about the mechanisms involved in striatal medium spiny neuron development. Such information is required in order to develop effective HD cell replacement therapies.

To achieve this, we used laser capture for microdissecting mouse striatal Germinal (GZ) or Mantle Zone (MZ) at different mouse developmental stages (E12.5, E14.5, E16.5 and E18.5). Subsequently, we performed microarray analyses to compare the gene expression profiles of striatal progenitor and postmitotic cells during striatal development.

Using a linear fit algorithm analysis, 3,636 DEGs (Differentially Expressed Genes) were obtained from the whole mouse genome. Gene ontology analyses revealed that MZ upregulated genes are mainly involved in ion transport, synaptic transmission and neural differentiation processes, while GZ upregulated genes participate in cell cycle related processes.

To further study the genes involved in striatal neuron generation, we used clustering techniques (hierarchical and k-means) to classify the DEGs into different expression patterns. Two main cluster types with high expression in either the GZ or MZ were identified. Remarkably, genes previously implicated in striatal development followed the expected expression pattern, providing further validation of present results. In addition, our data identified interesting groups of genes that display expression patterns similar to those of the reference genes, suggesting a potential role in striatal neuron development. Finally, Metacore software enabled us to identify new pathways that are involved in striatal development.

In conclusion, our study describes new genes and pathways that may play a crucial role during striatal development. This information can be extremely useful to better understand the mechanisms of medium spiny neuron generation which, in turn, will allow us to develop new strategies for cell differentiation and cell replacement therapies in Hungtington's disease.

ALTERATIONS OF THE FOREBRAIN IN THE *LHX2*-KNOCKOUT MOUSE

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The LIM homeobox gene *Lhx2* is expressed in specific and comparable patterns in the forebrain of different vertebrates. In mouse, *Lhx2* is essential for the development of three structures that evaginate from the secondary prosencephalon: the neurohypophysis, the retina, and the telencephalon (Porter et al., 1997; Zhao et al., 2010). In the telencephalon, *Lhx2* is critical for the correct patterning of the pallium, and in *Lhx2-/-* mice there is agenesis of the hippocampal formation and severe malformation of the neocortex (Porter et al., 1997), while the paleocortex and pallial amygdala appear relatively unaffected (Vyas et al., 2003). Data in zebrafish show additional roles of *Lhx2* in the formation of the diencephalon, ventral telencephalon and forebrain commissures (Seth et al., 2006; Peukert et al., 2011), but such roles remain mostly unexplored in mouse. We re-analyzed the role of *Lhx2* in forebrain development by studying the mRNA expression of a battery of developmental regulatory genes in the embryonic forebrain of *Lhx2-/-* mice. The genes studied include *Lhx6*, *Lhx9*, *Lmo3*, *Lmo4*, *Dlx5*, *Nkx2.1*, *Sp8*, *Er81*, *Lef1*, *Nhlh2*, *Tbr1*, *Pax6* and *Shh*. Our data show that the forebrain of *Lhx2-/-* mice shows severe malformations which include not only those previously reported but, in addition, new alterations in the telencephalon, hypothalamus and diencephalon. In the telencephalon, the pallio-subpallial boundary is misplaced ventrally, and the ventrolateral pallium is notably enlarged. Thus, it appears that *Lhx2* controls the size of the pallial sectors at both sides of the cerebral cortex, i.e. the cortical hem and the antihem/ventrolateral pallium. *Lhx2* also appears to control the relative size of different domains in the diencephalon (such as thalamus versus prethalamus). Thus, our study provides new results that help to better understand the role of *Lhx2* in forebrain development.
Supported by MINECO (BFU2012-33029) and a travel fellowship from UdL to A.A.

Áreas Temáticas
1ª: Desarrollo

CORRELATION BETWEEN MOLECULAR SPECIFICATION AND NEURONAL PHENOTYPIC DIVERSITY IN THE INTERPEDUNCULAR NUCLEUS

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The interpeduncular nucleus (IP) is a key limbic structure, highly conserved evolutionarily among vertebrates. IP receives indirect input from limbic areas of the telencephalon, relayed by the habenula via the fasciculus retroflexus. There are multiple studies focused on the chemoarchitecture and the connectivity of the IP, with complex results, due to the presence of multiple cell types across a variety of subnuclei.
We have recently demonstrated the origin, migratory pathway and final location of some of these cell populations in the subnuclei of the IP complex (Lorente-Canovas et al., 2012). These cell populations were characterized by the expression of distinct transcription factors (Pax7, Nkx6.1, Otp and Otx2) during chick brain development. Our hypothesis is that this transcriptional code may underlie the specification of the neuronal phenotype in the different subpopulations of IP.
In the present work, our objective is to analyze the correlation between the molecular expression of these transcription factors and the acquisition of a specific neuronal phenotype. We analyzed the expression of Pet1, L-enkephalin, Gad67, SP, Ser, bNOS, ChAT and VGlut2 in these molecularly identified subpopulations of the developing chick IP. In this study we have used chick embryos at different stages of development. Agarose-embedded tissue sections were analyzed by double in situ hybridization-immunostaining using different molecular and neurotransmitter markers.
We have identified three distinct Pax7-positive subpopulations within the IP, two of them expressing SP or Gad67, respectively; as well as two Nkx6.1 positive subpopulations, one of them being glutamatergic. Therefore, our results demonstrate the existence of further specific subpopulations in each molecularly characterized population of the complex structure of IP. Furthermore, we have shown a correlation between the multiple progenitor domains in the IP and the generation of neuronal phenotypic diversity.
We hope that these results may contribute to increasing our knowledge of the mechanism underlying patterning and generation of neuronal phenotypic diversity in the IP complex.

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Área Temática: Desarrollo

ONCOGENIC WNT/BCATENIN SIGNALING DISRUPTS THE APICAL-BASAL POLARITY IN NEURAL PROGENITORS CAUSING AN ABERRANT GROWTH OF THE NEUROEPITHELIUM

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Purpose: Activating mutations in the Wnt pathway effector βcatenin have been associated with tumorigenesis in a subset of medulloblastomas, the Wnt-subtype. Recently, a mouse model of Wnt-medulloblastoma shows that precursors situated in the dorsal brain stem can give rise to this subtype of tumors. However, how βcatenin contributes to the genesis of these tumors remains unclear. Our project is aimed to clarify the oncogenic mechanism of βcatenin in this subtype of medulloblastoma.

Materials and Methods: Neural tubes of HH10-12 chick embryos were electroporated, allowed to develop, dissected and processed for immunohistochemistry, luciferase assay, qPCR or FACS.

Results: Overexpression of active forms of βcatenin in chick central nervous system progenitors caused an aberrant accumulation of embryonic dorsal brainstem cells, characterized by ectopic germinative collections. These abnormal masses were found filling the IV ventricle reproducing the phenotype of Wnt-medulloblastoma mouse model. Short-term studies revealed that active forms of βcatenin initially induced an apical accumulation of polarity proteins such as atypical Protein Kinase C (aPKC), Zonula occludens-1 (ZO-1) and N-Cadherin, and later apical folds of the neuroepithelium (invaginations) that originated the ectopic germinative collections. In these invaginations, βcatenin expressing cells maintained the progenitor stage, but surprisingly, the proliferation of these cells was decreased and the cell cycle arrested at M phase. By dissecting transcriptional and structural functions of βcatenin, we demonstrated the requirement of Wnt canonical pathway for the generation of the phenotype. Moreover, qPCR assays revealed that Wnt/βcatenin pathway was up-regulating the expression of aPKC. Concordantly, overexpression of active forms of aPKC reproduced βcatenin phenotype.

Conclusions: These findings demonstrate that Wnt/βcatenin pathway controls the apical-basal polarity in neural progenitors trough the transcriptional regulation of aPKC. This aPKC misregulation disrupts the cell cycle blocking cells in M phase which seems to cause an aberrant growth of the neuroepithelium in the developing hindbrain. Our data provide novel model for the oncogenic mechanism of beta-catenin in the Wnt-sybtype medulloblatoma.

IMPLICATION OF ACs IN SONIC HEDGEHOG SIGNALING

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Objective: Sonic Hedgehog (Shh) signaling is essential for the development of many tissues, including neural tube where it controls patterning and cerebellum where it promotes cerebellar granule neuron precursors (CGNPs) proliferation. Shh pathway is negatively regulated by cAMP-dependent protein Kinase (PKA). How is PKA regulated by cAMP, is not totally described yet. Our study is focused on finding out which of the nine transmembrane Adenylyl Cyclases (ACs) isoform is the responsible for the cAMP increase leading to PKA dependent inactivation of Shh pathway.

Methods: In situ hybridization was performed using probes for the nine different isoforms of ACs in developing cerebellum. The overexpression studies were conducted by transfection of AC5 and AC6 expression vectors in two different models: mouse primary cell culture (CGNPs) and chick neural tube. Proliferation was analyzed by BrdU incorporation.

Results: First, we report by in-situ hybridation studies that AC6 is the isoform which expression is highest in the external granular layer (EGL), where CGNPs proliferate in response to Shh. In addition, over-expression studies conducted in CGNPs and neural tube revealed that AC5 and AC6 are able to attenuate Shh-induced proliferation. Finally, we show that AC5 and AC6 localize at the cilia, the organelle required to start Shh signaling in CGNPs.

Conclusions: Collectively, these findings suggest that the Shh pathway could be regulated exclusively by a single AC isoform. Interestingly, AC6 as AC1 and AC5 are the only isoforms that respond to Gi protein. Therefore, our results are in line with previous findings which demonstrate that inactivation of PKA occurs through inhibitory G protein (Gi).

Áreas Temáticas:

1. Desarrollo

5. Trastornos y reparación del sistema nervioso

Implication of ACs in Sonic Hedgehog signaling

EFECTOS NEUROGÉNICOS TRAS EL BLOQUEO FARMACOLÓGICO DE LOS RECEPTORES CANNABINOIDES CB1 Y CB2 EN RATAS TRATADAS CON COCAÍNA

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El sistema endocannabinoide juega un papel crucial en la modulación de numerosos procesos relacionados con la neurogénesis, neuroinflamación y muerte celular mediante la modificación de la actividad de los receptores cannabinoides tipo 1 y 2 (CB1 y CB2). De hecho, nuestro grupo, ha descrito la presencia de varias enzimas cannabinoides (DAGL, NAPE-PLD, FAAH, MAGL) en regiones neurogénicas del cerebro adulto. Sin embargo, todavía se desconoce muchos aspectos de estos procesos celulares en un contexto de adicción a drogas.

Objetivo. Evaluar el papel de los receptores CB1 y CB2 tras la administración de cocaína mediante el bloqueo farmacológico de los receptores CB1 (Rimonabant) y CB2 (AM630) en procesos de proliferación celular en regiones neurogénicas del cerebro adulto, astrogliosis y apoptosis (estriado, hipocampo e hipotálamo).

Material y métodos. Empleamos ratas macho de la cepa Wistar (250-300 gr) que recibieron una administración única o repetida de cocaína (20 mg/kg), Rimonabant (3 mg/kg) y AM630 (3 mg/kg) durante 5 días, siendo evaluadas en un Open-Field durante 30 min. Tras el test de actividad locomotora, analizamos el número de células con 5-bromo-2-deoxiuridina (BrdU) en la zona subventricular (SVZ), zona subgranular del giro dentado (SGZ) y zona ependimaria-parenquimática del hipotálamo, o que expresan caspasa 3, GFAP e Iba1 en estriado dorsal, hipocampo e hipotálamo, mediante inmunohistoquímica.

Resultados. Los resultados muestran que la administración aguda de cocaína disminuyó el número de células BrdU+ y aumentó el número de células caspasa 3+, GFAP+ e Iba1+ en las regiones cerebrales analizadas. La administración repetida de cocaína disminuyó el número de células BrdU+ en SVZ, aumentó el número de células caspasa 3+ en el hipocampo y células GFAP+ e Iba1+ en el hipocampo e hipotálamo. El tratamiento con Rimonabant y AM630 en ratas con administración repetida de cocaína aumentó el número de células BrdU+ en las regiones neurogénicas y disminuyó el número de células caspasa 3+ en el hipocampo, de células GFAP+ en el hipocampo e hipotálamo y de células Iba1+ en el estriado.

Conclusiones. Las alteraciones neurogénicas que tienen lugar durante el proceso adictivo a cocaína son disminuidas mediante la modulación farmacológica de los receptores cannabinoides CB1-2, disminuyendo la neuroinflamación y muerte celular asociadas a los efectos repetidos de la droga. Por tanto, el bloqueo farmacológico de los receptores CB1-2 podría constituir un nuevo uso terapeútico en el tratamiento adictivo a psicoestimulantes.

ASCL1/MASH1 PROMOTES BRAIN OLIGODENDROGENESIS DURING MYELINATION AND REMYELINATION

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Oligodendrocytes are the myelin-forming cells of the central nervous system. They differentiate from oligodendrocyte precursor cells (OPCs) that are produced from progenitors throughout life, but more actively during the neonatal period and in response to demyelinating insults. An accurate regulation of oligodendrogenesis is required to generate oligodendrocytes during these developmental or repair processes. We hypothesized that this regulation implicates transcription factors (TFs) which are expressed by OPCs and/or their progenitors. Ascl1/Mash1 is a proneural TF previously implicated in embryonic oligodendrogenesis and operating in genetic interaction with Olig2, an essential transcriptional regulator in oligodendrocyte development. Herein, we have investigated Ascl1 contribution to oligodendrocyte development and remyelination in the postnatal cortex. During the neonatal period, Ascl1 expression was detected in progenitors of the cortical subventricular zone (SVZ) and in cortical OPCs. Different genetic approaches to delete Ascl1 in cortical progenitors or OPCs reduced neonatal oligodendrogenesis, showing that Ascl1 positively regulated both OPC specification from SVZ progenitors as well as the balance between OPC differentiation and proliferation. Examination of remyelination processes, both in the mouse model for focal demyelination of the corps callosum (CC) and in multiple sclerosis (MS) lesions in humans, indicated that Ascl1 activity was up-regulated along with increased oligodendrogenesis observed in remyelinating lesions. Additional genetic evidence indicated that remyelinating oligodendrocytes derived from Ascl1+ progenitors/OPCs and that Ascl1 was required for proper remyelination. Altogether, our results show that Ascl1 function modulates multiple steps of OPC development in the postnatal brain and in response to demyelinating insults.

1ª: Desarrollo
2ª: Trastornos y reparación del sistema nervioso

COLONIZATION OF THE OPTIC NERVE HEAD AND PECTEN ANLAGE BY MICROGLIAL PRECURSORS DURING QUAIL RETINA EARLY DEVELOPMENT

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Microglia are cells of mesodermal origin that populate the central nervous system during development. In the avascular quail retina, microglial cells come from precursors of amoeboid phenotype entering the embryonic retina from the region of the optic nerve head and base of the pecten (ONH/BP) between days 7 and 16 of incubation (E7-E16). By using organotypic cultures of retinal explants, we have previously showed that these microglial precursors are already present in the primordial ONH and pecten anlage, which derive from the fusion of the optic fissure edges, from around E3, i.e., 4 days before they enter the retina. Our aim was to study the colonization pattern of the ONH/BP region of quail embryos between E3 and E6 by microglial precursors and their relationship with the development of other cell types present in this area. For this purpose, whole-mount and cryosectioned retinas of these developmental stages were immunolabeled with the following antibodies: QH1 (which recognizes all developmental phases of macrophage/microglial cells in the quail, as well as endothelial cells), anti-vimentin, TUJ1 (neuron-specific class III beta-tubulin) and anti-active caspase-3. In addition, interspecific chick-quail yolk sac chimeras (chick embryo grafted on quail yolk sac) surviving until E3.5-E7 (developmental ages matching E3-E6 in the quail) were analysed. This study revealed that QH1-positive cells with a macrophage phenotype and originated in the yolk sac were present in the ONH/BP area from E3. We identified two routes of entry of QH1-positive cells into the ONH/BP area: some cells entered from the meninges surrounding the ONH while others migrated from peripheral retina into the ONH/BP region and entered the optic fissure together with blood vessels contributing to pecten vasculature.

Supported by grant BFU2010-19981 from the Spanish Ministry of Economy and Competitiveness.

Áreas Temáticas:
1ª: Desarrollo
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

GENOARCHITECTONIC PECULIARITIES IN A PRETECTAL POPULATION DERIVED FROM THE VENTRAL JUXTACOMMISSURAL PRETECTUM

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The pretectal region is the alar plate of prosomere 1, placed between thalamus and mesencephalon. In previous work we reported that the pretectum of tetrapods (birds, mammals and amphibians, at least) is organized genoarchitectonically into three anteroposterior domains (precommissural, juxtacommissural and commissural regions - PcP, JcP o CoP) and these appear each subdivided into various dorsoventral subdomains. Some 30 pretectal nuclei were characterized genoarchitectonically in the chick (Ferran et al., 2009), but the derivatives from some domains were not sufficiently defined. The aim of the present study was to map additional transcription factors, enzymes and membrane/vesicular carriers that may contribute to clarify nuclei poorly identified in our previous studies in chicken.

To that aim, we performed single and double chromogenic in situ hybridization between stages HH32-HH45 of development and postnatal stages, mapping mRNA expression of the chicken genes *Enc1*, *FoxP1*, *Tac1*, *Vglut2*, *Gad67*, *Six3*, *Meis1* and *Meis2*. For additional molecular characterization we also used selected genoarchitectonic markers previously found useful for identifying the PcP-, JcP- or CoP-derived nuclei. We discovered a previously unidentified *Tac1*-expressing group of cells that is located in the ventral JcP subdomain. Curiously, this new pretectal nucleus does not express *FoxP1* and *Six3*, which are general markers of JcP-derived nuclei. Moreover, typical markers of PcP- or CoP-derived formations (*Bhlhb4* and PAX7, respectively) were also absent in these cells. This ventral JcP subdomain displays nevertheless *Six3* expression at early stages (HH24-28). These data elaborate upon our earlier identification of pretectal nuclei, highlighting specific properties in a region that was hard to understand previously.

OVER-EXPRESSION OF GLYCOPROTEIN ANOSMIN-1 INCREASES NEUROGENESIS AND OLIGODENDROGLIOGENESIS IN THE ADULT CNS *IN VIVO*

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The extracelular matrix glycoprotein anosmin-1 plays relevant roles in cell migration, axon outgrowth and guidance and the formation of axons collaterals during the development of the CNS. This protein binds to other extracelular matrix proteins and to FGFR1, modulating FGF-2 activity. In the present work, we show how the over-expression of anosmin-1 gives rise to a significant increase in neurogenesis and oligodendrogliogenesis in the adult CNS. The increased neurogenesis in the SVZ leads to selective changes in the number of interneuronal populations in the olfactory bulb, without any major changes in other structures of the olfactory system. Its physiological consequence is a problem in the olfactory memory without affecting olfactory perception or discrimination. On the other hand, the increased oligodendrogliogenesis in the adult gives rise to an increased number of OPCs and mature myelin-forming oligodendrocytes, as well as enhanced myelin formation and thicker myelin sheaths; on the contrary, there were no changes in the percentage of myelinated axons or the morphology of axons and internodes in the corpus callosum. Together with this, we show larger Ranvier nodes and a faster conduction velocity in vivo. All together, our results confirm the relevance of anosmin-1 in cell proliferation, as well as the relevance of FGF-2 for the generation of newborn neurons and oligodendrocytes in the adult CNS.

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SENC

15º Congreso Nacional de la Sociedad Española de NeuroCiencia

Tema 2

Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

Posters

SUBCELLULAR ASSOCIATION BETWEEN GIRK2, $GABA_B$ RECEPTORS, RGS7 AND Gβ5 PROTEINS IN THE HIPPOCAMPUS

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The GIRK channels mediate the inhibitory postsynaptic effect of neurotransmitters and abuse drugs. These channels are insensitive to voltage and only activated through G proteins. G proteins-mediated signalling is one for the most widely used trans-membrane mechanisms in mammalian organism. Regulator of G protein signalling (RGS) protein serve as negative regulators of G protein-coupled receptor signalling by stimulating GTP hydrolysis on the Gα subunits to promote their inactivation. There is now compelling evidence that all member of the R7 RGS protein family exists as complexes with type 5 G protein β subunit (Gβ5). However, little information is available on their cellular and subcellular localization, and their spatial relation with GIRK channels and GABAB receptors. Using expression systems and co-immunoprecipitation approaches, we show that GIRK2 interact with RGS7 and Gβ5 in HEK 293 FT cells. Using immunoelectron microscopy, RGS7 and Gβ5 were located in CA1 pyramidal cells at postsynaptic and presynaptic sites. The use of quantitative approaches at the EM level have shown that RGS7 and Gβ5 immunoparticles were detected both at intracellular sites (56% for RGS7 and 75% for Gβ5) and along the plasma membrane (44% for RGS7 and 25% for Gβ5). Of the immunoparticles detected along the plasma membrane in the *stratum radiatum* of the CA1 region, most were detected at presynaptic sites (5%) in axon terminals, likely Schaffer´s collaterals, establishing excitatory synapses with spines. In dendritic spines, RGS7 and Gβ5 showed the same distribution relative to the glutamate release site, also very similar to the distribution described for $GABA_B$ receptors and GIRK channels. Our molecular, cellular and subcellular studies suggest that RGS7 and Gβ5 are forming macromolecular complexes with GIRK and $GABA_B$, and suggest that RGS7-Gβ5 complexes may be critical in the regulation and activation of GIRK channels.

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REGIONAL AND SUBCELLULAR LOCALIZATION OF STRUCTURAL AND AUXILIARY SUBUNITS OF BK CHANNELS

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The large-conductance and Ca^{2+}/voltage-gated potassium channel (BK) performs important physiological roles. In the brain, a major function of the BK channel is to down-regulate neurotransmitter release at the presynaptic site and modulation of firing rates. The BK channel is a tetramer formed by the structural and functional α subunits that can assemble with an auxiliary β subunit. There are four identified β subunit genes (β_1-β_4), which show tissue-specific expression. BK channels are widely expressed throughout the brain, but little is known about their regional and subcellular distribution. Thus, we investigated the subunit composition of α-β in identified brain regions and cell types, focusing on the β_3 subunit. Using the histoblot technique, we found that both subunits are widely expressed in the brain showing mostly overlapping expression pattern; high levels of expression were detected in the neocortex, thalamus, striatum, hippocampus and cerebellum. Using the high-resolution pre-embedding immunogold technique in combination with quantitative approaches in the cerebellum, we observed that the α and the β_3 subunits were located in Purkinje cells at postsynaptic (60% for α and 77,5% for β_3) and presynaptic sites (40% for α and 22,5% for β_3). At postsynaptic sites, immunoparticles for the α subunit were detected both at intracellular sites (39%) and along the plasma membrane (61%), while for the β_3 subunit we found 48% at intracellular sites and 52% along the plasma membrane. Furthermore, 67% of immunoparticles for the α subunit and 51% for the β_3 subunit were concentrated in dendritic spines of Purkinje cells. Altogether, these data show similar patterns of subcellular localization for both α and β_3 subunits in Purkinje cells, suggesting that this auxiliary subunit is intimately associated with the main structural and functional subunit in this cell type.

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INHIBITION OF ENDOGENOUS PHOSPHODIESTERASE 7 PROMOTES OLIGODENDROCYTE PRECURSOR SURVIVAL AND DIFFERENTIATION IN MOUSE AND HUMANS: *IN VITRO* REMYELINATION ASSAYS

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During development, oligodendrocyte precursors (OPCs) are generated in specific sites within the neural tube and then migrate to colonize the entire CNS, where they differentiate into myelin-forming oligodendrocytes. Primary demyelinating diseases, such as multiple sclerosis (MS), are characterized by the death of oligodendrocytes and the consequent loss of myelin. The CNS reacts to demyelinating damage by promoting spontaneous remyelination, an effect mediated by endogenous OPCs, cells that represent approximately 5-7% of the cells in the adult brain. Numerous factors influence oligodendrogliogenesis and oligodendrocyte differentiation, including morphogens, growth factors, chemotropic molecules, extracellular matrix proteins and intracellular cAMP levels. In the present work, we demonstrate that, in postnatal and mature cerebral cortex of the mouse, OPCs express phosphodiesterase-7 (PDE7), enzyme which metabolizes cAMP. We investigated the effects of different PDE7 inhibitors (the well known BRL-50481 and two new ones, TC3.6 and VP1.15) on OPC isolated from brain cortex from P0 and P15 mice. While none of the PDE7 inhibitors analyzed altered OPC proliferation, TC3.6 and VP1.15 enhanced both OPC survival and differentiation. These effects were mediated via ERK intracellular signaling pathway. PDE7 expression was also observed in OPCs isolated from adult human brains. The differentiation of these OPCs into more mature oligodendroglial phenotypes was also accelerated under the treatment with both new PDE7 inhibitors.

Moreover, we have performed remyelination assays *in vitro* following demyelination of organotypic cerebellar slices. These new roles for PDE7 may further improve our knowledge of myelination and facilitate the design of therapeutic remyelination approaches for the MS treatment.

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Áreas Temáticas:

1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Trastornos y reparación del sistema nervioso

ALTERED RESPONSIVENESS OF CORNEAL COLD-THERMORECEPTIVE NERVE TERMINALS IN LONG-TERM EYE DRYNESS IS ASSOCIATED WITH CHANGES OF VOLTAGE-GATED NA^+ AND K^+ CURRENTS

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Impaired tear secretion reduces ocular surface wetness and evokes unpleasant eye dryness sensations whose peripheral origin remains unclear. Reduction of basal tearing in guinea pigs by exorbital main lacrimal gland removal was used as a dry eye disease (DED) model. 4 weeks later, ongoing spontaneous activity at basal temperature (34ºC) and response to cooling down to 20ºC in corneal cold thermosensitive nerve terminals were recorded. $[Ca^{2+}]_i$ changes, voltage-activated Na^+ and K^+ currents as well as cold-activated currents were recorded in retrogradely-labeled dissociated trigeminal ganglion corneal neurons. Lacrimal gland removal reduced tearing rate by 70% and produced a neuropathy of deep and superficial corneal nerve endings. Ongoing firing frequency of corneal cold sensitive nerve terminals at 34ºC and peak firing frequencies evoked by cooling ramps to 20ºC were higher in DED compared with control corneas (13.2±1.0 *vs.* 10.3±0.8Hz, and 31.1±1.8 *vs.* 25.3±1.8Hz respectively; $p<0.01$). Cold threshold was significantly reduced in DED terminals (-2.2±0.1 *vs.* -3.1±0.2ºC, $p<0.01$). Cold thermosensitive neurons from control and operated animals showed similar $[Ca^{2+}]_i$ responses to a cooling ramp or to menthol and in the characteristics of the I_{cold} currents. In contrast, TTX-Sensitive I_{Na} amplitude was significantly larger in DED (-63.8±18.2 *vs.* -6.0±5.0 pA/pF at -40mV; $p<0.01$) and mid-point of activation was significantly shifted towards more hyperpolarized voltages compared to controls ($V_{0.5}$= -42.8±1.9 *vs.* -29.7±2.8mV; $p<0.01$). TTX-Resistant I_{Na} current amplitudes were not significantly increased (-69.5±13.8 and -50.9±17.7 pA/pF at -20mV, respectively) but mid-point of activation was significantly shifted ($V_{0.5}$= -29.0±2.4 in DED *vs.* -21.9±3.0mV in controls; $p<0.05$). Rapidly and slowly-inactivating K^+ currents were significantly reduced in corneal cold neurons of tear-deficient eyes compared with controls: $K_{A,fast}$ 9.8±3.6 *vs.* 24.2±8.8 pA/pF at +40mV and $K_{A,slow}$ 23.4±3.9 *vs.* 45.4±9.8 pA/pF at +30mV; $p<0.001$. Non-inactivating K^+ currents were similar in both groups. K^+ currents had similar voltage dependency of activation and inactivation, except for inactivation of $K_{A,fast}$. We conclude that long-term ocular dryness enhances cold thermoreceptor firing due to an increase of Na^+ currents together with a reduced expression of K^+ currents and without significant changes in cold evoked currents mediated by TRPM8. Na^+ and K^+ current changes likely underlie the enhanced excitability exhibited by corneal cold sensory fibers of tear-deficient eyes, whose increased activity may be responsible of dryness sensations accompanying DED in humans.

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Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Trastornos y reparación del sistema nervioso

MODULATION OF NEURONAL EXCITABILITY AND VOLTAGE GATED SODIUM CHANNELS CONCENTRATION AT THE AXON INITIAL SEGMENT BY GSK3, β-CATENIN AND EXTRACELLULAR MOLECULES

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Axon physiology and neuronal excitability depends on axon initial segment integrity and the modulation of axon initial segment (AIS) ion channels expression and action potential fine regulation. The AIS contains a high concentration of sodium (Na_v), potassium (K_v) and calcium (Ca_v) voltage gated ion channels, which generate and control action potentials. Different neurodegenerative, psychiatric and brain trauma diseases have been related to AIS alterations, however, little is known about the cellular and molecular mechanisms that finely modulate the functional expression of ion channels at the AIS plasmatic membrane, neither in response to physiological changes nor in pathological conditions. The main objective of our work has been to elucidate which mechanisms are involved in AIS function physiological and pathological conditions. Our experimental approaches have been performed in long-term cultured hippocampal neurons, cultured brain slices and a model of in vivo brain injury, combining pharmacological treatments, plasmids nucleofection or biolistic transfection (Gene Gun) and electrophysiological techniques.

The results that will be presented show an important role of GSK3 and beta-catenin in the modulation and maintenance of sodium channels concentration at the AIS. Moreover, we will present data about the role of extracellular molecules which concentration is increased after brain injury causing a reduction of AIS proteins and ion channels concentration in a model of brain injury. Our data indicate that suppression or inhibition of its receptor protects axon initial segment integrity and neuronal excitability.

In conclusion, the identification of these molecules and signaling pathways opens new research lines on AIS study, and the possibility to developed therapeutic agents that modulate the function of these molecules after brain injury or neuropsychiatric diseases.

Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Trastornos y reparación del sistema nervioso

SYNAPTIC INTEGRATION OF CALLOSAL INPUTS TO PYRAMYDAL AND NON-PYRAMIDAL NEURONS OF LAYER II/III OF THE RETROSPLENIAL AGRANULAR CORTEX

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Objective: We have studied the integration of synaptic responses evoked by callosal axons in PV positive – fast spiking interneurons (PV-FS) and in pyramidal neurons (Pyr) of layer 2/3 of the retrosplenial agranular cortex.

Methods: We used whole-cell recordings with patch clamp electrodes of PV-FS and Pyr neurons in coronal slices of C57BL6 mice (P18-23). Contralateral callosal projecting neurons were stimulated with a bipolar concentric electrode placed on the homotopic contralateral cortex.

Results: EPSPs evoked by callosal axons were faster and had larger amplitude in PV-FS than in Pyr. This was due to a combination of larger amplitude (43.9±8.6 vs. 12.5±1.0 pA; n=19-15) and faster time course (rise time 0.51±0.05 vs. 1.20±0.09 ms; half amplitude decay time 1.53±0.20 vs. 5.06±0.29 ms) of single axon EPSCs and to a larger degree of convergence of callosal axons on PV-FS (estimated to be 3.5:1, PV-FS:Pyr). These differences, in addition to a faster membrane time constant, compensated the lower input resistance of PV-FS. We studied the temporal summation of callosal EPSPs with trains of four stimuli at 15-130 Hz. Due to the faster and larger EPSPs and larger EPSC depression, PV-FS showed less temporal summation and the firing of action potentials was predominantly at the beginning of the train; in contrast, the slower and smaller EPSPs in Pyr had clear temporal summation that gave rise to firing predominantly at the end of the train. The combination of all these differences caused that the proportion of PV-FS neurons that fired in response to callosal stimulation was larger than Pyr neurons (4/10 vs. 0/10 with single pulse stimulation). In addition, the latency to the first spike evoked by callosal stimulation was shorter and had less variability in PV-FS than in Pyr neurons (10.79±1.92 vs. 33.06±14.27); therefore, PV-FS neurons fired earlier and more synchronously than Pyr neurons in response to callosal inputs.
Conclusions: Our data describe the different activation of PV-FS and Pyr neurons in response to callosal inputs and stresses the importance of the activity pattern of the callosal projecting neurons to recruit different neuronal populations of the cortical network.

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1st: Neuronal excitability, synapses and glia: cellular mechanisms
2nd: Systems Neuroscience

ADENOSINE A_{2B} AND A_3 RECEPTORS IN THE MICE NEUROMUSCULAR JUNCTION

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In neuronal contacts, neurotransmitter release is finally controled by the functional confluence of several metabotropic receptor-mediated signaling pathways modulated in an activity-dependent manner. In the neuromuscular synapse, both nerve terminal and muscular activity contribute to the build up of extracellular adenosine which seems to modulate presynaptic ACh release through adenosine purinergic receptors (P_1Rs) both A_1R and A_{2A}R subtypes.

In a previous study we identified by immunocytochemistry A_1R and A_{2A}R receptors in the nerve endings at the mouse neuromuscular junctions (NMJs). A_1R can reduce spontaneous quantal leak of ACh and the collaboration of A_1R and A_{2A}R may protect synaptic function by reducing depression during repetitive activity. Here, we investigate by western blotting the presence in striated muscle and the localization by immunohistochemistry of A_{2B}R and A_3R receptors in the three cells of the adult neuromuscular synapse (neuron, glia and muscle). In electrophysiological experiments (in muscles paralyzed by blocking the voltage-dependent sodium channel of the muscle cells with μ-CgTx-GIIIB), we inhibited respectively A_{2B}R and A_3R with the selective antagonists MRS1706 and MRS1334 and analysed the size and frequency of the miniature endplate potentials (MEPPs), evoked potentials (EPPs) and also paired pulse facilitation and prolonged-activity (40Hz for 2 minutes of supramaximal stimuli) depression. The data show the presence and localization of these receptors in a specific pattern in the cells that make the neuromuscular synapse. However, a direct role in ACh release can not be observed.

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Área temática: Excitabilidad neuronal, sinapsis y glia: mecanismos celulares.

PRESYNAPTIC MEMBRANE RECEPTORS IN ACETYLCHOLINE RELEASE MODULATION ON NEUROMUSCULAR SYNAPSE

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Transmitters, co-transmitters, neurotrophic factors and other local signaling molecules co-ordinate the molecular machinery of the synapses in response to changing activity demands. In the neuromuscular synapse, presynaptic muscarinic ACh autoreceptors (mAChRs) and purinergic autoreceptors (P1Rs) directly couple ACh release and adenosine (ADO) secretion, respectively to regulate the nerve ending release mechanism itself. In addition, trophic factor receptors (TFRs) such as presynaptic neurotrophin receptors and presynaptic receptors for trophic cytokines can respond to target-derived mediators and cooperate in the local control of neurotransmission. Thus, the final functional outcome of the synapses can be built by the confluence of several metabotropic receptor-mediated signaling pathways.

In the last few years we have investigated the role of mAChRs, P1Rs and TFRs in the modulation of ACh release in the NMJ. Here, we brings together previously published data and some new results, and proposes an outline of the receptor-mediated molecular mechanism for controlling ACh release in the adult. We have focused on the immediate role in ACh release (spontaneous and evoked generation of synaptic potentials and activity-dependent short-term plasticity) of up to nine receptors acting locally (deafferentated muscle *ex vivo*) at the presynaptic component of the NMJ.

We identified several links between P1Rs, mAChRs and TFRs. We found a close dependence between mAChR and trkB metabotropic receptor pathways and observed that the mAChR group needs to operate correctly if trkB is also to operate correctly (and *vice versa*). Likewise, the functional integrity of mAChRs depends on P1Rs operating normally.

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Área temática: Excitabilidad neuronal, sinapsis y glia: mecanismos celulares.

INVOLVEMENT OF SERINE KINASES IN THE MODULATION OF ACH RELEASE IN THE NEUROMUSCULAR SYNAPSE

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The final functional outcome of the synapses can be built by the confluence of several metabotropic receptor-mediated signaling pathways. These receptors are coupled to intracellular pathways that converge on a limited repertoire of effector kinases to phosphorylate protein targets and materialize structural and functional changes. The kinases from the serine-threonine family, in particular protein kinase C (PKC) and A (PKA), are the most ubiquitous. These kinases have been involved in transmitter exocytosis and we have investigated the role of the serine kinases in the modulation of ACh release in the neuromuscular junction (NMJ).

We observed that both PKC and PKA potentiated release when they were specifically stimulated but in basal conditions PKC seemed not to be directly involved in transmitter release. However, PKA stimulation caused the PKC to couple to release, and PKA inhibition prevented PKC stimulation and coupling to ACh output. There was, therefore, some dependence of PKC on PKA activity in the fine control of ACh release. The interaction between mAChR and trkB receptors with PKC and PKA is complex. mAChRs reduce and trkB favours PKA coupling to release. However, mAChRs favour PKC coupling, which seems to be necessary for trkB to operate normally.

In summary, synapse operation is largely the logical outcome of the confluence of several metabotropic signaling pathways on intracellular kinases. Changes in the operation of any of these receptors and kinases affects the normal coupling of the other molecules to transmitter release. These multiple links highlight the complexity of the mechanisms required to fine-tune synaptic function.

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Área temática: Excitabilidad neuronal, sinapsis y glia: mecanismos celulares.

INVOLVEMENT OF KAINATE RECEPTORS IN CEREBELLAR CLIMBING FIBER TO PURKINJE CELL SYNAPTIC REFINEMENT

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During the last decade the participation of Kainate receptors (KARs) in excitatory synaptic transmission in several brain areas has started to be clarified. Cerebelar Purkinje cells (PC) receive two types of excitatory inputs, one through parallel fibers and the other through climbing fibers. By stimulating granular cell layer, EPSC showing pair pulse facilitation can be recorded, corresponding to the activation of Parallel fibers-PC synapses. A single climbing fiber (CF) can also be activated resulting in EPSC with striking characteristics: large amplitude all-or-none responses which present significant pair pulse depression.

We have previously shown that part of the synaptic component at CF-PC synapses is mediated by GluK1/GluK4 Kainate receptors (KARs). Unexpectedly, GluK4 or GluK1 KO mice (i.e. lacking functional KARs) showed a significant reduction of the CF-PC EPSC amplitude mediated by AMPA receptors compared to WT animals (WT EPSC Ampl=3.15$\pm$0.41 nA; GluK4(-/-)=1.54$\pm$0.22 nA; GluK1(-/-)=1.24$\pm$0.08 nA). To explore which synaptic parameters could account for this phenotype we applied "the multiple-probability fluctuation" (MPF) analysis to estimate the quantal parameters that describe synaptic efficacy: the number of release sites (n), the probability of release (p) and the amplitude of the response to a single released vesicle (q). Also, to evaluate possible defects in motor performance we carried out two different behavior tests, the rotarod and treadmill. While WT animals were able to run and reach 40 rpm speed in the treadmill, GluK4 KO animals performed poorly this task. They also present defects in the rotarod performance.

These results indicate that GluK1/GluK4 synaptic KARs are involved in the synaptic maturation of CF-PC synapses, having a role in cerebellar function in adult mice.

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Áreas Temáticas: Seleccione las **2** áreas temáticas que más se ajusten a su trabajo en orden de prioridad:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Desarrollo

POTENCIACIÓN A LARGO PLAZO INDUCIDA POR ESPIGAS DE CALCIO EN NEURONAS PIRAMIDALES DE CAPA V DE LA CORTEZA DE BARRILES

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La modulación colinérgica juega un papel importante en el procesamiento de la información sensorial facilitando la adquisición y almacenamiento de la información en la corteza. Dado que hemos publicado que acetilcolina facilita la generación de espigas de calcio en las neuronas piramidales de capa V de la corteza de barriles, ahora mostramos que estas espigas inducen LTP en las sinapsis excitadoras de las neuronas piramidales. Registrando los EPSCs mediante estimulación eléctrica en las dendritas basales en presencia de picrotoxina y generando un EPSP seguido de una espiga de sodio y una espiga de calcio por aumento de la intensidad de la estimulación observamos que al repetir la espigas de calcio 60 veces a una frecuencia de 0.2 Hz se induce la potenciación a largo plazo (>40 minutos) del EPSC (214.5 ±21.8 % con respecto al control). Esta potenciación se bloquea con (i) D-APV indicando que es NMDA dependiente, (ii) Nifedipina, demostrando que es necesaria la activación de los canales voltaje dependientes de calcio tipo L, (iii) QX314, sugiriendo que las espigas de sodio también son cruciales para este mecanismo y (iV) quelando el calcio citosólico de la neurona registrada con BAPTA sugiriendo un locus de expresión de la plasticidad postsinaptico. Además, los receptores metabotrópicos de glutamato y acetilcolina están involucrados en la inducción de la plasticidad sináptica inducida por las espigas de calcio porque esta LTP se bloquea con MCPG y Atropina respectivamente. Por consiguiente nuestros resultados demuestran que las espigas de calcio están implicadas en la inducción de plasticidad sináptica en las neuronas piramidales de capa V y podrían participar en el control del aprendizaje y la memoria en la corteza de barriles.

1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

2ª: Neurociencia de sistemas

LOS ENDOCANABINOIDES FACILITAN LA GENERACION ESPIGAS DE CALCIO EN LA CORTEZA DE BARRILES

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La corteza de barriles es una estructura clave en el procesamiento de información somatosensorial de los roedores, y en ella, la acetilcolina es capaz de favorecer la inducción de espigas de Ca^{2+}en las neuronas piramidales de la capa V. En este trabajo, hemos estudiado la posible participación de los endocanabinoides en la generación de las espigas de Ca^{2+} en neuronas de capa V de la corteza de barriles. Nuestros resultados demuestran que el 78% de las piramidales registradas presentan espigas de Ca^{+2} al prefundir WIN55,212-2 (10μM), un agonista de los receptores cannabinoides, estando ausentes en la mayoría de las células registradas (28%) en presencia del antagonista selectivo de los receptores CB_1, AM251 (10 μM). Para localizar los receptores CB_1 involucrados en la generación de la espiga de Ca^{+2} hemos aplicado WIN55,212-2 (20μM) en diferentes puntos a lo largo del árbol dendrítico de las neuronas registradas, demostrando que los cannabinoides solo inducen espigas de Ca^{+2} cuando se aplican localmente en capas IV y V, pero no cuando se aplican en las capas VI, II/III y I. Además hemos analizado la modulación de los IPSCs por endocannabinoides liberados por despolarización neuronal, fenómeno conocido como DSI (Depolarization-induced Suppression of Inhibition). Nuestros resultados muestran que el 33,3% de las células registradas presentan DSI (reduciéndose las amplitud del IPSC un 35.91%). También hemos analizado la modulación de los EPSCs por endocannabinoides liberados por despolarización (DSE). El 40% de las células registradas mostraron DSE, observándose una reducción de su amplitud del 26,97%. Por último, mostramos que los endocanabinoides liberados por estimulación antidrómica en capa VI facilitan la generación de espigas de Ca^{2+} en neuronas de capa V. De esta manera, nuestros resultados sugieren que durante la activación sincrónica de las neuronas piramidales de capa V, la despolarización puede liberar endocanabinoides que alterarían el equilibrio excitación-inhibición facilitando la generación de la espiga de Ca^{+2} en la corteza de barriles.

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THE HUMAN AMYGDALOID COMPLEX: CELULAR ARCHITECTURE AND DOPAMINERGIC INNERVATION

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The amygdaloid complex (AC) is associated with the perception of fear and consequent anxiety related behaviors. Each AC nuclear group has particular internal and external networks and encodes different aspects of fear. Dopamine exerts its action either directly over the AC pyramidal projection neurons or by modulating the nonpyramidal interneurons (IN) which, in turn, modulate the output neurons. The activity of parvalbumin (PV) and calretinin (CR) positive (+) GABAergic IN populations is strongly affected by dopaminergic (DA) inputs. To elucidate the mechanisms of DA modulation in primates we investigate the DA innervation and its relation with the neuronal types in every AC territory. For that purpose the following studies were performed in every AC nuclear groups, nuclei and nuclear subdivisions: 1) stereological quantification of absolute number and density of neurons and glia; 2) stereological estimation of absolute number and density of the PV+ and CR+ IN, calculating the proportion of these IN with respect to the total neurons; 3) stereological quantification of the dopamine transporter (DAT)+ axon absolute length and density, and ratio between length of axon and neuron number; 4) confocal microscopy analysis of contacts between DAT+ axons and PV+ and CR+ IN. There is selective DAT+ innervation in the human AC, which in the central nucleus (main output station) is twofold greater than in the basolateral group (main entrance structure). The distribution of CR+ and PV+ IN is heterogeneous and the percentage of CR+ IN with respect to the total neurons outnumbered that of the PV+ IN (13.30% for CR and 0.26% for PV; mean AC). Contacts between DAT+ axons and PV+ or CR+ IN were scant, suggesting that neither of the two IN populations is their main target. Determining the distribution and postsynaptic targets of the DA terminals are needed to understand dopamine neuromodulation in the AC.

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1. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2. Neurociencia de sistemas

AGE-RELATED ALTERATIONS IN GUINEA PIG CORNEAL NERVE MORPHOLOGY AND EPITHELIAL WOUND HEALING

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Purpose. To analyze the morphological changes of corneal innervation and corneal epithelial wound healing in young (1-2 months) and adult (8-9 months) guinea pigs.

Methods. 20 dissected eyes were fixed and cryoprotected. Whole-mount corneas were incubated with neuronal class III β-tubulin antibody and Alexa fluor 488. A set of corneas was incubated in ABC with diaminobenzidine. Corneas were drawn with camara lucida and photographed with confocal microscope. Corneal epithelial wound healing was studied at 1 and 7 months in 8 animals. Epithelium debridation was performed with a 2mm-diameter piece of paper soaked in n-heptanol. Lesions were stained with fluorescein and photographed regularly until complete closure. Images were analyzed with image processing software. Epithelial migration rate (EMR, in μm/h) and estimated time of healing (ETH, in hours) were calculated. Tear secretion level was measured in both groups of guinea pigs with use of phenol red thread test.

Results. Density of subbasal nerve leashes decreased significantly in 8-9 months animals compared to 1-2 months, while their length significantly increased. Also subbasal nerves leashes appeared less branched and the number of epithelial nerve terminals was reduced in adult guinea pigs. EMR was delayed and ETH was significantly increased in adult animals comparing to the young ones. Tear secretion was comparable in both groups.

Table. 1	**1 month**	**7 months**
EMR (μm/h)	69.0±4.5	51±5,1 **
ETH (h)	22.3±0.5	31,9±3,1 **

***p<0.001, t-test*

Conclusions. The corneal nerve architecture in guinea pig changes with time exhibiting the reduction in the subbasal and epithelial nerve density. This might contribute to the decrease of the neuropeptides level and leads to a neurotrophic slowdown of epithelial wound healing.

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Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Neurociencia de sistemas

NeCaB1 MODULATES TRAFFICKING AND AFFINITY OF GluK5 CONTAINING KAINATE RECEPTORS

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Fast excitatory synaptic transmission is mainly mediated by glutamate receptors in the Central Nervous System. This family of receptors comprises three different members: AMPA, NMDA and kainate. Among these, kainate receptors (KARs) are the less understood from a physiological point of view. An attempt to unveil important aspects of KARs physiology is to elucidate the protein interactome around these receptors. Hence, our lab used a yeast two-hybrid screening to identify possible partners of GluK5 subunits by using its C-terminal domain (CTD) as bait. Consequently, we identified Neuronal Calcium Binding Protein 1 (NeCaB1) as an interactor of GluK5 CTD.

We further verified the interaction between NeCaB1 and GluK5 by co-inmunoprecipitation in HEK cells and in pull-down assay. Moreover, we found that binding of NeCaB1 to GluK5 CTD is Ca^{2+} dependent in that interaction is disfavored in the presence of Ca^{2+}. Bimolecular fluorescence complementation (BiFC) further demonstrated interaction between these two proteins *"in vivo"* and served to narrow down the interacting segment.

The increased affinity for glutamate of GluK1/GluK5 heteromeric KARs as compared to homomeric GluK1 receptors served as a readout for detecting GluK1/5 heteromeric receptors at the plasma membrane. Therefore, we found that NeCaB1 promotes GluK5 containing KARs in the cell surface when internal Ca^{2+} was reduced to a minimum. In addition, we observed that NecaB1 increases the density of membrane GluK5 containing receptors at low Ca^{2+} levels. Unexpectedly, NeCaB1 was also found to increase the affinity of GluK5 containing KARs, either in combination with GluK1 or GluK2, in a Ca^{2+} dependent manner.

Altogether, these data demonstrate that NeCaB1 binds to CTD of GluK5 subunit containing KARs promoting their trafficking and increasing their affinity depending on environmental Ca^{2+}, indicating that NeCaB1 could dynamically determine the kind of KARs at synapses according to synaptic activity, constituting a kind of homeostatic plasticity.

Consolider,MinisterioEconomiaYCompetitividadAndGobiernoVasco.

Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.
2ª: Neurociencia de sistemas.

CONTRIBUTION OF TRPA1 CHANNELS TO MECHANOTRANSDUCTION IN MICE KNEE JOINT SENSORY NEURONS

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Joint pain accompanying arthritis and osteoarthritis is the most prevalent form of human pain. Join pain originates at the peripheral axonal terminals of primary sensory neurons located in the cephalic and dorsal root ganglia. Several cellular, neurophysiological and behavioral experimental models have been used to explore the neurophysiological mechanisms of joint nociception. Nonetheless, the transduction processes associated to the excitation by innocuous and noxious mechanical forces of joint sensory terminals under normal or pathological conditions are still incompletely understood. Several candidate molecules have been identified as potential sensory transducers in different somatic tissues, including various members of the TRP ion channels family, but their role in joint mechanosensation remains to be established.

We developed an experimental preparation to record electrophysiologically single afferent nerve fibres from the saphenous nerve of anesthetized mice in response to mechanical stimulation of the knee joint. Experiments were performed in wild type and knock-out mice for TRPA1, a channel associated to mechanotransduction. Conduction velocity and mechanical thresholds were similar in both types of animals. In contrast, we observed a significant reduction (around 44%) in the innocuous and noxious movement-evoked firing frequency in $TRPA1^{-/-}$ compared with WT animals. Reduction was more pronounced in C (51%) than in Aδ (39%) fibres. Calcium imaging was performed in retrogradely-labelled knee joint DRG neurons of WT and $TRPA1^{-/-}$ mice responding to hypoosmotic stimulation. The proportion of hypoosmotic-sensitive neurons was similar in both populations. However, response amplitude was smaller in $TRPA^{-/-}$ neurons. The data suggests that TRPA1 channels contribute to mechanotransduction in joint nociceptive afferents. Moreover, these experimental models open the possibility of using genetically modified mice to analyze the contribution of ion channels and other proteins to the transduction and modulation of innocuous and noxious mechanical stimuli in joint nociceptors.

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Áreas temáticas:

1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Neurociencia de sistemas

HIGH- AND LOW-AFFINITY GABA RECEPTORS ACT DIFFERENTLY MODULATING RECRUITMENT THRESHOLD OF OCULAR MOTONEURONS

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The release of γ-amino butyric acid (GABA) neurotransmitter into the synaptic gap produces rapid inhibition of postsynaptic neuron through low affinity receptors. Recent findings in cerebellum and hippocampus suggest that this neurotransmitter can also reach the perisynaptic region and tonically modulate neuronal excitability by high affinity receptors. The aim of this study was to determine whether ocular motor neurons are capable to modulate cell excitability via GABA-dependent receptors and mechanisms. Motoneurons were recorded intracellularly by the patch clamp technique. Membrane potential, input resistance, time constant, voltage threshold, rheobase and firing frequency were quantified before and during slice perfusion with gabazine ($GABA_A$ receptor antagonist, 20μM) or GABA (100μM). The results demonstrate the presence of a gabazine-sensitive tonic current (~25pA). The blockage of this current did not modify the membrane potential and slightly increased input resistance of motoneurons. The increase in this parameter produced a decrement in recruitment threshold that was more pronounced in high-threshold motoneurons. GABA application produced a holding current (~300pA), a hyperpolarization of the membrane potential (-5mV) and a great diminution of the input resistance (~50%) in the population. This decrement in resistance led to a decrease in time constant and an increase in the threshold of recruitment. In conclusion, the data suggest that the effect of GABA on the low-affinity receptors is similar in all motoneurons, while the capacity of modulation of the excitability through the high affinity receptors increases with recruitment threshold.

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Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Neurociencia de sistemas

EFECTOS DE LA ISATINA, UN INHIBIDOR ENDÓGENO DE LA MAO, SOBRE LOS NIVELES DE AMINOÁCIDOS NEUROTRANSMISORES EN EL NÚCLEO ESTRIADO. UN ESTUDIO *IN VIVO* MEDIANTE MICRODIÁLISIS CEREBRAL

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Objetivos

La isatina (indol-2,3-diona) es un inhibidor endógeno de la monoamino oxidasa que presenta una amplia variedad de efectos biológicos. Diversos investigadores han demostrado que la administración de isatina incrementa los niveles cerebrales de dopamina y se ha evidenciado que los niveles de isatina en orina incrementan en relación al grado de severidad de la enfermedad de Parkinson. Actualmente no existen estudios en los que se evalúen los efectos de la isatina sobre los aminoácidos neurotransmisores.

El objetivo de este estudio fue determinar, en ratas conscientes y en libre movimiento, los efectos de la isatina sobre los niveles *in vivo* de aspartato, glicina, glutamato y taurina en el núcleo estriado.

Material y métodos

La isatina (en una concentración de 10 mM) fue administrada durante 1 hora directamente en el núcleo estriado de ratas hembras Sprague-Dawley (250-300 g, 5/grupo) por medio de la sonda de microdiálisis. Los niveles de aminoácidos, obtenidos mediante microdiálisis, fueron determinados mediante HPLC con detección por fluorescencia (derivatización con OPA-NAC). El análisis estadístico se realizó por ANOVA y análisis multivariante Student-Newman-Kleus.

Resultados

La administración intraestriatal de isatina (10 mM) ha incrementado significativamente los niveles extracelulares de: aspartato (194,3 ± 29,6 %), glicina (140,7 ± 16,3 %) y glutamato (461 ± 114,7 %), en comparación con los niveles basales (considerados el 100 %). No se han observado modificaciones significativas sobre los niveles de taurina.

Conclusiones

Estos resultados muestran que la isatina perfundida en el núcleo estriado de ratas incrementa los niveles de aminoácidos neurotransmisores en dicho núcleo, siendo su mayor efecto sobre el glutamato (incremento superior a cuatro veces).

Finalmente, estos resultados sugieren que la isatina además de producir modificaciones en los niveles estriatales de dopamina puede interactuar con otros sistemas neurotransmisores. Son necesarios más estudios que nos permitan determinar los mecanismos mediante los cuales actúa la isatina.

Áreas Temáticas:

1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Trastornos y reparación del sistema nervioso

POSTSYNAPTIC ACTIVITY SETS THE SIGN OF THE MUSCARINIC LONG-TERM PLASTICITY OF $GABA_A$ INHIBITION

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Acetylcholine (ACh) regulates forms of plasticity that control cognitive functions, but the underlying mechanisms remain largely unknown. ACh controls the excitatory/inhibitory balance through modification of intrinsic excitability and synaptic excitation and inhibition at CA1 hippocampal pyramidal cells (PCs), known to participate in circuits involved in cognition and spatial information. However, the regulation by postsynaptic activity of the control of synaptic inhibition by ACh has not been sufficiently investigated. In rat PCs ACh combined with depolarization induced a long-term enhancement of perisomatic $GABA_A$ inhibition ($GABA_A$-LTP) mediated through an increased activation of $\alpha_5\beta\gamma_2$ subunit-containing $GABA_A$ receptors. The $GABA_A$-LTP required activation of M1-muscarinic receptors and an increased Ca^{2+}. A Type 1 endocannabinoid receptor (CB_1R)-mediated presynaptic LTD partially counteracted the postsynaptic $GABA_A$-LTP. Without PC depolarization ACh induced a presynaptic CB_1R-mediated LTD, revealing that postsynaptic activity gates ACh effects from presynaptic LTD to postsynaptic LTP. These results provide key insights into mechanisms potentially linked with cognitive functions and spatial information.

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Áreas Temáticas:

1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Neurociencia de sistemas

AXONAL INJURY DIFFERENTIALLY REGULATES EXPRESSION OF NR2A AND NR2B NMDA RECEPTOR SUBUNITS IN NEONATAL HYPOGLOSSAL MOTONEURONS

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Traumatic injury of a motor nerve at neonatal ages elicits massive loss of synaptic inputs and death of a high proportion of lesioned motoneurons. Our preliminary hypothesis is that an altered balance of signaling between NR2B- and NR2A-containing NMDA type glutamate receptors (NMDARs) could represent an early change preceding these regressive events. We specifically aimed to determine whether axonal injury induces a gain in the expression ratio of NR2B to NR2A subunits in neonatal hypoglossal motoneurons (HMNs). RT-PCR and *western blot* analysis revealed that the ratios of NR2B to NR2A RNAm and protein levels were significantly reduced from postnatal day 3 (P3) to P8 in the hypoglossal nucleus (HN). Interestingly, XIIth nerve crush at P3 apparently occluded this developmental change and resulted in an increase of the NR2B/NR2A ratio in the lesioned HN relative to the control side at P8. Accordingly, immunofluorescence labelling of brainstem sections showed that the staining intensity for NR2B increased in HMN cell bodies on the injury side, whereas NR2A immunoreactivity dramatically declined. Finally, to analyze surface expression of NR2B and NR2A we performed *whole-cell patch clamp* recordings of HMNs using subtype-selective antagonists. The combined analysis of excitatory postsynaptic currents evoked by afferent stimulation, miniature events and whole-cell current responses to NMDA pulses further shows an overall reduction in the amount of surface NR2A along with an increased contribution of NR2B to NMDAR-mediated currents in lesioned HMNs. Our functional analysis indeed demonstrates that the relative gain in NR2B function is prominent both, in synaptic and extrasynaptic membranes. We conclude that axonal injury differentially regulates expression of NR2 subunits in neonatal HMNs. The relative increment in the surface expression of NR2B-containing NMDARs in axotomyzed HMNs could promote the emergence of intracellular signaling pathways involved in the dismantling of afferent synaptic contacts and activation of the death program.

PRESYNAPTIC SILENCING OF GLUTAMATERGIC NERVE TERMINALS BY CANNABINOID CB1 RECEPTORS

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Cannabinoid receptors are the most abundant G protein-coupled receptors in the brain and they mediate retrograde short-term inhibition of neurotransmitter release, as well as long-term depression of synaptic transmission at many excitatory synapses. Presynaptic silent synapses fail to release neurotransmitter in response to a strong depolarization and Ca^{2+} influx. Then, the goal of this work was to determine whether cannabinoid, CB1, receptors induce presynaptic silencing at glutamatergic synapses. The dynamics of the synaptic vesicle cycle was studied in cerebellar granule cells transfected with vGlut1-pHluorin. The exocytosis was estimated as the depolarization-induced increase in fluorescence, while endocytosis and acidification lead to a decrease due to fluorescence at low pH. We found that prolonged stimulation (10 min) of cannabinoid receptors with the agonist HU-210 induced silencing of previously active synapses. Increasing cAMP with forskolin prevented silencing, via activation of the Exchange Protein directly Activated by cAMP (Epac). CB1R-induced synaptic silencing is a transient phenomenon as nerve terminal awake in 20 min in the absence of activity and more rapidly after increasing cAMP with forskolin or activating Epac proteins. Furthermore, increasing endocannabinoid levels by inhibiting the endocannabinoid degrading enzyme monoacylglycerol lipase with JZL184 greatly enhanced the number of silent synapses. Finally, by combining functional and immunocytochemical approaches, we observed a strong correlation between the release capacity of the nerve terminals and RIM1α protein content, but not that of Munc13-1 protein. In conclusion, CB1R activation induced presynaptic silencing at glutamatergic nerve terminals.

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THE UBIQUITIN-SPECIFIC PROTEASE USP36 MODULATES TRKA UBIQUITINATION AND SIGNALLING

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Binding of nerve growth factor (NGF) to its receptor TrkA induces its internalization, endosomal trafficking and subsequent lysosomal degradation or recycling to plasma membrane. Reversible ubiquitination orchestrated by the action of E3 ubiquitin ligases and deubiquitinating enzymes mediates endocytic trafficking of cell surface receptors. Previously, we have demostrated that TrkA receptor is ubiquitinated by the E3 ubiquitin ligase Nedd4-2. In this study, we sought for deubiquitinases that may be involved in TrkA receptor recycling. We have developed a screening using siRNA technology (Dharmacon siGENOME® SMARTpool® siRNA Library) in PC12 6/15 cells, a cellular model that express TrkA.

We have identified ubiquitin - specific protease 36 (Usp36) as a protein related with TrkA modulation in PC12 6/15 cells as Usp36 depletion increases ubiquitination and activation of TrkA. These results have been further confirmed using shRNA expression driven by lentiviral vectors. To verify celular localization of Usp36 subcellular fractionations and immunofluorescences have been carried out. Co-immunoprecipitation and ubiquitination assays *in vitro* and *in vivo* have been performed in HEK293 cells to confirm that Usp36 interacts with and reduces ubiquitination levels of TrkA. However, *in vitro,* Usp36 does not deubiquitinate TrkA. To clarify this disagreement we have performed experiments addressing whether Nedd4-2 and Usp36 compete to affect TrkA ubiquitination. All these data together suggest an indirect effect of Usp36 on TrkA ubiquitination and signaling, probably mediated by Nedd4-2.

Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Trastornos y reparación del sistema nervioso

IMMUNOELECTRON LOCALIZATION OF THE TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE 1 AT INHIBITORY SYNAPSES IN THE MOUSE DENTATE GYRUS

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The transient receptor potential vanilloid type 1 (TRPV1) is a non-selective cation channel that acts primarily as pain sensor in the periphery but also modulates neurotransmitter release and synaptic plasticity in the brain. TRPV1 function must lay on its anatomical distribution in the peripheral and central nervous system regions involved in the physiological roles of the channel. However, the anatomical localization of TRPV1 is well established in the periphery, but in the brain it is a matter of debate. We have recently shown that TRPV1 is highly concentrated in postsynaptic dendritic spines to asymmetric perforant path synapses in the outer 2/3 of the ML, being poorly expressed at the excitatory hilar mossy cell synapses in the inner 1/3 of this layer. However, the TRPV1 distribution at inhibitory synapses in the dentate molecular layer is still an open question.

To investigate this, we have used TRPV1 antibodies combined with a highly sensitive pre-embedding immunogold method for high resolution electron microscopy. TRPV1 immunoparticles were observed in dentate granule cell dendrites receiving symmetric inhibitory synapses.

The silver-intensified gold particles were mostly confined to postsynaptic membranes and distributed at a relative short distance from the inhibitory synaptic contacts. Importantly, the TRPV1 pattern distribution at inhibitory synapses disappeared in the molecular layer of TRPV1-knockout mice.

These findings give additional knowledge on the fine TRPV1 localization in the rodent hippocampus by means of high resolution electron microscopy.

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Areas temáticas:

1º.- Excitabilidad neuronal, sinapsis y glia: mecanismos celulares.
2º.- Neurociencia de Sistemas

CELLULAR MECHANISMS OF SPIKE TIMING-DEPENDENT LONG-TERM DEPRESSION IN THE HIPPOCAMPUS

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Aims

Spike timing-dependent plasticity (STDP) is a strong candidate for a synaptic mechanism involved in cortical development and learning and memory. The main objective of this work was to determine the cellular mechanisms of this form of LTD.

Materials and Methods

We conducted experiments in hippocampal slices prepared from P9-P16 mice using the whole-cell configuration of the patch clamp technique. To induce t-LTD, a post-pre pairing protocol (with the presynaptic activity ocurring 18 ms after a postsynaptic action potential) was applied after a stable EPSP baseline period of 10 min. EPSP slope was monitored for at least 30 min after the pairing protocol.

Results

We found that a post-before-pre pairing protocol induced robust t-LTD (75 ± 5% of control, n = 9). This t-LTD was completely blocked by the NMDA receptors antagonist D-AP5 (50 μM, 118 ± 7%, n = 7). The loading of the postsynaptic neuron with the use-dependent non-competitive NMDA receptor antagonist MK-801 failed to prevent the induction of t-LTD (76 ± 4 %, n = 6) indicating that t-LTD induction is mediated by non-postsynaptic NMDA receptors. To determine the subunit composition of NMDA receptors mediating this form of LTD we performed experiments in the presence of subunit-preferring NMDA receptor antagonists. t-LTD was prevented by PPDA (93 ± 8%, n = 5) but not by NVP-AAM077 (73 ± 6%, n = 6) or Ro 25-6981 (76 ± 5%, n = 5) indicating that requires NMDA receptors containing GluN2C/D subunits but no GluN2A or -2B. Finally, to determine the possible involvement of CB1 receptors in t-LTD, we performed experiments in the presence of the antagonist AM251 (3 μM). In this situation t-LTD induction was completely prevented (104 ± 8%, n = 9).

Conclusions

t-LTD induction requires the activation of non-postsynaptic GluN2C/2D subunit–containing NMDA receptors and CB1 receptors.

Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Desarrollo

LA INHIBICIÓN DE GSK3 GENERA UN AUMENTO EN EL NÚMERO DE SINAPSIS

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Abstract
La sobreactivación de la fosfoinosítido 3-kinasa (PI3K) conlleva un incremento en el número de sinapsis tanto en *Drosophila* (Martín-Peña et al., 2006) como en roedores (Cuesto et al., 2011). La activación de AKT, diana principal de PI3K, conduce a la inhibición por fosforilación de la Glucógeno Sintasa Kinasa 3 (GSK3), enzima implicada en multitud de procesos neuronales como son: migración, guía axonal, proliferación celular y plasticidad sináptica. Con el objetivo de caracterizar la implicación de GSK3 en el proceso sinaptogénico se ha estudiado el resultado de la inhibición directa de la actividad de GSK3 usando dos inhibidores farmacológicos: AR-A014418 (AR) y SB415286 (SB), o indirectamente tras la activación de AKT con el péptido activador de PI3K (PTD4-PI3KAc, Cuesto et al., 2011). Nuestros resultados indican que la inhibición de GSK3 aumenta la densidad sináptica hasta un efecto máximo aproximado del 30% en cultivos de 21 días tras 48 horas de tratamiento. En paralelo, los niveles de Sinapsina aumentan aproximadamente en un 60%. En esos mismos cultivos, la inhibición de GSK3 regula la formación de espinas de modo concentración-dependiente. Sin embargo, en cultivos de 12 días la inhibición química de GSK3 reduce la densidad sináptica aproximadamente un 25% (SB a 25 μM) del mismo modo que decae la expresión de Sinapsina. Nuestros datos muestran que: i) GSK3 participa en la vía sinaptogénica regulada por PI3K, ii) GSK3 está implicada en la formación de espinas y iii) La inhibición de GSK3 tiene un efecto potenciador o inhibidor de la sinaptogénesis en función del estado de desarrollo del cultivo.

Áreas Temáticas:
Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.
Trastornos y reparación del sistema nervioso.

CELLULAR MECHANISMS OF SPIKE TIMING-DEPENDENT LONG-TERM POTENTATION IN THE HIPPOCAMPUS

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Aims

Spike timing-dependent plasticity is a hebbian synaptic learning rule that underlies cortical development and memory. Our goal was to determine the cellular mechanisms of this form of LTP at Schaffer collateral-CA1 synapses of the hippocampus.

Materials and Methods

We performed experiments in the CA1 region of hippocampal slices prepared from mice (P4-P28) using the whole-cell configuration of the patch clamp technique. To induce t-LTP, a pre-post pairing protocol (with the presynaptic activity ocurring 5 ms before a postsynaptic action potential) was applied after a stable EPSP baseline period of 10 min. EPSP slope was monitored for at least 30 min after the pairing protocol.

Results

We found that this protocol induced robust t-LTP after the first postnatal week (P8-P18: 159 ± 2 % of control, n = 7; P21-P28: 182 ± 3 % of control, n = 5) but the same failed to induce t-LTP in the first postnatal week (92 ± 3 % of control, n = 5). t-LTP was completely blocked by the NMDA receptors antagonist D-AP5 (50 μM, 96 ± 2 %, n = 5). The loading of the postsynaptic neuron with the use-dependent non-competitive NMDA receptor antagonist MK-801 prevented the induction of t-LTP (100 ± 11 %, n = 6) indicating that t-LTP induction is mediated by postsynaptic NMDA receptors. To determine the subunit composition of NMDA receptors mediating this form of LTP we performed experiments in the presence of subunit-preferring NMDA receptor antagonists. t-LTP was prevented by NVP-AAM077 (103 ± 7%, n = 6) but not by PPDA (162 ± 3 %, n = 5) indicating that t-LTP requires NMDA receptors containing GluN2A subunits but no GluN2C/D.

Conclusions

t-LTP emerges in the second postnatal week and requires the activation of postsynaptic GluN2A subunit-containing NMDA receptors.

Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.
2ª: Desarrollo.

AMPA RECEPTOR SEQUESTRATION FROM SYNAPSES BY MAP1B LIGHT CHAIN

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Synaptic connections in the brain are continuously fine-tuned by adding or removing neurotransmitter receptors in response to neuronal activity. Still, little is known about the molecular and cellular mechanisms governing this form of synaptic plasticity. Rearrangements in the actin cytoskeleton are thought to be critical for the structural and functional changes of synapses during plasticity; nevertheless, recent evidence indicates that the microtubule cytoskeleton may also participate in the mechanisms underlying synaptic plasticity.

We have explored the potential role of a microtubule associated protein, MAP1B, in synaptic function and plasticity at hippocampal synapses. MAP1B associates with microtubules and promotes their assembly, but also interacts with actin, and so, it is an attractive candidate to participate in events that require cooperation between both cytoskeletons.

By using electrophysiological recordings on organotypic hippocampal slice cultures, we determined that the over-expression of MAP1B light chain (LC) was accompanied by a decrease in AMPA-receptor mediated synaptic transmission. Immunofluorescence and confocal microscopy experiments indicated that this depression was not due to a decreased number or size of dendritic spines. Instead, we found that $GluA_2$-containing AMPA receptors could not constitutively cycle towards synapses in the presence of MAP1B LC; as a consequence, their accumulation at synapses in basal conditions was prevented. Using microtubule co-sedimentation assays, we obtained evidence pointing to a possible sequestration of AMPA receptors in microtubules as a result of MAP1B LC over-expression. Moreover, we determined that the microtubule-dependent transport of transferrin receptor was substantially impaired in the presence of the light chain of MAP1B.

In conclusion, we present evidence for a possible role of MAP1B LC as a limiting factor for AMPA receptor transport in dendrites, which in turn would determine synaptic strength by controlling AMPA receptor presence at synapses.

Áreas temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

ACTIVITY-DEPENDENT PLASTICITY OF ASTROCYTE PROCESS AND DENDRITIC SPINE INTERACTIONS

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Structural relations between neural elements are fundamental in brain function. Plasticity of structural-functional relationships between dendritic spines and astrocytic processes, which enwrap synapses and regulate synaptic transmission, may be relevant in experience-dependent synaptic function but remains unexplored.

We have found that astrocytic processes display a basal movement that increase after activating synapses using stimulation paradigms that induce long-term potentiation of synaptic transmission. We show that stimuli that induce hippocampal long-term synaptic potentiation rapidly enhance the motility of synapse-associated astrocyte processes, which depends on presynaptic activity and requires astrocyte calcium signal. Sensory stimuli that increase astrocyte calcium induce similar plasticity in somatosensory cortex in vivo. The structural remodeling is accompanied by changes in the astrocyte-mediated modulation of synaptic transmission. Therefore, structural relations between astrocytic processes and dendritic spines undergo activity-dependent changes with metaplasticity consequences on synaptic activity regulation. Present results indicate that structural relationships between astrocytic processes and dendrites are activity-dependent regulated by synaptic plasticity and they have metaplasticity consequences on the astrocytic regulation of synaptic transmission and plasticity.

Therefore, these results reveal novel forms of synaptic plasticity based on structural-functional changes of astrocyte-neuron interactions.

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Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Neurociencia de sistemas

ADENOSINE RECEPTOR A1 INHIBITS NEURONAL DIFFERENTIATION FROM MULTIPOTENT STEM CELLS

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Objectives. The main objective of this work was to investigate the role of extracellular adenosine and its receptors in modulating neuronal differentiation of neural stem cells (NSC) from adult subventricular zone (SVZ).

Material and methods. Neuronal differentiation was evaluated on a single clonogenic neurosphere by immunofluorescence as a ratio between βIII tubulin (early neuronal marker) vs total cells, or by cytofluorimetry. Functional genomic analysis of differentiation was performed using an array containing genes relevant to neurogenesis. Differential expression of adenosine receptors was analyzed by qRT-PCR. *In vitro* results were confirmed *in vivo* by intracerebroventricular (icv) infusion of adenosine analogues.

Results. We found that adenosine reduces neuronal differentiation of adult NSC. The effect was most evident on the transient amplifying cells (C cells) as demonstrated by citofluorimetry assay. Adenosine A1 receptor (Adora1) mRNA was the most upregulated in contrast to the majority of genes represented in the macroarray used for screening. Upregulation of Adora1 was confirmed by qRT-PCR and Western blot, while the involvement of these receptors in the negative modulation of neuronal differentiation was further assessed using the specific agonist CPA and antagonist PSB36. Neurons generated during differentiation in the presence of adenosine showed an inhibition of vesicular transport to the synaptic terminal, which could be one of the causes of neuronal differentiation reduction. This modulation was confirmed also *in vivo* after icv infusion of CPA. This agonist significantly reduced the number of DCX^+ (neuroblast marker) /$BrdU^+$ cells in the olfactory bulb (OB) whereas no significant changes were observed in the SVZ with $GFAP^+$ (multipotent marker) /$BrdU^+$ or $nestin^+$ (C cells marker) /$BrdU^+$, demonstrating that activation of Adora1 in animals reduces neuronal differentiation in the SVZ and further migration to the OB.

Conclusions. Here we demonstrated that adenosine negatively modulates neuronal differentiation of neural stem cells from adult subventricular zone through the activation of Adora1.

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1ª. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª. Desarrollo

MODULATION OF TRPV1 CHANNEL BY HYALURONIC ACID

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Peripheral pain starts with the activation of the distal nerve terminals of nociceptor sensory neurons. These neurons express a variety of transduction channels including TRPV1, a polymodal channel activated by noxious heat, protons, chemical pungent compounds as capsaicin, and a large number of endogenous inflammatory substances. Nociceptor endings, in particular those located in the knee joints, are surrounded by hyaluronic acid (HA), an anionic glycosaminoglycan of the extracellular matrix. In pathological processes of the joints like osteoarthritis usually accompanied by intense pain, intraarticular HA is degraded and diluted, conversely, intra-articular injection of HA causes a long-lasting decrease of joint pain. We explored the hypothesis that this analgesic effect is due to the modulation by HA of the activity of TRPV1 channels in nociceptor nerve endings.

In heterologous systems (SH-SY5Y VR1 cells and HEK-293 cells transfected with TRPV1) as well as in dissociated DRGs neurons from adult mice, we measured: changes in intracellular calcium concentration $[Ca^{2+}]_i$ and in membrane currents, measured in whole-cell patch clamp configuration, in response to heating pulses or capsaicin. Exposure to HA decreased significantly the amplitude of the $[Ca^{2+}]_i$ responses evoked by heat and capsaicin, as well as the amplitude of bradykinin-sensitized capsaicin responses. Whole membrane currents in HEK-293+TRPV1 and spiking firing rate in DRG neurons, evoked by capsaicin were also reduced by HA. Single-channel recordings evidenced that this effect was due to a decrease of the opening probability of TRPV1 channels, while the amplitude of single channel currents was unaffected. In behavioral experiments, intra-plantar injection of HA reduced nociceptive responses to heat (hot plate test). Altogether, these findings suggest that HA is able to modulate the activity of TRPV-1 channels, thereby explaining the reduction of pain produced by injection of HA in the joints.

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Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Trastornos y reparación del sistema nervioso

1–42 β-AMYLOID PEPTIDE REQUIRES PDK1/NPKC/RAC 1 PATHWAY TO INDUCE NEURONAL DEATH

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Small GTPases of the Rho family are key players in complex signaling networks that control normal activity in most of cell types. Like other small GTPases, It is well established that RhoGTPases control cytoskeleton dynamics in neurons, thereby modulating synaptic plasticity. In fact, AD entails progressive dendritic spine loss, synaptic dysfunctions and morphological changes in dendrites. In normal brain, neuronal RhoA attaches to synapses and dendritic microtubules, however, in AD RhoA expression is decreased in synapses and increased in dystrophic neurites.

This neuropathology is associated with massive accumulation of two types of protein aggregates; senile plaques that are constituted mostly by 1-42 β Amyloid ($A\beta_{1-42}$) peptide and by neurofibrillary tangles containing hyperphosphorylated Tau protein. There is mounting evidence that the $A\beta_{1-42}$ peptide mediates many aspects of the pathogenesis of AD. *In vitro* this peptide is toxic to endothelial cells, smooth muscle cells, astrocytes, neurons and oligodendrocytes. The mechanisms by which $A\beta_{1-42}$ peptide exerts its cytotoxic action are not fully understood. Currently, there are ongoing efforts to discover the signaling pathways that are mediated by the $A\beta_{1-42}$ peptide. Several signaling cascades may be involved in cell damage and they appear to be activated by the $A\beta_{1-42}$ peptide, including oxidative stress generation, impaired Ca^{2+} homeostasis and mitochondrial dysfunction, generation of NO, and microglia activation. We have studied how the $A\beta_{1-42}$ peptide uses the cellular machinery for signal transduction leading to neuronal cell death in the cell line SN4741, in primary embryonic cortical neurons from rats as well as in neuronal organotypic cultures of hippocampus and entorhinal cortex. This signaling cascade involves specifically the Rac1 GTPase, which is regulated upstream by the PI3Kinase/PDK1/nPKCs pathway. This novel molecular characterization identifies nPKCs and Rac1 as potential therapeutic targets to block neuronal death program induced by the β-amyloid peptide.

Tipo de presentación: Póster
Áreas temáticas: 2 y/o 5

IDENTIFICATION OF TWO POPULATIONS OF HIPPOCAMPAL SPINES DEPENDING ON THEIR ACTIN POLYMERIZATION RATE

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Dendritic spines are small protrusions of postsynaptic membrane where it takes place the majority of glutamatergic synaptic inputs. Their head surface, dynamics and density have been related to synaptic plasticity and learning. Spine structure relays on a dynamic actin cytoskeleton. The objective of our work was to analyse the actin dynamics of singles spines employing FRAP (fluorescence recovery after photobleaching) technique both in organotypic and hippocampal neurons in cultures transfected with actin-GFP.

Our results indicate that, i: Spines have a mobile fraction between 0.2 and 1 with a mean average value of 0.7 ± 0.2. ii: Variability in mobile faction values is an intrinsic property of the spine and do not correlates with the distance from soma or the age of the culture. iii: Hippocampal spines in culture can be segregated in two populations considering their recovery time constant and mobile fraction, which moreover correlates with the spine head area. Small spines present faster recovery times and lower mobile fractions, while larger spines present slower recovery times and higher mobile fractions. iv; Hippocampal spines from organotypic slices are characterized by a single population of spines with an average recovery time of 26 ± 2.

This results support the idea of the existence of spines with a different set of actin binding proteins contributing to the regulation of spine plasticity according to their shape and therefore also with their role on the learning process.

Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.
2ª: Desarrollo

Ca^{2+} DYNAMICS IN THE ENDOPLASMIC RETICULUM OF CORTICAL ASTROCYTES: DIRECT MONITORING OF Ca^{2+}-INDUCED Ca^{2+}-RELEASE (CICR)

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Astroglia Ca^{2+} homeostasis is controlled by Ca^{2+} fluxes through plasma membrane and from organelles, mostly from the endoplasmic reticulum (ER). Expression of voltage-operated Ca^{2+} channels has been found in cultured astrocytes. ER possesses inositol 1,4,5-trisphosphate receptors ($InsP_3R$) that can be activated upon stimulation through a variety of metabotropic G-protein coupled receptors, mainly ATP and glutamate in these cells. By contrast, physiological significance of ryanodine receptors (RyRs) in astrocyte ER is controversial. In this work, we have first checked the expression of RyR3 and then studied its activation by direct monitoring the ER Ca^{2+} dynamics in mouse cortical astrocytes. We have developed a new probe based on the photoprotein aequorin and optimized it to measure high $[Ca^{2+}]$, as expected for the ER. This new Ca^{2+} probe was selectively targeted to the ER lumen of primary astrocyte by infection with herpes-virus amplicons carrying the aequorin gene. Infected cells expressed aequorin correctly in the ER, as demonstrated by co-localization with ER markers. Resting $[Ca^{2+}]_{ER}$ averaged 400 μM and a maximal dose of ATP provoked a complete and reversible ER discharge, indicating that the new ER probe was fully functional in these cells. Caffeine provoked a Ca^{2+} release smaller than ATP. Plasma membrane Ca^{2+} entry stimulated by depolarization with high K^+ elicited a fast and robust Ca^{2+} release, which was dose-dependent in the 40-120 mM range of $[K^+]$. This response was fully abolished by external Ca^{2+} removal or by addition of Ni^{2+}. Moreover, ryanodine inhibited both caffeine and K^+ effects. Ionotropic glutamate receptor activation also caused ER Ca^{2+} discharge. In permeabilized cells, the $InsP_3R$ inhibitor heparin completely blocked $InsP_3$-induced Ca^{2+} release, while did not affect the caffeine response. Importantly, external Ca^{2+} provoked transient and reversible ER Ca^{2+} release, convincingly demonstrating the presence of CICR in astrocytes.

DOPAMINE D_2 AND ACETYLCHOLINE α7 NICOTINIC RECEPTORS HAVE SUBCELLULAR DISTRIBUTIONS FAVORING MEDIATION OF CONVERGENT SIGNALING IN THE MOUSE VENTRAL TEGMENTAL AREA

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Alpha7 nicotinic acetylcholine receptor (α7nAChRs) mediate nicotine-induced burst-firing of dopamine neurons in the ventral tegmental area (VTA), a limbic brain region critically involved in reward and in dopamine D_2 receptor (D_2R)-related cortical dysfunctions associated with psychosis. The known presence of α7nAChRs and Gi-coupled D_2Rs in dopamine neurons of the VTA suggests that these receptors are targeted to at least some of the same neurons in this brain region. To test this hypothesis, we used electron microscopic immunolabeling of antisera against peptide sequences of α7nACh and D_2 receptors in the mouse VTA. Dual D_2R and α7nAChR labeling was seen in many of the same somata (colocalization over 97%) and dendrites (co-localization over 49%), where immunoreactivity for each of the receptors was localized to endomembranes as well as to non-synaptic or synaptic plasma membranes often near excitatory-type synapses. In comparison with somata and dendrites, many more small axons and axon terminals were separately labeled for each of the receptors. Thus, single-labeled axon terminals were predominant for both α7nAChR (57.9%) and D_2R (89.0%). The majority of the immunolabeled axonal profiles contained D_2R-immunoreactivity (81.6%) and formed either symmetric or asymmetric synapses consistent with involvement in release of both inhibitory and excitatory transmitters. Of 160 D_2R-labeled terminals, 81.2% were presynaptic to dendrites that expressed α7nAChR alone or together with the D_2R. Numerous glial processes inclusive of those enveloping either excitatory- or inhibitory-type synapses contained also single labeling for D_2R (n=152) and α7nAChR (n=561) . These results suggest that classic antipsychotic drugs, all of which block the D_2R, may facilitate α7nAChR-mediated burst-firing by elimination of D_2R-dependent inhibition in neurons expressing both receptors as well as by indirect pre-synaptic and glial mechanisms.
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Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Neurociencia de sistemas

SOMATODENDRITIC TARGETING OF M5 MUSCARINIC RECEPTOR IN THE RAT VENTRAL TEGMENTAL AREA: IMPLICATION FOR MESOLIMBIC DOPAMINE TRANSMISSION

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Muscarinic modulation of mesolimbic dopaminergic neurons in the ventral tegmental area (VTA) plays an important role in reward, potentially mediated through the M5 muscarinic acetylcholine receptor (M5R). However, the key sites for M5R mediated control of dopamine neurons within this region are still unknown. To address this question we examined the electron microscopic immunocytochemical localization of antipeptide antisera against M5R and the plasmalemmal dopamine transporter (DAT) in single sections through the rat VTA. M5R was located mainly to VTA somatodendritic profiles (71%; n=627), at least one-third (33.2%; n=208) of which also contained DAT. The M5R immunoreactivity was distributed along cytoplasmic tubulovesicular endomembrane systems in somata and large dendrites, but more often located at plasmalemmal sites in small dendrites, the majority of which did not express DAT. The M5R-immunoreactive dendrites received a balanced input from unlabeled terminals forming either asymmetric or symmetric synapses. As compared with dendrites, M5R was less often seen in axon terminals, comprising only 10.8% (n=102) of the total M5R-labeled profiles. These terminals were usually presynaptic to unlabeled dendrites, suggesting M5R activation can indirectly modulate non-DAT containing dendrites through presynaptic mechanisms. Our results provide the first ultrastructural evidence that in the VTA, M5R has a subcellular location conducive to major involvement in postsynaptic signaling in many dendrites, only some of which express DAT. These findings suggest that cognitive and rewarding effects ascribed to muscarinic activation in the VTA are primarily credited to M5R activation at postsynaptic plasma membranes distinct from dopamine transport.
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Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Neurociencia de sistemas

ROLE FOR NEONATAL D-SERINE SIGNALING: PREVENTION OF PHYSIOLOGICAL DEFICITS IN ADULT PICK1 KNOCKOUT MICE

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NMDA glutamate receptors play key roles in brain development. Nonetheless, it is unclear whether and how neonatal deficits in NMDA-receptor-mediated neurotransmission affect adult brain functions and behavior. Likewise, the role of D-serine in NMDA receptor-mediated synaptic plasticity during development remains elusive. Behavioral deficits in *Pick1* (a multifunctional scaffold protein that interacts with the D-serine synthesizing enzyme) knockout mice suggested altered prefrontal cortical function. Then, we tested the excitability of prefrontal pyramidal neurons with whole-cell current-clamp signals obtained in brain slices of these mice. We examined the number of action potentials evoked with intracellular current injections before and after bath application of NMDA (4 μM), as a readout of cell excitability, and the modulation of NMDA responses by D1 dopamine receptors by adding the selective D1 agonist SKF38393 (2 μM). Resting membrane properties of prefrontal pyramidal neurons were not affected in *Pick1* knockout mice. The number of action potentials evoked by a constant-amplitude current pulse was markedly increased by application of NMDA (from 6.6 ± 0.5 to11.4 ± 2.8 spikes) and with the combined addition of NMDA and SKF38393 (to 13.1 ± 4.8) in wild-type mice. In *Pick1* knockout mice, however, these increases in the number of action potentials were significantly attenuated. Unlike untreated *Pick1* knockout mice, NMDA elicited strong increases in evoked action potential firing in slices from D-serine-treated *Pick1* knockout mice (500 mg/kg, i.p. daily to pups beginning on postnatal day 3, for 2 weeks). The number of action potentials rose from 3.9 ± 1.2 to 7.4 ± 3.2 with NMDA administration and slightly increased to 8.9 ± 3.6 with the addition of SKF38393 to NMDA. These results indicate a novel role for D-serine during brain development, with significant influence on adult brain function and behavior.

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A ROLE FOR GSK3 IN THE MAINTENANCE OF BASAL SYNAPTIC TRANSMISSION

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Glycogen synthase kinase-3 (GSK3) is a threonine-serine kinase with pleiotropic actions in the central nervous system. Although GSK3 has been implicated in neurological disorders characterised by synaptic dysfunction, including Alzheimer's disease, its actions at the synapse remain obscure. Here, we have examined the role of GSK3 in modulating AMPA receptor-mediated current in CA1 hippocampal neurons. Using whole-cell and field potential recording in rat hippocampal slices, we have found that acute application of structurally unrelated selective inhibitors of GSK3 (AR-A014418 and CHIR-99021) leads to a run-down of evoked AMPAR-mediated currents. Consistent with this observation, overnight application of these inhibitors produces a decrease in AMPA/NMDA ratio of CA1 pyramidal neurons. Further, we used a virally-expressed dominant negative protein to show that acute depression of AMPAR-mediated currents is independent of activity of Rab5a, whose role in endocytosis of AMPA receptors is a candidate for modulation by GSK3. Thus, our findings suggest that GSK3 is required for the maintenance of basal synaptic transmission.

Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Neurociencia cognitiva y conductual

Cux1 AND *Cux2* SELECTIVELY TARGET BASAL AND APICAL DENDRITIC COMPARTMENTS OF LAYER II-III CORTICAL NEURONS

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A number of recent reports implicate the differential regulation of apical and basal dendrites in autism disorders and in the higher functions of the human brain. They show that apical and basal dendrites are functionally specialized and that mechanisms that regulate their development have important consequences for neuron function. The molecular identity of layer II-III neurons of the cerebral cortex is determined by the selective expression of Cux1 and Cux2. We previously showed that although their expression coincides and their protein structures are similar, the two Cux proteins are necessary and non-redundant for normal dendrite development of layer II-III neurons.
We demonstrate, using *in vivo in utero* electroporation and morphological analysis, that expression of *Cux1* and *Cux2* differentially promote the development of basal and apical dendrites, respectively. As the mechanisms that underlie these complementary functions are unknown, we studied the *in vivo* gain and/or loss of function of Cux1 and Cux2 proteins to characterize their role in the development of apical and basal dendrites. We show that Cux1 and Cux2 differentially regulate the branching of basal and apical compartments, respectively. These selective effects explain the additive functions of *Cux* genes as well as the functional diversification of layer II-III neurons into different subpopulations, possibly with distinct connectivity patterns and modes of neuron response.

Conclusions: Our data suggest that by selectively regulating basal and apical dendrites, Cux1 and Cux2 can promote the integration of layer II-III neurons in the intracortical networks in highly specific ways.

PROPAGATION OF ELECTROPHYSIOLOGICAL RESPONSES IN THE CEREBRAL CORTEX OF AN ANIMAL MODEL OF DEVELOPMENTAL DISORDERS: THE *Lis1/sLis1* MOUSE

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The *Lis1/sLis1* mutant mouse is a model human lissencephaly and of other developmental disorders of the central nervous system. It has both prenatal and postnatal cortical anomalies. We have studied the propagation of evoked epileptiform discharges along the superficial part of layer 2/3. Experiments were done in coronal slices (350 □m thickness) from rostral (anterior cingulate cortex) and caudal (retrosplenial cortex) levels. The epileptiform discharges were evoked by brief current pulses applied to layer 1 in the presence of 10 □M bicuculline and modified ACSF (with 5 mM K^+ and 1.2 mM Ca^{++}) and were recorded with pairs of extracellular electrodes at fixed distances from the stimulus electrode. The velocity of the dorso-ventral propagation of evoked epileptiform discharges away from the stimulation site at P15 was slower in *Lis1/sLis1* (35.4±2.5 mm/s,n=24) than in WT (47.6±2.3 mm/s, n=15; p<0001) in rostral slices and 46.2±3.3 mm/s(n=20) vs 78.3±14.3mm/s (n=10; p<0.050) in caudal slices. This slower propagation in *Lis1/sLis1* was transient since at P10 and at P20 the propagation velocity was similar in both *Lis1/sLis1* and WT animals: ≈30 mm/s (rostral) at P10 and P20 and ≈18 mm/s (caudal) at P10. The application of 1□M CNQX produced a larger decreased of the latency of these responses in *Lis1/sLis1* (12.6±4.7 ms, n=8) than in WT (20.4±2.8 ms, n=12; p<0.05) cortex. We studied the interhemispheric propagation time (IPT) of these epileptiform discharges in coronal slices in which most callosal axons were intact. The IPT was measured as the difference in the latency of responses recorded simultaneously with two electrodes placed in homotopic sites of both hemispheres. In caudal slices the IPT was longer in *Lis1/sLis1*(39.0±7.9 ms, n=12) than in WT (6.5±4.9 ms, n=6; p<0.05); in rostral slices the IPT was similar. These results suggest the existence of anomalies in the excitatory connections of the cingulated cortex of the *Lis1/sLis1* animal, both in local short-range and in long-range interhemispheric connections. Supported by the Spanish Ministry of Science and Innovation: FEDER (BFU-2010-27326) and CONSOLIDER (CSD2007-00023); Generalitat Valenciana: PROMETEO (2009/028).

Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.
2ª: Desarrollo.

AUTISTIC-LIKE BEHAVIOR IN A MOUSE MODEL WITH IMPAIRED β-NEUREXIN-1 FUNCTION

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Objectives: Autism spectrum disorders (ASD) are characterized by communication impairment, defects in social interaction and restricted and stereotyped patterns of behavior. Affected children are often diagnosed with ASD after a period of normal development, suggesting a postnatal disease mechanism. Neurexins are presynaptic proteins that bind to postsynaptic neuroligins. Mutations in neurexin and neuroligin genes have suggested a role for the neurexin-neuroligin pathway in autism. Recently, we have reported synaptic deficits of β-neurexin-1 as a risk factor for autism (1). Moreover, mutations in neurexin genes in other mental diseases, as schizophrenia, suggest that dysfunction of neurexins in particular brain areas could lead to different brain disorders.
To understand the role of β-neurexin-1 in the pathological mechanism of autism, we have generated transgenic mice with impaired β-neurexin-1 function as an animal model for autism.

Materials and Methods: *In vitro* studies were performed in hippocampal neurons isolated from rat. For *in vivo* studies we generated a transgenic mouse line that expresses an HA-tagged β-neurexin-1 mutant that lacks the cytoplasmic domain (HA-βNrx1ΔC) under the control of the inducible TRE promoter. We crossed the TRE-HA-βNrx1ΔC mice with CAMKII-tTA animals to inhibit the function of β-neurexin-1 in postnatal neurons.

Results: We show that HA-βNrx1ΔC works as a dominant negative mutant as it binds to neuroligins at synapses but inhibits vesicle release. In HA-βNrx1ΔC/CAMKII-tTA mice, HA-βNrx1ΔC is expressed in postnatal cortex and striatum, two brain areas affected in autism. Analysis of cortical synaptosome fractions demonstrates that HA-βNrx1ΔC is incorporated into presynaptic terminals *in vivo*. In behavioral tests, HA-βNrx1ΔC/CAMKII-tTA mice show autistic-like behavior with deficits in social interaction, increased self-grooming and lack of discrimination of social odors.

Conclusions: Postnatal inhibition of β-neurexin-1 recapitulates the core symptoms of autism. This suggests that dysfunction of β-neurexin at postnatal stages might play a role in the onset of autism.

Áreas temáticas:
1st: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2nd: Neurociencia cognitiva y conductual

SPHINGOMYELIN INFLUENCES DENDRITIC SPINE SIZE THROUGH THE MODULATION OF ACTIN

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Understanding the relevance of lipids in synaptic plasticity is increasingly acknowledged, but little is known about the molecular mechanisms involved. These mechanisms are critical for the characterization of many lipidoses as for the development of therapies for these diseases. Here we reveal sphingomyelin (SM) as a key modulator of the dendritic spine actin cytoskeleton, an important contributor to synaptic plasticity. We show that increased SM levels in neurons of acid sphingomyelinase knockout mice (ASMko), which mimic Niemann Pick disease type A (NPA), result in reduced spine number and size and low levels of filamentous actin. Mechanistically, high SM levels decrease membrane attachment and activity of RhoA and its effectors ROCK and ProfilinIIa. This, in turn, is due to the reduction of the levels of metabotropic glutamate receptors type I (mGluR1/5) that prevent RhoA binding to the synaptic membrane. Notably, pharmacological activation of the neutral sphingomyelinase, which we reveal is present at synapses, rescues the aberrant phenotypes. Altogether, these data demonstrate the influence of SM in dendritic spine physiology and contribute to our understanding of the cognitive deficits of NPA patients, opening new perspectives for therapeutic interventions.

Running title: Sphingomyelin influences dendritic spine actin cytoskeleton

Key words: actin/ dexamethasone /RhoA/ sphingomyelin/ vitamin D

EL ÁCIDO LISOFOSFATÍDICO, FOSFOLÍPIDO DERIVADO DE MEMBRANA, MODULA LA EFICACIA DE LAS ENTRADAS SINÁPTICAS GLUTAMATÉRGICAS Y GABAÉRGICAS SOBRE MOTONEURONAS

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El ácido lisofosfatídico (LPA), un fosfolípido derivado de membrana, como molécula de señalización extracelular al unirse a varios receptores (LPA_{1-6}) acoplados a proteínas G. Aunque el LPA y la mayoría de sus receptores se expresan en el sistema nervioso central, se conoce muy poco sobre su papel en la modulación de la transmisión sináptica. El objetivo de este trabajo ha sido determinar el papel del LPA como modulador de la transmisión sináptica excitatoria (AMPAérgica) e inhibitoria (GABAérgica) en motoneuronas (MNs), y describir las cascadas moleculares implicadas. Mediante *q*RT-PCR se observó que el ARNm para el receptor LPA_1 es el predominantemente expresado en MNs. Además, estudios inmunohistoquímicos revelaron una estrecha relación anatómica del LPA_1 y estructuras sinápticas excitatorias e inhibitorias sobre MNs. Registros de *whole-cell patch clamp* demostraron una acusada y reversible disminución (~70 %) en las corrientes evocadas AMPAérgicas y GABAérgicas en MNs del núcleo hipogloso (MNHs) tras la aplicación de LPA (10 □M) Estudios electrofisiológicos y de microscopía electrónica sugieren que el LPA reduce el *pool* de vesículas listas para ser liberadas en terminales excitatorios. En base a los resultados obtenidos, proponemos un mecanismo de acción presináptico del LPA sobre las entradas glutamatérgicas , donde el LPA actuaría presumiblemente sobre el receptor LPA_1 acoplado a la proteína $G_{i/o}$, con la subsecuente activación de la fosfolipasa C y la qinasa de la cadena ligera de la miosina. Por otro lado, se observó que el papel del LPA sobre la transmisión GABAérgica implica un mecanismo postsináptico mediado por LPA_1-RhoA-Rho kinasa-calcineurina. Además, por *western-blot* y fraccionamiento celular se ha observado que la reducción inducida por LPA en las corrientes GABAérgicas estaba, al menos en parte, mediada por la defosforilación y posterior retirada de membrana de la subunidad γ_2 del receptor $GABA_A$. En conjunto, los mecanismos observados sugieren que el LPA tendría un importante papel a la hora de establecer cambios en la eficiencia sináptica de las MNs en diversos desórdenes fisiopatológicos que cursan con cambios en las concentraciones de dicho fosfolípido. Asimismo, el hecho de que el LPA se sintetice en una forma dependiente de actividad lo identifica como un posible factor de comunicación autocrina y/o paracrina acoplando actividad neuronal y sináptica.

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1º: 1.Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2º: 5. Trastornos y reparación del sistema nervioso

FUNCTIONAL CHARACTERIZATION OF DIFFERENT SUBPOPULATIONS OF TRPM8-EXPRESSING COLD THERMORECEPTORS IN ADULT MOUSE TRIGEMINAL GANGLIA

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Primary sensory neurons are morphologically and functionally diverse. TRPM8, a cold- and menthol-activated transient receptor potential channel, is the principal sensor of environmental cold in peripheral thermoreceptors. However, the molecular determinants that define the excitability of these receptors remain poorly defined.

Using a transgenic mouse line that labels TRPM8(+) neurons with the yellow fluorescent protein YFP, we characterized adult TRPM8(+) trigeminal ganglion neurons (TGN) electrophysiologically and their responses to physiological stimuli, in short-term cultures.

All recorded neurons were intensely fluorescent for YFP, with diameters (ø) ranging from 12 to 30 μm. TRPM8(+) neurons showed three discharge patterns to 1-s depolarizing current pulses: tonic (regular AP firing), phasic (1-3 AP at the start) and tonic/phasic (adapting or bursting AP firing). We found a significant correlation between neuronal size and firing pattern: small neurons (ø ≤ 18 μm) were tonic while intermediate and large ø neurons were mostly tonic/phasic or phasic. All TRPM8(+) neurons depolarized during cooling ramps to 20ºC: small neurons were low-threshold (34-26ºC) while larger neurons were high-threshold (≤ 26ºC). Interestingly, the potassium channel blocker 4-AP (100 μM) transformed large, phasic, high threshold neurons, into tonic neurons more sensitive to cold. Cadmium and nifedipine, calcium channel blockers, reduced excitability of TRPM8(+) neurons during cooling. Preliminary results indicate that small neurons present greater density of L-type calcium currents.

In summary, cellular size predicts many functional characteristics of TRPM8(+) cold thermoreceptors. Moreover, voltage-gated potassium and calcium channels influence their excitability. Changes in their expression may be relevant to the development of cold pain symptoms observed in some in neuropathies.

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REDES FUNCIONALES ASTRO-NEURONALES SELECTIVAS ASOCIADAS A LAS VÍAS DIRECTA E INDIRECTA EN EL ESTRIADO

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Evidencias recientes han demostrado la existencia de comunicación entre neuronas y astrocitos. Sin embargo, se ignora si en el estriado existe dicha comunicación, sus propiedades y consecuencias fisiológicas.
Utilizando registros de pares de neuronas y de imagen de calcio en rodajas de estriado dorso-lateral de ratón, hemos estudiado las propiedades de la señal de calcio astrocitaria inducida por actividad neuronal y sus consecuencias sobre la excitabilidad celular y la transmisión sináptica glutamatérgica.
Hemos encontrado que la despolarización neuronal, que provoca la liberación de endocannabinoides (ECBs), genera elevaciones de calcio en astrocitos adyacentes acompañadas de la aparición de corrientes lentas de entrada (SICs) mediadas por activación de receptores NMDA postsinápticos, y de una potenciación heteroneuronal de la transmisión sináptica mediada por activación de receptores metabotrópicos de glutamato de tipo I. Estos efectos no se observan en presencia de antagonistas del receptor CB1, y en ratones $CB1R^{-/-}$ e $IP_3R2^{-/-}$ con déficits en la señal de calcio.
Por tanto, ECBs liberados por neuronas aumentan el calcio astrocitario por activación de receptores CB1. Esta señal de calcio estimula la liberación de glutamato por astrocitos, que 1) activa receptores de NMDA postsinápticos generando SICs, y 2) potencia la neurotransmisión glutamatérgica por activación de receptores mGluR tipo I presinápticos.
En neuronas de proyección identificadas (ratones Drd1a-td-Tomato y Drd2-eGFP), la generación de SICs y la potenciación mediadas por activación astrocitaria ocurren exclusivamente en neuronas homotípicas (pertenecientes a la misma vía, directa o indirecta, que la neurona estimulada) pero no en neuronas heterotípicas.
Estos resultados demuestran la presencia de comunicación específica entre astrocitos y neuronas pertenecientes a las vías directa e indirecta, e indican la existencia de redes astro-neuronales funcionales selectivas para cada una de las vías de proyección estriatal.

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Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Neurociencia de sistemas

LA REDUCCIÓN DE LOS NIVELES DE GS, GI Y G□13 EN NEURONAS RECEPTORAS OLFATORIAS CAUSA DEFECTOS EN LA PERCEPCIÓN OLFATORIA DE *DROSOPHILA MELANOGASTER*.

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En Drosophila se ha demostrado que el dímero entre los receptores olfativos (OR) y su co-receptor general (Orco) puede actuar como canal iónico no selectivo estimulado por olor. Sin embargo, también se han detectado vías mediadas por proteínas G en recepción olfatoria similares a las de la olfacción en vertebrados. Las subunidades alfa de las proteínas G, Gs y Gi son el activador y el inhibidor, respectivamente, de la adenilato ciclasa (AC), que produce el segundo mensajero AMPc, que al activar los canales iónicos dependientes de nucleótidos cíclicos produce la despolarización de la célula. Por su parte, los dímeros □□ de las proteínas G también pueden tener un papel en la transducción.

En este trabajo hemos estudiado el efecto del silenciamiento de Gs, Gi y G□13 en neuronas receptoras olfatorias adultas sobre la percepción olfatoria. Se utilizan RNAs de interferencia (RNAi) restringiendo su expresión espacio-temporal por un sistema de expresión Gal4/UAS controlado con un Gal80 termosensible.

Se mide la respuesta electrofisiológica tanto en neuronas individuales (Registros en Sensila Única, SSRs) como en órgano olfatorio completo (Electroantenogramas, EAGs), así como sus efectos en la percepción olfativa a través de pruebas de comportamiento de Drosophila en laberinto en Y.

La reducción de los niveles de Gs, Gi y G□13 en neuronas olfativas de Drosophila conduce a diferencias tanto en las respuestas electrofisiológicas como en el comportamiento olfativo. Sin embargo, estas diferencias no son generalizadas y dependen de la proteína afectada, así como del olor y la concentración probados. Todo ello sugiere que el efecto observado de las proteínas G en la recepción olfatoria no es debido a un deterioro general de las neuronas sino a que juegan un papel específico en la transducción olfatoria en Drosophila.

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Áreas Temáticas:
2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.
3. Neurociencia de sistemas.

COOPERATIVITY BETWEEN CALMODULIN-BINDING SITES IN Kv7.2 CHANNELS.

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The interaction of calmodulin (CaM), is essential for the surface expression of Kv7.2 the main component of the neuronal M channel. Mutations that interfere with CaM binding or the sequestering of CaM prevent this M-channel component from exiting the endoplasmic reticulum (ER), which reduces M-current density in hippocampal neurons, enhancing excitability and offering a rational mechanism to explain some forms of benign familial neonatal convulsions (BFNC). Previously, we identified a mutation (S511D) that impedes CaM binding while allowing the channel to exit the ER, hinting that CaM binding may not be strictly required for Kv7.2 channel trafficking to the plasma membrane. Alternatively, this interaction with CaM might escape detection and, indeed, we now show that the S511D mutant contains functional CaM-binding sites that are not detected by classical biochemical techniques. Surface expression and function is rescued by CaM, suggesting that free CaM in HEK293 cells is limiting and reinforcing the hypothesis that CaM binding is required for ER exit. Within the CaM-binding domain formed by two sites (helix A and helix B), we show that CaM binds to helix B with higher apparent affinity than helix A, both in the presence and absence of Ca(2+), and that the two sites cooperate. Hence, CaM can bridge two binding domains, anchoring helix A of one subunit to helix B of another subunit, in this way influencing the function of Kv7.2 channels.

NEURON-ASTROCYTE COMMUNICATION MEDIATED BY ENDOCANNABIOID/mGLUR SIGNALING AT TRIPARTITE SYNAPSES

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Endocannabinoid signaling play key roles in brain function, and cannabinoid effects in brain physiology and drug-related behavior are thought to be mediated by receptors exclusively present in neurons. We are investigating the astrocyte calcium responsiveness to neurotransmitters and its modulatory consequences on synaptic transmission and plasticity in rodent hippocampal slices

We have recently shown that hippocampal astrocytes express functional CB1Rs that may be activated by endocannabinoids released by neurons (Navarrete and Araque, Neuron, 2008). We have found that ECBs released by hippocampal pyramidal neurons transiently increase the probability of transmitter release at single CA3-CA1 synapses (Navarrete and Araque, Neuron 2010). But ECBs system is considered unable to induce the long-term potentiation (LTP) of synaptic transmission.

Using electrophysiological and Ca^{2+} imaging techniques in mice brain slices we have investigated whether the coincidence of ECB signaling and neuronal activity could induce long-term changes in synaptic transmission. We recorded pairs of pyramidal neurons and monitored Schaffer collateral-evoked synaptic transmission at single synapses. We depolarized one neuron to stimulate ECB release and monitored synaptic activity in the other neuron, which was mildly depolarized to mimic postsynaptic activity. Pairing both depolarizations induced a long-lasting enhancement of the synaptic efficacy. This LTP requires the coordinated activity of the three elements of the Tripartite Synapse: 1) ECB-evoked astrocyte calcium signal that stimulates glutamate release; 2) postsynaptic nitric oxide production; and 3) activation of protein kinase C and presynaptic type 1 metabotropic glutamate receptors (mGluRs). Consequently, ECBs can trigger LTP through stimulation of astrocyte-neuron signaling, revealing novel cellular mechanisms of endocannabinoids.

Therefore, these results show that endocannabinoid-mediated astrocyte-neuron communication have important consequences on synaptic physiology. They also indicate that the astrocyte calcium signal evoked by endogenous stimuli (neuron-released endocannabinoids) modulates synaptic plasticity.

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Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Neurociencia de sistemas

REGULATION OF $GABA_A$ RECEPTOR EXPRESSION AND FUNCTION IN OLIGODENDROCYTES

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Oligodendrocytes are endowed with neurotransmitter receptors whose levels and properties vary during development, maturation and myelination. However, knowledge about how neuron-to-oligodendrocyte interactions regulate those changes is scant. Here, we studied the presence of neurotransmitter receptor in oligodendrocyte progenitors (OPCs) and $O4^+$ oligodendrocytes (OLs), cultured alone or in the presence of dorsal root ganglion (DRGs) neurons, by electrophysiological and intracellular Ca^{++}($[Ca^{++}]_i$) recordings using patch-clamp and fluorimetry respectively. Cells were challenged at different days in vitro (1–12 DIV) with GABA, glutamate (Glut), and ATP. Both OPCs and OLs cultured alone showed inward currents to these transmitters (all at 1 mM and -80 mV). Thus, at 1DIV responses to GABA were very large (870 ± 100 pA) while those of Glut and ATP were moderate (40 ± 12 and 15 ± 3 pA, respectively). After 2DIV GABA sensitivity drastically diminished (36 ± 15 pA) in both cell types, while that of Glut and ATP remained constant. In contrast, the amplitude of GABA responses remained large when OPCs and OLs were co-cultured with DRG neurons. GABA responses were mediated by $GABA_A$ receptors (EC50 = 55 ± 12 μM), were inhibited by Zn^{++}, but were not affected by La^{+++}, indiplon, loreclezole or THIP. In addition, $GABA_A$ receptor-mediated responses in OPCs or OLs, alone or in co-culture, induced an increase in $[Ca^{++}]_i$ with the same temporal pattern depicted by electrophysiology, was dependent on extracellular Ca^{++}, and inhibited by blocking both voltage-dependent Ca^{++} channels and Na^+–K^+–$2Cl^-$ cotransporter with nifedipine and bumetanide respectively.
These data indicate that neuron-OL interactions regulate the expression in OL of excitatory $GABA_A$ receptors lacking δ and γ subunits. These receptors may be relevant to OL development and maturation and to neuron-OL signalling.

Supported by Ikerbasque, PASPA-UNAM, and MINECO

Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Trastornos y reparación del sistema nervioso

TARGETING THE BH3-ONLY PROTEINS AND p53 PATHWAY TO PROTECT OLIGODENDROCYTE PRECURSOR CELLS FROM AMPA-INDUCED DAMAGE

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Extracellular glutamate accumulation in the CNS and subsequent overstimulation of its ionotropic receptors is deleterious to oligodendrocytes and their precursors (OPCs), induces myelin damage and contributes to the pathophysiology of multiple sclerosis and diseases undergoing myelin destruction. Here we have investigated the role of BH3-only proteins BID and PUMA in OPC apoptosis after AMPA receptor activation. Cultured OPCs subjected to brief and moderate AMPA receptor stimulation increase the levels of active form of BID (tBID), which translocates into mitochondria leading to its disruption and releasing apoptogenic molecules. In turn, tBID inhibition with BI6C9 prevents the rise of tBID levels, abolishes mitochondrial dysfunction and protects from excitotoxic insults both OPCs and mature oligodendrocytes. Since BID is a specific substrate of caspase-8, we examined the role of its intrinsic inhibitor c-FLIP$_{L/S}$, and we detected that AMPA induces an early and transient decrease in c-FLIP$_{L/S}$ levels, preceding caspase-8 activation and BID cleavage. This gives c-FLIP$_{L/S}$ an important role in the early regulation of AMPA-induced apoptosis and suggests that its modulation soon after the insult can rescue oligodendrocytes from dying.
Additionally, the induction of cell death by p53 occurs via both target gene activation, like PUMA, and transcription-independent mechanisms at the mitochondria level. Indeed, we observed that a moderate activation of AMPA receptors in cultured OPCs elevated the levels of p53 and PUMA proteins, and of phosphorylated-p53, a state that favors its mitochondrial insertion. Preincubation of OPCs with pifithrin-μ, inhibited p53-mitochondria association, reduced p53/PUMA overexpression and prevented AMPA-induced excitotoxicity. This beneficial effects were not observed using pifithrin-□, an inhibitor of p53 accumulation in the nucleus.
Together, these results show c-FLIP/tBID and p53/PUMA pathways as key regulators of AMPA-induced mitochondrial dysfunction and cell death in cultured OPCs and suggest that modulation of these death pathways may help in developing novel oligoprotective drugs with therapeutic potential.

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Áreas Temáticas: Seleccione las **2** áreas temáticas que más se ajusten a su trabajo en orden de prioridad:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Trastornos y reparación del sistema nervioso

SPHINGOMYELIN MODULATES TYPE I METABOTROPIC GLUTAMATE RECEPTOR TRAFFICKING AND FUNCTION AT SYNAPSES

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Synaptic function relies on neurotransmitter receptor trafficking. This in turn depends on the dynamics of membranes from which lipids are major components. However, little is known about the influence of lipids in neurotransmitter receptor physiology. Our results in mice that lack the acid sphingomyelinase (ASMko) and show aberrantly increased levels of sphingomyelin at their synapses unveil this lipid as a key modulator for Type I metabotropic Glutamate receptor (mGluR1/5) physiology. ASMko synapses show reduced total and surface levels of mGluR1/5. The internalization rate of these receptors is increased in the absence of ASM, while their degradation is not impaired. Addition of sphingomyelin to synaptosomes and cultured hippocampal neurons from wild type mice mimics these effects confirming the direct role of this lipid in the aberrant phenotypes. Electrophysiology experiments offer insight on the functional implications. Because ASMko mice are a model for Niemann Pick disease type A (NPA) our results could contribute to explain the severe cognitive deficits of NPA patients.

Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Trastornos y reparación del sistema nervioso

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15º Congreso Nacional de la Sociedad Española de NeuroCiencia

Tema 3

Neurociencia de sistemas

Posters

UPREGULATION OF GLUTAMATERGIC NEUROTRANMISSION IN THE COCHLEAR NUCLEI OF THE *Igf1* NULL MICE

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Insulin-like growth factor 1 (IGF-1) is an activity-dependent peptide that plays an important role in the development and maturation of the nervous system, and modulates synaptic plasticity and neuronal function in the adult brain. Mice lacking the *Igf1* gene suffer from hearing loss, increases in their auditory thresholds and latencies, abnormalities in stria vascularis and the cochlear innervation as well as significant decreases in the number and size of spiral ganglion neurons. An issue that remains unknown however, is whether these cochlear abnormalities in IGF-1 deficient mice may result in an imbalance between excitation and inhibition in the cochlear nuclei, the first central relay station in the central auditory system. Accordingly, the expression and distribution of the vesicular glutamate transporter 1 (VGLUT1) and the vesicular inhibitory transporter (VGAT), specific markers for labeling excitatory and inhibitory terminals, were examined in the cochlear nuclei of a 4-month-old mouse model of IGF-I deficiency and neurosensorial deafness ($Igf1^{-/-}$ homozygous null mice) in comparison with $Igf1^{+/-}$ heterozygous and $Igf1^{+/+}$ wild type animals. The results demonstrate significant increases in the overall mean gray levels and the immunostained area of VGLUT1 but not VGAT immunostaining in the cochlear nuclei of $Igf1^{-/-}$ when compared to $Igf1^{+/-}$ and $Igf1^{+/+}$ animals. In conclusion, these findings provide evidence of an upregulation of the glutamatergic neurotransmitter system in the cochlear nuclei of IGF-1 null mice that may reflect a compensatory synaptic mechanism due to an IGF-1 deficient cochlea.

Áreas Temáticas:
1. Neurociencia de sistemas.
2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.

AGE-RELATED HEARING LOSS IN WISTAR RATS: MODIFICATIONS IN AUDITORY BRAINSTEM RESPONSES

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Animal models are essential to understand the mechanisms involved in pathologies of the sensory system. Since Wistar rats are a widely used experimental model in the auditory system, the goal of this study was to characterize in detail age-related changes in their auditory brainstem responses (ABR) in order to determine whether this rat strain could be used as a model of age-related hearing loss (ARHL). To do so, evaluations of ABR recordings at 0.5, 1, 2, 4, 8, 16 and 32 kHz were performed in adult male wistar rats which were distributed in three groups according to age (6-8, 12-14 and 18-20 months-old). The results demonstrated that there was a significant increase in the auditory thresholds at all frequencies tested as the age increased. Additionally, at all frequencies evaluated, there was a significant decrease in wave amplitudes and an increase in latencies in older animals compared to that in younger rats. These findings are consistent with previous studies in other animal models of ARHL and suggest that Wistar rats are also an excellent model for studying this sensory disability providing valuable information regarding the development or improvement of therapies that could benefit patients with this kind of sensory impairment.

Áreas Temáticas:
1. Neurociencia de sistemas.
2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.

AGE-RELATED HEARING LOSS IN WISTAR RATS: MODIFICATIONS IN SYNAPTIC VESICULAR TRANSPORTERS IN THE COCHLEAR NUCLEUS

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Age-related hearing loss (ARHL) is one of the most frequent sensory impairments in elderly people, and a source of important individual and socio-economic consequences. The understanding of the cellular mechanisms that occur in the central auditory pathway of these patients will help us to improve the diagnosis and treatment of this disability. Accordingly, the goal of this study was to characterize in detail age-related modifications in excitatory and inhibitory vesicular transporters in the cochlear nucleus (CN) of Wistar rats. To do so, auditory brainstem responses (ABR) recordings and immunohistochemistry for the vesicular glutamate transporter 1 (VGLUT1) and the vesicular GABA transporter (VGAT) in the CN were performed in adult rats which were distributed into three groups according to age (6-8, 12-14 and 18-20 months-old). The results demonstrated that there was a significant decrease in the mean gray level and in the immunostained area of both VGLUT1 and VGAT immunostaining in the CN as the age increased. These findings suggest that an age-related decline in excitatory terminals in the CN may influence the magnitude of the auditory evoked responses while the reduction in inhibitory terminals could be part of a synaptic homeostatic mechanism that may help to reset neuronal responses to progressively compensate for the altered primary inputs.

Áreas Temáticas:
1. Neurociencia de sistemas.
2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.

NEURONAL TUNING TO INSTANTANEOUS STIMULATION INTERVAL IN THE BARREL CORTEX

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Neurons in the barrel cortex (BC) are sensitive to whisker identity, but also to dynamical (temporally varying) stimulus features, and this additional feature selectivity is essential to their function. For example, whisker-mediated tactile discrimination is thought to depend on the temporal pattern of whisker motion fluctuations evoked upon contacting an object, and BC neurons encode the physical parameters (e.g. velocity) of fluctuations in the sequence. Here we asked whether BC neurons have the capacity to integrate such sequences of whisker fluctuations over time, and thus to encode specific temporal stimulation patterns.

We addressed whether BC neurons are tuned to single intervals exclusively (implying sensitivity to instantaneous frequency), or to sets of several successive intervals (implying integration over time). We performed juxtacellular recordings in anesthetized mice. Stimulation was applied with a piezoelectric wafer and consisted of different sequences of stimuli applied at periodic and non-periodic intervals. We found that neurons carry overwhelmingly more information about the latest single intervals than about earlier intervals or interval sequences. Our results place limits on the amount of temporal integration that can be carried out by neurons in the primary somatosensory barrel cortex. We are currently extending these analyses to neuronal responses during sequence discrimination in awake animals.

Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Neurociencia cognitiva y conductual

STIMULUS-SPECIFIC ADAPTATION IN THE AWAKE AND THE ANESTHETIZED INFERIOR COLLICULUS OF THE MOUSE

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The ability to detect unexpected sounds within the environment is an important function of the auditory system, as a rapid response may be required for the organism to survive. Recent studies found a decreased response to repetitive stimuli (standard), but a maintained response to rare or less frequent sounds (deviant) in individual neurons in the inferior colliculus and at higher levels. This phenomenon, known as stimulus-specific adaptation (SSA; Ulanovsky et al., 2003) has been suggested to serve for change detection and as a single neuron correlate for mismatch negativity.

Thus far, all the experiments on SSA have been performed in an anesthetized preparation; therefore it is unknown whether or not anesthetic agents affect SSA. For this reason we recorded single unit responses in the inferior colliculus of male CBA/J mice under an oddball paradigm to elicit SSA in awake and anesthetized animals. Details of the anaesthetized preparation are as usual in our laboratory (Malmierca et al., 2009; Duque et al., 2012) so that it is induced and maintained with urethane (1.5 g/kg). The surgery and recording procedures were performed on the same day of the surgery. For the awake preparation, we have adapted a head restraint technique long used in bats to mouse (Bryant et al., 2009; Muniak et al., 2012).

Our preliminary results show that the fundamental response properties of IC neurons such us the temporal or spectral response properties and the degree of inhibition may be different during the anesthetized and awake preparations. However, the levels of SSA found during the awake preparation are of the same magnitude than those found in the anesthetized one. These findings demonstrate the validity and the importance of all the previous experiments on SSA and opens the possibility of working in awake behavioral tasks and with genetically modified organisms. Financed by BFU2009-07286, EUI2009-04083. DDD held a fellowship from the Spanish MEC (BES-2010-035649).

1ª: Neurociencia de sistemas
2ª: Neurociencia cognitiva y conductual

CAMBIOS DENDRÍTICOS EN LOS NÚCLEOS DEL TRIGÉMINO POR PRIVACIÓN CRÓNICA DEL TACTO HÁPTICO Y SU MODIFICACIÓN POR ENRIQUECIMIENTO AMBIENTAL

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El cerebro es capaz de adaptarse estructuralmente a las modificaciones del entorno incluso durante la edad adulta. En los últimos años se ha demostrado que esta plasticidad dependiente de las entradas sensoriales o la experiencia no es exclusiva de la corteza cerebral, sino que se produce también en niveles subcorticales. El sistema trigeminal de roedores permite estudiar este tipo de plasticidad, sin lesionar la vía, induciendo una pérdida selectiva del tacto activo (háptico) mediante el recorte repetido de las vibrisas. Hemos aplicado este procedimiento en las vibrisas derechas de ratas adultas durante dos meses, y marcado posteriormente las neuronas trigeminotalámicas del núcleo principal (Pr5) y las intersubnucleares que proyectan a ese núcleo desde el núcleo caudal (Sp5c-Pr5), mediante depósitos de dextrano biotinilado en el tálamo y el propio Pr5, respectivamente. La privación induce una notable disminución de la longitud dendrítica total en las neuronas de Pr5 contralaterales, lo que hace desaparecer la asimetría entre lados que éstas presentan normalmente, y una disminución en el número de nodos y espinas en las neuronas de Sp5c-Pr5, bilateralmente. Estos cambios morfométricos no ocurrieron cuando se expusieron las ratas a un entorno enriquecido durante todo el período que duró el recorte de vibrisas. En estas condiciones, el sumatorio de los cambios inducidos en ambos hemisferios generó asimetrías por mayor complejidad en los árboles dendríticos y aumento de las espinas en neuronas contralaterales al recorte de vibrisas en las dos poblaciones neuronales estudiadas. Estos resultados permiten concluir: 1, que las dendritas de las neuronas trigeminales del tronco del encéfalo siguen abiertas a cambios plásticos dependientes de la experiencia durante la vida adulta, y 2, que estos cambios respaldan que las adaptaciones estructurales homeostáticas no se limitan a la corteza, sino que existen también en niveles más inferiores de las vías sensoriales.
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Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Sistemas homeostáticos y neuroendocrino

PROPIEDADES FUNCIONALES DE LAS PROYECCIONES DE LA CORTEZA SOMATOSENSORIAL A LOS NÚCLEOS PRINCIPAL Y ESPINAL CAUDAL DEL TRIGÉMINO

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El flujo de información sensorial en las estaciones de relevo subcortical está controla por la acción de conexiones topograficas desde la neocorteza. Proyecciones desde la corteza somatosensorial primaria (SI) al complejo nuclear trigeminal se han demostrado en mono, gato y roedores. Las zonas orofaciales de SI y corteza somatosensorial secunadaria (SII) proyectan somatotópicamente a los núcleos trigeminales sugiriendo una modulación de la transmisión somatosensorial orofacial a centros superiores cerebrales. El propósito de este trabajo es determinar las propiedades funcionales de las proyecciones corticofugales somatosensoriales a los núcleos trigeminales principal (Pr5) y espinal caudal (Sp5C) en la rata. Se realizaron registros unitarios en 45 animales en Pr5 o Sp5C aplicando trenes de estimulación en SI y SII; las vías anatómicas entre SI-SII y los núcleos Pr5 y Sp5C se estudiaron en 9 animales mediante la inyección de los trazadores fluorescentes retrógrados FluoroGold (FG) y Fast Blue (FB) respectivamente en dichos núcleos. Los resultados fisiológicos muestran que la corteza SI y SII facilita o inhibe las respuestas táctiles en Pr5 mediante la activación de receptores glutamatérgicos del tipo NMDA y por la activación de receptores $GABA_A$. En cambio, solo produce inhibición de las respuestas nociceptivas en Sp5C mediante la activación de receptores glicinérgicos y $GABA_A$. Los resultados anatómicos muestran una distribución homogénea de neuronas marcadas con FG en las cortezas cingular, motora, SI, SII e insular; las neuronas marcadas con FB son menos numerosas y se localizaron selectivamente en zonas rostrales motoras, agrupadas en la zona de barriles de SI, mas abundantes en niveles dorsales de SII y homogéneamente distribuidas en corteza insular, siendo mas del 90% las neuronas marcadas con ambos trazadores. Nuestros resultados indican que tanto el núcleo Pr5 como el Sp5C reciben proyecciones desde las cortezas motoras, SI, SII e insular que modulan las respuestas somatosensoriales.

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DINÁMICA CORTICAL DURANTE TAREAS ÓCULOMOTORAS: EL VECTOR DE INVERSIÓN ANTISACÁDICO

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Para comparar la dinámica corticales de diferentes tareas óculomotoras se registraron el EEG y los movimientos oculares en 21 voluntarios. Se realizó un bloque de tareas prosacádicas (sacádico hacia la diana), antisacádicas (al lado opuesto) y no-go (mantener la posición). El tipo de tarea la indicó el color del cuadrado central (S1) presente durante 1900-2500 ms (periodo instructivo). S1 desapareció durante 370 ms (gap) y apareció la diana (S2) a 8º a izquierda o a derecha aleatoriamente. La tarea antisacádica presentó mayores latencias y errores. Los periodos instructivo y gap se caracterizaron por una variación contingente negativa (CNV) y una mayor negatividad durante el gap, ambas mayores en la tarea antisacádica. El análisis de componentes principales mostró que la CNV se instauró durante el periodo preparatorio y continuó hasta el gap, y permitió separar actividades fronto-centro-occipitales relacionadas con la CNV, de otra fronto-central asociada al gap. El periodo instructivo se caracterizó por una desincronización fronto-centro-occipital en beta mayor en la tarea antisacádica que en no-go, y una sincronización parieto-occipital en alfa mayor en no-go. El gap se caracterizó por un incremento de potencia espectral y de coherencia entre ensayos en theta, mayores en la tarea antisacádica en la corteza fronto-central. El periodo ejecutivo (post-S2) se caracterizó por una negatividad en corteza parietal y una sincronización en la banda de 5-10 Hz que fueron contralaterales al estímulo. Exclusivamente en la tarea antisacádica, se produjo un desplazamiento progresivo de estas actividades hacia el hemisferio homolateral (vector de inversión) que fue máxima 50 ms más tarde. Estos resultados sugieren que la preparación de tareas de mayor demanda requieren un elevado control top-down por corteza frontal; y que la dinámica oscilatoria del vector de inversión en antisacádicos está representada por un aumento de potencia espectral de 5-10 Hz en corteza parietal. Financiación: SAF-2009-10560, P09-CVI-4712

1ª: Neurociencia de sistemas
2ª: Neurociencia cognitiva y conductual

INFLUENCIA DE LA CORTEZA VISUAL SOBRE LAS PROPIEDADES ESPACIALES DE LAS CÉLULAS TALÁMICAS EN EL ANIMAL DESPIERTO

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Objetivo: El presente estudio tiene como objetivo analizar la influencia de las conexiones de retroalimentación desde la corteza visual primaria (V1) sobre la organización de los campos receptores de las células del núcleo geniculado lateral (NGL) en animales despiertos, mediante la combinación de registros extracelulares y estimulación magnética transcraneal (TMS).

Material y métodos: Realizamos registros extracelulares (n=45) en el núcleo geniculado lateral del tálamo de primates despiertos (*Macaca mulatta*) entrenados para mantener la fijación en una ventana de 0.6 grados a la vez que ignoran los estímulos que se les presentan. Estudiamos la respuesta de las células a la presentación de enrejados (estáticos y en movimiento) de distinto tamaño sobre el campo receptor de la célula. Comparamos la respuesta en condiciones control con aquella obtenida inmediatamente después del bloqueo de la salida cortical por TMS (0.8Hz, 4 minutos) y a distintos intervalos de tiempo hasta conseguir la recuperación de la respuesta.

Resultados: El efecto más común fue una reducción, tanto en la actividad espontánea, como en la producida por estimulación visual tras la aplicación de la TMS (72% de las células). Este efecto fue más pronunciado sobre el estímulo de menor tamaño, provocando una alteración de la organización centro/periferia. Además, en 17 células, 37.7% de la muestra total, el bloqueo cortical provocó el desplazamiento del centro del campo receptor (hasta 6 grados) acompañado de un aumento del componente inhibitorio.

Conclusiones: Estos cambios ponen de manifiesto que los campos receptores son estructuras dinámicas y que su organización en cada momento puede depender del equilibrio entre distintas conexiones excitadoras e inhibidoras moduladas por la acción de la corteza.

Áreas Temáticas:
1ª: Neurociencia de sistemas
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EFECTO DE LOS CAMPOS MAGNÉTICOS ESTÁTICOS SOBRE LA CORTEZA VISUAL DEL ANIMAL DESPIERTO Y ANESTESIADO

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Objetivo: Analizar el efecto que produce un campo magnético estacionario aplicado en la corteza visual primaria sobre la respuesta de las neuronas corticales en animal anestesiado, así como el correlato psicofísico de dichos efectos en una tarea de detección de estímulos en primate despierto.

Material y métodos: Se realizaron registros extracelulares en la corteza visual de 2 gatos anestesiados y paralizados en condiciones control y durante la aplicación de un campo magnético estático sobre la zona de registro. Para inducir el campo magnético se utilizó un imán cilíndrico de Neodimio de 6 cm de radio, con una intensidad de campo de 0.6 Teslas medidos en la superficie del mismo. Los registros incluyeron respuesta espontánea y durante estimulación visual.

En primates despiertos (*Macaca mulatta*), estudiamos el efecto del mismo tipo de imanes sobre la resolución de una tarea de detección de estímulos. La tarea se realiza en condiciones control y después de haber estado sometida la corteza al campo magnético durante periodos de entre 20 y 60 minutos.

Resultados: En los animales anestesiados el campo magnético produjo una disminución de la respuesta en todas las células estudiadas, n=15. El efecto fue progresivo y en algunos casos produjo el silencio total de la célula estudiada. La duración del efecto fue variable manteniéndose hasta una hora después de haber retirado el imán.

A nivel psicofísico el campo magnético estático genera un escotoma reversible, cuya posición retinotópica varía en función de la localización del imán. La extensión varía dependiendo del tiempo de exposición al campo.

Conclusiones: Los campos magnéticos estáticos empleados (considerados de alta intensidad) producen un intenso efecto supresor sobre la actividad neuronal, llegando incluso a silenciar las neuronas afectadas, lo que se traduce en un efecto psicofísico que afecta inequivocamente a la percepción visual

Áreas Temáticas:

1ª: Neurociencia de sistemas
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UNA BANDA DE ALTA FRECUENCIA ESPECÍFICA DEL SUEÑO REM: DINÁMICA CORTICAL

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La actividad electroencefalográfica (EEG) supone una de las principales variables fisiológicas empleadas en la caracterización de estados neurofuncionales. En el presente estudio se caracterizó en detalle el EEG de alta frecuencia en diferentes estados de alerta en la rata. Se prepararon cinco animales con electrodos de registro de la actividad EEG, la musculatura del cuello y bobinas perioculares para la detección de movimientos oculares mediante el seguidor magnético de la posición ocular. Tras la identificación de cada estado de alerta, se efectuó un análisis espectral de la actividad EEG en el que se identificó un incremento en la banda de frecuencia entre 120 y 140 Hz que fue específico de la fase de movimientos oculares rápidos del sueño (REM). Para estudiar la dinámica cortical y la localización de dicha banda se prepararon nueve animales con 18 electrodos de EEG. Se encontró que las oscilaciones de alta frecuencia mostraron una mayor potencia espectral en la región centro-parietal, justo por delante de lambda, y ocurrieron en brotes sincrónicos con las oscilaciones en la banda theta. Las oscilaciones de alta frecuencia presentaron una alta coherencia en todas las localizaciones dentro de un mismo hemisferio pero no entre hemisferios. Por último, se implantaron otros cuatro animales con electrodos a nivel de corteza e hipocampo para comparar la alta frecuencia y su relación con theta. La potencia de alta frecuencia fue mayor en hipocampo que en corteza y se encontró una coherencia alta entre cada hipocampo y la corteza homolateral, sugiriendo un posible origen hipocámpico. Sin embargo, lesiones electrolíticas a nivel de hipocampo no provocaron cambios a nivel cortical en la alta frecuencia, demostrando que la oscilación de alta frecuencia cortical tiene un generador independiente de hipocampo. Financiado por el MICINN (SAF2009-10560) y la JJAA (P09-CVI-4712) con cofinanciación FEDER.

1. Neurociencia de sistemas
2. Sistemas homeostáticos y neuroendocrino

REGULACIÓN DE LOS ESTADOS DEL CICLO VIGILIA SUEÑO POR LA HISTAMINA AL ACTUAR EN EL TEGMENTO PONTINO

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La histamina tiene un papel promotor del estado de vigilia. Aunque hay estudios que sugieren que podría tener también un papel regulatorio sobre el sueño REM(REM). En la región del tegmento pontino se encuentran estructuras implicadas en la generación tanto de la vigilia como del REM que reciben proyecciones histaminérgicas. Para determinar los efectos de la histamina en el ciclo vigilia sueño (CVS) al actuar sobre la región dorsal del tegmento pontino oral (TPD, implicada en los mecanismos de vigilia) y sobre la region ventral del tegmento pontino oral (vRPO, estructura generadora de REM) hicimos microinyecciones de histamina (20-30nl en ss) en ambas regiones utilizando 11 gatos adultos con electrodos implantados para registros poligráficos crónicos de sueño y con una o dos cánulas dirigidas a las regiones de estudio a través de las cuales se realizaron las microinyecciones; éstas fueron seguidas de registros poligráficos de 6 horas de duración. Las microinyecciones de histamina en la región del TPD produjeron un aumento significativo del estado de vigilia durante las tres primeras horas de registro. Estos efectos fueron bloqueados por la administración previa de pirilamina (1mg/kg i.p). Mientras que, las microinyecciones de histamina en el vRPO produjeron cambios significativos en todos los estados del CVS, entre la segunda y la quinta hora de registro, producidos por un bloqueo especifico de la entrada a REM. Por el contrario, en la primera hora, en algunos de los animales se produjo una entrada rápida a REM semejante a la que se observa en los ataques de narcolepsia. Nuestros resultados indican, por tanto, que la histamina tiene un efecto facilitador y consolidador del estado de vigilia al actuar sobre la región del TPD, pero que también está implicada en la regulación del REM al actuar sobre la región del vRPO.

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Áreas Temáticas:

Neurociencia de sistemas
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CHARACTERIZATION OF NEOCORTICAL LAYER V PYRAMIDAL NEURONS CONTAINING A SACULAR ORGANELLE AT THE AXON INITIAL SEGMENT

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The axon initial segment (AIS) is a complex domain of the proximal axon that participates in the regulation of axonal transport mechanisms and the acquisition and maintenance of neuronal polarity. In addition, the AIS is critical for the initiation and propagation of action potentials due to the expression of a high density of voltage-gated Na^{+}, K^{+} and Ca^{2+} channels. Differences in AIS organization regarding subunit composition and localization of such channels are thought to contribute to the electrophysiological variability between neuronal populations, and the corresponding differences in action potential initiation and/or propagation. Regulation of Ca^{2+} concentrations of the AIS is considered essential to maintain its structure and function, and in the morpho-functional reorganization of the AIS after chronic depolarization. In the AIS of an uncharacterized population of cortical layer V pyramidal cells the presence of a giant saccular organelle has been recently described (Sánchez Ponce et al., 2011 *Cereb Cortex*) although its functions remain unknown. Through tract-tracing methods and immunocytochemistry here we show that this saccular organelle is present in the AIS of subpopulations of layer V pyramidal neurons projecting to various subcortical, non-thalamic targets, including corticospinal neurons, that express SMI32 and some of which are under the Thy-1 gene promoter. In addition, our results demonstrate that the giant saccular organelle expresses the inositol 1,4,5-triphosphate receptor 1 (IP_3R1) and the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) 2, both in rodent and human neocortex. These results suggest that the saccular organelle is involved in Ca^{2+} dependent regulation of the AIS structure, function and plasticity in layer V neurons which give rise to subcortical non-thalamic descending projections.

Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

FACILITACIÓN A LARGO PLAZO EN LA CORTEZA DE BARRILES DE LA RATA ANESTESIADA

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La experiencia sensorial tiene un amplio efecto sobre las características de la respuesta de las neuronas corticales. La estimulación de las vibrisas o la deprivación sensorial induce efectos a largo plazo como procesos de facilitación o depresión en las respuestas sensoriales o modificaciones en el campo receptivo. En este trabajo se registraron neuronas de las capas 2/3 y 5/6 de la corteza de barriles en ratas anestesiadas con uretano para determinar si la estimulación repetitiva de una vibrisa puede inducir una facilitación de la respuesta a largo plazo. Para este fin, estimulamos repetitivamente una vibrisa con pulsos de aire (20 ms) a frecuencias entre 1 y 8 Hz. Las respuestas control (0.5 Hz) aumentaron durante al menos 30 minutos después de una estimulación repetitiva con 60 pulsos a 5 u 8 Hz. La facilitación a largo plazo en las capas 2/3 y 5/6 se debió a la activación de los receptores NMDA ya que esta facilitación se vio reducida tras la aplicación de APV o MK801. Esta facilitación aumentó el campo receptivo. La aplicación de eserina intraperitonealmente aumentó el número de neuronas que se facilitan por la estimulación repetitiva, sugiriendo que la Ach favorece la generación de la facilitación a largo plazo. La aplicación de muscimol en la capa 2/3 disminuyó la respuesta y la facilitación de las neuronas de la capa 5/6, indicando que el proceso de facilitación se genera en las neuronas de la capa 2/3 propagándose a las neuronas de la capa 5/6. Nuestros datos muestran que una estimulación fisiológica repetitiva en una vibrisa a la frecuencia de exploración del entorno usada habitualmente por el animal, induce una facilitación de la respuesta sensorial a largo plazo de las neuronas de la capa 2/3 y que se extiende a lo largo del barril.

COMPONENTE TALÁMICO DE LA REORGANIZACIÓN DEL SISTEMA SOMATOSENSORIAL INMEDIATAMENTE DESPUÉS DE UNA LESIÓN MEDULAR

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Nuestro grupo ha demostrado que una lesión medular completa a nivel T9-T10 produce un cambio en el estado cortical que a su vez modula las respuestas corticales (Aguilar et al., J Neurosci 2010). Por otro lado, en un reciente estudio hemos demostrado que además de esta reorganización dependiente del estado, existe otro mecanismo de reorganización independiente del estado (Humanes-Valera et al., enviado). Sin embargo, no sabemos si este mecanismo tiene un origen subcortical. El objetivo del presente trabajo es determinar el componente talámico de la reorganización del sistema somatosensorial inmediatamente después de una lesión medular.

Se obtuvieron registros extracelulares en el tálamo ventral posterior lateral (VPL) de ratas Wistar anestesiadas con uretano en situación control e inmediatamente después de una lesión medular completa a nivel T9-T10.

Los resultados principales fueron los siguientes: 1) las respuestas talámicas evocadas por estímulos de alta intensidad (5mA) en la extremidad delantera aumentaron significativamente (9,4%) después de la lesión; 2) este aumento de las respuestas no mostró una dependencia significativa del estado del sistema; 3) en un grupo de animales en los que también se registró la corteza somatosensorial, el aumento de las respuestas talámicas (7.5%) fue significativamente menor que el aumento de las respuestas corticales (23,8%).

En conclusión, parte de la reorganización cortical observada inmediatamente después de una lesión medular completa tiene un origen subcortical.

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Áreas Temáticas:

1ª: Neurociencia de sistemas
2ª: Trastornos y reparación del sistema nervioso

EFECTOS DE LA LESIÓN MEDULAR SOBRE LA ACTIVIDAD CORTICAL DE ONDA LENTA EN ANIMALES ANESTESIADOS.

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La lesión medular produce reorganización cortical a largo plazo que en ocasiones se asocia con patologías como el dolor neuropático y el miembro fantasma. En estudios anteriores de nuestro laboratorio se ha demostrado que una lesión medular completa en ratas anestesiadas causa un cambio de estado en la actividad espontánea cortical. El objetivo del presente trabajo es determinar si una lesión medular produce efectos sutiles sobre la actividad neuronal cortical (previamente en onda lenta) que no impliquen un cambio de estado general.

Se utilizaron ratas anestesiadas con uretano, manteniendo constante el estado cortical en onda lenta (<1Hz) antes y después de la lesión medular. Se realizaron registros extracelulares en la corteza somatosensorial primaria sobre la representación de la garra delantera y la garra trasera en dos grupos experimentales: animales REAL, en los que se realizó una lesión medular completa a nivel de T9-10, y animales SHAM, en los que no se realizó ninguna lesión. Se cuantificó la generación de estados activados durante la onda lenta así como la descarga neuronal durante los mismos, antes y después de lesión “real” o “sham”.

Después de la lesión se produjo una disminución de la frecuencia de estados activados durante la onda lenta y una disminución de la actividad multiunitaria en cada estado activado. Ambos cambios fueron significativos tanto en la representación de la garra delantera (intacta) como en la representación de la garra trasera (desaferentada), pero fueron más acentuados en la representación de la garra trasera. Los cambios generales en las dos representaciones corticales podrían estar mediados por los sistemas de neuromodulación del tronco del encéfalo, mientras que los cambios específicos en la representación cortical desaferentada podrían ser debidos directamente a la desaferentación tálamo-cortical.

En conclusión, dentro del mismo estado cortical general, la lesión medular completa produce cambios sutiles sobre la excitabilidad neuronal.

Fondo de Investigación Sanitaria del Instituto de Salud Carlos III (PI11/02451), cofinanciado por FEDER
International Foundation for Research in Paraplegia (P120)

Áreas Temáticas:

1ª: Neurociencia de sistemas
2ª: Trastornos y reparación del sistema nervioso

CAMBIOS CORTICALES DEPENDIENTES E INDEPENDIENTES DEL ESTADO

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La lesión medular produce desaferentación sensorial y una gran reorganización cortical de la zona desaferentada a largo plazo. Sin embargo, se conoce poco acerca del papel de la corteza intacta adyacente a esta reorganización cortical. Hemos demostrado en un trabajo previo que después de una lesión medular se produce un cambio inmediato del estado cortical que a su vez modula las respuestas corticales (Aguilar et al., J Neurosci 2010). En el presente estudio pretendemos determinar si existen mecanismos que subyacen al aumento de las respuestas de forma independiente del estado. Para ello se controló el estado cortical mediante dosis de anestesia verificando que no existiera cambio de estado antes y después de lesión medular y por tanto todo cambio en la actividad evocada fuera independiente de estado. Los resultados obtenidos muestran que existe un incremento de las respuestas corticales de la zona intacta independientemente del estado de la corteza somatosensorial. Las respuesta evocadas durante actividad de onda lenta aumentaron después de lesión al comparar tanto las obtenidas durante periodos silentes (down-state) como durante periodos activados (up-state). También se observó un aumento de las respuestas corticales de la zona desaferentada al estimular la garra delantera tras lesión medular. Finalmente se determinó la existencia de una relación entre el incremento de las respuestas en la corteza desaferentada y el incremento de las respuestas corticales de la zona intacta, lo que sugiere que pueden representar diferentes puntos de vista de la misma reorganización funcional independiente del estado cortical después de lesión medular. Nuestros resultados muestran que existen cambios funcionales en la corteza somatosensorial inmediatamente después de lesión medular. Estos cambios inmediatos se deben a mecanismos dependientes e independientes del estado cortical que pueden contribuir a una reorganización cortical en la zona intacta inmediatamente después de una lesión medular.

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Áreas temáticas:

1ª Neurociencia de sistemas
2ª Trastornos y reparación del sistema nervioso

CANNABINOIDS MODULATE INFORMATION TRANSMISSION OF SENSORIMOTOR CIRCUIT OF THE BASAL GANGLIA

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The CB1 cannabinoid receptor which is densely located in the basal ganglia is known to participate in the regulation of movement activity. The aim of this study was to determine the effect of cannabinoids (Δ^9-tetrahidrocannabinol (Δ^9-THC), and WIN 55,212-2) on spontaneous and cortically evoked activity in the substantia nigra pars reticulata (SNpr) by extracellular recording techniques in anaesthetized animals.

Administration of Δ^9-THC (0.5 mg/kg, i.v.) stimulated (by 127 ± 14%) 6 of 11 SNpr recorded neurons, whereas it inhibited (by 73 ± 14%) the remaining 5 neurons. After Δ^9-THC the regularity of neuron activity was increased in all recorded neurons and the firing pattern changed toward a more bursting discharge. On the other hand, administration of WIN 55,212-2 (125-250 µg/kg, i,.v.) increased the firing rate of SNpr neurons (by 125 ± 7%, n=6) and the regularity of neuron activity, whereas did not modify the firing pattern. Previous administration of the cannabinoid receptor antagonist AM 251 (1 mg/kg, i.v.) completely blocked the effects induced by both agonists. Moreover, when AM 251 (1 mg/kg, i.v.) was administered alone also induced an increase (13 of 21 SNpr recorded neurons) or a decrease (remaining 8 neurons) in firing rate.

After Δ^9-THC or WIN 55,212-2 administration, the inhibitory component of the cortically evoked response (activation of the direct striatonigral circuit) and the late excitatory response (activation of the indirect striato-pallido-subthalamo-nigral circuit) were decreased or completely lost. However, the early excitatory response (activation of the hyperdirect cortico-subthalamic circuit) was not modified by cannabinoids administration. Previous administration of AM 251 (1 mg/kg, i.v.) completely blocked these effects without any modification of the cortically evoked responses.

These results suggest that CB1 receptor activation modulates the sensorimotor transmission through the trans-striatal pathways. This modulation may be relevant in the understanding of involvement of the cannabinoid system in motor control.

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Áreas Temáticas:

1ª: Neurociencia de sistemas
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

VENTRAL MEDIAL NUCLEUS NEURONS CREATE A DENSE CANOPY OF SYNAPTIC BUTTONS IMMEDIATELY BENEATH THE PIAL SURFACE

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Among the diversity of the thalamocortical (TC) neuron population, the matrix type (M-type) is characterized by widely ramified axons that target the layer 1 of multiple cortical areas (multiareal subtype). The cortical layer 1 contains most of the apical dendritic distal tufts of several pyramidal neuron populations. Vertical and temporal integration of inputs from both M-type TC neurons, which target the apical dendrites of cortical pyramidal neurons, and core-type TC neurons, that act on their basal dendrites, is thought to promote an oscillatory activity of pyramidal cells. M-type neurons contribute to the dispersion of this oscillatory activity by their widely spread axons through layer 1 to synchronize multiple cortical areas. The ventral medial thalamic nucleus (VM) harbors numerous multiareal M-type TC neurons segregated in two functionally territories: the nociceptive lateral portion (VMl) and the motor medial one (VMm). This study aims at analyzing the topography of TC projections arising from small populations of VM neurons by using microiontophoretic injections of anterograde tracers combined with juxtacellular recordings. The location of terminal fields in layer 1 was analyzed using cortical flat maps and microphotographs of flattened hemispheres. The size of synaptic buttons located in different cortical layers and areas was also measured. Our data show that VM TC neurons innervate massively the upper part of layer 1, forming a dense mesh of terminals that spread up to 30μm down the piamater. TC neurons from VMl and VMm innervated different cortical layers and areas. VMm neurons preferentially target the layer 1 of frontomedial areas, while VMl reach uniformly layers 1, 2/3 and 5 of frontoparietal areas. We found also certain differences in the size of synaptic buttons located in motor and somatosensory areas. This study provides detailed data of the cortical tangential spread and laminar target specificity of VM TC neurons in mice.

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1. Neurociencia de sistemas
2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

NEURONAL TYPES OF THE VENTRAL TEGMENTAL AREA: A SINGLE CELL TRACING STUDY

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Projections arising from the ventral tegmental area (VTA) are at the core of forebrain circuits for reward and it is clear that drugs of abuse and addictive behaviors exert their effects by increasing dopamine (DA) levels in the VTA axon synapses. DA-releasing VTA neurons project to limbic forebrain structures, including the prefrontal cortex, amygdala and accumbens nucleus. Besides DA cells, VTA is populated by GABAergic (35%) and glutamatergic (3%) projecting neurons. VTA comprises the parabrachial pigmented (PBP), paranigral (PN), rostral VTA (rVTA) and ventral tegmental tail, being DA neurons scarce in the two last regions. To characterize the VTA neuronal types, we injected Sindbis viral vector into the VTA of mice and determined whether the infected neurons were or not dopaminergic by immunohistochemistry against tyrosine hydroxylase and confocal microscopy. We entirely reconstructed 30 labeled neurons using a microscope with a camera lucida and the axonal length in the major terminal fields provided by the axons of the labeled neurons were estimated by stereology. Different neuronal types abound in VTA: mesocorticolimbic, mesolimbic, mesocortical, mesostriatal, and mesorhombencephalic. In our sample, the two mesocorticolimbic neurons were located medially in PBP, while the 12 mesolimbic cells were scattered in the PBP, PN and rVTA. The two mesocortical and the two mesostriatal neurons were all situated in the lateral sector of PBP and rVTA, and the 12 mesorhombencephalic abounded in the lateral and caudal PBP, as well as in rVTA. The axons of mesocorticolimbic neurons are the most widely ramified but the length of terminal fibers at their main targets is less than half the length of terminals from mesostriatal neurons at the dorsal striatum. This single neuron study provides detailed knowledge of patterns of projection of neurons located in every subdivision of VTA, which is needed to understand certain psychiatric disorders and drug addiction.

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1ª: Neurociencia de sistemas
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

PROLONGED L-DOPA TREATMENT MODIFIES THE ELECTRICAL ACTIVITY OF ENTOPEDUNCULAR NUCLEUS AND SUBSTANTIA NIGRA PARS RETICULATA NEURONS IN 6-HYDROXYDOPAMINE LESIONED RATS

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The prolonged L-DOPA treatment leads to disabling motor complications including dyskinesia in Parkinson´s disease (PD) patients and animal models. The mechanisms that underlie L-DOPA-induced dyskinesia (LID) are not clear, although the implication of the basal ganglia output nuclei, the substantia nigra pars reticulata (SNr) and the internal segment of the globus pallidus (GPi, entopeduncular nucleus in rat, EP nucleus), has been proposed. Here, we studied the involvement of the EP nucleus and the SNr in LID, using single-unit extracellular recordings and behavioural approaches in hemi-parkinsonian rats chronically treated with L-DOPA. Additionally, we also investigated the possible correlation between subthalamic nucleus (STN) neuron activity and its target nuclei (EP nucleus and SNr) neuron activity. Our results show that L-DOPA chronic treatment modified the electrophysiological parameters of EP nucleus and SNr neurons in 6-hydroxydopamine lesioned rats. We did not find any correlation between abnormal involuntary movement (AIM) scores and the electrophysiological parameters of EP nucleus neurons recorded 24 h or 20-120 min after the last L-DOPA administration. Whereas, we found positive correlation between the limb and orolingual AIMs and the electrophysiological parameters of SNr neurons recorded 24 h after the last L-DOPA administration. These correlations disappeared after the acute L-DOPA challenge. Moreover, we also found positive correlations between STN neuron activity and EP nucleus or SNr neuron activity in dyskinetics rats. Altogether, these results show that in dyskinetic animals the electrical activity of EP nucleus and SNr neurons is altered, revealing a correlation with STN neuron activity.

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Áreas Temáticas: 1ª: Neurociencia de sistemas
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

ESTUDIO *IN VIVO* DE LOS EFECTOS PROTECTORES DEL GLUTATIÓN Y TROLOX SOBRE LA LIBERACIÓN ESTRIATAL DE DOPAMINA INDUCIDA POR PARAOXÓN

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INTRODUCCIÓN Y OBJETIVOS:
El paraoxón es el metabolito activo del paratión, un insecticida organofosforado altamente neurotóxico cuyo mecanismo de acción principal es la inhibición irreversible de la enzima acetilcolinesterasa. Esto produce una acumulación de acetilcolina en el Sistema Nervioso Central, provocando un cuadro de toxicidad colinérgica. En trabajos previos observamos que el paraoxón produce además un importante incremento en la liberación estriatal de dopamina. En base a ello, el objetivo de este trabajo ha sido estudiar los posibles efectos protectores del Glutatión (GSH) y el Trolox sobre la liberación estriatal de dopamina inducida por paraoxón, en ratas conscientes y en libre movimiento.

MATERIAL Y MÉTODOS:
El Paraoxón (5 mM), GSH (1mM) y Trolox (1mM) se administraron directamente en el estriado de ratas hembras Sprague-Dawley (250-300 g, 4-8/grupo) a través de una sonda de microdiálisis. Los niveles de dopamina fueron medidos mediante HPLC con Detección Electroquímica. El análisis estadístico se hizo por ANOVA/test de de Student-Newman-Keuls. Diferencias consideradas: $P < 0,05$; $P < 0,01$ y $P < 0,001$.

RESULTADOS:
La administración intraestriatal de Paraoxón 5 mM produjo un aumento en los niveles extracelulares de dopamina de 1066 ± 119%, respecto a los niveles basales. La infusión de Paraoxón 5 mM en ratas pre-tratadas con GSH o Trolox (1mM), originó incrementos en los niveles extracelulares de dopamina de 396 ± 90% y 689 ± 88%, respectivamente; siendo estos incrementos un 63% y 35% menores que aquellos producidos en ratas tratadas solo con Paraoxón.

CONCLUSIONES:
Estos resultados muestran que la administración de compuestos antioxidantes podría tener un efecto protector frente a la liberación estriatal de dopamina inducida por paraoxón. Esto podría ser debido a la unión del paraoxón con los grupos –SH libres del GSH, o a la capacidad reductora de estrés oxidativo del Trolox.

Áreas Temáticas:

1ª: Neurociencia de sistemas.
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.

GIRK CHANNELS MEDIATE THE ELECTROPHYSIOLOGICAL, BEHAVIORAL AND HYPOTHERMIC EFFECTS OF 5-HT$_{1A}$ AGONISTS AND CITALOPRAM

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Background: It is well-known that GIRK channels containing GIRK2 subunits play an important role controlling excitability of several brain areas. These channels are coupled to inhibitory G-protein coupled receptors, such as 5-HT$_{1A}$ receptor, which inhibits serotonergic neurotransmission.

Objective: To study the implication of GIRK channels in the electrophysiological, behavioral and hypothermic effects of 5-HT$_{1A}$ agonists and citalopram.

Methods: Electrophysiological studies included *in vivo* single-unit extracellular recordings and *in vitro* patch-clamp recordings of dorsal raphe neurons. Behavioral tests were tail suspension test (TST) and novelty-suppressed feeding test (NSFT). Functional status of 5-HT$_{1A}$ autoreceptors was assessed measuring the hypothermic response to the 5-HT$_{1A}$ agonist 8-OHDPAT. Experiments were performed using wild-type and GIRK2 mutant mice.

Results: *In vivo* dose-response curves of citalopram (0.5-3 mg/kg, i.p.) were significantly shifted to the right in GIRK2 homozygous mice compared to that obtained in wild-type and GIRK2 heterozygous animals. This inhibitory effect of citalopram was blocked with the 5-HT$_{1A}$ antagonist WAY100365 (25µg/kg, i.p.). Moreover, when GIRK channels were pharmacologically blocked with tertiapin (100pmol, i.c.v.) dose-response curves of citalopram were also significantly shifted to the right as well as basal firing rate and the proportion of burst-firing neurons were increased. Whole-cell patch-clamp experiments revealed that 5-HT$_{1A}$ agonist 5-CT (100nM)-induced current was smaller in GIRK2 mutant groups. As expected, the hypothermic effect of 8-OHDPAT (0.5mg/kg, i.p.) was greater in wild-type mice comparing to GIRK2 mutant genotypes. Mutant groups showed lower immobility time in the TST and lower latency to eat in the NSFT. Interestingly, in TST, citalopram (10 mg/kg, i.p.) was less effective reducing the immobility time in GIRK2 mutant genotypes.

Conclusion: Mutation of Girk2 gene reduces the 5-HT$_{1A}$-mediated signaling and improves the behavioral response to anxiety-related situations. Thus, GIRK channels could be candidates as a therapeutic target for neuropsychiatric disorders.

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Áreas temáticas.

1ª: Neurociencia de sistemas
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

HIGHER SENSITIVITY OF WISTAR KYOTO COMPARED TO WISTAR RATS TO THE STIMULATORY EFFECT OF $5HT_7$ RECEPTORS AGONIST, AS19 ON LOCUS COERULEUS NORADRENERGIC NEURONS

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One of the main drawbacks in major depression treatment is the onset of antidepressants to produce their therapeutic effect. Recently, it has been proposed the $5HT_7$ autorreceptor as a new target for depression treatment, since blocking this receptor accelerates the therapeutic response of some antidepressants. Studies of the noradrenergic nucleus, locus coeruleus (LC) have highlighted mechanisms underlying the ethiopathology of depression and cellular effects of antidepressants.

The goal of this study was to characterize the effect induced by the activation of $5HT_7$ receptors onto LC activity of Wistar Kyoto (WKY) rat, a proposed animal model of depression, and its outbred control, the Wistar (Wis) rat.

For that purpose, we studied the effect induced by a selective $5HT_7$ receptors agonist AS19 on the LC neuron activity in vivo, using single unit extracellular recordings, and in vitro, using whole-cell voltage-clamp recordings. In vivo, AS19 (2.5-10mg/kg i.v.) had a dose-dependent stimulatory effect on LC noradrenergic neurons, and the selective antagonist of $5HT_7$ receptor SB 269077 (1mg/kg i.v.) did not block neither reverse this effect. Conversely, intracerebroventricular administration of kynurenic acid, an unspecific glutamatergic receptors antagonist (1μmol) partially blocked the effect induced by AS19. When LC slices were perfused with AS19 (100 μM), neither the frequency nor the amplitude of spontaneous excitatory postsynaptic currents were altered. Similarly, AS19 did not change the basal current neither the inward current induced by glutamate (100 μM). Interestingly, the *in vivo* sensitivity to AS19 was greater in LC neurons from WKY compared to Wis rats. Overall, these results suggested that WKY rats are more sensitive to AS19 which stimulatory effect seems to be indirect and partially mediated by the excitatory amino acid system.

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Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

PLASTICITY OF GLUTAMATE AND GABA RECEPTOR SIGNALING PATHWAYS IN THE RAT INFERIOR COLLICULUS: CHANGES IN GENE EXPRESSION AFTER COCHLEAR LESION

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Relatively simple and reliable peripheral input manipulation makes central auditory circuits privileged models to unravel neuronal adaptations to changes in sensory experience. One of the aims of our current research program is to look at changes at the molecular, cellular and circuit levels in central auditory neurons, with special focus on neurotransmitter receptors and other ion channels, after interfering with inputs, in order to shed light on possible reactive/plastic mechanisms to altered auditory experience.

The inferior colliculus (IC) is the midbrain convergence center of the auditory pathway. Neurons in the IC receive both excitatory and inhibitory inputs ascending from more caudal nuclei in the brainstem, and descending from the auditory cortex. We are exploring how deafness (i.e. lack of auditory activity) affects the expression of glutamate and GABA receptor signaling pathways in the inferior colliculus. Towards this goal PCR Arrays containing 84 key genes involved in GABA and glutamate receptor-mediated signaling, are being used after experimental deafness (cochlear ablation) in the adult rat, in order to screen patterns of gene regulation after a short (one day), or more prolonged time (15 and 90 days) after sensory deprivation.

At one day postlesion, some genes undergo changes in their expression, most of them being down-regulated, especially VGlut-2. After 15 days of deafness, there is an overall down-regulation which particularly affects NMDA2A, Homer 1 and 2, Inositol triphosphate receptor 1, BDNF and GAD67. In general, genes with their levels of expression modified at 1 day postlesion show a trend towards recovery to control levels at 15 days postlesion. Another set of genes are seen to change expression at 15 days postlesion and seem to recover at 90 days. Thus, glutamatergic and GABAergic signaling in the IC is involved in a plastic/adaptive response which seems to involve an early and a late component. I n any case, ninety days after the lesion, the expression levels of these set of genes returns to control levels.

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Áreas Temáticas:

1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Trastornos y reparación del sistema nervioso

CALBINDIN CONTENT AND DIFFERENTIAL VULNERABILITY OF MIDBRAIN EFFERENT DOPAMINERGIC NEURONS IN MONKEYS

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Calbindin (CB) is a calcium binding protein reported to protect dopaminergic neurons from degeneration. Although a direct link between CB content and differential vulnerability of dopaminergic neurons has long been accepted, factors other than CB have also been appointed, particularly those related to the dopamine transporter. Indeed, several studies have reported that CB levels are not causally related to the differential vulnerability of dopaminergic neurons against neurotoxins. Here we have used dual stains for tyrosine hydroxylase (TH) and CB in 3 control and 3 MPTP-treated monkeys to disclose dopaminergic neurons in the ventral tegmental area (VTA) and in the dorsal and ventral tiers of the substantia nigra pars compacta (SNcd and SNcv) co-expressing TH and CB. In control animals, the highest percentages of co-localization were found in VTA (58%), followed by neurons located in the SNcd (34%). As expected, SNcv neurons lacked CB expression. In MPTP-treated animals, the percentage of CB+/TH+ neurons in the VTA was similar to control monkeys (62%), whereas most of the few surviving neurons in the SNcd were CB+/TH+ (88%). Next, we have elucidated the presence of CB within identified nigrostriatal and nigroextrastriatal midbrain dopaminergic projection neurons. For this purpose, two control monkeys received one injection of Fluoro-Gold into the caudate nucleus and one injection of cholera toxin (CTB) into the postcommissural putamen, whereas two more monkeys were injected with CTB into the internal division of the globus pallidus. As expected, all the nigrocaudate- and nigroputamen-projecting neurons were TH+, although surprisingly, all of these nigrostriatal-projecting neurons were negative for CB. Furthermore, all the nigropallidal-projecting neurons co-expressed both TH and CB. In summary, although CB+ dopaminergic neurons seem to be less prone to MPTP-induced degeneration, our data clearly demonstrated that these neurons are not giving rise to nigrostriatal projections and indeed CB+/TH+ neurons only originate nigroestrastriatal projections.

IS THE LOCUS COERULEUS INVOLVED IN SENSORY GATING OF THE ACOUSTIC STARTLE REFLEX?

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Objectives: To investigate if the locus coeruleus (LC) is involved in the sensory gating of the acoustic startle reflex, we study the projection pattern and neurochemical features of the coerulean projection to the cochlear root neurons (CRNs). Our aims include: to determine the noradrenergic receptors subtypes in the CRNs and search morphological and molecular evidences that might explain the gender differences observed in sensory gating of the acoustic startle reflex.

Methods: The experiments were conducted in male and female Wistar rats. We used anterograde and retrograde tracers combined with immunohistochemistry to detect tyrosine hydroxylase (TH) and dopamine beta-hydroxylase (DBH). Coronal sections were analysed under light and confocal microscopy. We used gene expression analysis (RT-qPCR) to study the noradrenergic system of CRNs. Neurolucida 3D reconstruction of male and female brainstem was used to search gender-specific differences.

Results: We observed labeled terminals on the CRNs after anterograde tracer injections in the LC. The neuronal projection from the LC to the CRNs was confirmed with a retrograde tracer. We showed a strong immunoreactivity for TH and DBH in the cochlear root nucleus. DBH immunopositive terminals colocalize with the anterograde tracer after its injection in the LC. The CRNs expressed the noradrenergic receptors α1A-C, α2A-C and β1-3 with a remarkable gender difference. The distribution of LC neurons varied between males and females.

Conclusions: The LC projects to the CRNs of both sides with a clear ipsilateral predominance. The LC-CRNs projection uses noradrenaline as neurotransmitter. The CRNs contain a noradrenergic receptor profile sufficient to modulate the acoustic startle reflex. There is a sexual dimorphism in expression levels of noradrenergic receptors as well as in the rostrocaudal distribution of LC neurons. The LC might be involved in sensory gating of the acoustic startle reflex through the LC-CRNs projection.

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Áreas Temáticas:
1ª: Neurociencia de sistemas.
2ª: Neurociencia cognitiva y conductual.

AFFERENT AND EFFERENT PROJECTIONS OF THE A5 NORADRENERGIC GROUP AND ITS POSSIBLE RELATION TO ACOUSTIC STARTLE REFLEX

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The noradrenergic group A5 (A5) is situated in the pons, between the rostral part of the facial motor nucleus and the caudal part of the lateral superior olive. The A5 is mainly related to the autonomic nervous system and analgesia control. The acoustic startle reflex (RAS) is a motor response triggered by an intense and unexpected acoustic stimulus and is characterized by a fast muscle contraction of the face and body. Furthermore, it has a vegetative component mediated by the autonomic nervous system that is manifested by an increase in blood pressure and heart rate. The fundamental neural circuit of the acoustic startle reflex is composed by: ganglion cells of the organ of Corti, cochlear root neurons, pontine reticular nucleus, and spinal cord motoneurons.The RAS shows various modulations such as habituation, sensitization, prepulse inhibition and fear potentiation. The modifications of the RAS and its modulations are important in clinical diagnostics of psychiatric and neurodegenerative illnesses such as schizophrenia and Parkinson's disease. Startle modulations are promoted by influences from several nuclei on the fundamental neural circuit of the startle reflex. In a previous study, after injection of retrograde tracer Fluoro-Gold in the cochlear root, it was evident the presence of retrograde labeled cells in the A5, showing us the possibility of this area to be involved in the modulation of the RAS with other nucleus. Therefore, the main objective of this work was to study the A5 connections with areas related to the RAS. Therefore, we conducted stereotaxic injection of neurotracers in A5 (anterograde-BDA n=6; retrograde-Fluoro-Gold n=4). We observe that A5 has reciprocal connections with the following nuclei: sub-coerulean, lateral paragigantocellular, dorsal raphe, locus coeruleus, inferior colliculus. Furthermore the A5 region receives afferents from amygdaloid complex and sends efferents to reticular pontine caudal nucleus and the cochlear root neurons.

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ÁREAS TEMÁTICAS:

1ª: Neurociencia de sistemas.
2ª: Neurociencia cognitiva y conductual.

CHRONIC RESTRAINT STRESS INCREASES THE DENSITY OF MUSHROOM SPINES IN NUCLEUS ACCUMBENS CORE: RELEVANCE FOR CROSS SENSITIZATION TO COCAINE

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Behavioral sensitization is an example of experience-dependent plasticity, induced by drug or stress, which has been suggested to involve cellular adaptations in excitatory transmission in the nucleus accumbens (NAc) (Wolf, 1998; Nestler, 2005; Kalivas and O'Brien, 2008, Esparza et al., 2012). Like repeated drug administration, repeated exposure to stressors induced enduring adaptations in dendritic branching, the shape and the number of spines in NAc (Robinson & Kolb, 2004; Shen et al., 2009; Christoffel et al. 2011). GABAergic medium spiny neurons (MSNs) are the predominant cells of the NAc and reside in two functionally and anatomically distinct subregions of the NAc: core and shell. Since the stress-induced changes in the neuronal architecture within NAc and their role in the cross-sensitization to cocaine are unknown, the purpose of the present study was to determine the structural changes that occur in NAc after seven daily restraint stress session (two hours) and cocaine. Three weeks after the last restraint stress, we analyzed the morphology and density of dendritic spines using DiI microinjections in fixed brain sections from rats 45 min after an acute injection of saline or cocaine (15 mg/kg i.p.). We observed an increase in the density of mushroom spines in NAc core twenty-one days after chronic restraint stress, either after saline or cocaine challenge. Meanwhile, the total density of dendritic spines in the NAc core was not modified in these animals. This finding in the morphology of dendritic spines in core reminds to that observed following chronic cocaine (Shen et al., 2009), and could be also associated to previous findings showing a key role of core in the stress-induced cross-sensitization to cocaine (Garcia-keller et al., 2013).

LAS VÍAS TALAMOCORTICALES TIPO "CORE" Y "MATRIX" RESPONDEN DE MODO DIFERENTE A LA DEPRIVACIÓN SENSORIAL POSTNATAL

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La información sensorial de las vibrisas de los roedores llega a la corteza somatosensorial primaria (S1) a través de dos núcleos talámicos: 1) el ventral posteromedial (VPM), cuyas neuronas (tipo "core") arborizan en la capa 4 de dominios restringidos ("barriles"), y 2) el núcleo posterior (Po), cuyas neuronas (tipo "matrix") arborizan en las capas 5a y 1 e inervan además mediante colaterales axónicas otras áreas corticales. El VPM recibe su aferencia directriz ("driver") del núcleo principal del trigémino, transmitiendo fielmente información mecanosensorial a la corteza. El Po recibe aferencias directrices tanto del núcleo espinal del trigémino como de la corteza cerebral, y su papel funcional es todavía controvertido. Estudios previos demostraron que la lesión neonatal del nervio infraorbitario (ION) altera profundamente las conexiones talamocorticales del VPM, pero el efecto sobre las neuronas del Po no se ha investigado.

En el presente estudio analizamos mediante cuantificación estereológica, en ratones C57BL6 adultos intactos o deaferentados por transección neonatal del ION, diversos parámetros de la arborización en S1 de los axones del VPM y del Po. En los axones del VPM se observa un borramiento de los "barriles" y una disminución en el número (43,9%) y tamaño (27,7%) de sus varicosidades en la capa 4 cortical. Por el contrario, los axones del Po mantienen su morfología normal, salvo una disminución (37,7%) en el tamaño de sus varicosidades axonales. Nuestros datos muestran, por tanto, que las neuronas del núcleo Po son menos dependientes de la integridad de los aferentes sensoriales para el desarrollo de sus conexiones corticales que las de VPM. La presencia de conexiones "driver" corticales en Po podría explicar esta diferencia.

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Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Desarrollo

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF TRIGEMINAL NEURONS INNERVATING THE EYE AND PERIOCULAR TISSUES

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The functional properties of the trigeminal ganglion (TG) neurons innervating the ocular and periocular tissues (cornea, sclera, bulbar and tarsal conjunctivas, eyelashes and superior and inferior eyelids) are incompletely known. These neurons mediate different forms of pain and discomfort arising from the eye as a consequence of surface dryness, contact lens wear, surgical interventions or inflammatory processes. The aim of this work was to characterize *in vivo* the firing characteristics of the different functional types of TG ocular sensory neurons.
Neurons of the right trigeminal ganglion of anesthetized *Wistar* male rats (300±25g) placed in a stereotaxic frame were recorded extracellularly with a tungsten electrode (2MΩ), first placed 9mm below the cortical surface and then lowered gradually at 10μm/min speed. The signal was amplified (1000x), filtered (150-10Khz) and recorded to 20Khz using an ADC interface and software for off-line analysis. Stimulation of the ipsilateral eye and surrounding skin was performed using von Frey filaments for mechanical stimuli and instillation of 20μl drops of menthol (100μM) and capsaicin (100nM) for chemical stimulation. Also, the corneal surface was subjected to variable conditions of humidity. Receptive fields of the units were mapped and stimulated electrically (0.1-2ms, 15V pulses) to measure CV.

Neurons with receptive fields in the cornea and the inferior and superior eyelid were identified. Corneal and superior eyelid units were located more anteriorly and medial to bregma (AP 0.9-1.2mm, L 1-1.5mm) than inferior eyelid units (AP 1.5mm, L 2mm). Eyelid neurons responded to punctate (1.0mN) and stretching mechanical stimuli. Corneal units increased their firing frequency under mechanical stimuli (threshold: 0.25mN), menthol, capsaicin and dryness conditions. These results indicate the feasibility of obtaining stable, long lasting recordings of the electrical activity of ocular TG neurons, thus opening the possibility of analyzing the modification of their response under different experimental conditions.

Áreas Temáticas:
1ª: Neurociencia de sistemas.

DOPAMINE D_4 RECEPTOR ACTIVATION COUNTERACTS NIGROSTRIATAL PATHWAY ACTIVATION BY MORPHINE: RELEVANCE IN DRUG ADDICTION

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Morphine induces dopamine release in the caudate putamen (CPu), which promotes stereotyped behavior and habit learning for drug-seeking and –taking. Nigrostriatal pathway stimulation by morphine is due to a removal of tonic inhibition arising from SNr GABA interneurons on SNc dopaminergic neurons through the mu opioid receptor (MOR). Long-term morphine exposure produces a series of adaptations in SNc dopamine neurons, which affect neuron excitability and dopamine output to CPu. We have previously shown that dopamine D_4 receptor (D_4R) stimulation counteracts acute and chronic morphine-induced accumulation of several transcription factors in the CPu (Gago et al., 2011 Brain Res.). Since D_4R is expressed in the SNr (Rivera et al., Brain Res. 2003), we postulate that a functional D_4R-MOR interaction at the midbrain level could exists.

We have investigated the role of D_4R in the morphine-induced nigroestriatal dopamine metabolism in the rat brain using biochemical and immunohistochemical techniques. We also have studied the influence of D_4R on morphine-induced morphological changes in SNc dopamine neurons using both immunohistochemical and image analysis techniques. Finally, we examined a possible underlying mechanism of the D_4R-MOR interaction at the SN level using *in vitro* quantitative receptor autoradiography.

We have found that D_4R activation restores dopamine metabolism in the nigroestriatal pathway after acute morphine treatment and prevents morphine-induced rise of tyroxine hydroxylase and dopamine transporter. Rats receiving a continuous treatment of morphine (6 days) showed SNc dopamine neurons with smaller size and higher circularity index compared with the controls animals. These morphine-induced morphological adaptatives changes were prevented when a D_4R agonist (PD168,077) was administered at the same time with morphine. Autoradiographic studies demonstrated that the D_4R agonist reduce the affinity of MOR. The present study provides evidence for the existence of a fully blocking effect of the D_4R on the activation of dopaminergic nigroestriatal pathway by morphine.

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Áreas Temáticas:

1ª: Neurociencia de sistemas
2ª: Trastornos y reparación del sistema nervioso

EL SISTEMA OCULOMOTOR DURANTE EL SUEÑO REM

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Los movimientos oculares rápidos, la actividad electroencefalográfica (EEG) de alta frecuencia y baja amplitud y la ausencia de tono muscular interrumpida por mioclonías son características que definen el sueño paradójico (REM). Recientemente hemos estudiado la cinética de los movimientos oculares durante esta fase y hemos demostrado que estos movimientos son generados principalmente por la actividad fásica de las motoneuronas del núcleo motor ocular externo (NMOE). El estudio de estas motoneuronas demostró que reciben una inhibición tónica interrumpida por activaciones fásicas relacionadas con las ondas ponto-geníiculo-occipitales (PGO). El origen de la actividad oculomotora a nivel premotor es desconocido durante el sueño REM. En el presente trabajo se ha caracterizado la actividad de neuronas premotoras extraoculares. Se prepararon 6 gatos para el registro crónico de los movimientos oculares y de la actividad unitaria en el puente. Específicamente se registraron las neuronas de brote y omnipausa de la formación reticular, relacionadas con la generación de los movimientos oculares sacádicos y las neuronas de los núcleos vestibular medial y prepositus hipoglossi relacionadas con la generación de la señal de posición. Tanto las neuronas de pausa como las de brote mostraron actividad coherente con las actividades fásicas durante REM y muy similar a la vigilia. Las neuronas vestibulares mostraron una disminución de actividad tónica pero permanecieron activas durante la fase REM. Las neuronas del prepositus hipoglossi no disminuyeron su actividad durante REM y mostraron patrones de descarga similares a su actividad durante la vigilia. Casi todas las neuronas estudiadas mostraron inhibiciones y activaciones fásicas durante las PGO. Como conclusión, el sistema premotor extraocular no estuvo inhibido durante REM y generó actividades similares a las de vigilia que no fueron transmitidas al ojo debido a la inhibición de las motoneuronas. Financiado por el MICINN (SAF2009-10560) y la JJAA (P09-CVI-4712) con cofinanciación FEDER.

1. Neurociencia de sistemas
2. Sistemas homeostáticos y neuroendocrino

BILATERAL SENSORY INTEGRATION IN THE MOUSE STRIATUM – A WHOLE CELL *IN VIVO* STUDY

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The basal ganglia are involved in various motor and reward-related functions, however, their role in sensory processing remains unclear. The striatum is the input layer of basal ganglia, receiving excitatory inputs from multiple cortical areas, including sensory, motor and prefrontal cortices from both hemispheres. In this study we aimed to answer how striatal neurons integrate bilateral sensory input. To answer this question we obtained *in vivo* whole-cell patch-clamp recordings from neurons in the dorsolateral striatum and in cortical layer V of the barrel field (BF) in anesthetized mice. We recorded the evoked responses during brief deflections of ipsi- and contralateral whiskers. Recorded neurons were loaded with neurobiotin, the different types of striatal neurons were identified by their morphological and electrophysiological properties. Moreover, we distinguished between direct and indirect pathway projection neurons (MSNs) according to their biochemical expression of D1 and D2 dopamine receptors. Our results show that all recorded projection neurons and interneurons responded to bilateral whisker stimulation. Direct pathway projection neurons (D1 MSNs) have bigger responses for the contralateral whisker activation and have longer latency differences between ipsi- and contralateral responses than indirect pathway neurons (D2 MSNs), suggesting different functional roles in bilateral sensory integration. Using anatomical tracing and TTX injections in cortical areas before and after the bilateral whisker stimulation, we showed that responses for the –ipsi or contralateral pathways were mostly decreased in the MSNs after blocking selectively its contralateral BF, disclosing that the dominant pathway mediating the whisker evoked responses is the cortical instead of the thalamic one. Whereas responses to contralateral whisker stimulation are mediated via ipsilateral corticostriatal projections from the BF to dorsolateral striatum, responses to ipsilateral whisker involve additional cortico-cortical projections from the barrel cortex to motor cortex and contralateral barrel cortex.

Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

DESDE LAS CORRIENTES DE ESPINAS DENDRÍTICAS AL POTENCIAL DE CAMPO: CONTRIBUCIÓN DE CORRIENTES ACTIVAS Y PASIVAS

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El potencial de campo local (LFP) es una variable mesoscópica que refleja los cambios rápidos de actividad durante el procesamiento de información en el cerebro. Es conocido que los LFPs resultan de la suma de corrientes sinápticas en el espacio extracelular, pero los mecanismos celulares son poco conocidos debido a problemas teóricos y experimentales de difícil solución. Si bien es claro que la generación de un LFP implica corrientes sincrónicas en gran número de espinas dendríticas, la corriente sináptica vuelve al espacio extracelular a través de la compleja morfología de las neuronas, pero no hay pistas de cómo esta doble corriente transmembrana de distinta distribución espacial contribuye a los LFP en una población.

En este trabajo, hemos desarrollado un modelo realista de la región CA1 del hipocampo construido a partir de unidades celulares compartimentalizadas, cada una con cientos de espinas a lo largo de su morfología. Tratamos de abordar las siguientes preguntas: ¿son igualmente eficaces las espinas dendríticas de diferentes dominios celulares? ¿Es el potencial generado por una sola espina análogo al obtenido en la población?

Los resultados muestran que las espinas individuales generan patrones de potencial de campo muy variables según su posición en la célula (eje principal, dendritas primarias o secundarias). Además, se encontró que diferentes zonas del árbol dendrítico contribuyen con distinto peso a registros cercanos y lejanos. También se obtuvieron diferencias cualitativas y cuantitativas en la distribución espacial de los potenciales de campo producidos por sinapsis individuales con respecto a los producidos por grandes grupos que simularon la activación de vías específicas a nivel poblacional.

Estos resultados son importantes para la interpretación subcelular de LFPs y su correspondencia con señales macroscópicas. Proponemos que este método de análisis puede ser extendido a estructuras con arquitectura y morfología celular similar, como la corteza.

2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
3. Neurociencia de sistemas

MODULATION OF CA1 INTERNEURONS BY THE NATURAL INPUT FROM THE CA3 REGION

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The CA1 region of the hippocampus receives input from different regions that make synaptic connections to both principal cells and local interneurons. Most of these connections are excitatory (glutamatergic), and originate in the CA3 region and the entorhinal cortex. Amongst other functions, local inhibitory networks are thought to perform gating operations, contribute to the formation of assemblies, and sculpt the output rate of principal neurons. There is acceptable knowledge of the anatomical substrate for these interactions, as well as from in vitro electrophysiological unitary studies. However, functional information in vivo is lacking because of the difficulties in evaluating population input from specific extrinsic populations. In particular, there is little insight on which interneurons are driven by which pathways in the intact animal and when.

We here used linear multielectrode recordings of the local field potentials (LFP) across CA1 strata of anesthetized rats to extract the population ipsilateral CA3 input to CA1 by spatially discriminating techniques (Independent Component Analysis, ICA). The fine temporal details of the isolated spontaneous Schaffer input could thus be correlated to the natural spike dynamics of CA1 interneurons simultaneously recorded in different strata. Interneurons were also classified according to standard criteria: position of the soma, theta oscillations phase spike-phase relation during theta waves, firing rates in different states, and spike autocorrelograms.

The st. pyramidale showed the highest proportion of Schaffer-related interneurons, while those in the st. lacunosum-moleculare showed no relation. Among positively correlated interneurons, we found higher correlation in those with the soma located in the st. pyramidale than others in the st. oriens and radiatum.

We propose that the additional parameter for the physiological classification of CA1 interneurons based on their driving by natural CA3 input may complement standard criteria and enhance the functional understanding of local networks.

2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
3. Neurociencia de sistemas

TESTING THE EFFECT OF ALCOHOL INTAKE ON BRAIN NETWORKS USING COMBINED RESTING-STATE FMRI AND MANGANESE-ENHANCED MRI IN RATS

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Functional reorganization of motivation and emotion circuits is hypothesized upon long-term alcohol use. However, a comprehensive and longitudinal description of what such reorganization means remains to be provided. Here we combine multimodal magnetic resonance imaging (MRI) techniques to investigate functional changes in brain networks induced by voluntary alcohol drinking in Marchigian Sardinian alcohol-preferring (msP) rats. We apply resting-state fMRI in anesthetized animals to investigate ongoing functional connectivity longitudinally in the same group of animals before and after one month of high alcohol consumption. In a different group of animals but otherwise same experimental conditions we apply manganese-enhanced MRI (MEMRI) to build alcohol-driven differential activity maps in anesthesia-free animals. In MEMRI, Mn^{2+} preferentially accumulates in active brain regions where it enhances the T1-weighted signal. Manganese chloride was administered as an acute subcutaneous injection or by using 7-day subcutaneous osmotic minipumps (total dose 80 mg/kg), both protocols yielding similar imaging results. MEMRI data reveal alcohol-driven increases in brain activity in very specific regions of the ventromedial corticostriatal circuits including the accumbens, prelimbic and infralimbic cortex and insula. Importantly, resting-state fMRI analysis reveals converging findings that point towards an important role of the ventromedial frontostriatal circuitry for the high drinking phenotype of msP rats. This result is in good agreement with recent findings from alcohol self-administration in post-dependent rats and suggests common neuroadaptations in both models resulting in a similar behavioral output. Resting-state fMRI also highlighted a prominent role of the thalamo-cortico-striatal loop in the functional reorganization of frontal networks driven by high alcohol consumption.

1ª: Neurociencia de sistemas
2ª: Trastornos y reparación del sistema nervioso

CROSS-FREQUENCY COUPLING OF LOCAL FIELD POTENTIAL OSCILLATIONS IN THE CORTICO-STRIATAL PATHWAY AFTER KETAMINE ADMINISTRATION

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Background

Activity may play a role not only in the physiology of movement, perception and cognition, but also in the pathophysiology of psychiatric and neurological diseases like schizophrenia.

Ketamine has been used for many years to elicit behavioral effects reminiscent of schizophrenia in both healthy humans and in animal models of the disease. Phase-amplitude coupling (PAC) interactions between neuronal oscillations have been suggested as a potential mechanism to regulate communications among different frequency ranges. The aim of this study was to characterize these interplays throughout time in the oscillatory activity of the motor cortex and the caudate putamen of freely moving healthy rats, and their modulation mediated by the administration of ketamine (NMDA antagonist).

Methods

We recorded local field potentials from motor cortex and caudate-putamen (CPU) in 15 awake rats in basal condition (saline: 0.1 ml/kg) and after the administration of ketamine (10mg/kg). Recordings were divided in epoch frames of 15 minutes each.

Frequency components were characterized by the Welch periodogram and cross-frequency coupling was assessed by means of a modulation index (MI). Correlations were measured by means of the Pearson correlation coefficient.

Results

In basal condition (saline injection), the phase of the delta-theta band (2-6 Hz) entrained the amplitudes of three different frequency bands: low-gamma (LG, 50Hz), high-gamma (HG, 80Hz), and high frequency oscillations (HFO, 150Hz). The two structures analyzed exhibited PAC.

After ketamine injection frequency of modulatory activity PAC remarkably increased specially in the HFO band. This effect was reduced across time.

Conclusions

Delta-theta to gamma-HFO cross-frequency coupling occurs in the Cortex and the CPU of the rat. The frequency and magnitude of these phase-amplitude interactions are highly modulated by ketamine.

Áreas Temáticas
1ª: Neurociencia de sistemas.
2ª: Neurociencia cognitiva y conductual.

REGIONALIZED DISTRIBUTION OF *DELTA-LIKE HOMOLOGUE 1* (*DLK1*) IN THE FOREBRAIN OF THE ADULT MOUSE

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Background: Delta-like 1 (DLK1) is a transmembrane protein implicated in the proliferation/differentiation of different cell types. Its expression in adult mammals is mainly restricted to endocrine glands such as the pituitary gland, where it acts regulating its peptidergic cells. Moreover, DLK1 is expressed in specific areas of the adult brain such as hypothalamic cell groups and several monoaminergic nuclei.

Objectives/Materials and methods: We analyzed the distribution of *Dlk1*-expressing cell groups in the forebrain of the adult mouse, using in situ hybridization, and in accordance with the updated prosomeric model.

Results: Overall, *Dlk1* mRNA was particularly abundant throughout the hypothalamus and in some subpallial structures such as the preoptic area, the diagonal band and the septum. We also detected *Dlk1* expression in pallial regions including some nuclei of the amygdaloid nuclear complex and the hippocampal formation; no expression was detected within the majority of cortical areas**.** In the hypothalamus, cell populations expressing *Dlk1* were found at the alar paraventricular and subparaventricular domains (intense signal at the suprachiasmatic nucleus being particularly noteworthy), as well as at the basal plate (posterobasal and anterobasal areas, arcuate and dorsomedial nuclei, perimamillary/periretromamillary band). In contrast, the basal retromamillary/mamillary areas were largely devoid of such cells. Caudal to the hypothalamus, we observed patchy distribution of *Dlk1* within the alar/basal components of the three segmental units of the diencephalon (prosomeres 1-3); though *Dlk1* signal was almost absent from the prominent nuclei of the thalamus**.**

Conclusions: The wide expression of *Dlk1* in the adult forebrain, not assessed previously, indicates that this molecule possibly plays a different role than that characterized during development. The hypothalamic expression of *Dlk1* within the paraventricular and arcuate nuclei, involved in the hypothalamic-pituitary axis, suggests a neuroendocrine role for *Dlk1* although a paracrine function must be considered as well, apparently executed at the adult adenohypophysis.

Área temática: 1ª Neurociencia de sistemas
2ª Sistemas homeostáticos y neuroendocrino

A LONGITUDINAL SLICE OF THE ADULT MICE SPINAL CORD FOR ELECTROPHYSIOLOGICAL RECORDING OF NOCICEPTIVE NEURONES

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Central sensitization underlying chronic pain is conceived as an increased excitability of neural circuits processing pain signals in the spinal cord. Although most of the evidence supporting spinal sensitization arises from studies of individual neurones, adjustments in the temporal correlation in the discharge of neurons might also occur and these need to be addressed with techniques allowing the simultaneous recording of a number of discrete elements.

The spinal cord was extracted from young adult mice (> 5 weeks) via a dorsal laminectomy performed under anaesthesia (i.p. urethane 2 g/ Kg). A single longitudinal slice (~500 □m thick) was sectioned in a vibratome which contained the superficial layers of the cord and dorsal roots corresponding to lumbar segments 1 to 5. The slice was maintained *in vitro* by continuous superfusion of ACSF at 22±1 ºC. Patch electrodes or a NeuroNexus multiple electrode arrangement (MEA) mounted on a motorised micromanipulator were carefully lowered down into the preparation to record single or multiple neurons.

We commonly obtained simultaneous recordings from ~ 6 neurons without affecting slice viability as evaluated by Trypan Blue staining. The distance travelled by the electrode as well as the trajectory of the electrode track marked by DiI demonstrated that a high proportion of the neurons recorded were located in superficial Laminae I and II. Spontaneous activity had an important degree of synchrony in most of the neurons recorded. Most neurons showed synaptic responses to the activation of $A_{\square}$ or C fibres and a lack of wind-up to repetitive stimulation. Whole cell recordings confirmed the presence of functional neurons in superficial layers of the cord, many of which showed a delayed firing to depolarising pulses.
The results obtained so far confirm the viability of the spinal slice preparation from adult mice to perform simultaneous recordings from multiple neurons.

ALTERED SPONTANEOUS OSCILLATORY ACTIVITY IN THE NEOCORTEX OF A MOUSE MODEL OF ALZHEIMER DISEASE

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Objetives: The slow and fast (beta-gamma) cortical rhythms have been widely associated with higher cognitive functions, and it is known that they are reflecting the network state and activation. For this reason, the study of generation, propagation and synchronization of these rhythms is a good approach to better understand diseases presenting cognitive deficits and dysfunctions in cortical networks such as Alzheimer's disease (AD). Our aim was to characterize the emergent cortical activity in the 3xTg-AD mouse model of AD at advanced age.

Material and Methods: Extracellular recordings were obtained from different areas of the neocortex of anesthetized 14 mo old 3xTg-AD mice and spontaneous oscillatory activity was registered. The signal was amplified with a multichannel system, digitized and acquired. The Up and Down states were singled out by setting a threshold in the log(MUA) time series.

Results: At advanced ages, the slow oscillation lost regularity in all areas. In comparison to WT mice, 3xTg-AD mice showed a decrease in the frequency of slow oscillation with both, longer Up and Down states. Particularly striking was the an increase in the power of beta (15-30Hz) and gamma (30-90 Hz) frequency bands during Up states in all recorded areas, the increase in gamma being more evident in prefrontal cortex.

Conclusions: The characterization of the emergent cortical activity of the 3xTg-AD mouse model shows differences between TG and WT mice, probably reflecting imbalanced network properties. The understanding and analyses of these alterations will allow us to establish a relationship between electrophysiological patterns and cognitive deficits, providing a useful tool to better understand the disease.

Áreas Temáticas:
1ª: Neurociencia de sistemas.
2ª: Trastornos y reparación del sistema nervioso.

V1 SINGLE NEURONS CAN DISTINGUISH BETWEEN MOTION INTHE WORLD AND VISUAL DISPLACEMENTS DUE TO EYE MOVEMENTS

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How do perceptual systems differentiate between self-motion and motion in the world? This major question in neuroscience has special importance in vision: we can easily distinguish between real world motion and comparable displacements of the image over the retina due to eye movements, even though externally and internally generated motion produce equivalent retinal stimulation.
Here we compared the responses of V1 neurons to microsaccades (small-magnitude involuntary saccades that occur while attempting to fixate) in the presence of a stimulus in the neuron's receptive field (ocular motion) with the responses to stimuli motions mimicking microsaccades (motion in the world). The experiments aimed to determine whether area V1 neurons can differentiate between internal and external motion, and also the contribution of retinal versus non-retinal sources to microsaccade-driven responses in area V1.
We found that neuronal responses to real microsaccades were generally biphasic: a quick and dramatic increase over baseline was typically followed by a smaller and slower trough below baseline, whereas responses to simulated microsaccades included an excitatory peak but no trough. These differential neural responses to real versus simulated microsaccades indicate, for the first time, that area V1 neurons can distinguish between internally and externally generated motion. Our findings also suggest that excitatory responses to real microsaccades result from the displacement of the visual stimulus over the classical receptive field, with the subsequent inhibition reflecting other sources. These results help to delineate and constrain the role that area V1 plays in information processing, visual stability and/or perceptual suppression.

Área Temática: Neurociencia de sistemas

DISTORTION OF PAIRWISE THETA SYNCHRONIZATION BETWEEN HIPPOCAMPAL STRATA IN TEMPORAL LOBE EPILEPTIC RATS

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Previous work has reported disruption of the hippocampal theta rhythm in rat models of temporal lobe epilepsy (TLE), but the spectral features of this distortion still remain unclear. Using multi-site silicon probes, we recorded local field potentials (LFP) and estimated current source densities (CSD) from different hippocampal strata of normal and TLE rats (kainate-treated) engaged in active exploratory behavior. We studied the pairwise relationship (coherence and phase locking value) of the theta oscillatory activity between different strata at the molecular layer (ML) of the dentate gyrus (DG) and the CA1 region. We observed distinct contrasts between epileptic and control rats at the LFP and CSD levels. We also observed differences between groups of the phase difference between ML and the SLM. While the differences between control and TLE rats were maximal at the middle of the ML, CSD signals displayed only local differences involving pairs of neighboring sites within the CA1 and DG regions. We show that the LFP contrast could be predicted from the CSD signal by a distributed model of known dipoles of different lengths and phases, consistent with actual differences between groups due to hippocampal atrophy. We emphasize the importance of estimating couplings from CSD instead of LFP to accurately target the neurophysiological correlates of theta rhythm dysfunction in TLE.

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Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF A MODEL OF CORTICAL DYSPLASIA IN MICE INDUCED BY IN UTERO INJECTION OF METHYLAZOXYMETHANOL.

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Cortical dysplasia (CD) comprises a range of malformations affecting both neocortex and hippocampus. About 21% people suffering from pharmaco-resistant epilepsy exhibited some type of focal CD but the underlying mechanisms are not yet clear. Here, we characterized electrophysiologically a mouse model of CD induced by in utero intraventricular injection of methylazoxymethanol (MAM) at embryonic day E14. MAM-injected adult mice expressed a range of cortical and hippocampal heterotopias. We studied the hippocampus of MAM-treated mice by combining acute and chronic electroencephalographic recordings. Using multi-site silicon probe recordings in anesthetized mice, we found an increased orthodromic response in the CA1 region of MAM-treated mice to CA3 stimulation when compared to saline-injected and normal mice. Paired-pulse facilitation was decreased in MAM-treated animals whereas no change was detected in paired-pulse inhibition. Importantly, we found a significant decrease of the power of theta (4-12 Hz), gamma (20-90 Hz) and ripple oscillations (100-200 Hz) in MAM-treated mice after correction of 1/f decay. Chronic electrocorticographic telemetry recordings confirmed impairment of the theta rhythm in periods of exploratory behavior and REM sleep. We conclude that the MAM-treated displayed a range of developmental anomalies of brain structure and function.

Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

MAGNETIC RESONANCE IMAGING IN AWAKE RATS.

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In vivo brain imaging techniques in general and magnetic resonance imaging (MRI) in particular, allow fundamental investigations on the functional organization of brain networks and their relation to context-dependent processes as short and long-term plasticity, neuromodulation and adaptation in physiological and pathological conditions. The use of in vivo neuroimaging techniques in rodents to map brain activity has so far been limited by the necessity of animal fixation, and therefore the requirement of anesthesia. Functional MRI (fMRI) experiments on awake rodents represent an essential step forward in the investigation of brain networks and will greatly enhance the translational validity of preclinical studies. In this work we present a fully validated behavioral training protocol based on instrumental conditioning and positive rewards, which allows to record fMRI data in awake, quiet, non-stressed and immobile rats. The fMRI signal obtained from trained awake animals is free from movement artifacts and very robust across sessions and animals. At the end of the training period and several fMRI sessions we perform a number of behavioral tests (Forced Swimming Test, Elevated T-Maze, Release-Cue Test) and measure stress hormones by immunoassay to evaluate the presence of non-desired behavioral adaptation to chronic stress. The results demonstrate normal levels of Corticosterone in fecal samples and the absence of any sign of learned helplessness. Finally, to investigate the impact of anesthesia on functional network organization we performed resting-state and electric-stimulation driven fMRI experiments in consecutive anesthetized and awake conditions. The obtained results demonstrate quantitative and qualitative effects of anesthesia on brain activity patterns. Interestingly, while the effect on electrically evoked fMRI signals were limited to a quantitative decrease in the activation of an otherwise constant neuronal network, the impact of anesthesia on resting-state fMRI was more dramatic and altered the connectivity pattern of specific brain regions. This new method will significantly enhance the applicability of fMRI and open new possibilities to preclinical investigations.

Áreas Temáticas:
1ª: (3) Neurociencia de Sistemas
2ª: (7) Nuevos métodos y tecnologías.

ANATOMICAL ORGANIZATION OF THE FRONTO-TEMPORAL PATHWAYS IN THE MACAQUE MONKEY (*MACACA FASCICULARIS*).

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Frontal and temporal cortex, including the medial temporal lobe (MTL: hippocampal formation, and the parahippocampal region) are critical for memory. In particular, fronto-temporal connections might facilitate the interaction between long-term and short-term memory. However, the anatomical organization of these networks is still largely unknown. We aimed at disclosing it by using tract tracing techniques in nonhuman primates. A total of 14 retrograde (WGA-HRP, DY, and FB) and 6 anterograde (BDA and ^{3}H-aminoacids) tracer injections were placed into different areas of the rostral part of the temporal lobe: temporal pole (n=3), perirhinal cortex (area 35 and 36, n=5); parahipocampal cortex (TF, n=1), inferotemporal cortex (area TE, n=5), and of the frontal cortex: orbitofrontal cortex (n=2) and dorsolateral frontal lobe (A9 and A46, n=4). Our results showed that: 1) the ventrolateral frontal cortex (A46, 12, and 45) has the heaviest projection to area TE, and much weaker projection to other MTL regions; 2) the orbitofrontal cortex (A13) projects heavily to the medial temporal lobe, but also to higher order visual processing area TE; 3) Areas 35 and 36 of the perirhinal cortex receive frontal afferents almost exclusively from the orbitofrontal cortex. In summary, our results indicate that fronto-temporal projections are organized topographically whereby dorsolateral prefrontal cortex (A46, ventral portion of the principal sulcus; A12 and, A45 in the ventrolateral aspect of the frontal lobe) is almost exclusively linked to unimodal visual association cortices (area TE). The hippocampus, entorhial cortex, and parahippocampal region (polysensory) have denser connections with orbitofrontal areas.

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Áreas Temáticas:
1: Neurociencia de sistemas
2: Neurociencia cognitiva y conductual

ENTORHINAL AFFERENTS TO THE DORSOLATERAL PREFRONTAL CORTEX IN THE NONHUMAN PRIMATE (*MACACA FASCICULARIS)*.

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The entorhinal cortex (EC) is important for declarative memory processing, and its connections with the dorsolateral prefrontal cortex (DLPFC) might be necessary for both working and declarative memory. However, tract-tracing studies in the nonhuman primate have showed that EC-DLPFC has only very scarce connections. This study was aimed to explore further EC-prefrontal connections in the nonhuman primate. Anterograde tracer injections (BDA, MW10,000 n=9) were placed at different levels of the EC in nine cases of *Macaca fascicularis* monkeys. Stereotactic coordinates for the EC injections were guided by MRI in each experiment. Anterograde injections in different locations of the DLPF were also used in this study (flurophores linked to dextran amines FDA, RDA, n=4), ^{3}H-aminoacids, and PHA-L (n=3, kindly provided by D.G. Amaral). Injections in the DLPC were placed by direct exposure of the cortex. Results showed that whereas the density of EC projections to orbitofrontal (OFC) and medial frontal (MPFC) cortex was high, those to the DLPFC were negligible. The projection to DLPFC originated mainly in the intermediate and caudal fields of the EC and ended mainly in layers I and VI of areas 8 and 9. Our results suggest that, at least in monkeys, long-term and short-term memory pathways may be different.

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Áreas Temáticas:
1: Neurociencia de sistemas
2: Neurociencia cognitiva y conductual

HETEROGENEOUS ACTIVITY OF HIPPOCAMPAL PYRAMIDAL CELLS DURING SHARP WAVES EVENTS

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Sharp-waves (SPW) recorded in the hippocampus during behavioral immobility and slow-wave sleep, are critical for consolidation of CA3-CA1 memory traces encoded during previous exploratory experience. It is proposed that the reactivation of specific CA3-CA1 pyramidal cell ensembles during SPW is locally controlled by fast GABAergic inhibition. Here, we discovered that CA1 pyramidal cells exhibited heterogeneous SPW-associated activity dominated by the level of feedforward inhibition hard-wired in the CA3-CA1 circuit. Using a novel approach for juxtacellular recordings in drug-free freely moving rats, we found that CA1 pyramidal cells differentially participate of spontaneous SPW events. While some cells (50%, 4/8) fired in most SPWs, the remaining 50% were mostly silent during consecutive events. This heterogeneity was not likely to be spatially-modulated. To gain further insights into the mechanisms of firing heterogeneity, we then implemented intracellular sharp recordings in urethane anesthetized rats. We found similar heterogeneous behaviour of CA1 pyramidal cells with 5/9 cells exhibiting leading depolarization during SPWs and 4/9 cells being strongly hyperpolarized. Analysis of the reversal potentials suggested that GABAa-mediated potentials mainly controlled the hyperpolarized response. Interestingly, the SPW-associated dominant synaptic responses followed similar dynamics when compared with synaptic activity elicited by direct CA3 stimulation. We found that CA1 synaptic sequences were consistent with feedforward activation of GABAergic interneurons by the CA3 pyramidal cells. Therefore, firing activity of CA1 pyramidal cells during SPW events is determined by the level of GABAa-mediated inhibition in the CA3-CA1 circuit.

Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

CHARACTERIZATION OF THE CORTICAL SLOW WAVE ACTIVITY AND ITS MODULATION BY SUBCORTICAL AFFERENTS

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Slow-wave sleep (SWS) is characterized by the presence of slow-wave activity (SWA) in the cerebral cortex: large-amplitude waves together with faster activities in the range of the alpha (spindles) and gamma ranges. While the frequencies of such oscillatory activities have been identified, their dynamics and interactions require further investigation.

Here we analyzed the electrocorticogram of 7 anesthetized rats during slow-wave activity. We studied how low- and high-frequency oscillations interact and give rise to the characteristic patterns of SWA. Rats were anesthetized with urethane and supplemented with small doses of ketamine and xylazine. Glass pipettes were used to inject carbachol in the pedunculopontine nucleus to determine the effects of activating the ascending pathways from the brainstem on the ipsilateral electrocorticogram (ECoG). Recordings were acquired during epochs of robust and stable cortical SWA before and immediately after the infusion of carbachol. ECoG was recorded via steel screws above the somatic sensorimotor cortex and referenced to the cerebellum. Signals were filtered at 0.3-1000 Hz, amplified x2000, digitized at 2.5 kHz and stored in a personal computer.

The analysis show that spindles and gamma oscillations coexist but present distinct temporal dynamics across the slow oscillation cycle. Interestingly, spindles and gamma are functionally coupled to the slow oscillations but also between each other. Carbachol administration caused a gain in the gamma oscillations together with a reduction in the amplitude of the spindles and a decoupling of the gamma oscillations that are dependent on the spindles. None of these changes affected the onset or shape of the slow oscillations, suggesting that slow oscillations and the phasic increments of faster oscillations occur independently.

Our data suggest that subcortical structures play a role in the regulation of SWA and provide novel insights into the dynamics of cortical slow oscillations.

Áreas Temáticas:
1ª: Neurociencia de sistemas

DESCENDING CONNECTIONS FROM THE SEPTAL AREA TO THE NUCLEUS INCERTUS IN THE RAT.

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Projections from the pontine tegmental nucleus incertus (NI) to the medial septum have been implicated in modulating hippocampal theta rhythm. Brainstem-induced hippocampal theta rhythm depends on the integrity of this nucleus, which is in turn influenced by various neural inputs.
Aims: The aim of this study is to identify novel projections, from the septal area to the nucleus incertus, which may provide feedback modulation of that circuitry.
Material and Methods: Retrograde tracer fluorogold injections were made in the nucleus incertus to label descending afferents. Double labeling immunofluorescence with antibodies against calcium binding proteins were used to characterize projecting neurons. In a different set of experiments, the anterograde tracer miniruby was injected in discrete septal regions to confirm descending projections to the nucleus incertus.
Results: Fluorogold injections into the nucleus incertus resulted in strong retrograde-labeling in the medial septum and septofimfrial nucleus. Double-immunofluorescence of fluorogold and neuronal markers indicated that nucleus incertus-projecting and choline acetyltransferase-positive cells occupied different compartments within the medial septum. Fluorogold was observed in some parvalbumin, calbindin and glutamic acid decarboxylase (GAD)67-positive neurons, indicating that the descending system is partially GABAergic. Most of fluorogold positive neurons were also positive for calretinin. Anterograde miniruby injection into the medial septum revealed descending fibers coursing via the medial forebrain bundle to the supramammillary nucleus, median and paramedian raphe and nucleus incertus. Anterogradely labeled, terminal-like varicosities display synaptophysin, indicating that medial septal inputs form synapses on nucleus incertus neurons. Anterogradely-labeled fibers also co-localized with GAD67-positive puncta and in some cases, these puncta made close synaptic contact with GAD-67-labeled neurons of the nucleus incertus.
Conclusions: These data provide evidence for the existence of an inhibitory descending projection from the medial septum to the nucleus incertus that may form a feedback loop to modulate ascending nucleus incertus projections to the medial septum, hippocampus.

Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Neurociencia cognitiva y conductual

REGULATION OF THE DOPAMINE TRANSPORTER BY GDNF USING AAV-TetON VECTORS

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The dopamine transporter (DAT) is a membrane glycoprotein expressed in dopaminergic (DA-) cells, which is responsible for dopamine (DA) uptake from the extracellular space. DAT is involved in the vulnerability of DA-cells in Parkinson's disease because it increases cytosolic levels of DA, and consequently oxidative stress. So, regulation of DAT activity can protect DA-cells against degeneration. Experimental studies show that the glial cell line-derived neurotrophic factor (GDNF) has trophic and protective effect on DA-cells, although the mechanisms remain unknown. Interestingly, GDNF administration can modulate some DA-markers, although both up- and down-regulation have been reported.

Subject: Bearing in mind the relevance of DAT in the vulnerability of DA-neurons, the aim of this study was to investigate whether DAT may be regulated by controlled release of GDNF.

Material and methods: Adult male rats (n=30) were injected in the left striatum with tetracycline-inducible adeno-associated virus (AAV)-1 vector expressing human GDNF cDNA (AAV-tetON-GDNF) or green fluorescent protein (AAV-tetON-GFP). Thereafter, rats were treated with doxycycline (dox) in a dose range of 0.05-6g/kg diet during 5 weeks. Striatal GDNF was quantified by ELISA. DAT activity, expression and interactions were studied by using morphological, biochemical and molecular techniques.

Results: At a dose of 1mg/kg diet, dox induced a moderate increase (x2.5) of striatal GDNF levels with respect to sham and AAV-GFP injected rats (310±34 pg/mg tissue vs 125±21 pg/mg/tissue). This treatment induced a DA uptake decrease of 50%. Immunohistochemistry and western-blot showed no DAT expression changes in whole striatal extracts and synaptosomal membranes. The analysis of samples under non-reducing conditions and in situ proximity ligation assay revealed the formation of complex DAT oligomers ranging between 130-160 kDa which accumulate ubiquitinated DAT.

Conclusion: Long-term controlled release of GDNF by using AAV-TetON vectors induces intra- and intermolecular changes in DAT interactions and DA uptake decrease.

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Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia de sistemas

QUANTITATIVE MAPPING OF MATRIX-TYPE THALAMOCORTICAL PATHWAYS IN THE MARMOSET MONKEY.

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Objectives: Concepts on thalamo-cortical (TC) cell function remain strongly influenced by the point-to-point topography of the "specific" TC pathways targeting cortical layer 4. However, such pathways in fact involve only a subset of all TC neurons, other TC neurons (M-type cells) characteristically target layer 1. In rodents, they are prevalent in many thalamic nuclei (Clascá et al. Eur. J. Neurosci., 2012), however in the primate thalamus they have never been systematically explored. Here, we map the distribution and prevalence of M-type neurons and the convergence of their axons in the cerebral cortex of the *Callithrix jacchus*
Methods: M-type cells were selectively labeled by epipial deposits of retrograde axonal tracers, and the labeled somata were mapped and quantified throughout the thalamus. Stereotaxic BDA microinjections were made in some of the labeled nuclei to label anterogradely axons of M-type cells. The tangential distribution of labeled axons across the cerebral hemisphere was reconstructed from serial sections.
Results: Primate M-type neurons are most abundant in the mediodorsal, ventral-anterior, ventral-lateral, medial-pulvinar, anterior-pulvinar, posterior and suprageniculate thalamic nuclei. TC axons originating in some of the above nuclei target widely separated cortical zones. M-type pathways are also highly convergent, and around one thousand different M-type TC cells innervate each sq. mm. of cortical layer 1. Our data show that the axons from small populations of pulvinar cells diverge to innervate over 20 separate domains in the frontal, parietal, temporal and cingulate areas, where they arborize mainly in layers 1 and 3.
Conclusion: The M-type neuron population is present in many nuclei of the marmoset thalamus. They are prevalent in some association and motor thalamic nuclei. Our data also suggest that mean number of individual M-type cells targeting a given point of layer 1 is about one-third of the number observed in rodents.
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Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Neurociencia cognitiva y conductual

DIFFERENT DIVISIONS FOR DIFFERENT BEHAVIOURS: AFFERENTS TO THE MEDIAL AMYGDALA IN THE MOUSE

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The medial amygdala (Me) is a heterogeneous structure composed of three divisions, the anterior (MeA), posteroventral (MePV) and posterodorsal (MePD) subnuclei, having a differential role in socio-sexual and anti-predatory behaviors and different projections (Pardo-Bellver et al., Front Neuroanat. 2012;6:33. doi: 10.3389/fnana.2012.00033). However, a description of their afferents is lacking. To fill this gap, we analyzed the afferents to the Me by means of iontophoretic injections of fluorogold restricted to its subdivisions in CD1 mouse.

Besides the afferents from the olfactory bulbs, the amygdalohippocampal area is the main common afferent to all Me subnuclei. Specific intraamygdaloid inputs to the MeA arise from the basolateral complex, (medial) central amygdala, olfactory amygdala and piriform cortex. Extra-amygdaloid inputs originate from the frontal-insular cortex, bed nucleus of the stria terminalis (BST) posterointermediate part, ventromedial hypothalamus, ventral premammillary nucleus, paraventricular thalamic nucleus and posterior intralaminar thalamus. The MePV receives intramygdaloid inputs mainly from the posterolateral and posteromedial cortical nuclei (PMCo), and only minor afferents from other amygdaloid nuclei. Its extraamygdaloid afferents are similar to the MeA, although the inputs from the frontal-insular cortex are lighter. The MePD shows a very different pattern of connections. Its major inputs arise from the PMCo and medial posteromedial BST, but it shows little inputs from other parts of the amygdala, cortical areas and thalamic or hypothalamic nuclei.

In summary, our results stress the anatomo-functional differences among the subnuclei of the medial amygdala. The MePD is specifically interconnected with vomeronasal, sexually dimorphic areas involved in reproductive behaviour. The MeA and MePV show similar afferents related to fear, but the MeA seems more related to structures involved in cognitive processes (basolateral, central amygdala and frontal cortex) whereas the MePV is more related to the vomeronasal cortex.

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EFECTOS ANTIDEPRESIVOS DE LA ESTIMULACIÓN CEREBRAL PROFUNDA EN EL MODELO DE LA BULBECTOMÍA OLFATORIA

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Introducción: La estimulación cerebral profunda (ECP) del área cingulada 25 (Cg25) es eficaz contra la depresión resistente al tratamiento. A pesar de ello, se desconoce su mecanismo de acción. En roedores, el área equivalente a la Cg25 es la corteza prefrontal infralímbica (CPF-IL). El modelo de bulbectomía olfatoria (OBX) en rata es un modelo de depresión que conlleva cambios comportamentales, neuroquímicos y neuroendocrinos, similares a los descritos en pacientes deprimidos. Este modelo sólo responde al tratamiento crónico con fármacos antidepresivos, lo que le aproxima mucho a la situación en humanos. En el presente trabajo se ha examinado los efectos comportamentales y neuroquímicos de una hora de ECP en ratas bulbectomizadas.
Material y métodos: Se realizó la bulbectomía a ratas Wistar macho y 28 días después se implantaron los electrodos y/o las sondas de diálisis. A las 48 h se realizaron los tests de comportamiento y/o la microdiálisis. La ECP se llevó a cabo utilizando electrodos implantados bilateralmente en la CPF-IL (1 h, 130 Hz, 200 μA y 0.1 ms). Mediante microdiálisis se examinó la liberación de serotonina (5-HT) y glutamato (Glu) en la CPF. Para determinar el efecto antidepresivo se estudió la hiperactividad en campo abierto, la preferencia de sucrosa, la hiperemocionalidad y la interacción social.
Resultados: Una hora de ECP en la CPF-IL, con parámetros similares a los utilizados en la práctica clínica, produce una reducción significativa de la hiperactividad, la anhedonia, la hiperemocionalidad y las alteraciones en interacción social propias del modelo OBX. Además, esta estimulación produce un incremento de la liberación de 5-HT y Glu en la CPF.
Conclusiones: El modelo de OBX es sensible a un tratamiento de corta duración (1 h) de ECP de manera similar a la observada con el tratamiento crónico con fármacos antidepresivos. Estos hallazgos refuerzan la validez predictiva de dicho modelo.

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Áreas Temáticas:
1. Neurociencia de sistemas
2. Neurociencia cognitiva y conductual

ERBB4 DELETION FROM FAST SPIKING INTERNEURONS GENERATES HIPPOCAMPAL RHYTHMS ALTERATIONS

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Interneurons are relevant in the control and coordination of pyramidal neurons, and contribute to creating the excitation/inhibitory balance in different networks. Interneurons are also involved in the generation of different oscillations in the brain underlying various cognitive processes. In particular, fast spiking interneurons have been linked to the generation of gamma oscillations in the neocortex and hippocampus. Alterations in gamma rhythms have been reported in schizophrenia, possibly linked to the cognitive deficits associated with this disorder. We have previously identified a role for neuregulin 1 and its ErbB4 receptor, encoded by schizophrenia-susceptibility genes, in the wiring of fast spiking interneurons. Here we have analyzed the contribution of fast spiking interneurons to the generation of gamma rhythms in the hippocampus by analyzing conditional mouse mutants lacking the neuregulin receptor ErbB4 from these cells. We found that loss of ErbB4 in fast spiking interneurons causes an increase of gamma power in the local field potential recorded from CA1 in freely moving mice, while no defects were observed at other frequencies. We are currently exploring the behavior of individual neurons in relation to the gamma cycle and the relationship between the abnormal increase in gamma rhythms and cognitive function, as measured through specific working memory tasks in the T-maze.

1. Neurociencia de sistemas
2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

GLUTAMATE IMMUNOREACTIVE NEURONS IN THE FOREBRAIN OF THE SIBERIAN STURGEON (*Acipenser baeri*).

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Glutamate is a major excitatory neurotransmitter in the central nervous system of vertebrates. However, studies on the distribution of glutamatergic neurons in vertebrates are scarce and they have mainly been carried out in rodents and lampreys. With the aim of investigating the early evolution of the glutamatergic system, we have studied the distribution of the putative glutamatergic neuronal populations in the forebrain of a basal ray-finned fish, the Siberian sturgeon *Acipenser baeri* (Chondrostei, Acipenseriformes). Our results with immunofluorescence techniques using a polyclonal anti-glutamate antibody revealed the presence of neurons immunoreactive to glutamate (Glu-ir) in different nuclei and regions of the forebrain such as the olfactory bulbs, the dorsal and ventral telencephalic areas, the preoptic region, the vascular organ of the terminal lamina (OVL), the hypothalamus, the prethalamus, thalamus and pretectum. Abundant Glu-ir cerebrospinal fluid-contacting (CSF-c) cells were observed in the preoptic area, OVL, hypothalamic lateral and posterior recess nuclei, posterior tubercle, and prethalamus. Comparison of the Glu-ir populations of the Siberian sturgeon with the distribution of glutamatergic cells reported in the sea lamprey reveals shared abundance of Glu-ir CSF-c cells throughout the forebrain, which is unlike their reported absence in mammals. The results enable us to conclude that the general distribution of putative glutamatergic populations in the sturgeon forebrain is roughly similar to that observed in lampreys and mammals, suggesting conserved evolution of glutamatergic systems in vertebrates, although some populations as those observed in the inferior hypothalamic lobes may be specific of jawed fishes. This work is funded by the Spanish Dirección General de Investigación-FEDER (BFU2010-15816) and Xunta de Galicia (10PXIB200051PR, CN 2012/237).

Áreas Temáticas:
1ª: Neurociencia de sistemas

CHARACTERIZATION OF TWO POPULATIONS OF TRPM8 COLD SENSORY NEURONS INNERVATING THE CORNEA. ROLES IN PAIN AND TEARING REGULATION

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Objetivos: Cold thermoreceptors containing the TRPM8 cold transduction channel have been postulated to be responsible for the maintainance of the lacrimal film integrity. A new population of TRPM8 trigeminal neurons and nerve terminals is described here by means of neurochemical analysis.

Material and Methods: Corneas and trigeminal ganglions (TG) were collected from adult transgenic TRPM8-EYFP and TRPM8-KO mice and studied using double immunostaining techniques against GFP, Peripherin, NF200, CGRP, SP and receptors to neurotrophins. Presence of TRPM8 was determined both by RT-PCR and Western Blot analysis. Basal tear production was measured using Phenol Red threads in wild type and KO mice. Fast Blue retrograde neuronal tracer was used to detect neuronal bodies in the TG projecting to the cornea.

Results: Two populations of corneal cold sensitive fibers were identified by their morphology in the cornea of TRPM8 wild type mice. These populations correspond to two types of $TRPM8^{+}$ neuronal bodies in the TG with different neurochemical characteristics. The TRPM8 neurons with higher expression of TRPM8 are primary small and unmyelinated. Neurons with low TRPM8 usually contain CGRP and trkA receptors. Their central and peripheral projections are considered as Aδ fibers. TRPM8-KO corneas present modifications in the morphology of their cold sensory fibers. Furthermore, their somas at the TG are smaller than in the wild type mice, exhibit a lower expression of TRPM8 and are immunonegative for trkA. Their projections are preferently unmyelinated C fibers. Basal tear secretion was lower in KO mice in comparison with TRPM8 wild type animals.

Conclusions: The special neurochemical characteristics that distinguish the two populations of TRPM8 cold sensory neurons are probably responsible for the different roles of each type of fiber. Both populations are sensitive to cold temperatures and could play an important role in the maintainance of basal tear flow. We think that each of them could detect different ranges of temperature, from innocuous cool to noxious cold. Moreover, they are separated by their modulation by nerve growth factor and some markers related with pain and inflammation.

Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Sistemas homeostáticos y neuroendocrinos

MOVEMENT-RELATED MODULATION OF THE UNITARY RESPONSES IN THE STRIATUM

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The striatum forms the main input to the basal ganglia, and as such is involved in control movement, reinforcement learning and action selection. Although striatal neurons have been classified into different categories according to various electrophysiological criteria, most of the work has been carried out on anesthetized or immobilised animals. One potential problem in interpreting these results is that they can be affected by the experimental conditions.
Here we compared the unitary responses of striatal neurons from 5 freely moving animals behaving under two well-controlled conditions: quiet rest vs. passive walking on a treadmill.
To do so, we studied striatal single unit activity collected from multiple electrodes using chronically implantable multielectrode arrays. Local field potentials and unit activity were continuously recorded (during rest and walking) from the dorso-lateral portion of the striatum using silicon probes with 16 contacts. Individual responses were then classified using a spike-sorting algorithm. Neurons were characterized by means of their morphology, firing rate (FR), coefficient of variation (CV), bursting index (BI) and the presence of long-range time correlations (LRTC). In addition, we also evaluated the degree of spike-field coupling of the neurons to the oscillations in the theta and gamma bands.
Results showed that movement induced a significant increase in FR and BI while CV decreased. LRTC were detected in both conditions but did not show differences between them. The majority of neurons showed a significant spike-field coupling with the theta and gamma oscillations, changing the strength of coupling but showing no differences in the phase of preference. Interestingly, neurons with the same morphology (i.e. same putative category) showed different firing features.
We have found that the firing pattern of striatal neurons show long-range correlations and depends on the motor activity, meaning that they could play different roles in despite of their morphology.

Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Neurociencia cognitiva y conductual

TRPM8 ION CHANNEL IS EXPRESSED IN A NEW AMACRINE CELL SUBPOPULATION

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Objetivos: To establish the cytoarchitecture and cytochemical pattern of a new subset of amacrine cells expressing the TRPM8 cold-transducing channel.

Material y Methods: Retinas from adult transgenic TRPM8-EYFP and TRPM8-KO mice, in which a fluorescent protein was attached to the cold-transducing channel TRPM8 were used. A total of ten animals were employed. Immunohistochemical staining for calcium binding proteins (Calretinin, Calbindin, Parvalbumin), and for markers to Tyrosine hyroxylase, Choline acetyltransferase, GABA, Glutamate and Glycine, was performed in whole mounted retinas and in transversal cryostat sections of TRPM8-EYFP and TRPM8-KO eye globes. Presence of TRPM8 was determined both by RT-PCR and Western Blot analysis.

Results: A population of TRPM8-positive cells is homogeneously distributed in the central and periferal retina of the wild type animals and is located at the level of the inner border of the inner nuclear layer (INL). They were identified morphologically as amacrine cells, and were never found grouped in clusters. Their dendritic processes were located in two densely packed layers coinciding with sublayers 2 and 4 of the inner plexiform layer (IPL) corresponding to the two outer layers of CR staining. Fluorescent cells were also seen in the ganglion cell layer (GCL), being classified as displaced amacrine cells of the GCL. These cells were $ChAT^{+}$ and contribute to form the inner $TRPM8^{+}$ sublayer at the IPL. TRPM8-KO mice lack these neurons or they are unable to express TRPM8. No other morphological alteration was observed and we could not determine an evident visual deffect.

Conclusions: A subtype of amacrine cells expressing TRPM8 channels was identified. It appears unlikely that TRPM8 channels expressed by these neurons is involved in cold detection. In contrast, the expression of ChAT strongly suggest that near a 60% of cells of this new subtype may be starburst-like cells and could be involved in directional selectivity.

Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

KNOCKOUT PARA LA POLIMERASA μ: UN MODELO PARA EL ESTUDIO DE POBLACIONES DE INTERNEURONAS EN EL HIPOCAMPO

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El hipocampo constituye el área fundamental en el procesamiento de la memoria y en el aprendizaje, los mecanismos por los cuales realiza dicha función todavía no se conocen en su totalidad. Durante el proceso de envejecimiento puede producirse un deterioro cognitivo que afecta al hipocampo, entre otras áreas cerebrales, y a los procesos de aprendizaje y memoria, relacionados con cambios en estas áreas cerebrales.
Un nuevo modelo para este tipo de estudios lo constituyen los ratones deficientes en polimerasa μ (Pol μ). Estos animales muestran un fenotipo de envejecimiento cerebral retardado, con una importante capacidad de aprendizaje preservada en ratones de edad avanzada, acompañada de un elevado grado de plasticidad sináptica en el hipocampo (Lucas et al., 2013).
Estudios cualitativos y cuantitativos muestran comportamientos diferentes de las dos principales poblaciones de interneuronas presentes en el hipocampo, las células parvalbúmina positivas (PV^+; células en cesto y en candelabro) y las células somatostatina positivas (SST^+; células HIPP y O-LM).
En nuestro laboratorio hemos analizado y cuantificado la distribución de estas poblaciones de interneuronas a lo largo del envejecimiento en la formación del hipocampo. Durante el envejecimiento los animales control presentan una disminución significativa de la población de interneuronas PV^+ en los campos amónicos, sin embargo, en los ratones *knockout* esta población se mantiene constante. Por otro lado la población de células SST^+ no varía significativamente con la edad en el grupo control, sin embargo, en los ratones *knockout* para la polimerasa μ esta población de interneuronas SST^+ se incrementa con la edad.
En conjunto, estos resultados indican que los mecanismos de reparación de ADN ejercen un importante papel en los cambios de plasticidad sináptica en el hipocampo durante el proceso de envejecimiento, en los que deben de estar implicadas de alguna manera tanto las interneuronas parvalbúmina positivas como las interneuronas somatostatina positivas.

Áreas Temáticas:
1. Neurociencia de sistemas
2. Neurociencia cognitiva y conductual

ROLE OF RGS14 PROTEIN IN VISUAL MEMORY AND THE REGULATION OF SYNAPTIC PLASTICITY IN PERIRHINAL CORTEX

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Though the concept of participation of perirhinal cortex and frontal cortex in the processing of object memory has long been appreciated, but recently our laboratory extended this to area V2 of visual cortex. We found that activation of area V2 neurons by overexpression of RGS14 protein led to an enhancement of object recognition memory. The memory enhancement was of such extent that it converted the short term memory of 45 minutes into long lasting long-term memory that could be traced even after many months. Here, we have tested the memory enhancer effect of RGS14 in perirhinal cortex (PRh), an area known to be involved in object memory processing, and further explored the relationship of behavioral memory performance with synaptic plasticity within this area. Stimulation of PRh with RGS14 protein produced an equally robust increase in object memory as was observed in area V2. In addition, we found that RGS14-mediated activation of PRh, (i) blocked the depotentiation induced by 1Hz stimulation during 10min; (ii) blocked the LTP induced by 20Hz stimulation while showed no effect at 100Hz stimulation; and (iii) reduced the LTD induced by the application of 20 μM of carbachol, a cholinergic agonist, during 10min, however no effect was observed at a higher concentration (50 μM). Furthermore, we also observed that phosphorylated isoforms of AMPA receptor 1 (iGluR1) were significantly reduced. Thus, our results indicate that iGluR1 reflects the level of synaptic plasticity (LTP and LTD) observed in RGS-animals but lack this correlation in behavioral outcome.
This work was supported by projects from MINECO, Junta de Andalucía y NIH.

Áreas Temáticas:
1. Neurociencia de sistemas
2. Neurociencia cognitiva y conductual

ALTERACIONES DEL SUEÑO EN UN MODELO DE FIBROMIALGIA INDUCIDA POR RESERPINA EN RATA

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Objetivos: Comprobar si el modelo de fibromialgia en ratas basado en la depleción de monoaminas centrales mediante la administración subcutánea de reserpina (Nagakura et al., 2009), provoca alteraciones en el patrón de sueño/vigilia similares a las descritas en pacientes con fibromialgia.

Material y Métodos: El estudio se realizó en ratas macho de la cepa Sprague-Dawley. Se implantaron crónicamente electrodos de campo en CA1 del hipocampo, la corteza somatosensorial primaria y el núcleo del rafe dorsal, así como un electrodo de EMG en los músculos dorsales del cuello.Todos los electrodos se concentraron en un pedestal de plástico multicanal. Tras un periodo de recuperación y habituación se procedió a administrar la reserpina (1mg/kg sc diario, durante 3 días consecutivos). A cada animal se le realizaron registros control en días previos a la administración de la reserpina.

Resultados: Los resultados mostraron un incremento del tiempo total de sueño, fragmentado por un mayor número de despertares. También se evidenció un aumento de actividad theta hipocámpica, tanto en sueño como en vigilia.

Conclusiones: Estos resultados muestran una destrucción de la arquitectura del sueño, que combinada con el estado de hipersomnia que presentan las ratas durante la vigilia, sugieren presencia de sueño no reparador, síntoma recientemente incorporado como criterio diagnóstico de la fibromialgia. Nuestros resultados refuerzan la validez del modelo de fibromialgia inducida por reserpina, propuesto por Nagakura et al., en 2009 y sugieren una alteración de las monoaminas en la génesis de la fibromialgia.

Áreas Temáticas:
1º Neurociencia de Sistemas
2º Trastornos y reparación del sistema nervioso

FIRING PROPERTIES OF THE NUCLEUS INCERTUS NEURONS RELATED TO HIPPOCAMPAL THETA WAVES

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Theta oscillations allow the hippocampus to open temporal windows for information processing. Previous studies from our group have shown that nucleus incertus contributes to the hippocampal theta rhythm generation. In fact, nucleus incertus itself shows oscillatory activity at theta frequencies, coupled with the hippocampus.
The present study aims to find the possible relationship between firing properties of nucleus incertus neurons and the generation of hippocampal theta rhythm, with the final purpose of correlating the unit activity of the nucleus with the hipoccampal field potential. Field potentials and unit recordings were obtained of urethane anesthetized female rats. Sensorial stimulation was applied as a model of hippocampal theta rhythm generation. Data analysis was made with self-developed MATLAB functions based on wavelet analysis methods.
In addition to the two previously neuronal types described by our group, we have found a third neuronal population with a distinct firing pattern. These neurons fired at non rhythmic low frequencies and shifted into rhythmic theta frequencies during stimulation. Additionally, the spikes of these neurons showed phase preference with hippocampal theta waves during stimulation and spontaneous theta periods.
In conclusion, our results show that the generation of hippocampal theta rhythm can be related to the change in the firing rate of this third population of nucleus incertus neurons.

1ª: Neurociencia de sistemas
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

EXPLORING OLFACTORY AND VOMERONASAL-INDUCED OSCILLATIONS IN THE MOUSE OLFACTORY SYSTEM AND THE CORTICOMEDIAL AMYGDALA

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The amygdaloid complex is involved in processing the emotional value of sensory stimuli. Its corticomedial region receives olfactory and vomeronasal information, which include chemical signals with a significant emotional value (sexual pheromones or predatory cues). To functionally explore this process we recorded the electrophysiological activity in the main (MOB) and accessory (AOB) olfactory bulbs and the medial and posteromedial cortical amygdaloid nuclei of freely-behaving female mice. In order to assess the correlation between the recorded signal and the exploratory behavior, five different stimuli were supplied: geraniol-scented bedding ("pure olfactory" stimulus); bedding soiled by castrated males, female- and male-soiled bedding (conspecific-derived stimuli); and rat-soiled bedding (heterospecific, putative predator). The local field potentials were off-line processed by continuous wavelet transform in the time-frequency domain. All of the chemical stimuli presented elicited predominant theta rhythmicity in the MOB, identifiable as a “sniffing” oscillation, and coupled in time with high gamma segments (> 60 Hz). This pattern has been described as olfactory processing in rodents. The local field potentials in the AOB also showed a constant peak frequency in the theta band, accompanied by a coupled very-fast gamma oscillation, present under exploratory behavior. In the medial and posteromedial cortical amygdaloid nuclei the theta rhythmicity is also present. However the gamma oscillations in the medial amygdaloid nucleus present a similar pattern correlated to that of the MOB, whereas the AOB oscillatory pattern is coincident with the gamma rhythmical activity of the posteromedial cortical amygdaloid nucleus. This double oscillatory gamma component, segregated between both olfactory bulbs and different amygdaloid areas, suggests a convergent and complementary processing integrated in the emotional circuits. We hypothesize that this convergence is involved in learning to associate neutral odors with biologically relevant vomeronasal cues.

Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

COLLATERAL-SPECIFIC PRODUCTION OF LOCAL FIELD POTENTIALS IN THE SCHAFFER-COMMISSURAL SYSTEM: COMPUTATIONAL AND EXPERIMENTAL STUDY

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Local field potentials (LFP) are contributed by extracellular summation of the synaptic currents elicited by inputs converging in a recording site. The net contribution of each pathway depends on multiple factors, such as the chemical nature of the input, the temporal structure, and the degree of spatiotemporal overlap to other inputs.
In this study we explored the relative contribution to LFPs of two major inputs to the CA1 pyramidal cell population, the Schaffer and commissural inputs from the ipsi and contralateral CA3 regions. These excitatory inputs constitute an interesting reciprocal system as they both originate in the same CA3 neurons and project through bifurcating axon collaterals to similar, but not identical synaptic territories on CA1 pyramidal cells of both hippocampi.

We used in vivo multisite linear recordings of LFPs in the anesthetized rat and simulated LFPs obtained by an aggregate CA1 pyramidal model with high degree of anatomical and functional detail. The contribution of different pathways to real and model LFPs was estimated after their separation in pathway-specific components using our implementation of independent component analysis (ICA) to intracerebral LFPs.
We found a striking different contribution to LFPs recorded in the CA1 from the two inputs to the same neurons: LFPs appear to be generated only from Schaffer collaterals, but not from their commissural counterparts, in spite of the afferent activity being nearly synchronous in both CA3. The model showed that the natural jitter between the inputs (±2 ms) was insufficient to allow their spatial discrimination along the somatodendritic domains, but rather than a poor efficiency of the ICA, we found that the particular asymmetrical geometry of pyramidal cells promoted the dominance of Schaffer currents visibility in LFPs. We conclude that neurons may elicit LFPs in some but not all of their target populations.

1. Neurociencia de sistemas
2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

ELECTRICAL STIMULATION OF THE CENTRAL AMYGDALA ELICITS A CHANGE IN THE OSCILLATORY PATTERN OF MEDIAL PREFRONTAL CORTEX

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Many limbic structures involved in neuropsychiatric diseases also play a major role in stress control. Actually, activation of the central amygdala (CeA) has an activating effect of the hypothalamic-pituitary axis, so what triggers stress responses, whereas prefrontal cortex appears to be critical for inhibition or termination of the stress response. Accordingly, limbic system dysfunction plays a major role in numerous neuropsychiatric disease states that involve stress or anxiety. Since many of these disorders appear resistant to current treatments, it is required to deepen the study the operation of the circuit.

In the present work we study the oscillatory activity of the medial prefrontal cortex in response to a stressful situation generated by electrical activation of the central amygdala.

With this objective, we explored the field activity in infralimbic cortex (IL) in urethane-anesthetized rats. Recordings were made in spontaneous conditions using a model of acute stress generation through electric stimulation CEA using stimulation parameters that emulate firing rates induced by stress (0.2ms pulses and 0.08 mA to 5 - 8Hz, for 200s; Forster et al, 2008).

The results show a predominance of slow waves (below 2 Hz) in control situation with desynchronized activity in the range 1-8 Hz. After CeA stimulation, a delayed increase in the proportion of low frequency waves (0.5-3Hz) and a decrease in the theta activity (3-12Hz) were observed. Interestingly, the stimulation resulted in a synchronization at a narrow band at 0.5 Hz.

In conclusion, our results showed a delayed response of mPFC as a consequence of CeA activation. Future studies are needed to elucidate where in the stress process (activation, expression or inhibition) is this effect related.

Thematic Areas:
1. Systems Neuroscience
Two. Cognitive and Behavioral Neuroscience

GENOARCHITECTURE OF THE AMPHIOXUS CENTRAL NERVOUS SYSTEM: A MODEL OF EARLY SUBDIVISIONS

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The building plan (bauplan) of the central nervous system of vertebrates is defined by basic anteriorposterior and dorsoventral subdivisions shared by all major taxonomic groups. These building blocks are constructed by specific genomic rules that generate regional identities. Consistent with the topological structural similarities, early developmental stages were found to largely share similar gene expression profiles in all studied vertebrate species. Under this framework, we analyzed around fifty gene expression patterns from amphioxus focussing on early neural tube closure, providing valuable information about amphioxus early bauplan. Comparing mainly key transcription factors, we proposed here an initial model suggesting that this basal chordate presents two initial divisions named: Archaencephalon and deuteroncephalon prototagmas, *Otx* and *Gbx* positive respectively. The archaencephalon is subsequently partitioned in proencephalon (rostral) and dimesencephalon (caudal). Finally, we detected that the proencephalon presents an additional antero-posterior partition. Our data shows that the genetic regionalization of the amphioxus early developing central nervous system is much more complex than previously anticipated. Comparison of gene expression patterns between amphioxus and vertebrates suggests that amphioxus presents a simplified chordate bauplan; however, its basic partitions seem to have clear homology with those of vertebrates.

Áreas Temáticas:

1ª: Neurociencia de sistemas

2ª: Desarrollo

VOMERONASAL AND OLFACTORY INPUTS TO SUPRAOPTIC VASOPRESSINERGIC DENDRITES IN THE MEDIAL AMYGDALA OF MICE

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In rodents, which use mainly chemosignals for intra-species communication, the medial amygdala (Me) is a key node of the socio-sexual brain network; it receives direct vomeronasal-olfactory inputs and is sensitive to sexual steroids. Surprisingly, the Me shows a minor arginine-vasopressin (AVP) innervation, even if AVP is known to play a fundamental role in modulating socio-sexual behaviours. However, we have observed thick AVP-immunoreactive processes in the ventral Me (MeAV and MePV) of mice (Otero-Garcia et al., Brain Str. Funct. DOI 10.1007/s00429-013-0553-3). We have hypothesised that these processes are dendrites belonging to neurosecretory cells of the adjoining supraoptic nucleus (SO). These dendrites could receive olfactory inputs (targeting the Me) that would mediate pheromone/semiochemical-induced AVP dendritic release in the Me, involved in modulating social interactions.

In this work we check these hypotheses in CD1 female mice. In Experiment 1 we combine immunodetection of AVP and the dendrite marker MAP2. We confirmed under confocal microscopy that the AVP-ir processes in the ventral Me are dendrites, viz. they were doubly labelled. Many of these dendrites could be followed up to their somata of origin in the SO. In Experiment 2 we labelled the efferents of the main (MOB) or accessory olfactory bulb (AOB) with fluorophore-tagged dextranamines. Then, double immunohistochemistry for AVP and synaptophysin (SPY) allowed checking whether contacts of olfactory axons with AVP-ir dendrites corresponded to synaptic loci. Axons from the AOB synapted AVP-ir dendrites in the Me probably belonging to SO cells. Preliminary results indicate that the MOB also projects to AVP cells, as it happens in rats. We are currently analysing whether different portions of the MOB project differentially to the AVP-ir dendrites in the Me. These results suggest that in mice olfactory and vomeronasal stimuli could activate magnocellular secretory cells and also promote local AVP dendritic release.

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1ª: Neurociencia de sistemas
2ª: Sistemas homeostáticos y neuroendocrino

ROLE OF ELECTRICAL SYNAPSES MEDIATED BY CONNEXIN-36 IN THE CONTROL OF RESPIRATION

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Breathing is a primal neural process by which pO_2, pCO_2 and pH are regulated. Brainstem neural circuits controlling breathing are organized as serially arrayed networks connected by both chemical and electrical synapses. Connexin-36 (Cx36) is the principal component of electrical synapses between inhibitory neurons of respiratory nuclei, including the rhythmic Bötzinger and pre-Bötzinger complexes and the CO_2/pH chemosensitive retrotrapezoid nucleus. The expression pattern of Cx36 is developmentally regulated with a peak at postnatal day 15 (P15) followed by a progressive decay with the age. To assess the role played by Cx36 electrical synapses in the control of respiration, the changes in the respiratory rhythmicity and in the chemoreflexes to hypercapnia and hypoxia during postnatal maturation were studied in wild type (WT) vs. Cx36 knockout (Cx36-KO) mice. Basal respiratory rate of WT and Cx36-KO mice was similar at P15, while in adults of 3-month old it decreased in WT and increased in KO animals. With regard the respiratory chemoreflexes, we found that the response of WT mice to hypercapnia and hypoxia increase both in frequency and amplitude during postnatal development, while mice lacking of Cx36 showed an abnormally enhanced hypercapnic and hypoxic response at P15 and did not change significantly with the age. Thus, adult Cx36-KO mice in comparison with wild type ones still showed a hypoxic response somewhat increased but a significantly lower sensitivity to hypercapnia. We conclude that Cx36-KO mice clearly show a respiratory phenotype with alterations in rythymicity and central chemosensitivity and that most of postnatal maduration of respiratory function can be accounted by the reduction of neuronal connectivity mediated by the electrical synapses of Cx36.

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Áreas Temáticas:

1ª: Neurociencia de sistemas

2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

CAJAL WAS RIGHT! MULTIPLE DIRECT PROJECTIONS FROM SUBCOLLICULAR AUDITORY NUCLEI TO THE THALAMUS

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Despite the general assumption that the main source of ascending projections to the auditory thalamus is the inferior colliculus (IC), several isolated reports suggest that the medial geniculate body (MGB) receives direct projections from auditory subcollicular nuclei.

To investigate the sources of ascending projections to the auditory thalamus, we made iontophoretic injections of the retrograde tracer FluoroGold into different regions of the auditory thalamus (the various subdivisions of the MGB and the adjacent nuclei) and analyzed the distribution of the neurons labeled in all subcollicular auditory nuclei.

Our results show that the auditory thalamus receives direct convergent projections from a surprisingly high number of neurons in many subcollicular nuclei, including the contralateral dorsal cochlear nucleus and lateral superior olive; the ipsilateral medial superior olive (MSO), superior paraolivary nucleus (SPON), and ventral nucleus of the lateral lemniscus (VNLL); and the dorsal nucleus of the lateral lemniscus (DNLL) and the nucleus sagulum/horizontal cell group (Sag/HCG) of both sides. In the cases with the most retrograde labeling, virtually all neurons in the DNLL and Sag/HCG were seen to innervate the ipsilateral or the contralateral thalamus, and the percentage of labeled neurons in the ipsilateral MSO, and SPON approached 50%. The single subcollicular nucleus that contributes the most to the innervation of the auditory thalamus is the VNLL. These data suggest that many of the axons from lower centers that innervate the auditory thalamus must be collaterals of axons that also innervate the IC. Moreover, our quantitative, multivariate analysis indicates that each nucleus or region of the auditory thalamus receives a different combination of inputs from subcollicular centers.

These findings support Cajal's "central acoustic tract", improve our understanding of the organization of the auditory pathway, shed light on the complex parcellation of the auditory thalamus, and provide novel morphological frameworks for future functional studies.
Support: ISCIII PI10/01803

Áreas Temáticas:
1ª: Neurociencia de sistemas
2ª: Neurociencia cognitiva y conductual

THE DORSAL TECTAL LONGITUDINAL COLUMN (TLCD), A UNIQUE GABAERGIC NUCLEUS THAT INHIBITS HIGHER ORDER THALAMIC NUCLEI OF A SPECIFIC SENSORY MODALITY

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The tectal longitudinal column (TLC) is a longitudinally oriented, long and narrow nucleus that spans the paramedian region of the midbrain tectum of a large variety of mammals (Saldaña et al., J Neurosci 27:13108–13116, 2007). Recent analysis of the organization of this region revealed another novel nucleus located immediately dorsal, and parallel, to the TLC. Because the name "tectal longitudinal column" also seems appropriate for this novel nucleus, we suggest the TLC described in 2007 be renamed the "ventral tectal longitudinal column (TLCv)", and the newly discovered nucleus termed the "dorsal tectal longitudinal column (TLCd)".

We have used a constellation of anatomical techniques to demonstrate that the TLCd differs from its surrounding structures (TLCv and superior colliculus) cytoarchitecturally, myeloarchitecturally, neurochemically and hodologically. The distinct expression of vesicular amino acid transporters suggests that virtually all TLCd neurons are GABAergic. The TLCd receives major projections from various areas of the cerebral cortex (secondary visual mediomedial area, and granular and dysgranular retrosplenial cortices) and from the medial pretectal nucleus. It densely innervates the ipsilateral lateral posterior and laterodorsal nuclei of the thalamus. Thus, the TLCd is connected with vision-related neural centers. The TLCd may be unique as it constitutes the only known nucleus made of GABAergic neurons dedicated to providing massive inhibition to higher order thalamic nuclei of a specific sensory modality.
Financial support: ISCIII PI10/01803.

Áreas Temáticas:
1ª: Neurociencia de sistemas
1. 2ª: Neurociencia cognitiva y conductual

SENC

15º Congreso Nacional de la Sociedad Española de NeuroCiencia

Tema 4

Neurociencia cognitiva y conductual

Posters

EFFECTS OF ENVIRONMENTAL ENRICHMENT ON ANXIETY RESPONSES, SPATIAL MEMORY AND CYTOCHROME C OXIDASE ACTIVITY IN ADULT WISTAR RATS

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We studied the effect of an environmental enrichment protocol in adult Wistar rats on the activity in the elevated zero maze (EZM), performance in the 4-radial arm water maze (4-RAWM) and we also examined the changes in the neuronal metabolic activity in brain regions related to stress and spatial memory through cytochrome c oxidase histochemistry (COx). Forty rats were randomly assigned to four groups: Control group (CO, n=10), Environmental enrichment group (EE, n=10) Environmental enrichment + spatial learning group (EE+SPL, n=10) and Spatial learning group (SPL, n=10). The EE and EE+SPL groups were housed (2 months) in large cages during three hours every day with various stimulating objects. SPL and EE+SPL groups were assessed in the EZM. The session consisted of 5 minutes and the animal was placed in the center of a closed quadrant. The 4-RAWM testing was performed for 4 days, six trials/day with a 30 s inter-trial interval. Each rat was allowed 60 s to reach the platform. After the completion of the learning task, rat brains were processed. Our EE protocol had anxiolytic effect in the EZM, since the animals spent more time and made more entries into the open quadrants, they had lower latency to leave the closed quadrant and low levels of defecation (p<0.05). Besides, EE group showed fewer working and reference (p<0.05) spatial memory errors in the first sessions of spatial training. EE reduced the COx activity in brain regions related to stress, such as, infralimbic cortex, paraventricular thalamic and hypothalamic nucleus, basolateral amygdala, and ventral hippocampus (p<0.05), but interestingly there were not differences between groups in dorsal hippocampus (p>0.05) more related to spatial cognition. These results suggest a beneficial effect of EE on spatial memory, maybe as a result of reducing stress and the COx activity in brain regions involved in stress.

Áreas Temáticas:

1ª: Neurociencia cognitiva y conductual

2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares**.**

DIFFERENTIAL EFFECTS OF ENVIRONMENTAL ENRICHMENT AND AEROBIC EXERCISE ON SPATIAL MEMORY AND CYTOCHROME C OXIDASE ACTIVITY IN AGED WISTAR RAT

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Cognitive stimulation and physical exercise are useful for cognition enhancement in the elderly. We studied the effect of environmental enrichment (EE) and aerobic exercise, both implemented during 2 months in 18 months-old Wistar rats, on the performance of a spatial memory task (RAWM) and cytochrome c oxidase activity (COx) of brain regions involved in spatial memory.

Rats were randomly assigned to six groups: Control group (CO, n=11), Spatial learning group (SPL, n=10), Exercise group (EX, n=8) and Exercise + spatial learning group (EX + SPL, n=8), Environmental enrichment + spatial learning group (EE + SPL, n=8) and Environmental enrichment group (EE, n=8). Regular exercise was performed on the Rotarod 3 times a week for 15 minutes/day. The enriched groups were housed in large cages during three hours every day with various stimulating objects. The RAWM was performed for 4 days, six trials/day with a 30 s inter-trial interval. Start locations were randomized and the platform was in the same arm throughout the entire task. Each rat was allowed 60 s to reach the platform. Animals were decapitated in order to assess the oxidative metabolism.

EE+SPL had better spatial performance respect to EX+SPL group, with lower latency to reach the platform in the third and four days and lower percentage of entries into the incorrect arms ($p<0.05$). However, we did not find significant differences between groups in the distance travelled across the days ($p>0.05$). Also, we found significant differences between groups in the prefrontal cortex, in where EE+SPL group had lower COx activity ($p<0.05$). In contrast, we did not find significant differences between groups in the dorsal hippocampus ($p>0.05$). The EE had a better effect on spatial memory respect to aerobic exercise, may be due to underlying processes, such as neurogenesis or synaptogenesis, whereas aerobic exercise is more involved in angiogenesis.

Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Neurociencia cognitiva y conductual.

EARLY BEHAVIORAL ABNORMALITIES ARE INDEPENDENT OF EXTRACELLULAR Aβ ACCUMULATION IN A NEW TRANSGENIC RAT MODEL OF ALZHEIMER'S DISEASE

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Contradictory results have been obtained between the amount of amyloid deposition and the degree of cognitive decline in Alzheimer's disease (AD). On the contrary, intraneuronal Aβ accumulation has been linked to the mild cognitive impairment that precedes AD onset. Leon *et al.* (2010) have developed a new AD transgenic rat model that express the human amyloid precursor protein (AβPP) with the Swedish and Indiana mutations (McGill-R-Thy1-APP). The heterozygous transgenic rat has the advantage of not developing amyloid plaque deposition during its entire lifespan.

Objective. To assess locomotor activity, emotional reactivity and cognitive performance at a young age in an AD transgenic rat model that only accumulates Aβ intracellularly.

Materials and methods. 3-month-old heterozygous male transgenic rats (+/-tg, n=10) and their male wild-type littermates (wt, n=9) were evaluated in a behavioral test battery that included: Elevated Plus Maze (EPM), Open Field (OF), Novel Object Recognition Test, Y-maze spontaneous alternation test and a spatial learning and reference memory protocol in the Morris Water Maze (MWM). After behavioral tests were completed, the intraneuronal Aβ accumulation was assessed by immunohistochemistry, using the antibody McSA1 which is specific for the human Aβ peptide.

Results. In EPM and OF tests, +/-tg rats displayed higher levels of anxiety than their wt littermates. In the MWM, +/-tg rats showed spatial reference memory impairment during the probe trial. In contrast, episodic-like memory, working memory and spatial learning were preserved. Immunohistochemistry results confirmed that amyloid-β accumulated intraneuronally in the hippocampus and cerebral cortex. Extracellular Aβ accumulation was not observed.

Conclusions. Our results indicate that this heterozygous line exhibits emotional and cognitive alterations as early as 3 months of age. Since rats display a more complex behavioral repertoire than mice, this transgenic model could be interesting for testing therapeutic interventions for behavioral alterations that take place during preclinical stages of AD.

Áreas temáticas:
1ª. Neurociencia cognitiva y conductual.
2ª.Trastornos y reparación del sistema nervioso.

VOLUNTARY ETHANOL CONSUMPTION (VEC) IN WISTAR RATS PRE-EXPOSED TO AVERSIVE EFFECTS OF ETHANOL: ROLE OF GUSTATORY THALAMUS

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This study explores possible differences in voluntary ethanol consumption between rats with lesions of the gustatory thalamus and non lesioned rats (sham) that had previous experience with the drug by intraperitoneal injections according to a procedure of ethanol-induced conditioned taste aversion (CTA). Thus, this study is aimed to collect data on the amount of alcohol consumed (g/kg) and preference levels for that substance in lesioned rats versus sham rats using an ethanol voluntary consumption procedure that is characterized by the exposure to a series of increased concentrations of ethanol (2%, 4%, 8% y 10%). The animals used in this study were part of a previous study in which rats with lesions of the gustatory thalamus and rats with false lesion (sham) were exposed to an ethanol-induced or a LiCl-induced CTA. The findings of this previous study indicated that there were not differences in ethanol-induced or LiCl-induced CTA between groups (sham vs. lesion). Both groups learned to avoid the conditioned stimulus associated with the visceral discomfort, independently of the aversive agent used (alcohol or LiCl). These results seem also to suggest that high-moderate doses of ethanol have aversive effects comparable to LiCl, independent of its rewarding effects. Moreover, the obtained data through the VEC procedure reflected differences between groups in the level of general preference for ethanol though there were no differences in the g/kg consumed. Specifically, it was observed that Sham group showed marked differences in the levels of ethanol preference (a higher preference to the lowest concentrations and a rejection of the highest), whereas the lesioned group did not exhibited a distinctive preference level for different ethanol concentrations. These differences between groups are discussed in terms of taste deficits but can not rule out that the pre-exposure to ethanol, through an ethanol-induced CTA, has affected differently to the two groups. Future experiments will try to resolve this issue.

Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Sistemas homeostáticos y neuroendocrino

DISFUNCIÓN FRONTAL Y ANOMALÍAS ADAPTATIVAS: IMPLICACIONES DE UNA ALTERACIÓN UBICUA

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Objetivos:
Enfatizar en la relevancia neurocientífica –y neuroética- que implica el lóbulo frontal (LF) y las funciones ejecutivas (FFEE) a aquel asociadas, destacando las consecuencias que la alteración de este complejo anátomo-funcional conlleva en la integridad psíquica humana, al estar involucrado en la regulación cognitiva, afectiva y conductual.

Material y Métodos:
Revisión de publicaciones centradas en la neurociencia del LF (PubMed, Cochrane), realizando un tratamiento de los datos en clave clínica, destacando las numerosas anomalías funcionales que presenta la alteración de esta región cerebral, así como los variados contextos etiopatogénicos que las provocan.

Resultados:
Tras detallar una lista de las capacidades cognitivas que denominamos como FFEE, nuestro estudio expone que: 1) Existe un desarrollo filogenético tardío del LF, destacándose evolutivamente en el homo sapiens, en coherencia con expresiones culturales de mayor sofisticación. 2) La maduración ontogenética del LB se desarrolla con posterioridad respecto a otras regiones cerebrales, derivando en un desarrollo retardado de las FFEE. 3) Destaca la sensibilidad del funcionamiento ejecutivo durante el proceso de envejecimiento, en coherencia con la involución temprana de la región frontal, que resulta significativa a partir de la séptima década de vida. 4) El LF y las FFEE ejecutivas presentan una notable susceptibilidad al daño cerebral adquirido (hipoxia, traumatismos craneoencefálicos, etc.). 5) Dentro de los signos clínicos englobados en patologías vinculadas a alteraciones del neurodesarrollo, destacan las anomalías frontales y/o ejecutivas como rasgos con más incidencia en la capacidad adaptativa del paciente. 5) Se conocen tres síndromes neuropsicológicos vinculados, respectivamente, a tres áreas prefrontales (dorsolateral, orbitofrontal y frontomedial). 6) Aparecen estudios que postulan la ubicación de la capacidad ética humana en la conectividad frontal.

Conclusiones:
Las anomalías frontales –y ejecutivas-, presentes en numerosas patologías y disfunciones neurológicas, afectan significativamente el funcionamiento neuropsicológico humano, comportando importantes alteraciones en su capacidad adaptativa.

Áreas Temáticas
1ª: 4. Neurociencia cognitiva y conductual
2ª: 8. Historia, Docencia, Divulgación y Ética

INHIBITION OF nNOS NOT ONLY PREVENT BUT REVERSE SENSITIZATION TO COCAINE

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Behavioral sensitization it is known as the increased sensitivity to locomotor stimulating effect after repeated psychostimulants administration, and is believed to be relevant to drug addiction and craving in humans. Repeated cocaine induces behavioral sensitization in a 50% of treated male Wistar rats and it can modulate synaptic plasticity in the hippocampus which is an important brain region for some associative learning processes occurring during addiction. Nitric oxide is a neurotransmitter involved in a broad range of effects in the central nervous system including synaptic plasticity and complex behavioral responses among others. We have previously demonstrated a key role of nNOS/NO/sGC/cGMP signaling pathway in the development of cocaine sensitization and in the associated enhancement of hippocampal synaptic plasticity (HSP). In the present work, we attempted to determine whether the inhibition of nNOS after sensitization reverse this behavioral effect and the associated hippocampal synaptic plasticity. We administered 5 daily cocaine injections (i.p.) to 35 days old Wistar rats, followed by 5 daily 7-nitroindazole (nNOS inhibitor) injections (i.p.). We tested development of cocaine sensitization (in vivo) and the threshold for LTP in hippocampus (in vitro) as indicator of synaptic plasticity. The results show that inhibition of nNOS after repeated cocaine administration reversed behavioral sensitization and the highest level of plasticity induced by repeated cocaine. Therefore, considering that nNOS/NO/sGC/cGMP signaling pathway in the brain can initiate, contribute or exacerbate addictive behaviors, the interference of this pathway, even after development of cocaine sensitization, could be a useful tool to reduce susceptibility to relapse.

FUNCTIONAL CONTRIBUTION OF EXTRAHIPPOCAMPAL NETWORKS IN TWO DIFFERENT SPATIAL WORKING MEMORY TASKS IN WISTAR RATS

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Spatial working memory is seen as a critical element for basic cognitive functions. Interference or cognitive flexibility, show important differences between aged and adult rats, as the difficulty of the task increases. We aim to study the interference between distracting and relevant information. For that, we examined the differences between adults (3 months old) and middle-aged (18 months old) rats in two tasks with different levels of difficulty using the Morris Water maze with allocentric cues. The simple task consisted of four testing sessions, with one daily session comprised of one sample and one retention trials; the other task (multisession) consisted of three testing sessions, with three daily session. In this complex spatial task each daily session trial consisted on one sample and retention trials, but inter-session intervals were 5 minutes. In each session the platform changed the location.

Also, we examined the changes in metabolic oxidative activity in brain regions with the cytochrome c- oxidase histochemistry. We evaluated Cingulate cortex; Prelimbic cortex; Infralimbic cortex; Bed nucleus of the StriaTerminalis; CA1; CA3; Dentate Gyrus; Retrosplenial cortex; Central and Basolateral amygdala; Supramammillary and Medial mammillary nucleus. Moreover, we used the Discriminant function analysis to discriminate between all the brain regions in the two different learning tasks. Behavioral results showed that first, spatial working memory tasks results more easily for the adult group than for the middle-aged. In the case of multisession task, is much more difficult for both groups although adult group did this task better than middle-aged. On the other hand, Discriminant analysis showed that the most relevant regions to differ between the learning groups were the Medial mammillary, involved in the interference process, and the set of Suprammamillary, CA3 and CA1.

Áreas Temáticas:

1ª: Neurociencia cognitiva y conductual

2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares**.**

SIZE DOES NOT ALWAYS MATTER: Ts65Dn DOWN SYNDROME MICE SHOW CEREBELLUM-DEPENDENT MOTOR LEARNING DEFICITS THAT CANNOT BE RESCUED BY SAG-TREATMENT

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Humans with Down syndrome (DS) and Ts65Dn murine model of DS both show a reduced volume of the cerebellum due to a significant reduction in the density of granule neurons. Recently, cerebellar hypoplasia in Ts65Dn mice was rescued by a single treatment with SAG, an agonist of the sonic hedgehog pathway, administrated on the day of birth. In addition to normalizing cerebellar morphology, this treatment restored the ability to learn a spatial navigation task, which is associated with hippocampal function. It is not clear to what extent this improved performance results from restoration of the cerebellar architecture or a yet undefined role of sonic hedgehog (Shh) in peri-natal hippocampal development. The absence of a clearly demonstrated deficit in cerebellar function in trisomic mice exacerbates the problem of discerning how SAG acts to improve learning and memory. Here we show that phase reversal adaptation and consolidation of the vestibulo-ocular reflex is significantly impaired in Ts65Dn mice, providing for the first time a precise characterization of cerebellar function deficits in this murine model of DS. However, these deficits do not benefit from the normalization of cerebellar morphology following treatment with SAG. The lack of improvement at this functional assay and the unchanged synaptic plasticity properties of Purkinje cells by SAG treatment support the possibility that a direct effect of Shh pathway stimulation on the hippocampus might explain the benefits of this potential approach to the improvement of cognition in DS.

1. Neurociencia cognitiva y conductual
2. Trastornos y reparación del sistema nervioso

KAINATE RECEPTOR SUBUNIT COMPOSITION AND MENTAL DISORDERS

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Kainate receptors genes have been regarded as susceptibility genes in several mental disorders like depression, schizophrenia, bipolar disorder or mania. Indeed, the expression of one of the kainate receptor subunits, GluK4, has been found to be altered in patients of schizophrenia. To study how a change in expression of GluK4 may result in one or several behaviors related to mental illness, we have used two difference transgenic lines. On one hand, we used the GluK4 deficient mice and, on the other, we generated transgenic mice that overexpress GluK4. The overexpression of GluK4 was driven by the promoter of CaMKII, which expression starts at postnatal states and is restricted to the forebrain. To easily immunolocalize the protein, GluK4 was tagged with 5 myc sequences at the N-terminal. In situ hybridization and inmunocytochemical studies revealed high expression of the myc-GluK4 in the cortex and hippocampus, presenting lighter expression levels in striatum and thalamus. Biochemical experiments proved that the myc-GluK4 protein is delivered to the membrane and undergoes the same posttransductional modifications than the native protein.

Behavioral studies of the overexpression of GluK4 revealed less activity in the open field and elevated place maze, where the transgenic mice spent significantly less time exploring the open arena and open arms than their control littermates. On the other hand, GluK4 deficient mice showed an increase of activity in the open field reflecting a reduced anxiety phenotype. Interestingly, overexpressing GluK4 mice showed a 25% increase in the body weight. These data from both mice with altered GluK4 expression levels clearly implicate this subunit in mental disorders and prove that mood disorders may result from the change in activity of a single kainate receptor subunit

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1ª: Neurociencia cognitiva y conductual
2ª Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

TIMING–STRENGTH SYNAPTIC EVOLUTIONS AND FUNCTIONAL DISTRIBUTION OF HIPPOCAMPAL SYNAPSES INVOLVED IN CLASSICAL EYEBLINK CONDITIONING

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Aims

Although it is generally assumed that the hippocampus is involved in some types of associative learning, the specific contribution of synapses present in its intrinsic circuit or comprising its main inputs and outputs are still poorly defined. We have addressed this important question here, studying the evolution of the timing-strength synaptic patterns and the functional mapping of synaptic-learning state functions in different hippocampal synapses during the acquisition of an associative learning task.

Materials and Methods

In this study, we have recorded activity-dependent changes in synaptic strength of nine different hippocampal synapses during trace eyeblink conditioning in behaving mice. Selected analytical-experimental approach of multisynaptic state functions and evolution patterns enabled us to determine the timing and intensity of hippocampal synaptic changes across the acquisition process.

Results

The analysis of the timing-strength synaptic patterns allowed us to propose the functional distribution of the nine synapses included in the present study, which did not coincide with their sequential distribution according to anatomical criteria and connectivity. These results confirm that this type of associative learning is a multisynaptic process in which the contribution of each synaptic contact is different in strength and takes place at a different acquisition moment across the learning process. In addition, the proposed approach could also be applied to the selective stimulation of relevant synaptic nodes across hippocampal circuit.

Conclusions

We expect that a map of synaptic-learning state functions relating the acquisition of new motor and cognitive abilities and the underlying synaptic plastic changes will be offered in the near future for different types of learning tasks. Moreover, depending on the specific and timed activation of the multiple synaptic contacts, the hippocampus can be involved in many different functions, such as spatial orientation, object recognition, and many other forms of memory acquisition and retrieval.

Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Neurociencia de sistemas

MODULATION OF CDK5 ACTIVITY AMELIORATES COGNITIVE DYSFUNCTION IN HUNTINGTON'S DISEASE

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Cognitive impairment is an early clinical feature of Huntington's disease (HD). Unfortunately, the molecular mechanisms underlying these defects remain unclear. Learning and memory formation are modulated by pre- and post-synaptic signaling events. Particularly, Cdk5 a serine/threonine kinase whose activity is primarily restricted to the nervous system has been involved in many neurodegenerative diseases such as Alzheimer or Parkinson´s Disease. Indeed our group has demonstrated aberrant Cdk5 activity in the striatum of different HD models and HD human brain. These studies point out Cdk5 as an important modulator of neuronal dysfunction in several neurodegenerative disorders and highlight the importance of therapeutic strategies aimed to inhibit Cdk5 activity to slow or prevent HD progression**.** In this study we report altered Cdk5/p35/p25 expression in the striatum, hippocampus and cortex of HD knock-in mutant mice ($Hdh^{Q7/Q111}$) and HD patients. To determine whether altered Cdk5 activity could contribute to cognitive decline in HD we generated a new transgenic mice that expressing mutant huntingtin but heterozygous for Cdk5 ($Hdh^{Q7/Q111}$; $Cdk5^{+/-}$). The genetic modulation of Cdk5 levels in $Hdh^{Q7/Q111}$ mutant mice restored cortico-striatal learning deficits and improved performance in spatial and memory learning tasks, which suggests that both altered cortico-striatal and hippocampal function in HD could involve aberrant Cdk5 activity. Altogether, these findings suggest that modulation of Cdk5 activity or signalling in HD may contribute to restore synaptic plasticity and learning defects in this devastating disorder.

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Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Trastornos y reparación del sistema nervioso

EFECTO DEL NATALIZUMAB EN UN MODELO DE ENCEFALOMIELITIS AUTOINMUNE EXPERIMENTAL

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La principal causa de enfermedad neurológica invalidante no traumática es la esclerosis múltiple (EM). Enfermedad desmielinizante de naturaleza autoinmune que afecta al sistema nervioso central (SNC). Los datos muestran la existencia en daño oxidativo que juega un papel relevante en su patogénesis. Si bien, en la actualidad no existe tratamiento curativo, uno de los fármacos con mejores resultados es el natalizumab (Tysabri, Biogen Idec, Inc. and Elan Pharmaceutical, Inc.). Anticuerpo monoclonal humanizado dirigido contra alfa-4-beta-integrina, inhibidor selectivo de la adhesión de monocitos al endotelio vascular es utilizado en el tratamiento de la EM recurrente-remitente (EM-RR). Para el estudio de este proceso han sido diseñados diferentes modelos experimentales que permiten un acercamiento a EM-RR para un mejor conocimiento de los mecanismos moleculares subyacentes en el proceso y su evolución.

Objetivo: Analizar del efecto de natalizumab sobre la inmovilidad y estrés oxidativo presente en la encefalomielitis alérgica/autoinmune experimental (EAE).

Material y Métodos: Fueron utilizadas ratas Dark Agouti que se inocularon con glucoproteína oligodendrocítica de mielina (MOG), desarrollando un cuadro de EAE. Después de 14 días de recibir la glucoproteína y manifestar el cuadro, algunos de los animales fueron tratados con natalizumab (5 mg/kg peso ip, cada diez días durante 21 días).

Resultados: Los animales inoculados con MOG sufrieron una parálisis progresiva y significativa de las extremidades que aconteció de forma paralela a un incremento en el daño oxidativo tanto en cerebro como en médula espinal y sangre. Estos procesos fueron parcial y significativamente revertidos tras el tratamiento con natalizumab.

Conclusiones: i) MOG desencadena un procesos de EAE acompañado de intenso daño oxidativo en tejido nervioso y periférico; ii) Natalizumab evita y revierte en el animal con EAE la evolución del procesos de igual forma que ocurre en su administración clínica; y iii) Natalizumab reduce los niveles periféricos de marcadores de daño oxidativo en EAE, similar a la apreciada en los pacientes.

FEARLESS MICE? A BEHAVIOURAL ANALYSIS OF THE RESPONSE OF MICE TO RATS AND THEIR CHEMOSIGNALS

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Defensive reactions to predators can be facilitated by detection of their chemosignals, known as kairomones. In mice, predator kairomones are said to be vomeronasal (e.g. proteins in rat's urine) or olfactory stimuli, such as trimethylthiazoline (TMT; putative faecal fox kairomone). Olfactory and vomeronasal kairomones would elicit defensive responses by activating a neural system that includes the amygdala.

To explore this possibility we have reinvestigated this issue in our lab. In Experiment 1, we characterise the response of CD1 and C57BL/J6 mice to a drop (15 µl) of rat urine presented in a compartment of a two-chamber test cage. Using video-track software (SMART, Panlab) we analysed: a) location of the animals throughout the 5-minute tests; b) locomotor activity (track length); and c) freezing (movement slower than 1 mm/s). Each mouse was tested in a control situation (saline), a test with the stimulus (three groups: Sprague-Dawley urine, Wistar urine and saline) and a second control. Data were analysed using ANOVA with SPSS. Although both strains of mice behaved differently, they did not show freezing or avoidance to rat urine (of either strain). In addition, rat urine did not induce contextual memory: no freezing or avoidance of the stimulus compartment appeared in the second control. This strongly suggests that mice are nor fearful about rat urine. Thus, in Experiment 2 we tested the behavioural response of CD1 and C57BL/J6 mice to an anaesthetised Sprague-Dawley rats, using Aura hamsters or stuffed rat toys (IKEA) as controls. Mice reacted similarly to rats and hamsters, with no freezing or subsequent contextual fear. Thus, rat chemosignals appear not to act as kairomones. Since TMT is also unable to induce fear in mice (Fortes-Marco et al., 2013 *Anat Record*, in press), our findings challenge the existence of predator kairomones in mice.

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FUNCTIONAL TOPOGRAPHY OF THE SOMATOMOTOR SUBTHALAMIC NUCLEUS IN A BEHAVING MONKEY

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To investigate the neuronal signal processing in the basal ganglia during goal-directed actions, we recorded neuronal activity of the subthalamic nucleus (STN) in a monkey (*Macaca fascicularis*) performing two types of tasks using a joystick and a screen. One was a reaching task with a visible target and the other was a seeking task with an invisible target. Both tasks consisted in 45 trials, respectively, and the latter task was accomplished less certainly than the former. After hitting the target, the animal was required to release the joystick and press a button for getting reward.

A total of 70 neurons were sampled in the STN. Among them, 41 neurons which were distributed in the caudal part of the STN showed modulations of activity related to physical movements after the target hit similarly in both tasks. In almost half of them (n=20, type1), the firing rate in the reaching period was significantly higher than in the seeking period, while only 3 neurons (type2) showed lower firing rate. The rest (n=18, type3) did not show significant difference of the firing rate between the reaching and seeking periods, and mainly located in the lateral half of the somatomotor STN. Moreover, in 6 neurons of type1 and 5 neurons of type3, the average firing rate of the first 10 trials was higher than the last 10 trials in the reaching and/or seeking periods, while only one neuron of type2 and another of type3 showed higher firing rate in the last 10 trials. These neurons were found mainly in the medial half.

These results suggest that neuronal activity in the somatomotor STN is modified by uncertainty of accomplishment in goal-directed actions, and some neurons in its medial part are affected by habituation of the actions.

Áreas Temáticas:

1ª: Neurociencia cognitiva y conductual
2ª: Neurociencia de sistemas

NEW INSIGHT ABOUT GHRELIN –DEPENDENT SYNAPTIC EFFICACY IN HIPPOCAMPUS

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Ghrelin is a 28 amino acid peptide, which is synthesized both peripherally and centrally. The hippocampus is involved in learning and memory processes and it is a brain region that expresses high levels of Ghrelin receptors. Previously, we have shown that intrahippocampal administration of Ghrelin improves memory retention in a dose-dependent manner, and also decreases the threshold to induce Long Term Potentiation (LTP) in this structure, suggesting that Ghrelin could increase neuronal excitability in hippocampus. A critical requirement of glutamate NMDA-receptors containing NR2B-subunits for the LTP induction has been suggested, since ifenprodil, a NR2B-specific antagonist, completely blocks LTP induction in hippocampal slices. Genetic overexpression of NR2B-subunits can lead to an enhanced hippocampal LTP and improved learning and memory. In this work, we combined electrophysiology, evoked glutamate release from synaptosomes, behavioral paradigms, immunohistochemical detection, pharmacological NMDA-receptors blockade and hippocampal cell cultures in order to examine if synaptic efficacy induced by Ghrelin in hippocampus could be related to A) changes in glutamate release from synaptosomes, B) modification in intracellular levels of calcium in cultured pyramidal neurons, C) changes in the expression of the NR2B-containing NMD-receptors. We also study if Ghrelin reverted the cognitive deficit and LTP impairment induced by inhibition of NR2B-containing NMDA-receptors.

These results add new insight about the effect of Ghrelin upon the increase of synaptic efficacy in hippocampus, showing the first evidence that Ghrelin increased in about a 30% glutamate-release from synaptosomes as well as raised intracellular calcium release in neurons using Fluo3-AM. In addition, we demonstrated that acute Ghrelin administration induced increases in NR2B-subunit expression, and also reversed the memory deficits and LTP impairment induced by blockade of NR2B-containing NMDA-receptors using the selective antagonist Ro-26181.

In conclusion we demonstrated that Ghrelin modulates different pre- and post-synaptic events that could explain the Ghrelin-induced hippocampal plasticity facilitation and memory improvements.

Áreas temáticas:
1°: Neurociencia Cognitiva y conductual
2°: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.

PAPEL DE LAS CELULAS GLIALES EN LOS PROCESOS DE APRENDIZAJE Y MEMORIA

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La perpetuación de las memorias a partir de una memoria poco duradera, a menudo se explica mediante la síntesis de nuevas moléculas y cambios en la plasticidad sináptica. Este proceso se conoce como *consolidación* y puede revertirse mediante *reactivación*, recuperándose el estado lábil inicial. Esto conduce a un proceso similar al anterior conocido como *reconsolidación*.

La mayoría de las investigaciones sobre aprendizaje y memoria revelan el papel neuronal. Sin embargo, estudios recientes manifiestan la implicación de la glía en tales procesos.

Resultados de evitación pasiva demuestran la dependencia de las neuronas hipocampales al lactato liberado por los astrocitos en el proceso de consolidación. Defensa frente al estrés oxidativo, liberación de gliotransmisores, formación de sinapsis y otras funciones de los astrocitos amplían el foco de estudio hacia la cooperación entre glía y neuronas.

Nuestro objetivo es estudiar el papel de las células gliales, especialmente la microglía en los procesos que dan lugar al aprendizaje y la memoria.

Conductualmente hemos utilizado pruebas de reconocimiento de objetos y evitación pasiva para analizar los procesos de adquisición, consolidación y reconsolidación en ratones ante tres situaciones farmacológicas diferentes que modifican la función glial. En un primer experimento observamos el efecto de la D-serina, un coagonista de los receptores NMDA, liberado principalmente por células gliales. Posteriormente, estudiamos el comportamiento frente a la administración de minociclina, un inhibidor de la activación y proliferación de microglía. Y finalmente repetimos ambos paradigmas frente a la administración conjunta de ambas sustancias. Estos estudios se han complementado a nivel bioquímico estudiando la variación en el tiempo de la expresión de diferentes marcadores gliales ante una tarea de aprendizaje, así como los cambios morfológicos de la microglía y astrocitos reflejados mediante inmunohistoquímica.

La conclusión de este estudio es que las células gliales participan activamente en los procesos de almacenamiento de la información.

1ª: Neurociencia cognitiva y conductual
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

SENSORIMOTOR GATING AND SPEECH DISORDERS SHARE UNDERLYING FEATURES IN PARKINSON'S DISEASE?

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Objectives: We conducted a study in patients with Parkinson's disease (PD) and their corresponding controls, with the main objective to assess the alteration in this sensorimotor gating using the Auditory startle reflex (ASR) and its modulation through the Prepulse Inhibition (PPI), and temporal patterns of speech, correlating these factors.

Methods: 40 Patients diagnosed with PD and 23 controls took part of the study. After obtaining written informed consent, the ASR and PPI were evaluated using the SRH-Lab system (San Diego, CA). Voice recordings were register of a specific text given to the subjects with a professional recorder (Sony PCM-M10) and temporal patterns of speech were analyzed using the program Praat 5.1.42. Statistical analyses were performed using the SPSS program.

Results: Comparisons conducted in this study suggest significant differences in voice spectrographic records and in PPI measurements in patients with PD relative to controls.

Categorized depending on the average of prepulse inhibition, is associated to the kinesthetic stiffness, (defined as the percentages of non-speech periods and the number of pauses)**,** being higher at lower levels of PPI. Variations in the voice fundamental frequency of every 5 cycles (Jitter) are greater at lower levels of PPI. Finally, the minimum values of the fundamental frequencies of the voice decrease on those patients who have lower values in PPI 120.

We can't correlate changes in voice recordings in PD patients with the amplitude of the ASR with the data collected. However, ASR latency of PD subjects had significant correlation with several spectral noise features and Jitter.

Conclusions: PPI reflects an early stage of information processing, and the correlation with an alteration of the voice in PD patients could give us a clue for the mechanism underlying both processes.
Overall, simple and non-invasive tests such as ASR and voice analysis may identify early stages of PD.

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1ª: Neurociencia cognitiva y conductual

2ª: Nuevos métodos y tecnologías

ESTUDIO ANATÓMICO Y CONDUCTUAL DEL HÁMSTER EPILÉPTICO GASH:SAL EN REPOSO Y TRAS ESTIMULACIÓN AUDIOGÉNICA REPETITIVA

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Objetivos: para avanzar en la caracterización del hámster GASH:Sal (Genetic Audiogenic Seizure Hamster) como modelo de epilepsia, analizamos la morfometría del Complejo Olivar Superior (COS). Por otra parte estudiamos la evolución de las crisis epilépticas y los patrones de activación neuronal tras la inducción de las mismas de forma repetitiva (kindling).

Material y métodos: Empleamos hámster GASH:Sal y hámsteres dorados como control. Evaluamos estereológicamante el Núcleo Medial del Cuerpo Trapezoide (MNTB) y la Oliva Lateral Superior (LSO), mediante técnicas morfométricas y de reconstrucción tridimensional, de secciones teñidas con Nissl e inmunoteñidas para Calbindina. Los análisis comportamentales tras el kindling (dos estimulaciones acústicas diarias durante 10 días) se realizaron sobre las grabaciones de las crisis epilépticas, evaluando los diferentes ítems neuroetológicos con el programa ETHOMATIC. Las áreas cerebrales activadas se estudiaron mediante un estudio cuantitativo de la expresión de c-FOS en el encéfalo.

Resultados: El GASH:Sal muestra una disminución significativa del volumen y de las neuronas tanto del MNTB como de la LSO en comparación con sus controles. El kindling provoca un aumento en la complejidad de las crisis, correlacionado con un incremento en la activación neuronal de varias áreas encefálicas, fundamentalmente de regiones troncoencefálicas y límbicas como: colículo inferior, hipocampo, hipotálamo y amígdala.

Conclusiones: El GASH:Sal presenta disminución en el tamaño de los núcleos del COS estudiados, así como de sus neuronas constituyentes, lo cual podría estar relacionado con la naturaleza sonora del estímulo desencadenante.

El kindling aumenta la complejidad y severidad de las crisis, e involucra núcleos relacionados con el miedo, el dolor, el procesamiento del estímulo auditivo, el control de la musculatura y de las respuestas vegetativas entre otros.

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Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Trastornos y reparación del sistema nervioso

BDNF INDUCES STRIATAL-ENRICHED PROTEIN TYROSINE PHOSPHATASE DEGRADATION THROUGH THE PROTEASOME

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Striatal-enriched protein tyrosine phosphatase (STEP) has an important role in synaptic plasticity and neuronal function through the modulation of key signalling molecules (ERK1/2, p38, Fyn, Pyk2, NMDA and AMPA receptors). Brain-derived neurotrophic factor (BDNF) also contributes to the regulation of synaptic plasticity in the brain. While BDNF signaling has an important role in learning and memory and in plasticity processes, STEP normally opposes the development of synaptic strengthening. Here, we tested whether BDNF regulates STEP levels. When treating primary cortical neurons with BDNF (10 ng/ml; 24 h) we found a significant reduction of STEP levels compared to control cultures. To determine whether this was related to protein degradation or transcriptional down-regulation we exposed cultures to BDNF for shorter periods. Significantly lower STEP levels were found in cultures treated with BDNF for 5 min up to 6 h. This effect was prevented by the tyrosine kinase inhibitor K252a, but not by the MAPK inhibitor PD98059, the calpain inhibitor ALLN or the PI-3K inhibitor wortmannin. In contrast, by inhibiting the PLCgamma pathway (U73122), PKC (chelerythrine chloride) or the ubiquitin-proteasome system (MG-132) we were able to block the reduction of STEP levels in BDNF-treated cultures. In this line, STEP ubiquitination was higher in cortical neurons exposed to BDNF than in control cultures. To investigate the impact of BDNF-induced STEP degradation, we analyzed the phosphorylation level of two STEP substrates, GluN2B (Tyr^{1472}) and ERK1/2. The levels of pGluN2B (Tyr^{1472}) and pERK1/2 increased in cultures exposed to BDNF. However, they were significantly reduced in cultures where STEP degradation was prevented. Thus, BDNF-induced STEP degradation contributes to sustain high levels of pGluNB and pERK1/2. Importantly, we found that BDNF also induces STEP degradation in striatal and hippocampal neurons. In conclusion, our results show that BDNF effects are mediated, at least in part, through STEP degradation.

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Áreas Temáticas:
1. Neurociencia cognitiva y conductual
2. Trastornos y reparación del sistema nervioso

SEARCHING THE ORIGIN OF THE APPETITIVE CONDITIONING PLASTICITY

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Aims

Dopamine neurons of ventral tegmental area are activated by environmental cues that predict rewards. The ventral tegmental area is densely innervated by glutamatergic axons originated in many brain centres. It has been shown using in vitro patch-clamp that over the course of a cued-reward learning it is produced an increase in the synaptic strength of these glutamatergic synapses. Here, we wanted to elucidate which glutamatergic synapses are potentiated when animals associated a cue with a reward.

Materials and Methods

In order to identify potentiated glutamatergic synapses, we recorded activity-dependent changes in synaptic strength of the medial prefrontal cortex – ventral tegmental area and of laterodorsal tegmental nucleus – ventral tegmental area synapses. Mice had to learn a simple appetitive conditioning schedule during the simultaneous recording of the field excitatory postsynaptic potentials (fEPSPs) evoked in the ventral tegmental area when we stimulated in the medial prefrontal cortex and in the laterodorsal tegmental nucleus. Conditioning was carried out using a light, as conditioned stimulus, located over the feeder. On subsequent days, mice underwent Pavlovian conditioning in which a 10-s light presentation was followed by the delivery of a food pellet (20 mg).

Results

Animals establish the association between a cue and a reward in 7-10 sessions. Mice explored the feeder during more time when the cue (light) was on than when it was off. Preliminary results show that the amplitude of the fEPSP evoked in the ventral tegmental area when we stimulated in the medial prefrontal cortex is potentiated during the acquisition of this appetitive conditioning. In addition, the laterodorsal tegmental area – ventral tegmental area synapse did not change in strength during the acquisition of the appetitive conditioning.

Conclusions

We thought that, if we confirm these preliminary results, it could mean that for the enhanced of dopamine release to reward predictive cues it is necessary the potentiation of the synapse that carried information from the prefrontal cortex to the dopaminergic neurons located in the ventral tegmental area.

Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Neurociencia de sistemas

ROLE OF NPYY2 RECEPTOR IN ANXIETY: INTERACTIONS WITH THE N-TERMINAL GALANIN FRAGMENT (1-15)

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Previously, we demostrated that Neuropeptide-Y receptors interacts differentlly with Galanin(GAL) and the N-terminal fragment [GAL(1-15)] at membrane level. In the nucleus of the solitary tract, GAL but not GAL(1-15) modified the action of NPYY1 agonist and on the contrary, GAL(1-15) but not GAL facilitates the cardiovascular action of NPYY2 agonist.

In this work, we studied the interaction between GAL(1-15) and NPYY2 receptor using unconditioned anxiety-like tests and autoradiography. First we analyzed the effect of NPYY2 agonist NPY(13-36) on the behavioral tests.

Groups of rats (n=8-10) were intracerebroventricularly injected with NPY(13-36) 3nM or vehicle. In a second set of experiments, NPY(13-36)(3nM) and GAL(1-15)(1nM) were injected alone or in combination. All groups were inyected fifteen minutes before starting 5-minute session in the open field (OF) or the elevated plus maze (EPM). We analyzed the number of entries and time in the central square of OF and the percentages of entries and time in the open arms of EPM.

We also analyzed the binding of the NPYY2 agonist [^{125}I]-PYY(3-36) (25pM) in the presence of GAL(1-15) at the doses of 0.3nM, 1nM, 3nM and 10nM.

NPY(13-36) significantly decreased the parameters examined in OF ($p<0.05$) and EPM ($p<0.01$) as compared with vehicle. The coadministration of NPY(13-36) and GAL(1-15) did not modify the effect of NPY(13-36) alone. In autoradiography studies, GAL(1-15) 3nM and 10nM increased the NPYY2 agonist binding in the CA3 region of the hippocampus by 8% ($p<0.01$).

These results show an anxiogenic effect of NPY(13-36) in behavioral tests. GAL(1-15) interacts with NPYY2 at receptor level but not in the inconditioned anxiety-like test. These results may be of relevance for NPYY2 and GAL(1-15) mediated actions in the central nervous system. This work has been supported by the Junta de Andalucia CVI646 and TV3-Marató 090130/31/3.

1ª: Neurociencia cognitiva y conductual
2ª: Neurociencia de sistemas

LA MADURACIÓN DE LAS NUEVAS NEURONAS HIPOCAMPALES ESTÁ INVOLUCRADA EN LOS PROCESOS COGNITIVOS

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La neurogénesis hipocampal adulta es el proceso continuo de generación de nuevas neuronas en el hipocampo adulto, un área cerebral relacionada con los procesos cognitivos. Estas neuronas recién generadas migran y maduran en la capa sub-granular del giro dentado (DG), hasta insertarse en circuitos funcionales. Ante todo esto, ¿cuál es el papel de la neurogénesis adulta en la función del hipocampo? Nosotros hemos estudiado el papel de la ablación de las neuronas inmaduras en las distintas fases de los procesos cognitivos.

Aprovechando que las neuronas inmaduras del DG son sensibles a la radiación ionizante, hemos desarrollado un protocolo de irradiación con rayos X sobre ratones despiertos e inmovilizados que provoca la ablación rápida de neuronas inmaduras (caracterizada por RT-PCR e inmunohistología) y que permite realizar pruebas conductuales pocas horas después de realizar la irradiación. Así, con ratones irradiados en distintos momentos con respecto a la sesión de entrenamiento de reconocimiento de objetos o evitación pasiva (paradigmas dependientes de hipocampo, no emocional y emocional respectivamente), encontramos que la ablación de nuevas neuronas inmaduras produce deficiencias de adquisición y memoria con una temporalidad diferente en función de la prueba utilizada. Además, realizamos la caracterización electrofisiológica *in vivo* del modelo de ablación.

Por lo tanto, nuestros resultados indican que las nuevas neuronas adultas, en el momento adecuado de acuerdo a la prueba de comportamiento, desempeñan un papel importante en los procesos cognitivos dependientes de hipocampo.

Áreas Temáticas:

1ª Neurociencia cognitiva y conductual
2ª Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

AN ENRICHED ENVIRONMENT REVERTS COGNITIVE AND NEUROVASCULAR DEFICITS PRODUCED BY THE KINASE INHIBITOR OF VEGFR-2 VANDETANIB IN SENSORY AREAS

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Abstract

An enriched environment has been shown to significantly facilitate recovery from brain injury due to important cortical changes that occur mainly during the critical period. Vandetanib, is a small-molecule tyrosine kinase inhibitor (TKI) that targets VEGF receptor (VEGFR2-3) signalling which apart from angiogenesis processes is involved in neuroprotective, neurotrophic and neurogenic pathways. Our aim is to investigate neurovascular and cognitive effects of Vandetanib oral-administration and the role of enriched environment to Vandetanib effects counteract during the critical period of the rat sensory systems development.

Long Evans rats were reared under two different conditions: standard laboratory condition (SC) and enriched environment (EE), both groups with Vandetanib administration and with vehicle administration from P21 to P28. Visuospatial learning was tested with Morris Water Maze. Then rats were perfused and brains were removed. Vascular density was measured by Butiril Cholinesterase histochemistry and neuronal density by NeuN inmunohistochemistry. Quantifications were performed by optical dissector method in layer IV of rat visual and somatosensory cortices.

Results showed a significantly higher neurovascular density in the visual cortex of the two groups reared in an enriched environment compared to standard reared groups. This significance could also be observed in control groups in relation to Vandetanib or vehicle administration. Interestingly, this effect was not seen in the two groups of rats that have been raised in enrichment environment (with and without Vandetanib). In the somatosensory cortex, vascular density increase in enriched environment animals but neuronal population remains in control levels. Overall, neuronal and vascular densities and the learning curves of the behavioral test were very similar in both of them; suggesting that rearing the animals in an enriched environment can reverse the effects of VEGF receptor inhibition during in the rat sensory areas.

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Áreas Temáticas:
1. Neurociencia cognitiva y conductual
2. Trastornos y reparación del sistema nervioso

PARTICIPACIÓN DE LOS NÚCLEOS DEL CEREBELO EN EL CONDICIONAMIENTO CLÁSICO DEL REFLEJO CORNEAL EN RATONES DESPIERTOS

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El condicionamiento clásico del reflejo corneal es uno de los modelos experimentales más utilizados para el estudio de los mecanismos neuronales que subyacen a la adquisición de nuevas habilidades motoras y cognitivas. En la actualidad, existen dos interpretaciones diferentes acerca del papel del cerebelo en la adquisición y almacenamiento de respuestas palpebrales aprendidas mediante condicionamiento clásico. Una teoría propone que el cerebelo es el sitio donde dicho aprendizaje tiene lugar y donde se almacena, mientras que la opuesta sugiere que el cerebelo participa en la realización de movimientos palpebrales tanto reflejos como aprendidos. A fin de analizar estas teorías antagónicas, se prepararon ratones para el condicionamiento clásico del reflejo corneal y el registro de la actividad unitaria de neuronas identificadas de los núcleos del cerebelo. Se implantaron electrodos crónicos a los ratones para el registro de la actividad eléctrica del músculo orbicular de los párpados. También se registró el movimiento de los mismos con ayuda de un seguidor magnético de la posición. Los animales se condicionaron con sonidos como estímulo condicionado y un choque eléctrico en la rama supraorbitaria del trigémino como estímulo incondicionado, y se prepararon para el registro de la actividad eléctrica de neuronas ubicadas en los núcleos interpósito y dentado del cerebelo. Se hicieron condicionamientos de traza y de demora. Las neuronas se identificaron mediante su activación antidrómica desde el núcleo rojo contralateral. Los resultados obtenidos indican que neuronas de los núcleos cerebelosos presentan frecuencias de potenciales de acción relacionadas con la activación del músculo orbicular de los párpados y con el movimiento de los mismos. Existen varios tipos de neuronas, clasificables según su actividad durante el condicionamiento. El cerebelo parece relacionarse más con la realización del movimiento aprendido que con su adquisición.

MOVEMENT PREPARATION FROM A PASSIVE PERSPECTIVE: HUMAN MOTOR SYSTEM PREPARATION TO OBSERVE A MOVEMENT

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Objective: The corticospinal tract excitability is modulated when preparing movements. Earlier to movement execution the excitability of the spinal cord increases waiting for supraspinal commands to release the movement. Movement execution and movement observation shares processes within the motor system, though movement observation research has focused on processes later to movement onset.

Methods: We used single and paired pulse TMS on M1 (n=12), and electrical cervicomedullary stimulation (n=7), to understand the modulation of the corticospinal system during the "preparation" to observe a third person's movement. Subjects passively observed a hand which would remain still or make an index finger extension.

Results: The observer's corticospinal excitability rose when "prepared to see a movement" vs. when "prepared to see a still hand". The modulation took origin at a spinal level and not at the cortico-cortical networks explored.

Conclusions: We conclude that expectancy of seeing movements increases the excitability of the spine. Our spinal cord gets prepared to observe movements.

Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Neurociencia de sistemas

IMMUNOHISTOCHEMICAL C-FOS EXPRESSION AND AUTORADIOGRAPHY TO STUDY GALANIN/NEUROPEPTIDE Y Y1 RECEPTOR-RECEPTOR INTERACTION IN THE AMYGDALA

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We have shown Galanin(GAL)/Neuropeptide Y Y1 receptor(Y1) interactions in the nucleus tractus solitarius and the arcuate nucleus. Since both peptides play an important role in mood disorders, the aim of this work was to study GAL/Y1 interactions in the amygdala(AMY), key nucleus for fear, mood, and motivation. We have combined the analysis of the expression of c-Fos immunoreactivity(c-Fos IR) with an autoradiographic study in the AMY. Groups of anaesthetized rats (n=4) received intracerebroventricular injections(icv) of GAL(3nmol) and the Y1 agonist Leu^{31}-Pro^{34}NPY(3nmol) alone or in combination, and were sacrificed 90 minutes after the injections. Immunohistochemical detection of c-Fos protein(1:5000) in AMY sections and stereological analysis were performed in: Basal(BA), lateral(LA), Central [lateral capsular subdivision(CeC), lateral intermediate subdivision(CeI), medial subdivision(CeM)] and the medial paracapsular intercalated(ITC) subnuclei of the AMY. For Autoradiography, rats (n=6) were sacrificed 15 minutes after icv injections of GAL(3nmol) and AMY sections were incubated with Y1 agonist [125I]-Leu31-Pro34-PPY (25 pM). Autoradiograms were analyzed using the NIH image analysis system. Student's unpaired t-test and ANOVA followed by Newman-Keuls were used, respectively. We observed within the AMY that GAL increased c-Fos IR in ITC and CeC; the Y1 agonist induced both, an increase of c-Fos IR in BA and CeC and a decrease of c-Fos IR in LA and ITC. The coadministration of both peptides induced a specific effect in the ITC, significantly decreasing the c-Fos expression ($P<0,05$) induced by GAL or the Y1 agonist alone. Moreover, we observed that GAL significantly increased ($p<0,05$) the Y1 receptor agonist binding [I125]Leu31Pro34-PYY in the AMY. These results demonstrate an interaction between GAL and Y1 at the cellular and receptor level in the AMY and suggest that endogenous GAL and NPY system might interact to regulate emotional behaviours. Study supported by Spanish CVI6476 and TV3-Marató 090130/31/32.

1ª: Neurociencia cognitiva y conductual
2ª: Neurociencia de sistemas

EFFECT OF TIBOLONE ON MEMORY AND LEARNING IN AGING MOUSE

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Sex steroids exert different effects in the central nervous system (CNS), such as preserving neural function and promoting neuronal survival. Therefore, the age-related decrease in sex steroids may have a negative impact on neural function. Progesterone, testosterone and estradiol prevent neuronal loss in the CNS in different experimental animal models of neurodegeneration. However, hormone replace therapy may increases the incidence of endometrial, prostate and breast cancer. A strategy to reduce these latter is the use of tibolone (TIB), which has estrogenic and progestagenic metabolites. However, the role of TIB in the process of learning and memory in aging is unknown. The aim of this study was to evaluate the long term effect of TIB (0.01 and 1 mg/Kg orally daily for 12 weeks) on the memory and learning of male aging mice. For this purpose, three behavioral animal models (T maze, object and object-in-context recognition tasks) were employed. In the T maze there was an increased latency in both TIB group. In the object and object-in -context recognition tasks, administration of TIB mice increased the percentage of time spent investigating the novel object. These data suggest that TIB improve the memory and learning in aging mouse.

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Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Sistemas homeostáticos y neuroendocrino

A2A ADENOSINE RECEPTORS AS THERAPEUTIC TARGETS IN SCHIZOPHRENIA: BEHAVIOURAL CHARACTERIZATION OF A2A KNOCKOUT MICE

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Schizophrenia is a chronic and severe mental disorder with a presumed neurodevelopmental origin that lacks of an effective treatment. Hence, the identification of new potential pharmacological targets has great clinical interest. One promising candidate is the nucleoside adenosine that modulates dopamine and glutamate systems, both implicated in the pathophysiology of schizophrenia. Therefore, the aim of the present study was to use A2a receptor knockout (KO) mice with complete and specific inactivation of the A2a receptor (Ledent et al., 1997) as an endophenotype model of schizophrenia. To achieve this goal, we performed different behavioural paradigms to assess social, emotional and cognitive alterations in adult (3-6 months) male KO and wild-type (WT) littermates. Social interaction and nesting tests were used to evaluate social behaviour, tail suspension test constituted a measure for despair-like behaviour and the object recognition test and the passive avoidance paradigm were used to evaluate cognitive alterations. Our results showed an overall impairment in the social skills in KO mice evaluated with the social interaction test; although no differences were observed in the nesting test. Moreover, these animals showed a depressive like-response seen by an increase in the immobility time in the tail suspension test. Finally, both cognitive tests pointed out to learning alterations in KO animals. Either the object recognition test, a task dependent on the hippocampal formation and the passive avoidance paradigm, a test dependent on the prefrontal cortex and the amygdala showed learning and memory deficits in KO mice. All together, our results indicate that A2a KO mice exhibit many typical negative and cognitive hallmarks of schizophrenia and propose these animals as a new mouse model of the disorder.

EARLY LIFE INFLUENCES ON EMOTIONAL REACTIVITY, SOCIAL BEHAVIOR AND NEUROINFLAMMATION IN MICE

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Early life experiences play a key role in shaping brain and behavior. The aim of the present study was to evaluate the effects of different early rearing conditions on emotional reactivity, social behavior and neuroinflammation in male and female adolescent CD1 mice. "Maternal separation with early weaning" (MSEW) was used as a pattern of early life adversity. In MSEW, pups were separated from the dam various hours per day between postnatal day (PD) 2-16 and were weaned earlier on PD17. A model called "communal nest" (CN) was used as a model of social enrichment. In CN, three females share the care-giving behavior, and pups were weaned on PD25. Thus, at PD30 standard-reared animals and mice exposed to MSEW or CN were subjected to several tests to assess locomotor activity, anxiety- and depressive-like responses (elevated plus maze, tail suspension test and saccharin test), and social behavior (nesting test and social interaction test). Additionally, microglial activation was assessed by inmunofluorescence in the hippocampus, an important limbic brain area implicated in emotional responses. The results showed a hypolocomotor phenotype in MSEW male. Furthermore, although MSEW and CN mice exhibited increased anxiety levels on the elevated plus maze, MSEW mice showed the highest percentage of immobility in the tail suspension test. MSEW mice also showed significantly lower preference to saccharin solution. No differences were observed in social behavior. Finally, immunofluorescence studies showed higher activation of hippocampal microglia in females from MSEW and CN groups. Our results suggest that MSEW could be a good model to study emotional alterations after early life neglect and the existence of a link between emotional disorders and neuroinflammation. The present results cannot confirm the protective role of CN because mice subjected to this rearing condition pointed out increased anxiety and despair responses.

UN ESTUDIO ELECTROFISIOLÓGICO SOBRE LA ATENCIÓN EXÓGENA A DISTRACTORES EMOCIONALES PRESENTADOS EN LA PERIFERIA Y EN EL CENTRO DEL CAMPO VISUAL

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Dado que existen estímulos ambientales potencialmente relevantes para la supervivencia, en el cerebro se han desarrollado mecanismos eficientes para detectar estos estímulos con el fin de evitar posibles consecuencias dañinas para el organismo. Estos mecanismos hacen referencia a la *atención exógena*, la cual dirige automáticamente nuestros recursos cognitivos hacia estímulos con saliencia biológica. El objetivo de nuestro experimento fue estudiar si los distractores presentados en la periferia del campo visual capturan la atención exógena en la misma o diferente medida que los presentados en el centro del campo visual. Para ello registramos los potenciales relacionados con acontecimientos discretos (PRAD) mediante 59 electrodos distribuidos homogéneamente por el cuero cabelludo en 22 sujetos. La tarea se presentaba en el centro de la pantalla y consistía en categorizar dígitos, debiendo evitar en todo caso desplazar la mirada hacia los lados. Simultáneamente se presentaron distintas imágenes con diferente carga emocional (negativo, neutro, positivo) como estímulos distractores. Éstos podían aparecer en el centro de la pantalla o en la periferia (50% hemicampo visual izquierdo, 50% hemicampo visual derecho). Conductualmente se observó una mayor interferencia de los distractores emocionales negativos que de los neutros (mayores tiempos de reacción en la tarea de categorización de dígitos). Electrofisiológicamente, el componente P2 de los PRAD, tradicionalmente considerado un índice electrofisiológico de atención exógena, presentó una mayor amplitud ante los distractores emocionales negativos que a los neutros con independencia de su localización espacial (centro o periferia). En conclusión, la atención exógena es sensible a la saliencia biológica de la emoción con independencia de la localización espacial.

Área Temática: Neurociencia cognitiva y conductual.

PROTECTIVE ROLE OF B-CATENIN OVEREXPRESSION IN HIPPOCAMPAL PROGENITOR CELLS IN AN ANIMAL MODEL OF DEPRESSION

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It has been suggested that antidepressants may, at least in part, exert their neurogenic effects in the subgranular zone of the hippocampus by increasing levels of β-catenin. In fact, the regulatory effect of β-catenin (a key mediator in the Wnt signaling pathway) in neuronal differentiation/proliferation is proposed to be contributing to mood disorders pathophysiology within the context of neurogenesis and neuroplasticity in these diseases.

We generated conditional inducible mice overexpressing (TG) β-catenin in progenitor cells of the subgranular zone (SGZ) of hippocampus to further understand the role of β-catenin in depression/anxiety disorders. This transgenic line expresses CreERT under the control of the astrocyte-specific glutamate transporter (GLAST) promoter inducible by tamoxifen administration. We tested several depression and anxiety-related behavioral responses in a time-dependent way after one and two months of tamoxifen administration. Additionally, we compared the results obtained in a model of depression induced by chronic corticosterone.

TG mice exhibited less anxiety (reduced latency to feeding) in the novelty suppressed feeding (NSF) one month after tamoxifen injection ($p<0.05$), an effect that was more evident two months later (142.1±40.3 in TG *vs* 492±40.2 in WT; $p<0.01$). However, no differences were found in the forced swimming (FST), open field (OF) or sucrose consumption tests. In addition, corticosterone-induced anxiety in the NSF was attenuated in TG mice (539.4±34.3 in WT *vs* 301±80.6 TG). Regarding FST, chronic corticosterone-WT mice showed a significant increase in the immobility time compared with vehicle mice. However, corticosterone-TG mice did not exhibit increased immobility.

This differential pattern of responses suggests a "protective" role of β-catenin overexpression against the depression and anxiety effects induced by chronic corticosterone.

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Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia cognitiva y conductual

BEHAVIOURAL AND BIOCHEMICAL CHANGES IMPLICATED IN EPIGENETIC MODULATION IN SAMP8: EFFECT OF ORY-2001 (A DUAL LSD1/MAOB INHIBITOR).

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Alzheimer's disease (AD) is a progressive, irreversible neurodegenerative disorder culminating in dementia. Its etiology and pathogenesis are complex, and encompass many genetic and enviromental risk factors, changes in the expression of thousands of genes, and up-regulation of multiple pathogenic pathways.
SAMP8 mice are an accelerated aging mouse model which presents AD pathogenic pathways such as overproduction of beta-amyloid, tau kinases, inflammation and oxidative stress.
Overall, epigenetic changes during aging of SAMP8 are beginning to be reported. In this work, we focus on the participation of epigenetics in the senescence process ocurring in SAMP8 and the effect of ORY-2001, a dual inhibitor of Lysine-Specific Demethylase 1 (LSD1) and MAOB in behavioural and biochemical parameters. We determined a loss of memory and learning capabilities with age in SAMP8 by means of the Novel Object Recognition Test (NORT).
Preliminary analysis of untreated animals showed that several relevant proteins are differentially expressed in the brain of untreated SAMP8 vs SAMR1 as a control mice. Most notably, SAMP8 mice exhibited a slight increase on LSD1 and RCOR2 expression levels, pointing to the possible relevance of LSD1 inhibitors in this model. LSD1 and RCOR1/2 are present in repressive protein complexes recruited at REST (RE1 containing) target sites, and have been implicated in the down-regulation of BDNF expression in some neurodegenerative diseases. Confirming previous reports, BDNF has been found to be downregulated in SAMP8 vs SAMR1 mice.
Treatment with ORY-2001 dramatically rescues memory and learning capabilities of SAMP8 mice, as determined by the Novel Object Recognition Test (NORT) in 5 months-old mice treated for 8 or 16 weeks, suggesting that LSD1 is a potential target for AD treatment.
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Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual.
2ª: Neurociencia de sistemas.

HIPPOCAMPAL ARC AND N-CADHERIN DECREASE IN 5-HT$_{1A}$ OVEREXPRESSING MICE

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The activation of postsynaptic serotonin1A receptors (5-HT$_{1A}$) exerts anxiolytic actions when activated. The modulation of this serotonergic subtype is implicated in mood related disorders. Changes induced by its activation involve dendritic elongation and sprouting in neurons of forebrain regions. Here, we have used a mouse overexpressing (OE) the 5-HT$_{1A}$ receptor in serotonergic projection areas (prefrontal cortex and hippocampus) to characterize different signaling pathways related to synaptic plasticity. We analyzed using western blot the hippocampal protein expression of relevant signaling pathways: CREB, Akt, ERK, β-catenin, mTOR, and their phosphorylated state, proteins present in the synapse as N-cadherin, and the mRNA expression of early genes by qPCR. We also tested hippocampal proliferation by BrdU immunohistochemistry.

5-HT$_{1A}$ OE mice exhibit a decrease in the activated form of Akt (pAkt/Akt) (67.4±9.3 *vs* 100.0±12.5 for WT mice; $p<0.05$) from the PI3K pathway. Other elements of different pathways as pmTOR/mTOR (rapamycin/mTOR pathway), pERK/ERK (MAPK pathway), and β-catenin, were not modified. Downstream, the activation of the transcription factor CREB (cAMP response element-binding; pCREB/CREB) was not modified. One of the genes regulated by CREB is the brain derived neurotrophic factor (BDNF). However, both protein and mRNA BDNF expression and the activation of its receptor (pTrkB/TrkB) were not changed in the 5-HT$_{1A}$ OE mice. Other gene transcription regulated by CREB is the immediate early gene Arc, which mRNA expression was significantly decreased in OE mice (0.52±0.07 *vs* 1.00±0.03 for WT; $p<0.01$). The expression of N-cadherin was reduced (41.8±7.6 *vs* 100±19.2 for WT mice; $p<0.01$). There were no changes in hippocampal proliferation.

In conclusion, these mice show no changes in hippocampal proliferation, confirmed by the lack of changes in β-catenin. However, the decrease in N-cadherin together with Arc, suggests structural changes in the synapse which may explain the neurological alterations elicited by this transgenic mice.

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Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia cognitiva y conductual

HUE DISCRIMINATION IN ABSOLUTE PITCH POSSESORS

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Absolute pitch (AP) is a rare ability that consists in labelling automatically and without effort any tone of the chromatic scale in the absence of an external reference. Absolute pitch is a good model to study interindividual variability as it represents the possession of an exceptional capability in an otherwise normal population.
Recent reports have shown the existence of distinctive connexions among cortical areas and activity patterns in AP possessors. These differences provide a higher degree of efficiency in the functional connectivity among different clusters, including regions known to participate in multisensory integration. These evidences open the possibility to the idea that sensorial modalities different to sound perception may be processed differentially in AP possessors. Our objective was to develop a quantitative test to measure luminosity and hue discrimination together with a pitch test.
All participants were professional or advanced student musicians The pitch test consist in labelling pure tones corresponding to the occidental chromatic scale with their accidents between 130.37 and 1980 Hz. Subjects were categorized as AP1 if the mean deviation of their responses is less than 0.5 semitones, AP2 if the deviation is in the 0.5-1.5 semitones and non-AP if the mean deviation is above 1.5 semitones.
The hue test is a 16x16 matrix of rectangles composed of an ordered hue gradient created in the HVS space. During the test the hue of a random rectangle is replaced by the hue of another one present in the matrix, being the task to mark the hue in the correct matrix location. Our preliminary results, based on a limited set of data suggest similar discrimination abilities in AP and non-AP subjects.

Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Neurociencia de sistemas

A COGNITIVE-BEHAVIORAL MANAGEMENT OF BRUXISM.

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Bruxism is one of the most frequently parafunctions leading to dental problems. This, added to the lack of agreement for a suitable treatment strategy, makes non-invasive therapies such as myofeedback arise greater interest. Although different etiopathological factors have been described in literature, the psychological factor is becoming more relevant (stress and anxiety). Myofeedback has been used as a cognitive-behavior therapy for bruxism with successful outcomes. Due to the lack of similar studies, it seems reasonable to do further studies along these lines and during longer periods which include a greater number of subjects. 79 both bruxism and non-bruxism subjects underwent a medical history, a questionnaire, an intraoral and extraoral examination, a recording of the electromyogram (EMG) activity and a myofeedback test with visual alert. The test was performed in masseter and temporal muscles and was divided in three stages: an initial baseline recording, myofeedback recording, and a final baseline recording. A significant decrease of the EMG mean amplitude for the masseter muscle was observed between the initial recording and the myofeedback recording ($p<10^{-4}$), as occurred for the EMG mean amplitude in temporal muscle ($p=0.0013$), and EMG maximum amplitude in masseter ($p<0.001$) and temporal ($p<10^{-6}$) muscles. Also, a significant decrease in EMG maximum amplitude between initial recording and final recording was observed for the masseter muscle ($p=0.0236$) and temporal muscle ($p=3.01x10^{-6}$). It was not significant for the EMG mean amplitude, although it was observed a decreasing tendency. We also noticed a similar tendency along sessions. This study leads us to conclude that although it is observed a direct effect on the decrease of the EMG activity with the muscle relaxation during the myofeedback test, the small sample size and the limited number of sessions, encourages to do further research to confirm a long-lasting effect.

Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual.
2ª: Nuevos métodos y tecnologías.

CORRELATION BETWEEN NEUROPEPTIDE QUANTIFICATION, INTERNEURON SUBPOPULATIONS OF V1 LAYER IV AND COGNITION DURING THE CRITICAL PERIOD OF ENVIRONMENTALLY ENRICHED RATS

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Objectives: Enriched environment (EE) induces cellular, molecular and behavioural effects. Moreover, EE also increases neurogenesis in the hippocampus and increases the expression of angioglioneurins such as VEGF or BDNF. During the course of development, GABAergic interneurons contribute to key aspects of the functional maturation of the cortex in different ways. The maturation of GABAergic neurotransmission takes place in two steps and results from the dynamic interaction between developmentally directed gene expression and brain activity. A paradigmatic example of GABAergic modulation is the Layer IV of the visual cortex, the site where afferences from the thalamus arrive. Our aim was to investigate the effects of EE in visual and spatial learning and memory, the expression of neuropeptides and the subpopulations of GABAergic interneurons at the peak of the visual critical period.

Material and methods: Standard and environmentally enriched Long Evans rats were studied at P28. Spatial and visual memory were studied by the Object Displacement and Recognition Tests respectively. Neuropeptide expression was studied by Mass Spectrometry and Neuron subpopulations by Stereology on NeuN, SOM and PV immunochemistry.
Results
GABAergic interneurons are key regulators of the critical period, with a balance between SOM+ and PV+, that constitute the majority of the local networks at this layer. Our results show that EE improves visual but no spatial memory and increases the ratio of SOM+ interneurons in the Layer IV of V1, as PV+ subpopulation is significantly lower.

Conclusion: Apart from GABAergic neurotransmission, interneurons play a key role in neuromodulation, by the secretion of neuropeptides whose role still remains unknown. We have described a group of neuropeptides that are overexpressed in the visual system, that are related to the main interneurons in layer IV.

Áreas Temáticas: Seleccione las 2 áreas temáticas que más se ajusten a su trabajo en orden de prioridad:
1ª: Neurociencia cognitiva y conductual
2ª: Neurociencia de sistemas

SCOPOLAMINE-INDUCED SHORT-TERM MEMORY DEFICIT IN MICE: REVERSAL BY A NOVEL COMPOUND CODED ASS234.

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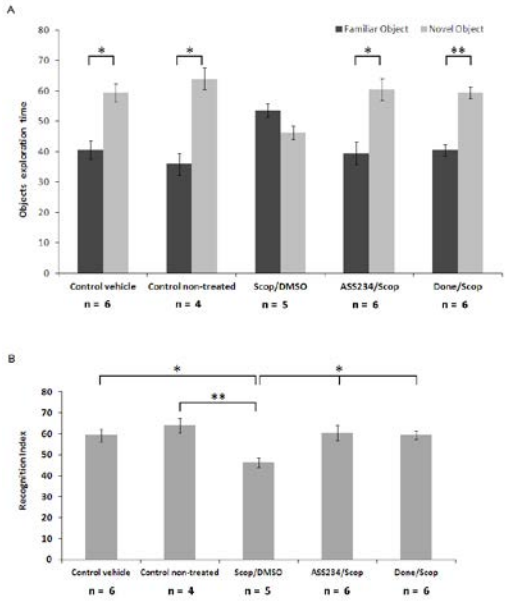
Background: Alzheimer´s disease (AD), the most common form of degenerative dementia, is a complex heterogeneous disease with multiple cellular changes implicated in its pathogenesis. Cholinergic impairment has been found responsible for cognitive decline and social performance observed in patients. Although stimulation of the cholinergic system results in the satisfactory improvement of AD cognitive symptoms, aproved treatments only provide transitory and modest healing in cognitive impairment without affecting the natural progression of the disease. Thus, drug discovery in the field of AD is currently directed to develop drugs to halt the progression of the disease. This study examines the effect in cognition of our hit compound ASS234, a multipotent acetylcholinesterase, butyrylcholinesterase (AChE, BuChE)/monoamine oxidases (MAO A and B) inhibitor with a potent amyloid beta-aggregation inhibitory action as well as antioxidant antiapoptotic properties.



Methods: The effect of ASS234 on the cognitive functions in C57BL/6J mice treated with scopolamine was evaluated using the object recognition task.

Results: Robust effect of single-dose ASS234 on cognition was consistently observed.

Conclusion: In the search of drugs with therapeutic potential for the treatment of AD, herein we report that ASS234 significantly lowers scopolamine-induced learning deficits in healthy adult mice, suggesting this multitarget molecule as a promising alternative drug of choice to treat the cognitive decline underlying in AD.

Figure: Illustrates the effect of combined administration of scopolamine/DMSO, donepezil and ASS234 on individual object exploration time (A) and RI (B) in T2. (A) Both donepezil and ASS234 significantly increase the novel object exploration time (**p<0.01 and *p<0.05, respectively). (B) Shows significant differences between Scop/DMSO and Control non-treated groups (**p<0.01). n Means the number of experimental animals per group. The percentage of the novel object recognition time was evaluated as a RI= (tNovel Object /[tNovel Object+tFamiliar Object]) × 100. (Supported by MICINN SAF2010-15173; SAF2012-33304; SAF2009-07271; EU COST Action CM-1103).

Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Trastornos y reparación del sistema nervioso

METHYLPHENIDATE INDUCES C-FOS IN RAT MEDIAL SEPTUM.

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The methylphenidate is a common treatment for the attention deficit with hyperactivity disorder. The septal area is considered a key component of the mechanisms underlying arousal and attention.
Aims: The aim of this study was to map the acute effect of methylphenidate over c-fos expression in the septal area and other brain areas including, the nucleus accumbens, habenula, hippocampus, amygdala and nucleus incertus.

Material and Methods: Rats were orally administered with MPD at doses of 1.3 and 2.7 mg/Kg. c-fos expression in different areas was detected by immunohistochemistry. Double immunofluorescence was used to characterize neurons that were positive for c-fos using calcium-bindign proteins (palvalbumin, calretinin and calbindin 28kD) antibodies.

Results: We did not observed significative differences in any of the studied areas at the lower doses of 1.3 mg/Kg. However, at the high doses of 2.7 mg/Kg an increase in c-fos activation was observed in two septal nuclei: the vertical medial septum and the dorsal part of the caudal lateral septum.
c-fos activated cells occupied a lateral compartment within the medial septum which did not contained parvalbumin positive neurons, however a few of these cells were c-fos activated. Some c-fos activated medial septal neurons also displayed immunofluorescence to calretinin or calbindin 28kD.

Conclusion: These results show that one of the first targets of MPD treatment is the ventral part of the medial septum which is involved in the generation and maintenance of hippocampal theta rhythm. On the other hand, the dorsal part of the caudal lateral septum is involved in social behaviours that is also affected in attention deficit with hyperactivity disorder.

Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª. Neurociencia de sistemas

LONG-TERM BEHAVIORAL EFFECTS AFTER A CHRONIC ORAL EXPOSURE TO LOW DOSES OF CHLORPYRIFOS IN APOE2, APOE3 AND APOE4 TRANSGENIC MICE

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Chlorpyrifos (CPF) is one of the most widely used organophosphate pesticides over the world, in intensive agriculture and livestock. According to the literature, both acute and chronic exposures to CPF induce a wide range of neurotoxic effects in adult mammals. This pesticide induces cognitive impairments and neuronal damage, which suggest a possible relationship between CPF exposure and Alzheimer's disease (AD) or cognitive impairment in aged population. Genetics, gender or age can provide distinct protection or vulnerability to AD. According to this, being carrier of the ε4 allele of the apolipoprotein E (ApoE) gene is a well-established risk factor to develop AD. The present study aims to evaluate physical and behavioral effects in ApoE transgenic male mice carrying different polymorphisms of human ApoE (ε2, ε3, ε4) after a chronic oral exposure to low doses of CPF. We fed homozygous ApoE2, ApoE3 and ApoE4 mice with a diet supplemented with 2 mg CPF/kg, or the respective control diet, during 13 weeks. After that, animals were maintained without treatment during a six months washout period. Changes in subject's body weight were monitored. Learning and memory were assessed in a Barnes maze during the last treatment week and in a Morris water maze at the end of the washout period. We found a gradually significant increase in treated ApoE3 body weight compared to their respective control all along both treatment and post-treatment periods. General genotype effects were observed in all behavioral tests. Regarding Barnes maze results, treated ApoE3 showed an impaired retention compared to their respective control. Concerning Morris water maze results, an interaction between genotype and treatment during the acquisition period was detected. These results indicate differential acute and delayed effects of CPF exposure depending on ApoE genotype.

Thematic areas:
1º Cognitive and Behavioral Neuroscience
2º Disorders and nervous system repair

MOTOR LEARNING OF MICE LACKING CEREBELLAR PURKINJE CELLS

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El cerebelo es un centro de coordinación y aprendizaje motor cuya lesión conlleva la instauración de un síndrome motor caracterizado por ataxia o descoordinación motora y déficits en el aprendizaje motor.
En nuestro laboratorio poseemos una mutación atáxica en ratones: la mutación *tambaleante*, que a diferencia de otras, expresa su fenotipo en la vida adulta. Esta peculiaridad la hace especialmente útil y por tanto, constituye un modelo único para el estudio de los mecanismos celulares que subyacen en una ataxia de instauración adulta
Para caracterizar los circuitos neuronales afectados por la mutación *tambaleante* se han realizado experimentos conductuales motores (campo abierto, rotarod, prueba del alambre, prueba de la caida,..etc.) que han confirmado los problemas atáxicos de estos ratones. Las pruebas de aprendizaje motor (rotarod, prueba de la caída, prueba del alambre, prueba de la barra inclinada, ..etc.) muestran que los ratones tambaleantes son capaces de aprender nuevas tareas motoras, pero a un ritmo menor que el de los controles, probablemente debido a problemas en la ejecución motora. Estos resultados coinciden con los obtenidos en mutaciones similares en ratones que también afectan a las células de Purkinje del cerebelo (Porras-García *et al*., 2005; 2013).

Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Trastornos y reparación del sistema nervioso

MEMORIA DE TRABAJO DE EXPRESIONES FACIALES: DATOS CONDUCTUALES Y ELECTROFISIOLÓGICOS

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El objetivo del presente estudio fue analizar la influencia de la emoción sobre el rendimiento en una tarea N-back utilizando expresiones faciales como estímulos. En la primera fase, los participantes realizaron un entrenamiento N-back con caras neutras, siendo seleccionados, para una segunda fase, únicamente quienes alcanzaron un nivel 3-back. El propósito de esta primera fase fue doble: en primer lugar, familiarizar a los participantes con la tarea; en segundo lugar, asegurar un nivel de esfuerzo mnemónico similar en la segunda fase. En esta segunda fase se registraron la ejecución conductual y los potenciales relacionados con acontecimientos discretos (PRAD), mediante 59 electrodos distribuidos homogéneamente por el cuero cabelludo, mientras los participantes llevaban a cabo una tarea 3-back empleando como estímulos caras negativas (asco), positivas (alegría) y neutras. Los análisis preliminares (n=13) muestran un efecto tanto de la memoria, como de la interacción entre memoria y emoción en el componente P3 en zonas centrales, temporales y parieto-occipitales. En concreto, este componente presentó mayores amplitudes ante los estímulos correctamente recordados (el sujeto respondía 'sí' cuando el estímulo era el mismo que el presentado 3 ensayos atrás) que ante los no recordados (el sujeto respondía 'no' cuando el estímulo era diferente que el presentado 3 ensayos atrás). Asimismo, y dentro de los estímulos correctamente recordados, se observó un efecto de la valencia emocional, siendo la amplitud del componente P3 mayor en respuesta a caras negativas. En conclusión, los datos sugieren una modulación emocional de la memoria de trabajo que apunta a la valencia afectiva como factor clave, en congruencia con las teorías sobre la existencia de un sesgo de negatividad en el procesamiento cognitivo.

EFFECTS OF GABAERGIC AND CHOLINERGIC AGENTS IN ANXIETY, ATTENTION AND IMPULSIVITY IN APOE2, APOE3 AND APOE4 MICE

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Human apolipoprotein E (ApoE) is the main cholesterol transporter in the brain with three allelic variants in humans (apoE2, apoE3, apoE4). Humans and rodents carrying different human apoE isoform differ in anxiety, learning and memory. Dysfunctional cholinergic and gabaergic systems have been suggested to underlie some of apoE4-related deficits. The aim of this study was to characterize baseline attention and impulsivity in apoE transgenic mice and challenge them pharmacologically. The response to gabaergic drugs was first tested in an Open-Field. Adult apoE2, apoE3 and apoE4 female mice were administered 0.9% saline, alprazolam (0.06-0.12 mg/kg) or picrotoxin (0.5-1 mg/kg) by intra-peritoneal injection 30 min before testing. Attention and impulsivity were assessed in a 5-Choice Serial Reaction Time Task. Behavioral and pharmacological manipulations started once the animals showed a stable performance with a stimulus duration of 1s for 5 consecutive days (>50% correct, < 25% omission, > 80% accuracy). Sessions in which the inter-trial interval (ITI) was increased (7 - 10 s) and the stimulus duration was shortened (0.8 - 0.5 s) were performed once a week for eight weeks. Alprazolam (0.06-0.12 mg/kg), picrotoxin (0.25-0.5 mg/kg) and cholinergic antagonist scopolamine (0.8-1.6 mg/kg) were injected 30 min prior testing twice a week during six weeks using a Latin square design. Increase in premature response was observed in long ITI sessions, especially when it lasted 10 s, which also decreased accuracy and increased perseveration. A decrease in attentional performance was also observed when the stimulus duration shortened to 0.5 s. The scopolamine effects, decreased accuracy, increased omissions and premature responding, were more pronounced in apoE2 mice. ApoE3 and apoE4 mice showed different sensitivity to alprazolam in the Open-Field. Alprazolam decreased omissions with no effects on impulsive behavior. These results indicate GABA has an important role in attentional processes and suggests differences between apoE genotypes.
Project supported by the Spanish Government (Ministerio de Economía y Competitividad) (PSI2010-2174-C02-1).

Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Trastornos y reparación del sistema nervioso

LONG-TERM BEHAVIOURAL AND MOLECULAR EFFECTS OF INTERMITTENT ADOLESCENT ALCOHOL EXPOSURE IN MALE AND FEMALE RATS.

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Alcohol drinking, especially among adolescents and young adults, is a serious public health concern. In the present study we aimed to investigate the short and long-term effects of intermittent access to ethanol (20% in drinking water), using a 4 days drinking-in-the-dark procedure, during the adolescence period (postnatal days, pnd, 28 to 52). Male and female adolescent *Wistar* rats were given access to ethanol (or water) for 2-h sessions during three days, and for an additional 4-h session on the 4th day. Blood ethanol concentrations were measured 90 min. after the 4-h sessions of drinking on the first and the last weeks (pnd 31 and 52). Two days after ethanol withdrawal (pnd 54), animals were exposed to the elevated plus-maze (EPM) test of anxiety and cognitive function was evaluated in the novel object recognition test (at pnd 63). No differences in anxiety levels were observed; however, in the long-term, adolescent ethanol exposure induced a remarkable deficit in recognition memory. Present findings demonstrate that this protocol of intermittent access to ethanol during adolescence induced a deficit in recognition memory in male and female rats. At pnd 68, the animals were sacrificed and brain samples dissected, i.e. prefrontal cortex and hippocampus, to investigate possible underlying mechanisms by Western Blotting. Alcohol exposure induced a significant decrease in the expression levels of serotonin 2A receptor and dopamine D1 receptor in the prefrontal cortex and hippocampus of female animals. Similarly, a reduction in the levels of histone-3 acetylation was observed in the two brain regions, whereas a decrease in histone-4 acetylation was only evidenced for females' prefrontal cortex. In resume, future studies are needed to reveal the neurobiological mechanism underlying the alcohol-induced cognitive deficit, and to better understand the sexual dimorphisms observed in the effects of adolescent alcohol exposure.

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Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.

DEEP DISTURBANCES IN BODY TEMPERATURE AND WAKING/SLEEP RHYTHMS IN A CASE OF WILSON DISEASE

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Introduction: Wilson disease (WD), also known as hepatolenticular degeneration, is an autosomal recessive disorder in which copper metabolism disturbances result in copper accumulation and secondary damage in affected organs. It is a particularly rare disease with a prevalence of one in 40000 in most populations. Sleep disorders of unknown etiology are common in most patients.
Objectives: This study aims at reporting the case of a 34 years old female patient suffering Wilson's disease and with serious sleep disorders accompanied of an extreme eveningness chronotype (score 20 in Horne-Ostberg Morningness-Eveningness Questionnaire). To recognize the origin of the sleep disorders, thermometry, wrist actimetry and body position sensors (TAP) where used (according to the method of Ortiz et al., 2010), as indicators of circadian rhythm disturbances.
Material and methods: Body position and rest-activity rhythms were assessed over 14 days by actimetry (Hobo G Acceleration Data Logger, Massachusetts, USA). Sensors and pendants were placed on the non-dominant arm. The peripheral temperature rhythm was assessed continuously for the same period using a wrist temperature sensor (iButton DS1921H, Dallas, Maxim), placed over the radial artery. The subject was instructed to keep a sleep diary, annotating bed time, lights off time, sleep onset and offset, get up time, as well as time and duration of eventual naps. Sleep quality was evaluated with the Oviedo Sleep Questionnaire.
Results and conclusions: The method proved to be effective in detecting the temperature circadian rhythm, which was found to be extremely irregular, with highly disorganized sleep schedules and over 6h delay in sleep-wake rhythm.

1. Neurociencia cognitiva y conductual
2. Sistemas homeostáticos y neuroendocrinos

CLÍNICA, GENÉTICA Y RESONANCIA MAGNÉTICA FUNCIONAL: BIOMARCADORES DE DEMENCIA EN LOS PRIMEROS ESTADIOS DE LA ENFERMEDAD DE PARKINSON.

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El 80% de los pacientes con enfermedad de Parkinson (EP) sufren algún tipo de alteración cognitiva, alcanzando la demencia en el 30% de los casos. El proyecto ICICLE propone la búsqueda de biomarcadores de demencia en la EP combinando datos clínicos, genéticos, neuropsicológicos y resonancia magnética funcional en una cohorte incidental de 200 pacientes recién diagnosticados y 80 controles.

El protocolo incluyó una visita clínica, muestra de sangre, evaluación neuropsicológica y resonancia magnética funcional, incluyendo 3 tareas: la Torre de Londres, Rotación espacial y memoria. Los datos se analizaron con Statistical Parametric Mapping (SPM8) en Matlab, y regiones de interés (ROIs) *ad hoc* para cada tarea con Marsbar toolbox. El genotipado para rs4680 (COMT Val158Met), rs9468 (MAPT H1 vs H2 haplotipo) y rs429358 + rs7412 (ApoE genotipo 1-4) requirió un ensayo de discriminación alélica (sistema de detección HT7000, Applied Biosystems).

Se encontraron diferencias significativas entre pacientes y controles en tiempo de reacción, respuestas correctas y activación de las regiones de interés para cada tarea. Dentro del grupo de pacientes, se comprobó que las variaciones en los genes COMT, MAPT y ApoE correlacionan con los resultados de los tests y los patrones de activación cerebral. Se identificó la influencia de variables neuropsicológicas (fluencia verbal) en la habilidad de los pacientes para resolver las tareas.

Estos resultados indican que desde los primeros estadios de la enfermedad existen diferencias significativas entre pacientes y controles a nivel cognitivo (tanto en puntuación como en el patrón de activación cerebral). Las variaciones en los genes COMT, MAPT y ApoE son determinantes en las habilidades del los pacientes y en su correlación cerebral funcional. Estos datos coinciden con trabajos previos, confirmado por primera vez la interacción entre genética, clínica y activación cerebral en el diagnóstico temprano de alteración cognitiva en EP.

Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual.
2ª: Neurociencia de Sistemas.

MEMORY CONSOLIDATION OF ENHANCED INHIBITORY AVOIDANCE TRAINING DOES NOT REQUIRE mRNA SYNTHESIS ON DORSAL HIPPOCAMPUS

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It has been claimed that memory consolidation requires *de novo* mRNA synthesis, particularly of Immediate Early Genes, such as *zif268* and *arc*. On the other hand, it has been demonstrated that over-training protects memory against the amnesic effects of several treatments, including protein synthesis inhibitors that interfere with cerebral activity, particularly in the hippocampus. It is not known whether the amnesic effect of inhibitors of transcription occurs if subjects are subjected to over-training situations. Therefore, the aim of this work was to determine if the administration of 5.6-Dichloro-1-β-D-ribofuranosilbenzimidazol (DRB), an inhibitor of transcription, in the dorsal hippocampus (DH) produces amnesia in rats trained in an inhibitory avoidance (IA) task using a low (1.0 mA) or high (2.0 mA) intensity of reinforcement. To this end, DRB (80 ng/0.5 μL) or its vehicle, dimetil sulfoxide (DMSO, 1%), was administered bilaterally into DH 15 min before the training session. Forty-eight hours later, their retention latencies were measured. In an additional experiment under the same training conditions, we measured *arc* and *zif268* expression in DH using Quantitative Real-Time PCR, 30 min after training. Our results showed that pre-training DRB administration produced amnesia in the 1.0 mA group while the 2.0 mA group showed an intact memory. We found that only *zif268* increased significantly in HD in the 2.0 mA-vehicle group as compared with the other conditions. While *arc* increased in response to training in all groups, even those who were administrated with DRB. These data show that enhanced learning of IA training (high foot-shock group) blocks the DRB-induced amnesia in DH. The overtraining-protective effect is consistent with previous experiments from our laboratory where amnesia is prevented after interference with activity at both brain systems and cellular levels, suggesting plastic changes that ensure the preservation of highly aversively motivated memories.

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Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Neurociencia de sistemas

EL TRASPLANTE DE PRECURSORES GABAÉRGICOS EN EL HIPOCAMPO DORSAL DE RATONES NULOS PARA EL RECEPTOR LPA_1 DISMINUYE LAS RESPUESTAS ASOCIADAS A ANSIEDAD

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RESUMEN

El sistema GABAérgico está implicado en la conducta emocional de tal manera que cualquier defecto o alteración del mismo puede generar comportamientos y respuestas de tipo ansioso y depresivo. Durante los últimos años la terapia celular mediante el trasplante de progenitores de interneuronas GABAérgicas derivadas de la eminencia ganglionar media (EGM) en el hipocampo adulto se ha enfocado a corregir los defectos de hiperactividad y ansiedad que se presentaban en los modelos de epilepsia o de infarto cerebral. Por otra parte, la vía de señalización del receptor de ácido lisofosfatídico LPA_1, se ha configurado en los últimos años como un elemento relevante en la función hipocampal y su ausencia, en el modelo de ratón nulo (maLPA_1-null) genera alteraciones emocionales y cognitivas debidas a una neurogénesis hipocampal adulta defectuosa. Por ello, nos preguntamos si el trasplante de precursores interneuronales derivados de la EGM en el hipocampo dorsal de ratones adultos nulos para el receptor LPA_1 podría atenuar el comportamiento de tipo ansioso y la reducción de neurogénesis observado en estos animales, que actuarían como modelo neuropatológico. Para ello, examinamos a diferentes tiempos postrasplante el efecto de las células trasplantadas en la conducta usando test de campo abierto, laberinto en cruz elevado, test de natación forzada y test de actividad en rodillo. Nuestros resultados muestran que las células trasplantadas son capaces de restaurar el ambiente hipocampal hospedador así como de disminuir la respuesta de tipo ansioso, sin efectos anómalos en la actividad motora y manteniendo su correcta diferenciación. Estos resultados avalan, en definitiva, el uso de la terapia celular para el tratamiento de patologías que cursen con cuadros de ansiedad y/o depresión.

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THE THIN LINE BETWEEN LOVE AND HATE: MATERNAL AGGRESSION IS INDUCED BY THE ATTRACTIVE MALE SEXUAL PHEROMONE DARCIN IN MICE

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Female mice are aggressive towards intruders only during lactation (maternal aggression), male intruders eliciting especially violent attacks. Maternal aggression is known to be dependent on the vomeronasal organ (VNO), thus suggesting that it is elicited/enhanced by male pheromones. Therefore, during lactation the response of female mice to male pheromones changes from attraction (male pheromones are rewarding to non-lactating females) to aggression. This can be due to: a) changes in the expression of vomeronasal receptors in the VNO that allow detecting aggression-eliciting pheromones only during lactation; or b) changes in the circuitry of the socio-sexual brain that would modify the response of the dams to the same male pheromones.

To test these hypotheses, in Experiment 1 we performed aggression tests in three groups of FEMALES: dams, sensitized virgin females (sisters of the dams sharing pup care) and virgins having no contact with pups. Each female was tested against two MALE intruders: intact and castrated. Only dams were aggressive with other females showing only occasional attacks. And only intact males were attacked, although dams occasionally attacked castrated males. These data were supported by the statistical analysis. The lack of aggressiveness of sensitized females suggests that continuous interaction with pups is not enough to promote aggression, but it depends instead on endocrine/physiological changes related to parturition and lactation.
In Experiment 2 we tested whether the attractive urine-borne male pheromone darcin also induces maternal aggression. To do so, we performed aggression tests of dams and sensitized females (that served as controls) towards castrated male intruders swabbed with: a) urine of intact males; b) recombinant darcin (r-darcin); c) saline. Results indicate that urine and r-darcin promote similar, high levels of aggression to castrated males. Therefore, changes in the female brain would explain the different response to male pheromones during lactation.

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Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Sistemas homeostáticos y neuroendocrino

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15º Congreso Nacional de la Sociedad Española de NeuroCiencia

Tema 5

Trastornos y reparación del sistema nervioso

Posters

NITROSATIVE ALTERATION OF SERUM ALPHA-SYNUCLEIN AS POTENTIALLY ETIOLOGICAL FACTOR IN SPORADIC PARKINSON'S DISEASE

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Objectives. Nitrosative stress, where nitrosylation of tyrosine leading to 3-nitrotyrosine proteins or free 3-nitrotyrosine is the most prominent change, has been proposed as a pathogenic mechanism in Parkinson's disease (PD). However, levels of 3-nitrotyrosine proteins or amines in serum and cerebrospinal fluid (CSF) of patients with PD have not been studied.

Methods. Nitrosative stress-induced protein changes in serum and CSF were analyzed in PD patients (n=54) and control subjects without any neurological disorder (n=40), by using ELISA, western-blotting and mass spectrometry.

Results. The findings indicated the presence of nitrosative stress in serum and CSF of patients with early PD leading to selective increase of 3-nitrotyrosine proteins other than nitroalbumin, without free 3-nitrotyrosine. Among 3-nitrotyrosine proteins, nitro-α-synuclein (N-αSyn) was detected in serum, not CSF, and nitrosative stress was observed to affect selectively the sites of tyrosine nitrosylation of N-αSyn. Thus intensity of nitrosylation of Tyr125/136 residues was enhanced, and that of Tyr39 site was reduced, and the ratio between both parameters was significantly higher in early-PD patients relative to controls and advanced patients ($p<0.01$). A basal level of serum N-αSyn was detected in control subjects.

Conclusions. We propose that evaluating nitrosative stress through enhanced levels of 3-nitrotyrosine proteins in serum and CSF without changes in nitroalbumin, together with the anomalous profile of tyrosine nitrosylation of serum αSyn characterized by dominant nitrosylation of Tyr125/136 could serve for diagnosis of sporadic Parkinson's disease at early stages of the disorder.

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1. Trastornos y reparación del sistema nervioso
2. Nuevos métodos y tecnologías

DESACOPLAMIENTO APOPTOSIS-FAGOCITOSIS DURANTE LA EXCITOTOXICIDAD ASOCIADA A LA EPILEPSIA

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La fagocitosis microglial es esencial para el mantenimiento de la homeostasis cerebral, ya que es activamente antiinflamatoria e impide la liberación de contenidos intracelulares tóxicos, al menos in vitro. Contrariamente a la bien caracterizada respuesta inflamatoria, la respuesta fagocítica en condiciones neurodegenerativas in vivo es una gran desconocida. Como modelo de neurodegeneración utilizamos la inyección intrahipocampal de ácido kaínico (KA), que produce excitotoxicidad y convulsiones. A continuación evaluamos la eficacia fagocítica mediante la determinación del índice fagocítico (porcentaje de células apoptóticas fagocitadas), y el tiempo de eliminación (tiempo medio de degradación de las células apoptóticas), utilizando inmunofluorescencia y microscopía confocal. Al contrario de lo esperado, la eficacia fagocítica microglial se ve drásticamente reducida a las 6-24h tras la inyección de KA. A las 24h hay una pequeña compensación y fagocitos no profesionales, como los astrocitos, o los neuroblastos, fagocitan una pequeña proporción de células apoptóticas. El desacoplamiento entre apoptosis y fagocitosis resulta en la acumulación de células apoptóticas, que tardan hasta 3 días en comenzar a ser eliminadas, mientras que en condiciones fisiológicas su tiempo de eliminación es de 1.2-1.5h. Además el bloqueo de la fagocitosis correlaciona a lo largo del curso temporal con el desarrollo de la inflamación, determinada mediante RT-qPCR de un panel de citoquinas pro- y antiinflamatorias. Dado que en estudios previos no hemos encontrado efecto de la inflamación en la fagocitosis, estos resultados sugieren que la inflamación se desarrolla al menos parcialmente como consecuencia del bloqueo fagocítico, y sugieren que la fagocitosis puede ser una nueva diana farmaceútica para acelerar la recuperación tisular después de una lesión excitotóxica. Agradecimientos: Fundación Ikerbasque; Gobierno Vasco (S-PC12UN014); Ministerio de Economía y Competitividad (BFU-2012-32089).

Areas temáticas:
1ª . Trastornos y reparación del sistema nervioso
2ª . Excitabilidad neuronal, sinapsis y glia: mecanismos celulares

MAPPING METABOLIC BRAIN ACTIVITY IN THREE MODELS OF HEPATIC ENCEPHALOPATHY

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Cirrhosis is a common disease in Western countries. Liver failure, hyperammonemia and portal hypertension are the main factors that contribute to human cirrhosis that frequently leads to a neuropsychiatric disorder known as hepatic encephalopathy (HE). In this study, we examined the differential contribution of these leading factors to the oxidative metabolism of diverse brain limbic system regions frequently involved in memory process by histochemical labelling of cytochrome oxidase (COx). A total of 28 male adult Wistar rats were used (230-260 g at the start of the experiments). The animals were randomly distributed into 3 groups: portal hypertension (PH group, n=12), hyperammonemia (HA group, n=8) and animals with cirrhosis by administration of thioacetamide (TAA group, n=8). We have analyzed cortical structures such as infralimbic and prelimbic cortex, subcortical structures such as hippocampus and ventral striatum, at thalamic level like the anterodorsal, anteroventral and mediodorsal thalamus and, finally, the hypothalamus, where the mammilary nuclei (medial and lateral) were measured. The severest alteration is found in the model that mimics intoxication by ammonia, followed by the thioacetamide-treated group and the portal hypertension group. No changes were found at the mammilary bodies for any of the experimental groups. The results obtained show that there is a differential contribution of portal hypertension, hyperammonemia and liver disease to the brain metabolic dysfunction associated with HE. The most interesting finding is that the alterations in metabolic brain activity do not develop equally in the three models.

Áreas Temáticas:

1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia de sistemas

AGING WITHOUT APOLIPOPROTEIN D: MOLECULAR AND CELLULAR MODIFICATIONS IN THE HIPPOCAMPUS AND CORTEX

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A detailed knowledge of the cellular and molecular mechanisms underlying aging in the brain is fundamental to understand both, the natural process of its functional decline, and the basal conditions upon which neurodegenerative and other brain pathologies superimpose.

Of particular interest are the potential adaptability and plasticity present in the aged brain, and the contribution of endogenous protective mechanisms to such physiological processes. Apolipoprotein D (ApoD) is one of the few genes with a consistent and evolutionary conserved up-regulation in the aged brain. Increasing evidence supports a protecting role for ApoD upon stress or injury in both CNS and PNS. However, a thorough study of the effects of ApoD expression along the normal process of aging is still missing.

By using an ApoD-KO mouse we have uncovered unexpected processes affected by ApoD that contribute to the functional maintenance of the nervous system upon aging. We have focused our efforts in the cortex and hippocampus. Both ApoD and aging affect differentially to these brain regions. We use histochemical methods combined with markers of glial reactivity, oxidative and inflammatory damage, phospho-tau, ubiquitin and lipofuscin accumulation, as well as markers of myelination state. Using expression microarrays we find that in the hippocampus a set of genes, enriched in neurotransmission and mitochondrial function regulation, depend on ApoD for their response to aging. In contrast, the lack of ApoD specifically triggers changes in genes coding for membrane proteins, related to ion transport. Aging dependence on ApoD in the cortex differs from that of hippocampus in an enrichment of myelin regulation and inflammatory response related genes, while the lack of ApoD results in aging-triggered changes in lipid metabolism and insulin signaling genes. These findings predict novel roles for ApoD in the maintenance of functional circuits and brain metabolism during healthy brain aging.

MECHANISMS OF CELL DEATH AND NEUROPROTECTION IN HUNTINGTON'S DISEASE HUMANS' SKIN FIBROBLASTS

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Huntington's disease (HD) is a neurodegenerative disorder characterized by progressive motor, cognitive and psychiatric deficits, associated with predominant loss of striatal neurons and is caused by polyglutamine expansion in the huntingtin protein. Inhibition of the normal protein degradation is produced by blockade of the ubiquitin proteasome system (UPS). Epoxomicin, a proteasome inhibitor, increases the levels of proteins involved in neurodegenerative disorders. Furthermore, treatment with trehalose, a disaccharide inductor of autophagy, diminishes the amount of soluble huntingtin and huntingtin aggregates in some transfected cells.

We have investigated the effects of epoxomicin in skin fibroblast of control and HD patients (with similar age and number of glutamine repeats), and whether the stimulation of autophagy by trehalose, an alternative mechanism for elimination of abnormal proteins, reverts epoxomicin-induced damage.

At baseline, HD fibroblasts have increased the amount of ubiquitinized proteins, and higher levels of reactive oxygen species (ROS) and LAMP2A as an autophagy marker. We have observed a higher cell replication in HD patients than in controls of the same age. The replicative capacity diminishes more in HD fibroblasts than controls when 12 passages in culture is exceeded. Epoxomicin increases the activated caspase-3, HSP70 and ROS levels in both HD and controls. Treatment with trehalose counteracts the increase in ROS and activated caspase-3 levels induced by epoxomicin, and also increase the LC3 levels more in HD fibroblast than controls.

Trehalose could be a new therapeutic tool in Huntington's Disease.

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Áreas Temáticas:
1ª: 5 Trastornos y reparación del sistema nervioso
2ª: 3 Neurociencia de sistemas

TREHALOSE REDUCES OXIDATIVE STRESS IN FIBROBLASTS FROM A PATIENT WITH ATAXIA INDUCED BY CHIP DEFICIENCY

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Maintaining protein folding is essential for optimum protein performance and normal cellular function.The carboxy terminus of Hsc70 interacting protein (CHIP) possesses U-box-dependent ubiquitin ligase activity as well as cochaperone activity and plays an essential role in protein quality control by integrating the molecular chaperone machinery with the UPS. Previosly we have provided evidences that the inhibition of proteosome with epoxomicin has significant effects on the levels of proteins involved in neurodegenerative disorders. In addition, trehalose, a natural disaccharide with two glucose molecules, known to enhance autophagy, has been shown to be effective in protecting proteins against stress and as a chemical chaperone can effectively stabilize proteins from denaturation by heat shock, therefore it may also be able to suppress aggregation of denatured proteins.

Here, we examine the effects of trehalose on oxidative stress innate and induced by epoxomicin in skin human fibroblast from a new spinocerebellar ataxia caused by mutations in *STUB1*gene, which encodes for the C-terminal HSP70-interacting protein (CHIP) (Bettencourt et al.)

We have shown that human fibroblast deficient in U-box-dependent ubiquitin ligase activity, have increased sensitivities to oxidative damage and exhibit a decline in proteosome activity.Treatment with trehalose increase the number of autophagosomes, normalize the levels of p62, LC3 and LAMP2A. In addition trehalose increase the GSH and decrease the free radicals levels. Trehalose could be helpful in CHIP deficient ataxia.

Areas temáticas: 1ª.Trastornos y reparación del sistema nervioso.
2ª. Neurociencia de Sistemas

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NEURAL PROGENITOR CELL IMPLANTS MODULATE VASCULAR ENDOTHELIAL GROWTH FACTOR AND BRAIN-DERIVED NEUROTROPHIC FACTOR EXPRESSION IN RAT AXOTOMIZED NEURONS

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Axotomy of central neurons leads to functional and structural alterations which largely revert when neural progenitor cells (NPCs) are implanted in the lesion site. The new microenvironment created by NPCs in the host tissue might modulate in the damaged neurons the expression of a high variety of molecules with relevant roles in the repair mechanisms, including neurotrophic factors. In the present work, we aimed to analyze changes in neurotrophic factor expression in axotomized neurons induced by NPC implants. For this purpose, we performed immunofluorescence followed by confocal microscopy analysis for the detection of vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and nerve growth factor (NGF) on brainstem sections from rats with axotomy of abducens internuclear neurons that received NPC implants (implanted group) or vehicle injections (axotomized group) in the lesion site. Control abducens internuclear neurons were strongly immunoreactive to VEGF and BDNF but showed a weak staining for NT-3 and NGF. Comparisons between groups revealed that lesioned neurons from animals that received NPC implants showed a significant increase in VEGF content with respect to animals receiving vehicle injections. However, the immunoreactivity for BDNF, which was increased in the axotomized group as compared to control, was not modified in the implanted group. The modifications induced by NPC implants on VEGF and BDNF content were specific for the population of axotomized abducens internuclear neurons since the neighboring abducens motoneurons were not affected. Similar levels of NT-3 and NGF immunolabeling were obtained in injured neurons from axotomized and implanted animals. Among all the analyzed neurotrophic factors, only VEGF was expressed by the implanted cells in the lesion site. Our results point to a role of NPC implants in the modulation of neurotrophic factor expression by lesioned central neurons, which might contribute to the restorative effects of these implants.

Áreas temáticas
1º Trastornos y reparación del sistema nervioso
2º Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

MESENCHYMAL STEM CELL-MEDIATED ACTIVATION OF NATIVE OLIGODENDROCYTE PROGENITORS IN A CHRONIC DEMYELINATED MOUSE MODEL

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Objective: To investigate the therapeutic potential of mesenchymal stem cells (MSC) in a murine demyelinating model.

Material & Methods: Bone marrow-derived MSC were pre-incubated *in vitro* with iron nanoparticles and stereotaxically injected into both lateral ventricles of mice fed with cuprizone for 12 weeks, inducing an irreversible demyelinating state. After transplantation, all the animals were analyzed *in vivo* by MRI at different time points (0-90 days). The MRI images were processed to quantify myelin in the corpus callosum using image analysis software. Also, several mice were sacrificed at the same time points to perform immunohistochemistry analysis and corroborate the data obtained by MRI. In this regard, myelin density as well as several immature and mature oligodendrocyte markers were analyzed.

Results: The grafted stem cells were detected near the injection site as well as in several areas of the demyelinated corpus callosum. Oligodendrocyte progenitor cells were detected near the stem cells as early as 1 month after transplantation. Furthermore, increased myelin content was detected in the corpus callosum two months after treatment. The control mice and vehicle-injected group did not present significant differences in myelin at the various time points analyzed. This was corroborated by the increased number of cells in the corpus callosum expressing either immature or mature oligodendrocyte markers in the stem cell-treated mice.

Conclusions: The findings of this study revealed that MSC transplantation activates remyelinating processes throughout the corpus callosum of cuprizone-treated mice. This work might have major implications for the development of future therapeutic strategies for chronic demyelinating disorders.

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Áreas Temáticas
1ª: Trastornos y reparación del sistema nervioso.
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.

NEURONAL GALECTIN-4 IS REQUIRED FOR AXON GROWTH AND FOR THE ORGANIZATION OF AXONAL MEMBRANE L1 DELIVERY AND CLUSTERING

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Galectin-4 (Gal-4), mainly expressed in mammalian gastrointestinal tracts, binds specifically to clustered N-glycans with N-acetyllactosamine (LacNAc) epitopes at branch ends, making selection of distinct glycoproteins possible. Of relevance for cell polarity, it displays as well a high specificity toward the 3'-sulfated galactose headgroup of sulfatides. These binding characteristics underlie its pivotal role to organize cargo selection, and to facilitate apical delivery in polarized enterocytes.

Objective: We aimed to define the expression and the distribution of Gal-4 in primary neurons, and to investigate its putative role in axon development through the modulation of axonal glycoproteins.

Material and methods: Gal-4 expression was evaluated by immunofluorescence and WB analysis, in cultured primary E18 rat hippocampal and cortical neurons. N-glycosylation and sulfation were inhibited by pharmacological means. Gal-4/L1 interaction was demonstrated by IP after reversible crosslinking, and by antibody co-patching on living neurons.

Results: Gal-4 is expressed by hippocampal and cortical neurons being sorted to discrete segments of the axonal membrane in a microtubule- and sulfatide-dependent manner. Gal-4 knockdown retards axon growth, an effect that can be rescued by recombinant Gal-4 addition. This Gal-4 reduction, as the inhibition of sulfatide synthesis does, lowers the presence and clustered organization of axon growth-promoting molecule NCAM L1 at the axon membrane. Furthermore, we find that Gal-4 interacts with L1 by specifically binding to LacNAc branch ends of L1 N-glycans. Impairing the maturation of these N-glycans precludes Gal-4/L1 association resulting in a failure of L1 membrane cluster organization. In all, Gal-4 sorts to axon plasma membrane segments by binding to sulfatide-containing microtubule-associated carriers and, being bivalent, it organizes the transport of L1, and likely other axonal glycoproteins, by attaching them to the carriers through their LacNAc termini. This mechanism would underlie L1 functional organization on the plasma membrane, required for proper axon growth.

Áreas Temáticas:

1. Trastornos y reparación del sistema nervioso
2. Desarrollo

DISTRIBUTION OF NEUROTROPHINS IN ADULT RAT EXTRAOCULAR MOTONEURONS

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Extraocular motoneurons are unique regarding their lesser vulnerability in neuromuscular diseases such as amyotrophic lateral sclerosis (ALS). These neurons are particularly responsive to neurotrophins, as the exogenous administration of these molecules prevents the effects of axotomy. In fact, contrary to other motoneuronal populations, adult extraocular motoneurons constitutively express the NGF receptor, TrkA. Thus, we first aimed to characterize the presence of neurotrophins in the extraocular motoneurons of the adult rat, and second, we searched for possible differences with other motoneurons that suffer ALS-related degeneration.

For this purpose, we developed immunocytochemistry protocols against NGF, BDNF or NT-3 at oculomotor, trochlear, abducens, facial and hypoglossal nuclei. Antibodies against choline acetyl transferase (ChAT) were used for motoneuron identification. Immunoreactions were assessed by means of confocal microscopy. The percentage of total ChAT-positive cells that was positive against each neurotrophin was calculated, and statistical analysis was performed to detect differences between nuclei.

Approximately 95% of extraocular neurons were positive for BDNF or NT-3 immunostaining. However, these cells were not stained with the antibody against NGF. No differences were found in the percentage of positive motoneurons for each neurotrophin between the three extraocular motor nuclei.

Regarding the facial nucleus, 87% of cells were positive for BDNF, but only 3% were immunoreactive for NT-3. In the case of the hypoglossal nucleus, only 4% of motoneurons were positive for BDNF, and no positive cells were found for NT-3. NGF was absent from both nuclei.

Conclusions

1. Most adult rat extraocular motoneurons contain BDNF and NT-3, but not NGF.
2. Extraocular motoneurons present a different pattern of neurotrophin content when compared to that present in other brainstem motoneurons (either only BDNF or no neurotrophin).
3. These results might be related with the higher resistance of extraocular motoneurons to neuromuscular disorders, such as amyotrophic lateral sclerosis.

Áreas temáticas:

1. Trastornos y reparación del sistema nervioso
2. Neurociencia de sistemas

CATEPSINA D EN UN MODELO MURINO DE DEMENCIA FRONTOTEMPORAL CON MUTACIONES EN EL GEN DE LA PROTEÍNA TAU

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Nuestro grupo desarrolló un modelo murino de Demencia Frontotemporal con Parkinsonismo asociada al cromosoma 17 describiendo acumulación de tau hiperfosforilada y alteraciones lisosomales (Lim et al, MCN 2001). En el presente trabajo se analiza si estas alteraciones lisosomales tienen relación con la acumulación de tau. Como se ha descrito que la proteína tau podría degradarse en lisosomas por la enzima Catepsina D (Kenessey et al, J. Neurochem. 1997), hemos analizado si en las regiones cerebrales con mayor acumulación de tau, hipocampo y corteza, hay alteraciones de Catepsina D.

Objetivos concretos y metodología:

1- Análisis mediante Western Blot de la expresión de Catepsina D en ratones silvestres y transgénicos a distintas edades y de la proteína tau humana en los mismos ratones transgénicos.

2- Inmunohistoquímica de Catepsina D a nivel de microscopía óptica en los mismos ratones silvestres y transgénicos de 1. Análisis ultraestructural comparativo de estructuras Catepsina D positivas en hipocampo de ratones silvestres y transgénicos de 24 meses.

1- El hipocampo de ratones transgénicos mostró niveles más altos de Catepsina D que los ratones silvestres a todas las edades, mientras que los niveles de tau humana de ratones transgénicos disminuyen a 12 meses pero aumentan a 24 meses. En corteza de ratones transgénicos los niveles de Catepsina D descendieron durante el primer año, aumentando a los 24 meses, mientras que los niveles de tau se mantuvieron constantes.

2- La localización de Catepsina D en ratones transgénicos no varió en hipocampo, pero si en corteza. A nivel ultraestructural, los ratones transgénicos mostraron mayor cantidad de cuerpos multivesiculares y lisosomas secundarios Catepsina D positivos.

Estos resultados sugieren que la presencia de tau humana mutada activaría el sistema lisosomal como mecanismo compensatorio para reducir sus niveles, siendo el hipocampo más sensible a la presencia de tau mutada que la corteza.

Áreas temáticas:
1ª: Trastornos y reparación del sistema nervioso.

PARTIAL RECOVERY OF VISUAL FUNCTION AFTER COMPLETE OPTIC NERVE TRANSECTION IN THE LIZARD *GALLOTIA GALLOTI*

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Significant regeneration of retinal ganglion cell axons occurs after optic nerve transection through a permissive glial scar in *Gallotia galloti*. Although several of the cellular and molecular events underlying this process have been studied by our group, the functionality of the system has not been tested until now. The pupillary light reflex, accommodation and head orienting have been also used in other reptiles to test visual function (Dunlop et al., 2004). We examined 18 lizards at 3, 6, 9 and 12 months after transection. Our results revealed a tendency of eyelid closing within the first months after operation. Interestingly, by 6 months we detected a significant recovery of pupillary light reflex in two thirds of specimens including a robust response in 17% of them. However, visually guided behaviour recovery was observed only in 2 specimens, yet when presenting a prey (mealworm) in the right, affected eye, most lizards (89%) did not constrict the pupil to focus nor did they follow it as it moved, a behaviour which was detected in the unlesioned side. We conclude that a partial recovery of the visual pathway functionality takes place spontaneously in adult *G. galloti*, which could be enhanced by training or pharmacologically.

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Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia de sistemas

MOVILIZATION OF PROGENITORS IN THE SUBVENTRICULAR ZONE TO UNDERGO OLIGODENDROGENESIS IN THE THEILER´S VIRUS MODEL OF MULTIPLE SCLEROSIS: IMPLICATIONS FOR A REMYELINATING PROCESS IN LESIONS SITES

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Pathological loss of myelin in diseases like Multiple Sclerosis (MS) is usually followed by a phenomenon of remyelination, in which oligodendrocytes synthesize new myelin sheaths to envelope exposed around axons in the adult central nervous system (CNS). The importance of remyelination arises from the fact that this process not only restores saltatory conduction, but also protects axons from different insults and thus limits clinical disability in demyelinating diseases. In this scenario, the mammalian subventricular zone (SVZ) has garnered attention as a potential source of replacement cells after injury. This zone harbours stem cells and supports long-distance migration, and is activated in MS patients to promote gliogenesis. Although $NG2^+$ precursor cells are the first to react to demyelination and show the highest proliferation rate in comparison with other cells, the relative contribution of the SVZ with respect to the inflammatory demyelination induced by the Theiler´s virus infection and oligodendrogenesis has never been addressed. In the present study, we have investigated the behavior of the SVZ in Theiler´s Murine Encephalomyelitis Virus – Induced Demyelinating Disease (TMEV-IDD). We report that in this viral model for MS, there is a preclinical phase of the disease with demyelination in the corpus callosum that is followed later by an attempt of remyelination. This phase is accompanied by an activation of the SVZ with no apparent activation of $NG2^+$ precursors, and a strong and clear staining for $GFAP^+$ B cells close to the lateral ventricles of the brain that correlate with an increase of the proliferative rate in this area when measured by BrdU incorporation. Finally, we show that Theiler´s infection enhances the mobilization of SVZ progenitor cells to the surrounding demyelinated corpus callosum and generate mature APC^+ oligodendrocytes.

This work has been supported by grants from the MINECO SAF 2010-17501 and REEM (Red Española de Esclerosis Múltiple, RD0700100060)

Áreas temáticas:

1º Trastornos y reparación del sistema nervioso
2º Neurociencia de sistemas

CANNABIGEROL QUINONE ALLEVIATES EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS SYMPTOMATOLOGY

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Phytocannabinoids (pCBs) without psychotropic effects are considered of special interest as novel therapeutic agents in CNS diseases. These pCBs include cannabidiol (CBD), cannabigerol (CBG), Δ9-tetrahydrocannabivarin (Δ9-THCV) and cannabidivarin (CBDV). We have developed a series of new cannabinoid quinones, among them the CBG quinone (VCE-003) that shows PPARγ and CB2 receptors agonism. In addition we have found that VCE-003 activates the Nrf2/ARE pathway in neuronal cell lines. In the present study, we investigated the therapeutic potential of VCE-003 in the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS) by immunization con MOG $_{33-55}$. VCE-003 (5 mg/kg ip, daily) was administered to susceptible C57/BL6 mice at the onset of symptomatology. Clinical score and weights of mice were daily recorded until the day of sacrifice (28 days post-immunization). VCE-003 treatment delayed the onset of disease and ameliorated the symptomatology. Histological analysis of spinal cord of EAE mice treated with VCE-003 showed decreased microglia reactivity and reduced cellular infiltrates, in particular $CD4^+$ T lymphocytes. Double labeling with Neurofilament and the myelin protein, RIP indicated that VCE-003 diminished the axonal damage. Demyelination was evaluated by Luxol fast blue labeling. Changes in the expression of several cytokines, adhesion molecules and Nrf2-dependent genes were determined by qRT-PCR. The implication of PPARγ and CB2 receptors in the beneficial effects of VCE-003 in the EAE model of MS is being investigated by using specific receptors antagonists. To study the effect of VCE-003 in endothelial cells and the possible modulation of VCAM-1 production, an endothelial cell line, b.end 5, was treated with TNF-α and VCE-003. As VCE-003 diminished the induction of VCAM-1 in these cells, we are evaluating the involvement of NFκB in VCE-003 effects. Taken together our results support the potential of VCE-003 for the treatment of MS and other chronic inflammatory diseases.

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Áreas temáticas:
1ª: Trastornos y reparación del sistema nervioso.
2ª: Neurociencia de sistemas.

GLIAL CONDITIONED MEDIUM IMPROVES HUNTINGTIN PATHOLOGY IN VITRO AND IN VIVO

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PURPOSE

We have tested the effects of glia conditioned medium (GCM) in normal (Q7) and mutant (Q111) huntingtin striatal cells and in R6/1 mutant mice. We studied the protection of fetal and postnatal GCM on H2O2 (2 μM), glutamate (5 mM) and 3-nitropropionic acid (2.5 mM) related toxicity.

RESULTS

Fetal GCM protects from all these toxins, reducing the cell death and increasing the cell survival. Fetal GCM reduces the caspase fragmentation of the protein PARP, the expression of chaperone Hsp70 and the accumulation of ROS and polyubiquitinated proteins. In addition, in Q111 striatal cells treated with H2O2 for 24 hours, the intracellular GSH levels are higher in the presence of GCM. Notably, the 13-day and 2-month postnatal GCM, totally protects from H2O2 induced cell death in mutant striatal cells. GCM neuroprotective effects are more potent than those of the already identified neurotrophic factors such as bFGF, BDNF and GDNF.

We also tested the neuroprotective effects of GCM from fetus mice (E16) in the left striatum of HD R6/1 mice infused for 15 and 28 days with GCM through a striatal implanted catheter connected to an Alzet© pump (0.25 μl / h). Infusion of GCM reduced intraneuronal inclusions of huntingtin in the striatum, increased DARPP-32 positive neurons, increased TH+ neurons in the substantia nigra and increased autophagy.

CONCLUSSION

Our data suggest a neuroprotective effect of GCM in two different models of HD in vitro and in vivo. The putative effect of GCM in HD should be further investigated.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

LONG TERM EFFECTS OF LPS ADMINISTRATION ON SPATIAL MEMORY AND ADULT NEUROGENESIS

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Increasing evidences highlight a role for neuroinflammation and neurogenesis during Alzheimer's disease (AD) progression. The aim of this work is to reveal a possible impact of neuroinflammatory conditions in memory and adult neurogenesis in wild type (WT) and triple transgenic mouse model of AD (3xTg-AD).

4 month-old mice housed in mild-enriched cages (containing a malleable paper bag) were intraperitoneally injected with saline or lipopolysaccharide (LPS) and their performance on spatial memory analyzed with the Morris water maze (MWM) test 6 weeks later. Only 3xTg-AD mice, which performed similarly to WT mice during the last-day MWM trial session, were selected for this study.

LPS 3xTg-AD treated mice did not demonstrate any preference for the platform quadrant during MWM probe tests, indicating impaired spatial memory. In addition, 3xTg-AD mice showed a decrease in the number, volume and branching of doublecortin positive (Dcx+) cells (newly-generated neurons) in the dentate gyrus. Moreover, the branching of mid-size Dcx+ cells was slightly-reduced by LPS treatment in 3xTg-AD mice while increased in WT mice. Interestingly, a decrease in the quantity and volume of Dcx+ cells was also observed in LPS-treated WT mice. Furthermore, the number of PSD95+ puncta was reduced in Dcx+ cells of mice treated with LPS. Indeed, when WT mice were challenged with a more demanding MWM protocol they showed memory deficits similar to those observed in 3xTg-AD mice.

These data indicate that LPS treatment impairs hippocampal neurogenesis and spatial memory. Our results suggest that acute and sporadic neuroinflammatory events may influence the production of new hippocampal neurons and favour the development of strong memory deficits associated to Alzheimer's disease pathology.

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1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia cognitiva y conductual

BONE MARROW TRANSPLANTATION IN HINDLIMB MUSCLES OF SOD1 MICE EXTENDS LIFE SPAN AND IMPROVES MOTOR FUNCTION AND BIOLOGICAL MARKERS OF NEUROMUSCULAR INTEGRITY

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Objectives:
Amyotrophic lateral sclerosis (ALS) is a motoneuron degenerative disease, characterized by degeneration of upper and lower motor neurons; which leads to progressive paralysis and death from respiratory failure within 3-5 years of symptoms onset. In this work, SOD1 mutant mice were used to study the potential neurotrophic effect of bone marrow cells grafted into Quadriceps Femoris muscle.

Material and Methods:
Bone Marrow cells were grafted into *Quadriceps Femoris* of SOD1 mice and behavour, trophic factors, muscle genetic markers and lifespan were studied.

Results:
Bone marrow intramuscular transplants resulted in increased longevity with improved motor function and decreased motoneuron degeneration in the spinal cord. Moreover, certain muscle disease-specific markers, which are alternated in SOD1 mutant mouse, and may serve as molecular biomarkers for the early detection of ALS in patients, have been studied. Also, electromyography study is been doing.

Conclusion:
This work demonstrated that stem cell transplantation in the muscle increased motoneuron survival and showed the correction of muscular biomarkers of disease progression.

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Tematic Area:
1ª: 5. Trastornos y reparación del sistema nervioso

MORPHINE ALTERS THE EXPRESSION OF THE DOPAMINERGIC FACTORS THROUGH THE MODIFICATION OF SEVERAL miRNAs

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There are several studies regarding the molecular processes that lead to the appearance of addiction, nevertheless the molecular processes that lead to its appearance still remain unclear. Previous results from our Lab. showed that morphine modifies the pattern and levels of expression of the dopaminergic markers in the development of the central nervous system. It is known that cocaine modifies the levels of the microRNA 212,a small RNA linked to the addictive process. Our results showed that after morphine exposure the levels of both the miR-212 and miR-29a were altered, in both qPCR and ISH studies. miR-29a has been related with Notch activity, which is known to mediate dopaminergic differentiation at the early stages of central nervous system development, hence we propose a cross-talk between opioid activity and the Notch signaling cascade. As morphine binds opioid receptors, we analized if this interaction was mediated by the mu opioid receptor (oprm1, which is known as the receptor mainly responsible for morphine activity). To study this interaction we studied the levels of expression of these two microRNAs after morphine exposure and after the knock-down of oprm1 at three developmental stages (24, 48 and 72 hpf). Moreover, to analyze the cross-talk with Notch signaling cascade, we used DAPT, an inhibitor of the metalloproteases needed to activate this pathway. The expression patterns of the dopaminergic markers, such as *th, dat, pitx3* and *nurr1,* were studied both with qPCR and ISH. This study proposes an interaction between the Notch signaling cascade, the development of the dopaminergic system, and opioid activity.

NOTCH SIGNALING PATHWAY IS ACTIVATED IN MOTONEURONS OF SPINAL MUSCULAR ATROPHY

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Aims: Spinal muscular atrophy (SMA) is a neurodegenerative disease produced by low levels of Survival Motor Neuron (SMN) protein that affects alpha motoneurons in the spinal cord. Notch signaling is a cell-cell communication system well-known as a master regulator of neural development, but also with important roles in the adult central nervous system. As Notch signaling functions in mature astrocytes as an inducer of reactive gliosis, we hypothesized that SMN depletion could be related to an increased expression of Notch ligands in reactive astrocytes that, in turn, may result in the activation of the Notch signaling in the neighboring spinal cord motoneurons.

Materials and methods: SMN depletion was induced in the U87MG astroglioma cell line through lentiviral transduction with an shRNA sequence targeting SMN. The SMNΔ7 mouse model of SMA was also studied at P11. The expression of Notch1 receptor, its active intracellular domain (NICD), its ligands (Jagged1and Delta1) and Neurogenin 3 was assessed by western blotting and/or immunocytochemistry.

Results: SMN deficiency in the astroglioma cell line U87MG was associated to an increase in the expression of the main components of Notch pathway, namely its ligands, Jagged1 and Delta1, the Notch1 receptor and NICD. In the SMNΔ7 mouse we also found increased astrocyte processes positive for Jagged1 and Delta1 in intimate contact with lumbar spinal cord motoneurons. In these motoneurons an increased Notch signaling was found, as denoted by increased NICD levels and reduced expression of the proneural gene neurogenin 3, whose transcription is negatively regulated by Notch.

Conclusions: Both *in vitro* and *in vivo*, SMN deficiency results in increased expression of Notch ligands on astrocytes. It is suggested that their processes may activate the Notch signaling pathway in adjacent spinal cord motoneurons. These findings may be relevant to understand the impaired neuritogenesis of SMA motoneurons.

A "DOUBLE HIT" MURINE MODEL FOR SCHIZOPHRENIA SHOWS ALTERATIONS IN THE STRUCTURE AND NEUROCHEMISTRY OF THE MEDIAL PREFRONTAL CORTEX AND THE HIPPOCAMPUS

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Schizophrenia is a very complex psychiatric disorder in which both alterations in neurodevelopment and aversive experiences during adolescence seem to be important risk factors. Animal models reproducing these types of alterations mimic some of the symptoms in patients, constituting an approach to study the etiopathology of this disorder. Among these models, the perinatal injection of NMDA receptor antagonists and the postweaning social isolation rearing are among the most widely used. Each of them has reproduced different behavioral, structural and neurochemical alterations resembling those found in schizophrenic patients. Our aim is to combine them in a "double hit" model, which should produce a wider spectrum of alterations. Lister Hooded rats have been subjected to a single injection of MK-801 at P7 and have been socially isolated from postweaning to adulthood. We have found that these animals present increased weight gain and volume reductions in their medial prefrontal cortex (mPFC) and hippocampus. They also show an increased number of activated pyramidal cells and a decrease of parvalbumin expressing cells in the mPFC. The expression of the polysialylated form of the neural cell adhesion molecule (PSA-NCAM), a molecule related to neuronal structural plasticity and that of GAD67 are decreased in the mPFC. qRT-PCR analysis revealed that the mRNA of calbindin was decreased in the mPFC while, that of calretinin was increased. Alterations in the expression of the ERbB4 mRNA, a gene associated to schizophrenia, were also found in this region. All these structural and neurochemical alterations, specially in cortical inhibitory circuits, are similar to those found in schizophrenic patients and are more numerous than those found in each of the single models. Consequently, we consider that the present "double hit" model is a better tool to study the neurobiological basis of schizophrenia and to explore new pharmacological approaches to treat this disorder.

1ª: Trastornos y reparación del sistema nervioso
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

THE DECREASE ON NEURONAL VIABILITY INDUCED BY AMYLOID-BETA 1-40 AND 1-42 IS NOT ASSOCIATED WITH REACTIVE OXYGEN SPECIES PRODUCTION

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Amyloid-beta (Aβ) accumulation together with tau protein hyperphosphorylation are the main molecular events presumably responsible for neurodegeneration observed in Alzheimer's disease. In this context, it has been reported that Aβ accumulation promoted neuronal death presumably as a consequence of enhanced reactive oxygen species (ROS) production in mitochondria. In this work, we have used three Aβ peptides: Aβ 25-35, Aβ 1-40, Aβ 1-42, and we have studied their effect on ROS production and cell viability in rat neurons in primary culture. Our results showed that the three peptides significantly decreased neuronal viability, whereas only Aβ 25-35 induced a significant increase of ROS production. In addition, we have performed immunohistochemistry against Aβ in order to observe its localization within the neuron. Our images indicated that Aβ 25-35 was internalized into neurons reaching to perinuclear mitochondria as showed by its co-localization with MitotrakerRed, a mitochondrial marker. However, Aβ 1-40 and Aβ 1-42 localized in the neuron periphery and no co-localization with MitotrakerRed was found. In conclusion, other molecular mechanisms such as calcium release or synaptic transmission impairment must be involved in neuronal death induced by Aβ 1-40 and Aβ 1-42.

Área temática:

1. Trastornos y reparación del sistema nervioso.

INTRANASALLY ADMINISTERED CONJUGATED SERT-SIRNA RAPIDLY EVOKES ANTIDEPRESSANT ACTIONS AFTER ENDOCYTOSIS INTO SEROTONIN NEURONS

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The development of new treatments for depression is predicated upon identification of neural substrates and mechanisms that underlie its etiology and pathophysiology. Current antidepressants, which inhibit serotonin transporter (SERT), display limited efficacy and slow onset of action. Conversely, new findings support that SERT gene silencing by inhibitory RNA (siRNA) produces a more rapid and potent antidepressant response than pharmacological blockade of SERT. Here, we examined the effects of RNAi-induced SERT suppression on cellular and behavioral changes produced in an animal model of depression, focusing on signaling cascades involved in synaptic plasticity. We used a SERT-siRNA molecule conjugated with sertraline (C-SERT-siRNA) for specific intracellular delivery to serotonin (5-HT) neuron. Intranasal C-SERT-SIRNA administration for 7-day (2.1 nmol/day) to C57Bl/6J mice reduced SERT mRNA expression (~42%) after endocytosis in TPH-positive cells. This was accompanied by a selective and widespread reduction of SERT binding sites (~30-50%) in the most brain regions analyzed. Furthermore, decreased SERT expression and subsequent reduction in 5-HT_{1A}-autoreceptor function markedly increased extracellular 5-HT concentration in mouse forebrain. Increased cell proliferation (Ki-67-positive cells), neurogenesis (NeuroD-positive and DCX-positive cells) and dendrite maturation in dentate gyrus were also observed. Hippocampal neuronal plasticity was associated with augmented BDNF, VEGF, TRKb, neuritin and PSD95 mRNA levels. Remarkably, these effects occurred much earlier and were of greater magnitude than those evoked by fluoxetine (10 mg/kg/day, i.p., 28-day). Finally, C-SERT-siRNA (7-day), but not fluoxetine, reversed the behavioral dysfunction displayed by a depression mouse model related with the chronic corticosterone consumption in the tail suspension, novelty suppressed feeding and preference sucrose tests. Our results highlight the critical role of post-transcriptional SERT regulation and the utility of intranasal route for brain delivery siRNAs. Although many hurdles remain, the present data represent a step forward for the use of RNAi-based medicines to improve current limitations of antidepressant therapy.

1st. Trastorno y reparación del sistema Nervioso
2d. Nuevos métodos y tecnologías

ALPHA-SYNUCLEIN STAGING IN THE AMYGDALA OF A53T MICE MODEL OF PARKINSON'S DISEASE: CELL TYPES INVOLVED

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder. Clinically, it is characterized by motor (resting tremor, bradykinesia, rigidity and postural imbalance) and non-motor symptoms (e.g., constipation, olfactory dysfunction, REM sleep behavior, depression and anhedonia). The appearance of Lewy bodies (ubiquitin and alpha-synuclein aggregates) in particular brain areas takes place according to six neuropathological stages. It has been demonstrated that early sites of Lewy body formation (stage I) are the olfactory bulb, anterior olfactory nucleus as well as the dorsal motor nucleus of the vagus (dXII). The substantia nigra and the amygdala are involved in stage III prior to massive cortical spreading. This work analyzes the distribution and cell types containing alpha-synuclein aggregates in the basolateral, central and cortical amygdaloid nuclei of A53T homozygous transgenic mice model of 16, 30, 43 and 56 weeks. Alpha-synuclein positive-cells were quantified using immunohistochemical procedures and Image J software. Co-localization between alpha-synuclein and calcium-binding proteins -such as calbindin, parvalbumin, calretinin- and the neuropeptide somatostatin were analyzed by immunofluorescence techniques under confocal microscopy. Density of alpha-synuclein-positive cells was increased from 16 to 43 weeks in all amygdaloid nuclei. However, there was a decrease of alpha-synuclein-positive cells from 43 to 56 weeks. The basolateral nucleus showed higher levels of alpha-synuclein as compared to the central and cortical nuclei. The expression of alpha-synuclein protein was higher in the cortical than in the central nucleus. These data on the alpha-synucleinopathy in the different nuclei of amygdaloid complex across time are relevant to understand the progression of PD in the brain. Supported by Spanish Ministry of Economy and Competitiveness (BFU2010-15729).

1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia de sistemas

FRIZZLED RECEPTORS EXPRESSION IN THE DAMAGED SPINAL CORD: SPECIAL FOCUS ON SPATIO-TEMPORAL AND CELLULAR EXPRESSION PATTERN OF FRIZZLED 5

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Wnt proteins are a large family of molecules that are critically involved in central nervous system (CNS) development. Experimental evidences suggest a role for this family of proteins in many CNS disorders, including spinal cord injury (SCI), which is a major neuropathology owing to its high prevalence and chronic sensorimotor functional sequelae. Interestingly, most Wnt proteins and their inhibitors are expressed in the uninjured spinal cord, and their expression patterns are altered after injury. However, little is known regarding the expression of their better-known receptors, the Frizzled family, after SCI. Thus, the aim of the present study was to evaluate the expression of Frizzled receptors in the damaged spinal cord.

On one hand, we analysed the spatio-temporal mRNA and protein expression patterns of Frizzled receptors after contusive SCI using quantitative RT-PCR and single and double immunohistochemistry, respectively. Our results show that almost all of the 10 known Frizzled receptors were expressed in specific spatial patterns in the uninjured spinal cords. Moreover, the Frizzled mRNAs and proteins were expressed after SCI, although their expression patterns were altered during the temporal progression of SCI. On the other hand, analysis of cellular Frizzled 5 expression pattern by double immunohistochemistry showed that, in the uninjured spinal cord, this receptor was expressed in neurons, oligodendrocytes, astrocytes, microglia and NG2+ glial precursors. After injury, Frizzled 5 was not only still expressed in oligodendrocytes, astrocytes and NG2+ glial precursors but also in axons at all evaluated time points. Moreover, Frizzled 5 was expressed in reactive microglia/macrophages from 3 to 14 days post-injury.

In conclusion, our data suggest the involvement of Frizzled receptors in physiological spinal cord function and in the cellular and molecular events that characterise its neuropathology.

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Áreas Temáticas:

1. Trastornos y reparación del sistema nervioso
2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

AMELIORATION OF ISCHEMIC BRAIN DAMAGE BY PERITONEAL DIALYSIS

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Ischemic stroke is a devastating condition for which there is still no effective therapy. Acute ischemic stroke is associated with high concentrations of glutamate in the blood and interstitial brain fluid due to the inability of the tissue to retain glutamate within the cells of the brain, which ultimately provokes neuronal death. Moreover, the increased concentrations of interstitial glutamate exert further excitotoxic effects on healthy tissue surrounding the infarct zone. We investigated the hypothesis that peritoneal dialysis could decrease the blood levels of glutamate thereby accelerating brain-to-blood glutamate clearance and thus minimizing brain damage. Experiments were performed on adult male Sprague-Dawley rats The pMCAO was achieved by ligature and glutamate content in rat plasma, human serum and human peritoneal dialysate samples was assayed by fluorimetric determination by enzymatic method. We used blood-oxygenation-level dependent (BOLD) functional magnetic resonance imaging (fMRI) to determine tissue functionality.and we performed limb-use asymmetry test as a behavioral trial. In a rat model of stroke, this simple procedure reduced the transient increase in plasma glutamate, consequently decreasing the size of the infarct area. fMRI demonstrated that the rescued brain tissue remained functional. Moreover, in patients with kidney failure, peritoneal dialysis significantly decreased serum glutamate concentrations.
These data indicate that by decreasing the glutamate concentration in the blood, peritoneal dialysis effectively promotes brain-to-blood glutamate efflux, minimizing the ischemic increase in extracellular glutamate and the resulting tissue damage. Furthermore, our results indicate that the functional deficit produced by ischemic insult can be partially prevented by peritoneal dialysis.This work was supported by grants from the Spanish MINECO to JS-P (BFU2010/16947), IL (SAF2011-23354) and MAM (SAF2009-08145, SAF2012-33216 and CSD2010-00045); from Fondo Europeo de Desarrollo Regional (FEDER) ‘Instituto de Salud Carlos III’ (RD06/0026, RD12/0014) to IL, MT, JV and JS-P; from the ‘Comunidad de Madrid’ (CAM-I2M2 2011-BMD-2349) to IL, MT and JS-P; and NEUROSTEMCM to MAM (S2010/BMD-2336). Research in the laboratory of SC and JL is supported by grants from the Spanish MINECO (BFU2009-09938, BFU2011-24084 and CSD2007-00023).

ELECTROPHYSIOLOGICAL RETINAL ALTERATIONS IN PATIENTS RECEIVING HYDROXYCHLOROQUINE THERAPY

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Purpose: To evaluate the changes in multifocal electroretinography (mfERG) in patients receiving hydroxychloroquine.

Methods: mfERG recordings were performed in 9 control subjects and 19 patients receiving hydroxychloroquine. A number of retinal areas were stimulated by using a stimulus array of 61 hexagons. The overall stimulus pattern subtended an angle of approximately 30 degrees on either side of fixation. The first-order kernel mfERG responses were analyzed. Individual mfERG responses for the hexagons were grouped into five concentric rings centered on the fovea for analysis. The response latencies of the first negative peak (N1) and the first positive peak (P1), as well as the electrical densities for each individual ring were then measured. Informed consent was obtained in all participants, and the study was approved by our institutional review board.

Results: mfERGs of patients showed recordings with abnormal morphology of polyphasic low amplitude waves. Compared with the control group, N1 and P1 latencies were slightly higher (2%-8%), while the electrical density of the five rings was significantly ($P < 0.001$) lower (30% - 40%). According to the distribution of the reduction of density were observed three patterns, the first with decrease throughout the retina or full field loss pattern (R1-R5 rings; 12 patients), the second with predominant decrease in the central area (R1-R2 rings; 4 patients) and a third with predominant decrease in the peripheral region (R4-R5 rings; 3 patients).

Conclusions: The results show that all the patients studied presented alterations in electrophysiological characteristics of the outer retina. Of these alterations, is the decrease of electric density of responses the most outstanding. The pattern of mfERG loss more frequent was the full field. These results suggest that the observed alterations are mainly due to the underlying pathology and not treatment with hydroxychloroquine, since alterations due to the drug are predominantly parafoveal.

IGF-I GENE THERAPY PREVENTS GLIA ACTIVATION AFTER A STAB WOUND INJURY IN THE BRAIN

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Background: Traumatic brain injury (TBI) is a leading cause of mortality and morbidity worldwide. Despite extensive efforts to develop neuroprotective therapies for this devastating disorder there have been no successful outcomes in human clinical trials to date. Neuroinflammation is well established as a key secondary injury mechanism after TBI, and it has been long considered to contribute to the damage sustained following brain injury. It comprises a feature of many neurological disorders that is accompanied by the activation of glial cells and the release of pro-inflammatory cytokines and chemokines. Such activation is a normal response oriented to protect neural tissue and it is mainly regulated by microglia and astroglia. However, excessive and chronic activation of glia may lead to neurotoxicity and may be harmful for neural tissue . The role of glia in this process has been well recognized and they have been regarded as promising targets for development of anti-inflammatory therapies. Neurotrophic factors are small molecules that exert different functions in the central nervous system (CNS), among them insulin-like growth factor-I (IGF-I) exerts neuroprotective actions that are mediated at least in part by control of activation of glia.

Objective: In this study we have assessed the efficacy of IGF-I gene therapy in reducing the inflammatory response of astrocytes and microglia after a stab wound injury.

Methods: Wistar rats were injected IP with a recombinant adenovirus harbouring the cDNA of IGF-I as a therapeutic virus, or GFP as a control virus, fourteen days previous to stab wound injury in the cortex and hippocampus. Two days after injury rats were sacrified and gliosis was assessed by immunohistochemistry. The number of GFAP and vimentin immunoreactive astrocytes and the number of major histocompatibility complex-II (MHC-II) and Iba I immunoreactive microglia were estimated in the hippocampus and cortex in the lateral border of the wound.

Results: We found that IGF-I gene therapy decreased the number of total (IbaI positive cells) and reactive (MHCII positive cells) microglia in hippocampus and the number of reactive microglia in the cerebral cortex compared with the control group. The treatment did not significantly affect the number of GFAP or vimentin immunoreactive astrocytes.

Conclusion: These findings suggest that IGF-I gene therapy may represent a new approach to reduce microglia inflammatory reaction in this model of TBI.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Nuevos métodos y tecnologías

EFECTO NEUROPROTECTOR DE IGF-II EN SITUACIONES DE ESTRÉS NEURONAL INDUCIDO POR CORTICOSTERONA

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Introducción: El daño por estrés en tejido neuronal mediado por la presencia de elevados niveles de corticosterona, se relaciona con la producción de alteraciones en funciones cognitivas. Así, el estrés agudo induce un aumento importante en los niveles plasmáticos de corticosterona que se asocia con alteraciones de plasticidad neuronal. Datos previos de nuestro grupo demuestran un efecto neuroprotector a dosis bajas del factor de crecimiento similar a insulina (IGF-II) en ratas de edad avanzada, donde el estrés de tipo oxidativo se encuentra involucrado en el propio proceso senil y en el desarrollo de diferentes enfermedades neurodegenerativas.

Objetivos: En el presente trabajo se pretenden estudiar las propiedades antioxidantes de dosis bajas de IGF-II en cultivos primarios de corteza neuronal de ratas adultas, sometidos a elevadas concentraciones transitorias de corticosterona.

Métodos: Los parámetros de estrés oxidativo se determinaron mediante citometría de flujo y espectrofotometría.

Resultados: Las neuronas corticales incubadas con corticosterona mostraron un aumento de especies reactivas de oxígeno (ROS), del consumo de reserva antioxidante celular (etax), y de la peroxidación lipídica (LOOH) todo ello como consecuencia de un desequilibrio entre factores pro-oxidantes (ej. daño mitocondrial, NOS) y factores antioxidantes (ej. GSH, SOD). La co-incubación de corticosterona y dosis bajas de IGF-II reequilibra estos factores. Este efecto neuroprotector podría estar mediado al menos en parte por la interacción de IGF-II con sus receptores específicos, ya que su bloqueo origina de nuevo un desequilibro entre los factores antioxidantes/pro-oxidantes.

Conclusión: Las dosis bajas de IGF-II ejercen un efecto neuroprotector en situaciones de estrés oxidativo inducido por altos niveles de corticosterona.

Áreas Temáticas:
1ª: Área 5: Trastornos y reparación del sistema nervioso
2ª: Área 2: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

PASSIVE EXERCISE OF THE HIND LIMBS AFTER COMPLETE THORACIC TRANSECTION OF THE SPINAL CORD PROMOTES CORTICAL REORGANIZATION

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Physical exercise promotes neural plasticity in the brain of healthy subjects and modulates pathophysiological neural plasticity after sensorimotor loss, but the mechanisms of this action are not fully understood. After spinal cord injury, cortical reorganization can be maximized by exercising the non-affected body or the residual functions of the affected body. However, exercise per se also produces systemic changes – such as increased cardiovascular fitness, improved circulation and neuroendocrine changes – that have a great impact on brain function and plasticity. It is therefore possible that passive exercise therapies typically applied below the level of the lesion in patients with spinal cord injury could put the brain in a more plastic state and promote cortical reorganization. To directly test this hypothesis, we applied passive hindlimb bike exercise after complete thoracic transection of the spinal cord in adult rats. Using western blot analysis, we found that the level of proteins associated with plasticity – specifically ADCY1 and BDNF – increased in the somatosensory cortex of transected animals that received passive bike exercise compared to transected animals that received sham exercise. Using electrophysiological techniques, we then verified that neurons in the deafferented hindlimb cortex increased their responsiveness to tactile stimuli delivered to the forelimb in transected animals that received passive bike exercise compared to transected animals that received sham exercise. Passive exercise below the level of the lesion, therefore, promotes cortical reorganization after spinal cord injury, uncovering a brain-body interaction that does not rely on intact sensorimotor pathways connecting the exercised body parts and the brain.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia de sistemas

COMPARACIÓN ENTRE CÉLULAS TRONCALES MESENQUIMALES DE TEJIDO ADIPOSO ALOGÉNICAS Y XENOGÉNICAS EN EL INFARTO CEREBRAL AGUDO. PRUEBA CONCEPTO EN RATAS

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Introducción: La terapia con células troncales mesenquimales (CTM) ha demostrado ser útil en modelos animales**.**Sin embargo,se necesita más información sobre el tipo celular apropiado, siendo interesante realizar una prueba de concepto, administrando CTM obtenidas de tejido adiposo humano (CTM-TA_h) y rata (CTM-TA_r) para demostrar la seguridad de ambas en el modelo animal.

Objetivo: Analizar en el infarto cerebral seguridad y efectos sobre la recuperación funcional de la administración i.v. de CTM-TA_h (administración xenogénica) o CTM-TA_r (administración alogénica) en un modelo animal.

Material/Métodos: Modelo de oclusión de la arteria cerebral media permanente (OACMp) en ratas machos Sprague Dawley.Las ratas se distribuyen en 4 grupos (n=10):a)Sham:cirugía sin infarto; b)Control:cirugía + infarto;c)CTM-TAh: cirugía + infarto + i.v CTM-TAh ($2X10^6$ células); d)CTM-TAr: cirugía + infarto + i.v CTM-TAr ($2X10^6$ células).Analizamos:evaluación funcional por el test de Rogers; Volumen de lesión por resonancia magnética y H&E,migración/implantación celular por resonancia magnética e inmunohistoquímica;muerte celular (TUNEL), y formación de tumores.

Resultados: No se observa formación de tumores en ningún grupo de tratamiento.Los animales tratados con células troncales, CTM-TA_h y CTM-TA_r presentan mejor recuperación funcional que los controles a las 24h y 14 días ($p<0,05$),sin diferencias entre los grupos de tratamiento.No se observó migración ni implantación celular en los grupos de tratamiento, ni reducción en el volumen de lesión respecto a controles.Sin embargo, ambos tratamientos reducen la muerte celular respecto al grupo control ($p<0,05$).

Conclusion: Ambos tipos celulares han demostrado ser seguras, no produciendo efectos adversos en los animales y sin evidencia de formación de tumores e igual eficacia sobre la recuperación funcional.

Áreas Temáticas:
1ª Trastornos y reparación del sistema nervioso
2º Excitabilidad neuronal, sinapsis y glia: mecanismos celulares

SULFURO DE HIDRÓGENO EN EL INFARTO CEREBRAL REDUCE EL TAMAÑO DE LESIÓN Y POTENCIA LA RECUPERACIÓN FUNCIONAL EN UN MODELO ANIMAL

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Introducción: El sulfuro de hidrógeno (H_2S) induce un estado hipometabólico a concentraciones micromolares.En el infarto cerebral interferiría con los mecanismos de necrosis y apoptosis, influyendo en la regulación de los genes protectores.

Objetivo: Estudiar el efecto sobre la lesión y recuperación funcional de la exposición a H_2S después de infarto cerebral por oclusión permanente de la arteria cerebral media (OpACM) en rata.

Material/Métodos:Ratas machos Sprague-Dawley fueron distribuidas en 3 grupos:1-Sham: cirugía sin infarto;2-Control: cirugía+OpACM;3-Tratamiento:cirugía+OpACM+inhalación de 40 ppM H_2S.Analizamos:Evaluación funcional por test de Rogers y Rotarod;Volumen de lesión por resonancia magnética (RM) y H&E;Muerte celular por TUNEL;marcadores de protección (Nox4, SOD2) y reparación (proliferación celular (BrdU), GFAP, VEGF, Sinaptofisina) por inmunofluorescencia y western-blot.Los animales se sacrifican a las 24h ó 14 días.

Resultados:A las 24h y 14 días los animales tratados tenían una mayor recuperación funcional con disminución en tamaño de lesión (H&E) y muerte celular.Observamos reducción en el tamaño de lesión por RM a los 14 días ($p<0,05$).En cuanto a marcadores de protección cerebral, los animales tratados tuvieron niveles más bajos de NOx4 (24h y 14 días) y SOD2 (14 días) con respecto a los controles ($p<0,05$),pero,no se apreciaron diferencias en marcadores de reparación (BrdU, GFAP, VEGF y sinaptofisina) entre los animales control y tratados.

Conclusión:La exposición a sulfuro de hidrógeno de animales con infarto cerebral es eficaz en mejorar la recuperación funcional. Se relaciona a mecanismos de protección (reducción del volumen de lesión, muerte celular y niveles de Nox4 y SOD2), pero no parece hacerlo en los de reparación cerebral.

Áreas Temáticas:
1ª Trastornos y reparación del sistema nervioso
2º Excitabilidad neuronal, sinapsis y glia: mecanismos celulares

EFECTO DE LA ADMINISTRACIÓN DE CÉLULAS TRONCALES MESENQUIMALES EN INFARTO CEREBRAL SUBCORTICAL. MODELO EXPERIMENTAL EN RATA

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Introducción: A pesar de su alta incidencia (15-22% de los ictus isquémicos), el daño subcortical sobre las fibras nerviosas no se ha investigado lo suficiente, por lo que no se dispone actualmente de datos sobre su respuesta a tratamiento.

Objetivo: Analizar el efecto sobre la recuperación funcional de la administración de células troncales mesenquimales (CTM) en el infarto cerebral subcortical.

Material y métodos: El infarto subcortical en ratas se indujo mediante inyección de Endotelina-1 en la cápsula interna (0.25ug/uL). A las 24h se administró $2x10^6$ CTM (i.v.) (grupo tratado) y suero salino (grupo control). El tamaño de lesión (MRI) y la evaluación funcional motora (Walking-beam, Rotarod, Rogers) se analizaron durante 7 días tras el tratamiento. Los estudios histológicos de proliferación (Ki-67), muerte celular (TUNEL), inmunofluorescencia y western blot se llevaron a cabo tras el sacrificio (día 7).

Resultados: Los animales tratados con CTM mostraron una reducción del déficit funcional a las 72h (Walking-beam, $p<0,05$). Asimismo, el área de lesión se redujo a los 7d en comparación con los animales controles ($p<0,05$), observándose una disminución de la muerte celular ($p<0.05$), así como un incremento de la proliferación ($p<0.05$). Además, se observó un aumento de los niveles de marcadores de reparación cerebral como VEGF ($p<0.001$) y Lingo-1 ($p<0.05$) en el área de lesión a los 7d tras el tratamiento.

Conclusiones: El tratamiento con CTM tras infarto subcortical es eficaz en la recuperación funcional, reducción del área de lesión y muerte celular. Asimismo potencian los procesos de reparación cerebral.

Áreas Temáticas:
1ª Trastornos y reparación del sistema nervioso
2º Excitabilidad neuronal, sinapsis y glia: mecanismos celulares

EFECTO DE UN BLOQUEANTE DEL RECEPTOR DE ANGIOTENSINA TIPO 1 ADMINISTRADO PREVIAMENTE Y DURANTE EL INFARTO CEREBRAL. ESTUDIO EN RATAS

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Introducción: Bloqueantes del receptor de angiotensina I han sido testados en modelos animales de infarto cerebral, mostrando buena recuperación funcional y reducción del volumen de infarto.

Objetivo: Analizar en el infarto cerebral el efecto protector y reparador cerebral del pre-tratamiento y tratamiento con olmesartan (OLM) en infarto cerebral de una Oclusión permanente de la Arteria Cerebral Media (OpACM) en ratas.

Métodos: 40 ratas Sprague-Dawley: 1-Sham(cirugía sin OpACM); 2-Control (cirugía+OpACM); 3- pre-tratamiento OlM(pre-OLM)(OLM (10 mg/kg/día) durante 7d + cirugía+ OpACM+ OLM(10 mg/kg/día durante 14d); 4-OLM(cirugía+OpACM+OLM (10mg/kg/día durante 14d); Analizamos: recuperación funcional (test de Rogers y Rota-rod); tamaño de lesión por resonancia magnética (RM) y H&E; muerte celular por TUNEL; marcadores de reparación (GFAP, VEGF y BDNF) por inmunofluorescencia y western-blot.

Resultados: Los grupos OLM y pre-OLM mostraron mejora funcional en el test de Rogers comparado con el grupo control, reducción de tamaño de lesión y muerte celular a los 14d ($p<0,05$). El grupo pre-OLM mostró además una mejora funcional por test de Rota-rod a los 14d. Observamos una disminución en los niveles de NOX-4, VEGF y BDNF en el grupo pre-OLM comparado con el grupo OLM y control ($p<0,05$).

Conclusiones: El tratamiento previamente y durante el infarto cerebral fue efectivo sobre la recuperación funcional, tamaño de lesión y reducción de la muerte celular. Sin embargo, no encontramos un efecto del tratamiento sobre los marcadores de reparación cerebral.

Áreas Temáticas:

1ª Trastornos y reparación del sistema nervioso

2º Excitabilidad neuronal, sinapsis y glia: mecanismos celulares

CHRONIC CANNABIDIOL ATTENUATES THE BEHAVIOURAL AND GLIAL CHANGES INDUCED BY REPEATED TREATMENT WITH THE NMDA RECEPTOR ANTAGONIST MK-801 IN MICE

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Cannabidiol (CBD), a major non-psychotomimetic constituent of Cannabis sativa, may have antipsychotic-like effects. However, the antipsychotic properties of repeated treatment with CBD have not been investigated in animal models. Thus, we evaluated if a repeated treatment with CBD would attenuate the behavioral changes induced by chronic administration of MK-801, an NMDA receptor antagonist. In addition, we also analyzed cellular changes by using glial and neuronal markers. Methods: Male C57BL/6J mice (6 weeks of age at the beginning of the experiments) received daily ip injections of MK-801 (1 mg/kg) for 28 days, and the CBD treatment started on the 6th day after the start of treatment with MK-801 and continued until the end of the treatment (28th day). We investigated if repeated treatment with CBD (30 and 60 mg/kg) would attenuate the impairments induced by chronic treatment with MK-801 in social interaction (SI) and object recognition (OR) tests. Immediately after the OR, the animals were perfused. Immunohistochemical (IHQ) analyses were performed with NeuN (a neuronal marker), IBA-1 (a microglia marker) and GFAP (an astrocyte marker) expression in the prefrontal cortex, dorsolateral striatum, nucleus accumbens core and shell, and hippocampus. Results: Repeated treatment with MK-801 impaired SI as well as memory function as evaluated in the OR, and both effects were attenuated by CBD. Concerning the IHQ data, we observed that the treatment with MK-801 did not change on NeuN expression, but it increased IBA-1 expression in the prefrontal cortex, nucleus accumbens core and shell, and hippocampus, and also increased GFAP expression in the prefrontal cortex and dorsolateral striatum. These changes were attenuated by CBD. CBD, by itself, did not change any of the behavioral responses or cellular marker analyzed. Conclusion: These results indicate that repeated treatment with CBD is able to attenuate the psychotomimetic-like effects and changes in neuroinflammatory markers induced by chronic administration of the NMDA receptor antagonist, a model that seems to mimic certain signs shown by schizophrenic patients. The data support the view that CBD may have antipsychotic properties.

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PROGNOSTIC IMPACT OF THE -866G/A POLYMORPHISM IN THE PROMOTER OF UCP2 IN ISCHEMIC STROKE

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Background: Accurate prediction of functional outcome after stroke is currently elusive although it is conceivable that poor outcome may be related to increased oxidative stress and inflammation. The -866G/A polymorphism in the promoter of UCP2 gene has been reported to be associated with the production of reactive oxygen species and pro-inflammatory molecules. Indeed, it has been reported in animal models of cerebral ischemia that knocking out the UCP2 gene exacerbates neuronal death. In this study we analysed whether the UCP2 -866G/A polymorphism may be a prognostic genetic marker of functional outcome after ischemic stroke.

Methods: UCP2 -866G/A and 45-bp insertion/deletion (45-bp Ins/Del) polymorphisms were genotyped in 408 patients with acute ischemic stroke (mean age, 72.8 ± 12.1 years; males, 59.3%) included within 12 h of symptoms' onset. Stroke severity was evaluated by NIHSS. Functional outcome was evaluated using the modified Rankin scale (mRS). Infarct volume was determined by CT measurements between 4^{th}-7^{th} days. Serum IL-6 (inflammation marker) and 8-OHdG (oxidative stress marker) levels were measured on admission and 72 hours after stroke by ELISA. The main outcome variable was poor functional outcome (modified Rankin scale >2) at 3 and 12 months. Secondary outcome variables were early neurological deterioration (END: an increase ≥4 points in the NIHSS during the first 48 hours), infarct volume and correlation with molecular markers.

Results: The *GG* genotype was independently associated with poor functional outcome at both 3 months (OR, 2.87; 95% CI: 1.42-5.84, p=0.002) and 12 months (OR, 4.07; 95% CI: 1.78-9.32, p=0.001) after adjustment for age, NIHSS at admission, temperature at 24h and END. This polymorphic variant was also associated with infarct volume (B, 22.03; 95% CI: 5.43-38.62, p=0.009) and END (OR, 2.84; 95% CI: 109-7.39, p=0.032) after adjustment for confounding factors. Finally patients with the *GG* genotype showed higher levels of IL6 at 72 h and 8-OHdG at admission.

Conclusion: The *GG* genotype of the UCP2 -866G/A polymorphism may be considered a genetic marker of poor functional outcome and END after ischemic stroke.

Áreas Temáticas: 1ª: 5; 2ª: 7.

EL SISTEMA PURINÉRGICO COMO ESTRATEGIA NEUROPROTECTORA EN EL TRATAMIENTO DE LA LESIÓN TRAUMÁTICA DE LA MEDULA ESPINAL

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El ATP y otros agonistas purinérgicos son mediadores extracelulares que se liberan tras la lesión traumática en la medula espinal (LME) promoviendo procesos citotóxicos que extienden el daño inicial.

El objetivo principal de este trabajo es estudiar la capacidad de agonistas purinérgicos no clásicos, como la diadenosina tetrafosfato (AP4A), para: i) neutralizar la inducción de cascadas apoptóticas iniciadas por el trauma y ii) reducir los efectos deletéreos de la lesión en modelos murinos de LME.

Para estudiar los mecanismos neuroprotectores que se inducen tras el tratamiento con AP4A se evaluó la citoprotección de AP4A en líneas celulares de neuroblastoma murino (Neuro2a) y humano (SH-SY5Y) tras insulto pro-apoptótico empleando técnicas de citometría de flujo y western blot. Además, se hizo una caracterización farmacológica de los receptores purinérgicos implicados en la citoprotección por AP4A monitorizando los cambios en calcio intracelular mediante fluorometría con Fura2-AM. AP4A protege frente a estímulos pro-apoptóticos (staurosporina) y excitotóxicos (glutamato) en ambas líneas neuronales y reduce la activación de las cascadas de apoptoticas. Además, reduce la respuesta de calcio intracelular debida a ATP extracelular mediada por receptores P2X y P2Y.

Para evaluar el efecto de AP4A en los modelos murinos de LME, se realizaron lesiones en la medula espinal, tanto en ratón CB57-BL6 como en rata Wistar, y se evaluaron las funciones motoras a diferentes tiempos post-lesión y tras tratamiento intraperitoneal con AP4A o vehículo. En ambos casos AP4A reduce los daños motores derivados del trauma con mejoras muy significativas en parámetros claves de la locomoción como el movimiento y posición de las patas traseras o la coordinación con las delanteras.

En conclusión, la diadenosina tetrafosfato reduce los efectos lesivos de la LME reduciendo los efectos citotóxicos de la liberación masiva de ATP o glutamato mediada por calcio, disminuyendo la inducción de apoptosis y mejorando los déficits motores derivados del trauma.

Áreas Temáticas:

1ª: Trastornos y reparación del sistema nervioso.

THE P53 ARG72PRO POLYMORPHISM DETERMINES CIRCULATING LEVELS OF CD34$^+$ PROGENITOR CELLS AND PROGNOSIS AFTER INTRACEREBRAL HEMORRHAGE

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Objectives: There is evidence of a beneficial effect of circulating endothelial progenitor cells (CD34$^+$) in the repair of ischemic tissues. The number of CD34$^+$ cells is associated with good prognosis of intracerebral hemorrhage (ICH) patients. We have recently shown that the *Arg72Pro* polymorphism in the *Tp53* gene modulates neuronal apoptosis and determines functional prognosis in stroke patients. The polymorphic variant p53-Arg displays higher apoptotic capacity, the Arg/Arg genotype being associated with poor functional outcome after stroke. Here, we analyze the possible effect of this SNP on circulating CD34$^+$ progenitor cells and its impact on functional prognosis after ICH.

Materials and methods: A prospective cohort study of 78 patients with nontraumatic ICH was performed. *Tp53 Arg72Pro* was genotyped by PCR-RFLP. Circulating CD34$^+$ cell number and apoptotic rate (Annexin-V staining) were analyzed by flow citometry. Serum levels of BDNF, VEGF, Ang-1 and SDF-1α were also analyzed at 72 hours after admission.
Results: Patients harboring the Arg/Arg genotype have lower levels of circulating CD34$^+$ progenitor cells at 7 days after admission, which correlates with poor prognosis at 3 months after stroke. Moreover, levels of growth factors that promote cell mobilization from bone marrow, such as VEGF or SDF-1α, were significantly lower in Arg/Arg patients than in patients harboring the *Pro* allele.

Conclusions: The increased number of circulating CD34$^+$ cells after ICH only occurs in patients Pro$^+$ with good prognosis, whereas Arg/Arg patients maintain the same number of circulating CD34$^+$ at the time of admission. Once we discarded differences in the apoptotic rate of CD34$^+$ cells between genotypes, we propose that the reduced CD34$^+$ progenitor cells mobilization could determine the prognosis after ICH.

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Áreas Temáticas: Seleccione las **2** áreas temáticas que más se ajusten a su trabajo en orden de prioridad:
1ª: 5
2ª: (7)

ELECTRON TOMOGRAPHY REVEALS ARCHITECTURAL ALTERATIONS OF THE GOLGI COMPLEX ASSOCIATED TO HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a devastating neurodegenerative disorder caused by the expansion of a CAG triplet encoding a polyglutamine sequence in the amino-terminal end of the huntingtin protein. Normal huntingtin (htt) accumulates in the perinuclear region in a polarized form that overlaps with the trans-Golgi region and with clathrin-coated vesicles. This has led to suggest that normal htt could participate in protein trafficking between the Golgi complex and the extracellular space. In fact, some studies show that mutant htt interferes with the post-Golgi traffic of brain-derived neurotrophic factor (BDNF) and the traffic to lysosomes.

Electron tomography (ET) is currently the leading imaging technique for 3D visualization of cellular components at close-to-molecular resolution and has made possible significant advances in cell biology in the last few years. Using animal models of the disease, we have set up a protocol to obtain 3D images of neuronal subcellular architecture by ET. This protocol is combined with high-pressure freezing of brain tissue that ensures the preservation of cellular architecture in the closest-to-native possible conditions. 3D visualization of the Golgi complex in striatal neurons has revealed that Golgi stacks are found in discrete and independent volumes around the periphery of the nuclei in the brains of control mice. However, in HD models, Golgi stacks are elongated, polarized to one side of the nuclear periphery and cisternae appeared dilated. These hypertrophic Golgi complexes are composed of a network of stacks interconnected at different 3D-levels. We are working in the identification of the molecular alterations behind these architectural changes and the relevance that these could have for post-Golgi traffic and associated homeostatic mechanisms.

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Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Nuevos métodos y tecnologías

HUMAN BRAINSTEM ARE CHARACTERIZED BY HIGH AND CONSTANT EXPRESSION OF APOLIPOPROTEIN D DURING LIFETIME

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Aims: Apolipoprotein D (Apo D) is a lipocalin, which has a wide distribution in human, presenting its highest expression at the central nervous system (CNS). Studies on Apo D expression in CNS have focused on cerebral cortex, hippocampus and cerebellum, where it was found that the expression of Apo D is increased in specific areas in relation to certain neurodegenerative diseases and injuries. Futhermore, it has also been found a progressive increase associated with lesion and aging. However, no studies have been done yet on expression of Apo D in the brainstem.

Materials and method: In this work,we measured levels of Apo D protein in this human region by slot-blot and studied its relationship with aging. Then, we performed a more detail study on its expression in eleven brainstem nuclei of medulla oblongata and pons, quantifying its immunohistochemical signal and describing the immunostaing in cell types and neuropil.

Results: The more important result of the present study was that Apo D is highly expressed already in the youngest subjects and its expression remain throughout the brainstem during all decades studied. During aging, this expression shows little variations that are not statistically significant. This characteristic is different in all to that observed in other regions of the CNS. When we study the nuclei by function we found that predominantly motor nuclei have higher levels of Apo D than sensitive ones. These data are important in studies addressing this lipocalin and we can help find a role in brain aging and neuropathology.

Conclusions: Our results prove that a highly expression of Apo D during all stages of life, it is necessary to prevent neuronal death in most of nuclei of the brainstem.

Áreas Temáticas:

1ª: Trastornos y reparación del sistema nervioso

APOLIPOPROTEINS AS BIOMARKERS OF ALZHEIMER'S DISEASE PROGRESSION: APOJ VS APOD

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Aims:
Being Alzheimer's disease (AD) the most common cause of dementia in the elderly, the identification of the proteins implicated in the process is of paramount importance. Among the proteins involved in AD the apolipoprotein family attracts special attention, mainly Apo E and Apo J. Another apolipoprotein discovered in relation to aging and AD is Apo D. Apo D and Apo J expression is increased in AD brains in relation with the progression of the disease. It has been postulated that Apo J could participate stabilizing the soluble amyloid against the fibrillar one (main component of the mature senile plaques) and Apo D could act as a molecule carrier for the maintainace and repair of the cerebral parenchyma. Apo J as well as Apo D have been located in senile plaques but there are no reports about the location and the relationship between these proteins.

Materials and method:
In this work, we have compared Apo J and Apo D expression during AD progression, from Braak stage I to Braak stage VI, as well as their preferential regional location in the cerebral cortex, in paraffin samples using immunhistochemical techniques.

Results:
Our data show that while Apo J expression is higher than Apo D in Braak Stage I, the second one shows a better correlation with AD advance. So Apo J could be a good marker of the begining of the disease while Apo D could be useful to assess the progression. Regarding the topographical location Apo D shows preference for the lower layers of the cortex whereas Apo J is mainly located in the upper ones.
When we studied the presence of these two apolipoproteins in the senile plaques no colocation was found in spite of being both of them present in most of the cases. Apo J immunostaining is higher in the diffuse plaques altough Apo D presence prevails in the mature ones.

Conclusion:
The different spatial and temporal location of these proteins suggests that they could play different roles in AD initiation and progression.

Áreas Temáticas:

1ª: Trastornos y reparación del sistema nervioso
2ª: Nuevos métodos y tecnologías

VIABILIDAD NEURONAL EN UN MODELO MURINO DE DOLOR INFLAMATORIO CRÓNICO

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El incremento de la prevalencia de enfermedades asociadas con dolor crónico, así como sus consecuencias económicas y psicológicas ha despertado gran interés en los mecanismos moleculares y celulares que subyacen al dolor. Estudios previos han mostrado que tras la inducción de dolor neuropático en un modelo murino se produce la muerte celular de neuronas del Sistema Nerviosos Central (médula espinal y la corteza). La implicación de esta pérdida neuronal en la cronicidad del dolor no está demostrada.

El objetivo principal de este trabajo es el de caracterizar las regiones del cerebro, del troco del encéfalo y de la médula espinal implicadas en la modulación de la nocicepción crónica. Para ello hemos utilizado un modelo de dolor crónico inflamatorio, consistente en la inyección intraplantar de una solución de adjuvante Freud's, el cual, produce de forma inmediata edema e hiperalgesia termal, síntomas que se prolongan en el tiempo más allá de 3 semanas. En este modelo hemos estudiado, usando técnicas inmunohistológicas, el efecto que tiene la implantación del dolor crónico producido por inflamación en estas regiones.

Nuestros resultados muestran que hay una reducción en el número de neuronas en varias regiones tanto en la vía ascendente como descendente que modulan la nocicepción, en núcleos asociados a la nocicepción previamente descritos para el dolor neuropático como en otras nuevas.

Estos datos muestran que hay diferentes regiones anatómicas definidas en el cerebro, tronco del encéfalo y médula espinal de ratones que sufre dolor inflamatorio crónico que sufren disminución en el número de neuronas en comparación con ratones control sin dolor.

Áreas Temáticas: Seleccione las **2** áreas temáticas que más se ajusten a su trabajo en orden de prioridad:
1. Trastornos y reparación del sistema nervioso
2. Neurociencia cognitiva y conductual

EARLY IGF-I NEUROTROPHIC UNCOUPLING CAN BE USED AS A BIOMARKER OF DISEASE ONSET IN ALZHEIMER MOUSE MODELS

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Summary

Circulating insulin-like growth factor I (IGF-I) enters into the brain in response to neuronal activity in a process that we refer to as neurotrophic coupling (Nishijima et al., 2010). In Alzheimer's disease (AD) the blood-brain barrier function is compromised and worsens with disease progression (Zlokovic, 2011). Thus, we hypothesized that blood-borne IGF-I brain-uptake would be altered in AD and that this could be used as a biomarker of this disease. When acutely exposed to environmental enrichment, which induces IGF-1 brain entrance), wild type mice showed increased phosphorylation of IGF-IR in response to peripheral IGF-I input. However, using two different AD mouse models in presymptomatic stages we found that the central response to systemic IGF-1 was either significantly limited in animals with no Aβ accumulation (young APPswe) or completely abrogated in those with Aβ plaques (young APPswe/PS1dE9).

To explore a potential diagnostic use of this early loss of neurotrophic coupling we implemented an electrocorticogram (ECG)-based system in which control mice showed enhanced ECG activity in response to systemic IGF-I injection whereas young APP mice without any sign of pathology showed blunted ECG responses to IGF-I. Besides, APP/PS1 mice with AD pathology entirely lost it. The underlying mechanism probably involves inhibition by soluble Aβ of serum IGF-I transcytosis through brain endothelium and central IGF-I resistance (as determined by increased pS^{616} IRS-1 levels in AD animals, similar to that found in humans (Talbot et al., 2012)). Furthermore, healthy non-human primates also showed a similar pattern of electroencephalogram (EEG) activation after peripheral IGF-I injection. All these data suggest that loss of the EEG signature of serum IGF-I may be exploited as a biomarker of AD in humans, based on the fact that, as we here report, neurotrophic coupling deteriorates in AD before any sign of pathology is observed and is progressively impaired along time.

Áreas Temáticas:

1ª: Trastornos y reparación del sistema nervioso
2ª: Sistemas homeostáticos y neuroendocrino

MODULACIÓN DE LA ACCIÓN PRO-APOPTÓTICA DE RTN1C EN EL NEUROBLASTOMA HUMANO SH-SY5Y

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La muerte celular programada o apoptosis puede desencadenarse a partir de diferentes estímulos citotóxicos extra- o intracelular. El retículo endoplásmico (RE) participa en el mantenimiento de la homeostasis celular, sin embargo cuando existe estrés del RE y este persiste, se desencadena la respuesta apoptótica.

RTN1C pertenece a una familia de proteínas transmembrana localizadas principalmente en el RE que conservan un alto nivel de homología en su dominio C-terminal. Este estudio se centra en el papel de RTN1C en el proceso apoptótico inducido por el estrés del RE. Hemos identificado mediante co-inmunoprecipitación a RTN1C como una proteína de unión a la proteína *X-linked inhibitor of apoptosis protein* (XIAP), inhibidor natural de las caspasas. Utilizando mutantes de XIAP y RTN1C definimos las regiones de XIAP y RTN1C implicadas en la interacción. Estos resultados fueron apoyados por los estudios de co-localización endógena de RTN1C y XIAP con microscopia confocal.

La sobre-expresión de RTN1C conduce a una muerte celular caspasa dependiente que se incrementa frente a estímulos de estrés de RE como thapsigargina y este efecto se ve atenuado por la presencia de XIAP. XIAP regula la actividad pro-apoptótica de RTN1C por interferencia en la cascada apoptótica de las caspasas y por ubiquitinación de RTN1C debida a XIAP. De igual modo, hemos observado el papel de RTN1C en la respuesta de la vía NFκB y ante el estímulo TNFα y su implicación en la contribución del estrés del retículo.

Estos resultados ponen de manifiesto que esta interacción podría modular la respuesta de supervivencia de la célula frente a diferentes estímulos citotóxicos tanto en la vía del estrés del RE como su posible implicación en otras vías celulares.

Áreas temáticas:

1ª: Trastornos y reparación del sistema nervioso

WNT EXPRESSION IN THE SPINAL CORD OF ADULT MICE: MORE THAN A DEVELOPMENTAL MORPHOGEN?

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The Wnt family of proteins plays key roles during central nervous system development and has been implicated in several neuropathologies during adulthood, including spinal cord injury (SCI). However, Wnts expression knowledge is relatively limited during adult stages. Here, we sought to define the spatio-temporal expression pattern of the different Wnt family members after hemisection SCI in adult mice using quantitative PCR. Under physiological conditions, the mRNAs of most Wnt ligands, modulators and soluble inhibitors, as well as the Frizzled (Fz) receptors and co-receptors, are constitutively expressed in adult mice. Furthermore, following dorsal hemisection we found significant time-dependent variations in Wnt expression profiles. Finally, we performed double immunohistochemistry against Fz1 and Fz4 as representative members of the late and acute classes of up-regulated receptors, respectively. Our results showed that, in the uninjured spinal cord, Fz1 was expressed in neurons and oligodendrocytes, while Fz4 was expressed only in astrocytes. After injury, both receptors were still maintained in the same type of cells. In conclusion, we demonstrate that most members of the Wnt family of glycoproteins are constitutively expressed in the healthy spinal cord of adult mice and that their transcriptional spatio-temporal expression patterns change following SCI hemisection. Remarkably, we also show a differential expression of Fz receptors by neurons and glial cells, suggestive for specific Wnt expression patterns and thus diverse roles on each neural cell type. Further studies will help to in-depth characterize the role of all Wnt factors and receptors described, and eventually allow for the design of novel therapies.

This work was supported by FISCAM PI2008-39.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

NITRIC OXIDE PRODUCTION IS ASSOCIATED TO INCREASED LIPOPEROXIDATION AND activatION OF CASPASE-3 IN DEMYELINATED BRAIN REGIONS OF THE TAIEP RAT

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We previously showed that the increase in nitric oxide (NO) levels and NO synthase (NOS) expression correlate with the progression of reactive astrocytosis and demyelination in the brains of 6-month-old taiep rats. Increased levels of NO can result in high concentration of peroxynitrite and thus cause tissue damage, which consists of lipoperoxidation of the cytoplasmic membrane, such as the myelin, and of apoptotic and necrotic cell-death. The aim of this work was to study whether the increased NO production is associated with lipoperoxidation and cell death in the cerebellum and brainstem over the age (1, 3, 6, and 8 months) of taiep rats. The results were compared with those obtained in matched Sprague-Dawley (SD) rats. We measured the levels of nitrites (NO production), malonyldialdehyde, and 4-hydroxyalkenal (lipoperoxidation) in brain tissue homogenates. The three NOS isoforms and cleaved caspase-3 were evaluated by using ELISA and immunostaining techniques. Our results showed that NO production and lipoperoxidation increased in the cerebellum and brainstem as the age of the taiep rats increased compared to SD rats. The overexpression of nNOS and iNOS were in the Purkinje cells, magnocellular neurons, and in oligodendrocytes, whereas the glial cells showed strong cleaved-caspase-3 immunoreactivity. In summary our results suggest that NO plays a role in the demyelination and cell death in the taiep rat.

SUBACUTE ZINC AND L-NAME ADMINISTRATION CAUSED AN INCREASE OF NO, ZINC, LIPOPEROXIDATION, AND CASPASE-3 DURING A CEREBRAL HYPOXIA-ISCHEMIA PROCESS IN THE RAT

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Administration of either Zinc or N-ω-nitro-L-arginine-methyl ester (L-NAME) protects against oxidative stress and cell death induced by cerebral ischemia. However, the effect of the treatment with zinc and L-NAME remains unknown. This work aims to study the effect of administration of zinc and L-NAME on nitrosative stress and cell death in a model of transient cerebral ischemia. Male Wistar rat were intraperitoneally (ip) treated with ZnCl2 (2.5 mg/kg) each 24 h, for 4 days. At day 5, L-NAME (10 mg/kg; ip) was injected 1 hour before a 10-min common carotid artery occlusion (CCAO). The temporoparietal cortex and hippocampus were dissected out to determine zinc, nitrites, and lipoperoxidation levels at different times. Cell death was assayed using hematoxylin-eosin and active caspase-3 immunostainings. The subacute administration of zinc before the CCAO decreased the levels of zinc, nitrites, lipoperoxidation, and cell death in the late phase of the ischemia. The L-NAME administration in the rats treated with zinc increased the levels of zinc levels in the early phase, and increased the levels of zinc, nitrites, lipoperoxidation, and cell death by necrosis in the late phase. These results suggest that the combined use of these two therapeutic strategies increased the cerebral injury caused by CCAO, unlike the individual administration.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencias de sistemas

CB1-MEDIATED NEUROPROTECTION AT THE BASAL FOREBRAIN CHOLINERGIC AREAS

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The cholinergic basal forebrain cells (CBFC) are involved in the acquisition of memory and in learning processes that are consolidated in cortical areas (Cx) and hippocampus. The loss of CBFC in the nucleus basalis of Meynert (nbM) and in the medial septum (MS) is associated with the cognitive impairment in Alzheimer´s disease (AD). The endocannabinoid system that modulates the cholinergic signaling in these brain areas is also altered in *postmortem* brains of AD patients, but the reported observations are controversial. Some authors have described a decrease in CB1 receptor density in hippocampus and Cx, while others have reported an increase in CB1 and CB2 densities surrounding the senile plaques. The aim of our study was to analyze the effects of synthetic cannabinoid drugs in organotypic cultures of hemibrain slices containing CBFC from neonatal rats (P7). The effects of the treatment with the specific CBFC immunotoxin 192IgG-saporin, were evaluated as an *ex vivo* model of cholinergic lesion. Cell death was evaluated by propidium iodide uptake and immunohistochemistry was used to label CBFC ($p75^{NTR}$) and CB1 receptors. The toxicity induced by 192IgG-saporin was decreased after pre-treating the brain slices with WIN55,212-2 (1 nM - 10 nM), while this effect was partially reversed by the CB1 antagonist/inverse agonist, AM251 (1 μM). Both, the inhibition and the overstimulation on CB1 increased cell death in the CBFC 192IgG-saporin model. The organotypic hemibrain culture model of CBFC allows the pharmacological identification of neurolipids with neuroprotective effects.

Áreas Temáticas:

1ª: Trastornos y reparación del sistema nervioso

2ª: Neurociencia de sistemas

CONSECUENCIAS FUNCIONALES A LARGO PLAZO DE TRATAMIENTO POSTNATALES CON METILFENIDATO EN UN MODELO DE RATÓN PARA EL ESTUDIO DEL TRASTORNO POR DÉFICIT DE ATENCIÓN CON HIPERACTIVIDAD

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Resumen
Partiendo de la hipótesis de que los ratones modelo de TDAH tienen reducida la liberación de Dopamina y Noradrenalinala el **objetivo** del presente estudio consiste en investigar de qué modo el tratamiento diario (crónico) durante las tres primeras semanas de vida postnatal con Metilfenidato, afecta en el **adulto** a funciones motoras reflejas o exploratorias, así como a la adquisición de respuestas motoras condicionadas o a los procesos de aprendizaje y memoria en la atención.

Se parte de camadas CD1 tratadas con AR en las primeras etapas embrionarias del ratón, para estudiar las consecuencias durante su vida y desarrollo aplicando posteriormente Metilfenidato (fármaco) en la etapa postanatal del ratón (machos). Para ello se propone el modelo de ratón con déficit de atención e hiperactividad expuesto a Ácido Retinoico creado por nuestro grupo de características comportamentales similares a las humanas, interpretando las distancias.

Los resultados obtenidos demuestran que, una vez suprimido el tratamiento, en la etapa adulta, los animales mantienen los síntomas característicos del TDAH a dicha edad, manteniéndose el déficit atencional, así como la hipoactividad, Los resultados también demuestran que los tratamientos postnatales con el fármaco en ratones silvestres, afectan negativamente el desarrollo de las pruebas mencionadas, lo que indica que producen daños irreversibles en sus cerebros, afectando a las tareas que implican psicomotricidad, atención y memoria.

Conclusión
-El modelo retinoico (AR) puede ser un buen patrón para estudiar el TDAH, en cuanto a falta de atención, impulsividad y actividad se refiere.

-El tratamiento con Metilfenidato en los ratones AR no mejoran en el adulto los síntomas característicos del TDAH y afectan negativamente el desarrollo de las pruebas realizadas, lo que indica que producen daños irreversibles en sus cerebros, afectando a las tareas que implican psicomotricidad, atención y memoria, entre otras.

GLIAL LAZARILLO PROTECTS NEURONS FROM TYPE I SPINOCEREBELLAR ATAXIA (SCA1) DEGENERATION BY A MECHANISM INVOLVING THE CONTROL OF AUTOPHAGY FLOW AND OF LIPID PEROXIDE CLEARANCE

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Glial Lazarillo (GLaz) is a Drosophila homologue of Apolipoprotein D (ApoD), a lipocalin showing an increased expression with aging and neurodegeneration. GLaz/ApoD protects against oxidative stress and promotes axon regeneration after injury, but its mechanism of action is unknown. We hypothesize that GLaz/ApoD modulates membrane dynamics and oxidation. To contrast this hypothesis we study GLaz protective role on the polyQ-based Spinocerebellar Ataxia type I (SCA1) model in Drosophila.

Human polyQ-Ataxin1 expression in fly photoreceptors triggers GLaz up-regulation. Overexpressing GLaz in photoreceptors or in retinal support cells rescues neurodegeneration. When expressed in retinal support cells, the GLaz rescue is dependent on Lipocalin-receptor expression by photoreceptor neurons, and the GLaz-GFP fusion protein co-localizes with photoreceptor markers, suggesting the existence of receptor-mediated endocytosis of GLaz.

Upon neurodegeneration, oxidative stress and induction of autophagy coexist. To test whether the GLaz beneficial effects are mediated by the modulation of autophagy we quantified Atg8a expression and monitored the accumulation of ubiquitinated proteins and p62. Overexpression of GLaz reduces Atg8a induction, but concurrently reduces the accumulation of ubiquitinated proteins and p62. Upon autophagy stimulation by rapamycin treatment, the levels of SCA1-dependent accumulation of ubiquitinated proteins and p62 are further reduced by GLaz. In addition, GLaz decreases the SCA1-dependent induction of Gsts1, a SCA1 genetic modifier contributing to the clearance of lipid peroxides.

Our data support that GLaz enters the degenerating neurons by a receptor-mediated mechanism and promotes the resolution of autophagy, increasing its flow and helping to clear polyQ-induced protein aggregates. Moreover, the beneficial effects of GLaz are linked to lipid peroxide clearance, either directly or through receptor-mediated modulation of protective gene networks.

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ESTUDIO DE LOS MECANISMOS CELULARES DEL PAPEL NEUROPROTECTOR DE ApoD: PAPEL DE LA ENDOCITOSIS

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La apolipoproteína ApoD se expresa en el sistema nervioso durante su desarrollo y aumenta con el envejecimiento y la neurodegeneración. Estudios previos a nivel celular han demostrado que ApoD se sobre-expresa en respuesta a diversas formas de estrés (reducción de suero o tratamiento con el agente pro-oxidante paraquat). Nuestros datos sugieren que cuando una célula sufre estrés oxidativo, ApoD es endocitada. Nuestra hipótesis de trabajo es la siguiente: Tras su endocitosis, ApoD podría llegar hasta compartimentos subcelulares como los lisosomas, elemento crítico en el inicio de programas de muerte celular o supervivencia. Proteger la membrana lisosomal sería suficiente para explicar que ApoD pueda evitar la muerte celular en condiciones de estrés oxidativo.

Para poder estudiar el tráfico subcelular de ApoD ha sido necesario obtener una proteína que se pueda distinguir de la propia. Para ello, hemos desarrollado un sistema de expresión eucariota con el que obtenemos ApoD de ratón fusionada con dos secuencias de reconocimiento: polyHis-tag en el N-terminal y Strep-tag en el C-terminal. Hemos obtenido la proteína pura mediante cromatografía de afinidad y estamos usándola en el estudio del tráfico celular en células gliales de ratón silvestre y knock-out para ApoD.

Hemos encontrado ApoD dentro de la célula tanto durante su producción y secreción como durante su internalización. Una fracción importante de la misma co-localiza con marcadores lisosomales tanto en glioblastomas humanos como en astrocitos de ratón. Para conocer el papel de ApoD en los lisosomas estamos cuantificando la proteína que colocaliza con Lamp2 en situaciones de deprivación de suero y tratamiento con paraquat. Si la presencia de ApoD en los lisosomas condiciona su estado de oxidación y con ello la eficiencia del mismo en procesos como la autofagia nuestros datos podrán dar luz a un mecanismo de acción nuevo para una lipoproteína como controlador de la supervivencia celular.

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STUDY OF THE ENDOCANNABINOID SYSTEM ALONG THE CEREBELLAR NEURODEGENERATIVE PROCESS OF THE PCD MUTANT MOUSE

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Introduction: The PCD (Purkinje Cell Degeneration) mutant mouse suffer a postnatal neurodegeneration of the cerebellar Purkinje cells (PC´s). This neurodegenerative process has been related to both defects in the stability of the cytoskeleton and DNA repair. Additionally, the endocannabinoid system has been reported to enhance cytoskeletal integrity, especially through ligands of the peroxisome proliferator activated receptors subtype alpha (PPARα). Moreover, reported effects of PPARα on apoptosis make essential the study of the endocannabinoid system during this neurodegenerative process.

Objectives: The aim of the present study is to determine possible changes or adaptations of the endocannabinoid system in the neurodegenerative environment of the cerebellum of the PCD mouse, with particular emphasis on PC´s. Further, we also analyzed the effect of oleoylethanolamide (OEA, a PPARα agonist) on the neurodegenerative process of the PCD mouse.

Material and methods: To study the endocannabinoid system, we analyzed the expression of the CB1 and PPARα receptors in different cell populations of the cerebellar cortex. The studies were performed at P7, P15, P17, P22 and P30, taking into account the different stages of the neurodegeneration. OEA was administrated intraperitoneally at a dose of 10 mg/kg following 3 different experimental designs: 1) acute administration at P14, 2) acute administration at P16, and 3) continuous administration from P7 to P22. At P30 both PC´s survival and the expression of the CB1 and the PPARα receptors were analyzed.

Conclusions: Preliminary results suggest an enhancement in the expression of key elements of the endocannabinoid system, implicating a possible compensatory mechanism during the neurodegenerative process in PCD mice. In relation with OEA administration, only acute administration at P14 seemed to exert a clear neuroprotective effect.

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Áreas Temáticas:

1ª: Trastornos y reparación del sistema nervioso

2ª: Desarrollo

ROLE OF A PARTICULAR ASTROCYTE REACTION IN THE REPAIRMENT OF THE CEREBRAL CORTEX

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Objectives. In congenital hydrocephalus there is a defect in the development of the neuroepithelium/ependyma preceding ventriculomegaly and followed by a reaction of periventricular astrocytes. The role of such astrocytes that replaces the absence of ependyma was investigated using an animal model of the disease (hyh mouse) and in necropsies of human foetuses with hydrocephalus and controls.

Materials and methods. Histopathological examination, in vitro and in vivo and experiments using intracerebroventricular injections of tracers, and quantifications by real time RT-PCR of neuroprotective and neuroinflammatory factors were performed.

Results. The astrocytes were found forming an integrated cell layer of cells coupled with gap junctions containing connexin 43, projecting microvilli towards the ventricle surface, expressing high levels of aquaporin 4, and presenting an endocytic activity. The astrocyte cell layer and the ependyma also revealed similar behaviours for transcellular and paracellular transport of molecules. The astrocytes were found as a primary source of TNF-alpha and they also expressed the TNF-alphaR1 receptor, suggesting an autocrine control of the proper reaction. The quantification by real time RT-PCR of TNF-alpha and the TNF-alphaR1 performed in hyh mice with two different progressions of the disease, severe and compensated, showed a tight correspondence of their levels with the morbidity and probably with the neurodegenerative consequences of the disease (hypoxic conditions, myelin degeneration, neuronal death). In contrast TGF-beta1, which is a factor that has been implied in the origin of astrogliosis, did not revealed such correspondence. The study of the microglia has shown that these cells could be subtly activated or in an alert stage.

Conclusion. The obtained evidence suggests that reactive astrocytes could represent an attempt to re-establish brain homoeostasis at both sides of the ventricle surface.

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Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Desarrollo

EFFECTS OF HYPOXIA ON RETINAL GANGLION CELLS AND ASTROCYTES IN NEONATAL PIG RETINA

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Introduction: Neurodegeneration in retinal pathologies, such as glaucoma, is often exacerbated by hypoxic conditions. The consequences of the decrease in oxygen on the behavior of retinal ganglion cells (RGC) and retinal glia are not fully understood.

Objectives: The aim of the present study was to analyse the survival of RGCs and also assess the morphological changes in astrocytes in the neonatal pig retina under control and hypoxic conditions.

Materials and Methods: We induced mild hypoxia to neonatal pigs by decreasing the oxygen concentration to 12-14% for 90-120 minutes, following by re-oxygenation at a concentration of 21-35% for 240 minutes. Subsequently, eyes were enucleated and the retina carefully extracted and fixed for immunochemistry analysis. RGCs nuclei were labeled with anti-Brn3a and astrocytes with anti-GFAP specific markers. The number of RGCs/mm^2 was quantified in the central and peripheral retina and the morphology of astrocytes in these areas was analysed.

Results: We observed that, following hypoxia, retinal astrocytes adopt a more disorganised distribution with an increase in lateral process extension. There were no significant changes in the number of RGCs at this time point.

Conclusions: This study found that mild hypoxia induces rapid alterations in retinal glia morphology but had no immediate effect on the number of RGCs. The downstream consequences of these glial changes require further investigation. A deeper understanding of the relationship between RGCs and astrocytes in retinal pathologies may aid the effort to improve neuroregeneration.

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Áreas Temáticas:
1ª: 5. Trastornos y reparación del sistema nervioso
2ª: 2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

LACK OF CHAPERONE-MEDIATED AUTOPHAGY IN MOUSE BRAIN LEADS TO NEURODEGENERATION

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Chaperone-Mediated Autophagy (CMA) targets and degrades a select pool of cytosolic proteins in lysosomes. CMA maintains protein homeostasis by turning over damaged, toxic, and dysfunctional proteins, as well as proteins involved in neurodegenerative diseases, like alpha synuclein (involved in Parkinson's disease (PD)) and tau (in tauopathies and Alzheimer disease). CMA activity decreases in aging but the specific consequences of this age-dependent functional decline and it possible contribution to the aggravation of age-related diseases remains unknown.

To investigate the contribution of CMA to neuronal homeostasis and the possible relation between CMA failure in aging and neurodegeneration, we have developed conditional knock-out mice for the lysosomal CMA receptor, the Lysosomal Associated Membrane Protein 2A (L2A) using the loxP/Cre system. Using mice expressing cre under the Tyroxyne Hydroxylase promoter (THL2AKO), we have found that selective suppression of CMA in dopaminergic neurons results in diminished motor activity and shorter stride length than in the WT mice. These mice also present astrogliosis and decreased markers of dopaminergic neurons in the midbrain and striatum, along with accumulation of alpha synuclein oligomeric forms. Mice with CMA blockage in cortex and hippocampus neurons, generated by breeding with mice expressing cre uder the calmoduline IIa promoter, develop clasping behavior, as early as 3 months old and also show marked changes in cellular homeostasis, with accumulation of oxidized and lipid modified proteins and lipofucscin, but in this case the protein preferentially accumulated in form of protein inclusions is tau.

We have used differential proteomics (using the iTRAQ 4 plex system) of aggregate proteins to identify the specific subset of putative CMA substrates that contribute to the alterations in neuronal homeostasis and ultimately to the pathology observed in these models with deficient neuronal CMA function.

Our in vivo findings highlight the importance of CMA in maintaining neuronal proteostasis, and confirm the possible therapeutic value of restoration of normal CMA activity in old organisms in the prevention and /or reversion of the pathology in neurodegenerative diseases.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Nuevos métodos y tecnologías

SEXUAL DIFFERENCES IN GLIAL REACTIVITY AFTER BRAIN INJURY

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Different pathologies of the central nervous system (CNS) show sex differences in their incidence, symptomatology and neurodegenerative outcome. Glial cells may contribute to these sex differences. Indeed, recent studies have revealed a sex dimorphic response of primary astrocytes to an inflammatory challenge (Santos-Galindo et al 2011). The aim of the present study was to explore in detail glial reactivity in a model of cortical stab injury performed in intact adult male and female mice in the estrus phase of the estrous cycle. Brain injury resulted in an enhanced number of GFAP, vimentin and Iba-1 immunoreactive cells in both sexes. The astroglia response was similar in males and females. Animals of both sexes showed both an equal number of GFAP and vimentin immunoreactive cells and a similar ratio of reactive/non-reactive astrocytes (vimentin/GFAP). No sexual dimorphisms were found when comparing the proportion of Iba-1 positive cells with reactive phenotype in the whole lesion area. However, males showed a significantly higher number of total Iba-1 immunoreactive cells in the lesion border (from the edge of the lesion to 220µm away). Our findings suggest that certain populations of glial cells exhibit different behavior between males and females after a cortical stab injury.

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Santos-Galindo et al. Sex differences in the inflammatory response of primary astrocytes to lipopolysaccharide. Biology of Sex Differences 2011, 2:7

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Sistemas homeostáticos y neuroendocrino

SOCIAL BEHAVIOR AND ULTRASONIC VOCALIZATION IN DYSTROPHIN-DEFICIENT MDX MICE. IS DYSTROPHIN LOSS A PREDISPOSING FACTOR FOR AUTISM?

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Background: Recent reports suggest that Duchenne muscular dystrophy (DMD) patients are at higher risk for autism spectrum disorders (ASD). DMD is caused by mutations in the *dmd* gene affecting the expression of the cytoskeletal protein, dystrophin. DMD is associated with cognitive deficits, likely due to the loss of brain dystrophin, which is normally expressed in structures involved in autism (cerebellum, hippocampus, neocortex). DMD boys show deficits in social behavior, independent of their physical progression and cognitive abilities. However, a link between the *dmd* gene and ASD is unclear, due to the lack of genotype-phenotype studies in DMD patients.

Objective: We used the mdx mouse model of DMD to assess whether the lack of dystrophin, which is commonly lost by all DMD patients, can be associated with autistic-like behavior.

Method: Social behavior was evaluated in mdx and littermate controls (WT) in several paradigms assessing initiation, expression and adaptation of social interactions. Ultrasonic vocalizations were recorded during social interactions and during presentation of sexual olfactory stimuli.

Results and conclusions: Sociability and preference for social novelty are unaltered in mdx mice. However, mdx mice display mild alterations in social interactions and ultrasonic communication depending on the sex and genotype of intruders. Ultrasonic vocalizations are also affected, with fewer, shorter, and less intense calls when interacting with other mdx males or with anesthetized females. Furthermore, mdx and WT mice use a distinct repertoire of syllables, suggesting abnormal communication. This apparent function of brain dystrophin in social behavior and communication skills suggests that the *dmd* gene might be proposed as a predisposing risk gene for ASD.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia cognitiva y conductual

DÉFICITS MOTORES EN RATÓN NULO EN SMAD3 MODULADOS POR EL SISTEMA DOPAMINÉRGICO

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Objetivos: Recientemente nuestro grupo ha puesto de manifiesto como el ratón nulo en Smad3 presenta un síndrome parkinsoniano temprano con déficit cognitivo, degeneración neuronal, degradación dopaminérgica, y depósitos de alfa-sinucleína con modificaciones post-traduccionales similares a las observadas en la enfermedad de Parkinson, localizadas en regiones características de la enfermedad en humanos, como la substancia negra, cuerpo estriado y corteza motora, sugiriendo su interés como modelo de enfermedad de Parkinson. En el presente trabajo hemos abordado la posibilidad de que estas alteraciones moleculares y celulares se manifiesten con déficits motores semejantes a la enfermedad humana.

Material y métodos: Ratones macho deficientes en Smad3 se sometieron a una batería de test comportamentales para medir su función motora, como *footprint*, rotarod, suspensión de cola y actividad locomotora en campo abierto, asociado a un sistema de seguimiento por vídeo para el análisis automático de la exploración y actividad. Se trataron los ratones con diversos moduladores de la dopamina, como la Rasagilina (inhibidor selectivo de MAO-B), la Nomifensina (inhibidor de la recaptación de dopamina), el Quinpirole (agonista de receptores D2) y el SKF-38393 (agonista de receptores D1).

Resultados y conclusiones: Presentaremos los resultados de estas investigaciones donde se muestra un déficit motor muy marcado en la deficiencia de Smad3 que está modulado por el sistema dopaminérgico. Remarcamos especialmente, la importante mejora motora en el ratón nulo para Smad3, tras un tratamiento crónico con Rasagilina. Estos resultados confirman el papel central de la ruta de señalización intracelular de TGFbeta/Smad3 en la homeostasis dopaminérgica y sugieren a Smad3 como posible nueva diana terapéutica contra la enfermedad de Parkinson.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia cognitiva y conductual

APOMORPHINE-INDUCED DYSKINESIAS IN UNILATERAL 6-HYDROXYDOPAMINE-LESIONED RATS: QUETIAPINE DOSE-RESPONSE EFFECTS

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Levodopa-induced dyskinesias complicate long-term treatment of Parkinson's disease (PD). Its pathophysiology is unknown but the serotonergic system seems to have a role. $5HT_{2A}$ antagonists have attenuated dyskinesia in MPTP-monkeys, but data in PD patients are controversial. We investigated the effect of quetiapine, an atypical neuroleptic with 5-HT2A receptor antagonistic properties, on dyskinesia induced by apomorphine (APO) in the 6-hydroxydopamine-lesioned (6-OHDA) rat model. The nigro-striatal pathway was unilaterally lesioned with 6-OHDA. After a dose-increase protocol of APO administration (escalating doses 0,1, 0,2, 0,5 mg/kg, sc., over three 5-d blocks) animals developed dyskinesias. On day 16, APO (0,5 mg/kg) and quetiapine (20 mg/kg, ip.) or vehicle were co-administered for 2 weeks. Dyskinesias and rotational behavior in response to APO were measured on days 1, 5, 6, 10, 11, 15, 16, 20, 25 and 30. In addition, PET studies with the radioligands ^{11}C-PE2I (dopamine transporter) and ^{18}F-FDG (metabolism rate) were performed under the influence of drugs. Quetiapine reduced the total number of APO-induced rotations in comparison with animals treated only with APO, indicating a motor inhibitory effect of quetiapine which could be explained by its D_2/D_3 dopamine receptor antagonist activity. Surprisingly, quetiapine increased the global score of dyskinesias. This could be either a consequence of the reduction of the rotational behaviour, and/or due to the action of quetiapine on other receptors such as adrenoreceptors or histamine receptors. A more complex interaction between dopaminergic and serotoninergic system should also be considered. Dyskinetic animals had a reduction in ^{18}F-FDG uptake in the striatum which was associated with the severity of the dyskinesias. In conlusion, the striatum is a key structure in the expression of dyskinesias. Current studies using PET with ^{18}F-Altanserine ($5\text{-}HT_{2A}$ ligand) could help to understand the underlying action mechanisms of quetiapine and to deeply analyze the role of the serotonin system (DFG11/019, PI11/02109).

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia cognitiva y conductual

CHARACTERIZATION OF THE AUTOANTIGEN REPERTOIRE IN NEUROLOGICAL DISORDERS ASSOCIATED TO GAD AUTOIMMUNITY

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The presence of autoantibodies against the intracellular protein GAD65 has been described in different neurological diseases. How a unique autoantibody is related with such a broad spectrum of syndromes is an open question. We postulate two hypotheses (1) there are differences in the repertoire of GAD autoantibodies and (2) there are autoantibodies against other synaptic targets. To test these hypotheses we analyzed the largest cohort of GAD positive patients ever reported, including 96 patients who developed cerebellar ataxia, stiff-man syndrome, epilepsy, and/or limbic encephalitis. We first determined whether the antibodies reacted with epitopes of GAD67. These studies showed that serum of patients with higher titers of GAD65 antibodies also recognized GAD67. Overall 70% of all cases had antibodies against GAD65 and GAD67. Comparison among phenotypes showed that the titers of GAD67 antibodies were lower in patients with epilepsy. We next investigated whether the epitopes were conformational, showing that the antibodies of all patients with cerebellar ataxia recognized linear GAD65 but only ~50% recognized linear GAD67. To determine the presence of antibodies against other synaptic targets, serum samples of all 96 patients were tested using immunofluorescence with cultures of dissociated rodent live hippocampal neurons, showing that 9 had antibodies had reactivity against surface proteins (1 against NMDA Rc, 1 against GABA(B) Rc, and 7 are still uncharacterized).

Additionally, among patients whose serum did not react with cultures of live neurons, 10 cases from each clinical group were specifically tested for antibodies to glycine, GABA(A) and GABA(B) receptors; the 40 samples were negative for all 3 receptors. In summary, we show for first time the presence of GAD67 autoantibodies in LE and EP patients, demonstrate the relevance of epitope conformation for GAD67 but not GAD65 antibodies, and reveal the presence of additional antibodies to relevant synaptic receptors in 9% of the patients.

Áreas Temáticas:

1ª: Trastornos y reparación del sistema nervioso
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

IMPLICATION OF DOPAMINE IN THE RECRUITMENT OF IMMUNE CELLS

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ABSTRACT

Objectives: The aim of this work was to test whether dopamine (DA) might play a role in the infiltration of peripheral immune cells and leakage of the blood–brain barrier (BBB) observed in neuroinflammation, providing a link between peripheral and central inflammatory events.

Materials and methods: Male albino Wistar rats (250-270 g) were used for this study. Rats were anesthetized with chloral hydrate (400 mg/kg) and positioned in a stereotaxic apparatus to conform to the brain atlas of Paxinos and Watson. The dopaminergic toxin 6-hydroxydopamine (6-OHDA) was used to damage dopaminergic neurons in the substantia nigra (SN) and the striatum; alpha-methylparatyrosine (α-MPT) was used as inhibitor of tyrosine hydroxylase, the enzyme catalyzing the key step in the synthesis of DA. The effects of the treatments at different time points on astrocytes, microglia, osteopontin and TH-positive neurons and terminals were measured by inmunohistochemistry, as well as disruption of the BBB by immunofluorescence, content of DA by HPLC, and infiltration of macrophage and microglia by flow cytometry.

Results: Injection of 6-OHDA within the nigrostriatal pathway produced loss of dopaminergic neurons and terminals, loss of astrocytes, disruption of the BBB, activation of microglia and reduction of OPN immunoreactivity. Depletion of DA content by α-MPT reduced both the infiltration of peripheral macrophages and the increase of microglial cells induced by 6-OHDA into the nigrostriatal pathway.

Conclusions: Data obtained in this study suggest that DA could be relevant for sustaining inflammation and lymphocytes recruitment induced by 6-OHDA. Since it is thought that inflammation and microglial activation are involved in the initiation and progression of Parkinson's disease (PD), implication of DA in the degeneration of dopaminergic neurons induced through inflammatory processes should be considered.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

NEUROTOXIC EFFECT OF METFORMIN ON DOPAMINERGIC NEURONS

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ABSTRACT

Objectives: Metformin is a widely used oral antidiabetic drug with known anti-inflammatory properties due to its action on AMPK protein. This drug has shown a protective effect on various tissues, including cortical neurons. The aim of this study was to determine whether metformin is able to protect dopaminergic neurons of the substantia nigra (SN) in animal models of Parkinson's disease (PD).

Materials and methods: Two widely studied models of PD were used to induce damage in the dopaminergic neurons of the SN. Male albino Wistar rats (250-270 g) and C57BL mice (20-25g) were used for this study. Rats were anesthetized with chloral hydrate (400 mg/kg) and positioned in a stereotaxic apparatus to conform to the brain atlas of Paxinos and Watson. The injection of 2 μg of the inflammogen lipopolyssacharide (LPS) was used to induce a strong inflammatory response and to damage dopaminergic neurons in the SN. Mice were injected with MPTP (i.p.), an inhibitor of mitochondrial complex I, to damage dopaminergic neurons in the SN. In both experiments, a group of animals was treated with a daily oral dose of metformin (150 mg/Kg, seven days). Loss of astrocytes, activation of microglia and loss of TH was measured by immunohistochemistry, and content of DA was measured by HPLC. The levels of several proinflammatory cytokines were measured by RT-PCR.

Results: Metformin reduced microglial activation measured both at cellular and molecular levels. However, metformin did not protect dopaminergic neurons neither from injections of LPS within the nigrostriatal pathway of rats nor i.p. injections of MPTP in mice, but produced a bigger loss of dopaminergic neurons.

Conclusions: The data obtained in this study suggest that, contrary to other brain structures, metformin treatment could be deleterious for the dopaminergic system. This could be due to the inhibition of the mitochondrial complex I produce by this drug in the SN, a brain structure particularly sensitive to inflammation that is involved in PD.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

DEPENDENCE OF EXTRAOCULAR MOTONEURONS ON VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

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Vascular endothelial growth factor (VEGF) has been recently implicated in motoneuron survival, since a mutation in the promoter region of the gene in transgenic mice causes late onset degeneration of motor neurons, resembling amyotrophic lateral sclerosis (ALS). One of the hypotheses is that inadequate neurotrophic stimulation of motoneuron by VEGF may be the reason for motoneuronal degeneration. Interestingly, motoneurons of the oculomotor system are more resistant to neurodegeneration in ALS disease, when compared to other cranial or spinal motoneurons. Differences regarding neurotrophic dependence between extraocular motoneurons and other motoneuronal types could play an important role mediating the lesser vulnerability of these motoneurons.

Consequently, we were interested in unveiling the degree of dependence of extraocular motoneurons on VEGF, and compare it with other motoneurons sensitive to neurodegeneration. For this purpose, we performed Western blot in protein extracts from extraocular muscles in order to determine whether those muscles are a natural source of VEGF for extraocular motoneurons. The presence of VEGF receptor (VEGFR2 or Flk-1) has also been analyzed by immunohistochemistry in the three extraocular motor nuclei, and the expression level has been compared with that observed in other non-extraocular cranial motoneurons located in the hypoglossal and facial nuclei.

Expression of VEGF was demonstrated in extraocular muscles, and its receptor Flk-1 was extensively distributed in extraocular motor nuclei, suggesting that VEGF could act as a trophic factor for ocular motoneurons. Facial and hypoglossal motoneurons also contained VEGF receptors, but in a significantly lesser degree. VEGF itself was also present in motoneurons, so this factor could be acting not only in a retrograde manner, but also via autocrine. Therefore, differences in VEGF dependence could contribute to the different susceptibility between ocular and other motoneurons to neurodegeneration.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia de sistemas

THE EFFICIENCY OF INTEGRATION OF BONE MARROW CELLS INTO THE ENCEPHALON DEPENDS ON THE METHODOLOGY OF TRANSPLANTATION

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Since the late 90's, bone marrow-derived cells (BMDC) have been demonstrated to integrate physiologically into the encephalon, which has opened new research lines employing BMDC as therapeutic tools for different injuries of the central nervous system. The efficiency of this physiological integration is not very high, although brain damage seems to increase it, which supports its therapeutic employment. However, it is necessary to improve and accelerate this integration of BMDC to ensure their efficiency in putative cell therapies, especially when certain brain disorders have short time-courses.

Healthy GFP-labeled bone marrow cells were transplanted into mice suffering selective neurodegeneration to develop a cell therapy. Different methodologies were applied and compared on this work: some of them imply the reconstitution of the recipient's bone marrow by a healthy one, with different ablation (radiation or chemicals) and transplant (intravenous or intrahepatic) methodologies. A continuous support of BMDC without bone marrow ablation was also tested.

Radiation was the most suitable methodology for achieving the highest amount of bone marrow-derived neural cells because of the opening of the blood brain barrier. However, this integration was a long-lasting process not fully efficient for fast neurodegenerative diseases. Accordingly, we decided to increase the time-window for this integration bringing forward the age of transplant (newborns), but the time lapsed from the reconstitution of the recipient's bone marrow, to the arrival of the new BMDC to the brain is also too long. Finally, avoiding bone marrow reconstitution, but mimicking a continuous support of alogenic BMDC into the recipient's blood stream with chronic injections a better integration of BMDC into the brain was achieved.

The most suitable methodology for transplant among those analyzed implies the opening of the blood-brain barrier as well as the acceleration of the BMDC arrival into the encephalon bypassing the reconstitution of the recipient's bone marrow.

Áreas Temáticas:
1ª:Trastornos y reparación del sistema nervioso
2ª: Nuevos métodos y tecnologías

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ESTUDIO FARMACOLÓGICO EN EL HÁMSTER EPILEPTICO GASH:Sal

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Objetivos: Determinar el efecto anticonvulsivo y antiepiléptico de diversos fármacos antiepilépticos sobre el hámster GASH:Sal (Genetic audiogenic seizure hámster), y caracterizar la respuesta comportamental a estos fármacos mediante análisis neuroetológico.

Material y métodos: Se administraron tres fármacos antiepilépticos: Ácido Valproico (VPA), Levetiracetam (LEV) y Fenobarbital (PB) a los hámsteres GSH:Sal, determinando su potencia anticonvulsivante, su efecto sobre la severidad de las crisis y su toxicidad. Los análisis neuroetológicos se realizaron sobre las crisis grabadas, evaluando los diferentes ítems comportamentales con el programa ETHOMATIC.

Resultados: La acción aguda de PB y VPA es dosis-dependiente. En tratamientos agudos, el PB modifica la crisis desde los 5 mg/kg, bloqueándolas totalmente a los 20 mg/kg. El VPA comienza a ser eficaz en el 75% de los animales a los 300 mg/kg, siendo plenamente efectivo a los 500 mg/kg. Dosis elevadas de ambos fármacos eliminan los comportamientos tónico-clónicos, alargándose la carrera salvaje, hasta bloquear totalmente la crisis. El LEV no actúa de manera clara; su efecto no depende de la dosis, y elimina las crisis en un 50% a los 50 y 80 mg/kg. En las dosis agudas altas de PB y VPA se observaron efectos atáxicos, mientras que con LEV aparece sedación como efecto secundario. En administración crónica, el PB fue el único anticonvulsivo que anuló totalmente las crisis con 60 mg/kg/día. En condiciones crónicas, se observa ataxia y sedación con los tres fármacos, siendo los efectos más pronunciados con LEV.

Conclusiones: La administración de fármacos antiepilépticos al hámster GASH:Sal modifica la secuencia comportamental de las crisis audiógenas de manera dosis-dependiente, anulando la fase ~~de~~ convulsiva y prolongando la carrera salvaje, que pasa directamente a la inmovilidad postictal, la cual aumenta su duración. En administración aguda, el PB presenta la mayor eficacia terapéutica. Todos los fármacos ensayados, tanto en administración aguda como crónica, provocan ataxia y sedación de forma dosis-dependiente.

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Areas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia cognitiva y conductual

DAMAGE AND REPAIR OF RAT VESTIBULAR CALYX ENDINGS IN CHRONIC OTOTOXICITY

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Acute ototoxicity causes loss of equilibrium through degeneration of the vestibular sensory hair cells. In most mammalian species, this functional loss is permanent because hair cells do not regenerate. In contrast, highly variable degrees of functional recovery are observed if the exposure is discontinued in humans suffering chronic ototoxic exposure. The cellular and molecular events associated with this chronic dysfunction and recovery are not known. In this study, chronic ototoxicity was induced in male adult Long-Evans rats by drinking water exposure to 3,3'-iminodipropionitrile (IDPN) (20 mM, 4 weeks). A specific behavioral test battery was used to assess vestibular dysfunction. Animals were paired according to dysfunction ratings, and sacrificed at the end of the exposure or after 4 weeks of wash out. Data from scanning and transmission electron microscopy and confocal immunostaining were obtained from each single animal, to determine the relationship between vestibular function, tissue ultrastructure and molecular events. Animals with extreme dysfunction ratings (>20) showed ciliary coalescence, incomplete functional recovery, and loss of hair cells after 4 weeks of wash out. Animals with ratings of dysfunction in the 15 to 19 range showed complete behavioral recovery. In these animals, normal ciliary morphology was maintained and no hair cell loss occurred. At 4 weeks of exposure, many calyx endings surrounding type I hair cells were fragmented and partially retracted. In the terminals maintained in place, the septate juntions between the calyx afferents and the hair cells were lost, immunoreactivity of the septate junction protein caspr was dramatically reduced, and that of the KCNQ4 was mislocalized. These changes were no longer observed in the wash out animals with complete behavioral recovery. We conclude that synaptic uncoupling and repair are one major phenomenon involved in functional loss and recovery during chronic vestibular toxicity.
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Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

LITIO COMO TERAPIA NEUROPROTECTORA EN EL MODELO APPSL/PS1M146L DE LA ENFERMEDAD DE ALZHEIMER

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El litio se utiliza desde hace varias décadas en el tratamiento de trastornos bipolares y la depresión, y recientemente se debate su uso potencial en patologías neurodegenerativas como la enfermedad de Alzheimer (AD). Diversos estudios han puesto de manifiesto su efecto positivo como potente inhibidor de GSK3beta disminuyendo la fosforilación de tau, la producción de Abeta e incrementando plasticidad sináptica. Sin embargo, su posible efecto neuroprotector previniendo la muerte neuronal in vivo no ha sido aun demostrado ya que la mayoría de los modelos transgénicos de AD no presentan pérdida neuronal. Nuestro modelo APPSL/PS1M146L sufre una pérdida significativa de neuronas SOM/NPY en hipocampo y corteza entorrinal desde edades tempranas (6 meses) con un marcado desarrollo de distrofias axonales. En este trabajo hemos estudiado el posible efecto neuroprotector del litio en este modelo animal mediante tratamiento crónico en la dieta desde los 3 hasta los 9 meses de edad. Se han utilizado técnicas imnunohistoquímicas, western blots y análisis por RT-PCR, y además se ha determinado la carga amiloide, el grado de compactación y el tamaño de las placas. El resultado más relevante de este estudio fue la preservación de la población de interneuronas SOM/NPY tanto en hipocampo como corteza entorrinal en los animales tratados, mientras que en los no tratados existió una pérdida significativa de esta supoblación neuronal. El efecto neuroprotector del litio se manifestó también en una marcada disminución de tau fosforilado, distrofias axonales y marcadores sinápticos, junto con una mejora cognitiva de los animales utilizando el test de reconocimiento de objetos. Este efecto preventivo del litio parece estar asociado con cambios en la formación de placas de Abeta que podrían afectar a su toxicidad, ya que los animales tratados presentaron placas más pequeñas y apariencia más compacta. *Financiación: FIS PI12/01431 (AG) y FIS PI12/01439 (JV).*

Áreas temáticas: 1. Trastornos y reparación del sistema nervioso.
2. Neurociencia de sistemas.

PROCESO INFLAMATORIO Y MUERTE NEURONAL EN EL HIPOCAMPO HUMANO DURANTE LA PROGRESIÓN DE LA ENFERMEDAD DE ALZHEIMER

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Los cambios fenotípicos/funcionales de las células gliales que acontece durante la progresión de la enfermedad de Alzheimer (EA) no son conocidos. La activación de las células gliales puede inducir muerte neuronal mediante la producción de factores citotóxicos, desencadenando así una cascada de eventos patogénicos que finalmente llevan a la demencia. En este trabajo, hemos analizado la activación astroglial y microglial, la expresión de citoquinas inflamatorias y la neurodegeneración durante el curso de la EA en el hipocampo humano. Para ello, se han llevado a cabo RT-PCR, western blots y técnicas inmunohistoquímicas y estereológicas, en muestras postmortem de diferentes estadios de la enfermedad: estadíos leve-moderado (Braak II y Braak III-IV) sin signos de demencia y estadíos severos (Braak V-VI) con demencia, así como en controles no dementes. Nuestros datos demostraron la existencia de un proceso neuroinflamatorio significativo con activación microglial y, más pronunciada, astroglial, en los casos más severos de la enfermedad. La microglía activada presentó un fenotipo clásico M1, coincidiendo con el incremento en la expresión de los marcadores citotóxicos TNF-alfa y FAS-L en las muestras humanas con estadio Braak V/VI. Este ambiente citotóxico además coincide con la bajada del marcador neuronal NeuN, así como con una reducción tanto en la expresión como en la densidad celular, de las subpoblaciones de interneuronas SOM y PV. Además, las formas solubles de β-amiloide y/o tau procedentes de cerebro de pacientes severos indujeron in vitro la activación de células microgliales a un fenotipo clásico (M1) con expresión de TNF-alfa. Las células gliales activadas y citotóxicas, podrían estar participando en los procesos neurodegenerativos observados en los estadios más severos de la EA. Finalmente, la vulnerabilidad selectiva de estas interneuronas GABAérgicas, podría repercutir en los procesos de inhibición local induciendo déficits de memoria. Financiado por *FIS-PI12/01431(AG), FIS-PI12/01439(JV) y proyecto colaborativo CIBERNED PI2010/08 (JV y AG).*

1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia de sistemas

ESTRÉS OXIDATIVO Y DISFUNCIÓN MITOCONDRIAL EN LA ENFERMEDAD DE PARKINSON

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Objetivo:

La incidencia de enfermedades neurodegenerativas crece continuamente, debido al aumento de esperanza de vida de la población, siendo la Enfermedad de Parkinson (EP) la que se encuentra en segundo lugar. Aunque su etiología no es bien conocida, numerosas evidencias han asociado el estrés oxidativo y la disfunción mitocondrial con el daño de las neuronas dopaminérgicas de la sustancia negra. El objetivo de este trabajo es analizar estos dos procesos en pacientes con EP.

Sujetos y Métodos:

Se estudiaron un grupo de 150 pacientes con EP y otro grupo de 26 controles, con edades superiores a los 55 años. A partir de muestras plasmáticas y por medio de métodos espectrofotométricos, se determinaron los niveles de lactato (LAC) como marcador de disfunción mitocondrial y los niveles de ácido úrico (AU) y actividad de la superóxido dismutasa (SOD) como indicadores de las defensas antioxidantes.

Resultados:

Los niveles plasmáticos de LAC se encontraron incrementados ($P < 0,05$) en el grupo de pacientes, indicando la existencia de disfunción mitocondrial. Por el contrario, en el grupo de pacientes la actividad enzimática de SOD se encontró disminuida ($P < 0,05$), sugiriendo una reducción de las defensas antioxidantes. No se encontraron diferencias significativas en los niveles plasmáticos de AU, posiblemente debido a la influencia de los hábitos alimenticios y consumo de alcohol. En el grupo de pacientes, al comparar entre los diferentes estadios de la enfermedad o entre sexos no se encontraron diferencias significativas en ninguno de los parámetros bioquímicos analizados.

Conclusiones:

Los pacientes con EP muestran disfunción mitocondrial y tienen disminuidas las defensas antioxidantes, lo que puede influir en la progresión de la enfermedad. Una terapia dirigida a mejorar el estrés oxidativo podría ser muy útil para el tratamiento de la EP.

AGING-RELATED DYSREGULATION OF DOPAMINE AND ANGIOTENSIN RECEPTOR INTERACTION

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Aging is the most prominent risk factor for Parkinson's disease (PD), however it is not known whether the aging-related decrease in dopaminergic (DA) function leads to the aging-related higher vulnerability of DA neurons and risk for PD. The renin-angiotensin system (RAS) plays a major role in the inflammatory response, neuronal oxidative stress and DA vulnerability via type 1 (AT1) receptors, which is counteracted by AT2 receptors. Interactions between dopamine and angiotensin receptors have been reported in several studies, suggesting that both systems may directly counterregulate each other. In the present study, we investigated the effects of aging-related changes in DA system on the nigral and striatal RAS.

The effects of DA depletion were studied in transgenic mice (D1,D2 null mice and D2 overexpressing mice) and in the 6-hydroxydopamine model in young and aged rats. Real-time PCR and Western blot were used to study dopamine and angiotensin receptors levels. High performance liquid chromatography was used to quantify dopamine and its metabolites. Striatal DA terminals and TH expression in substantia nigra compacta were analyzed by optical density after TH-immunohistochemistry.

In the present study, we observed a counterregulatory interaction between dopamine and angiotensin receptors. We observed overexpression of AT1 receptors in the striatum and substantia nigra of young adult dopamine D1 and D2 receptor-deficient mice and young dopamine-depleted rats, together with compensatory overexpression of AT2 receptors or compensatory downregulation of angiotensinogen/angiotensin. We also observed downregulation of dopamine and dopamine receptors, and overexpression of AT1 receptors in aged rats, without compensatory changes in AT2 or angiotensinogen/angiotensin levels. These results suggest that, in addition to its role as an essential neurotransmitter, dopamine may play an important role in modulating oxidative stress and inflammation in the substantia nigra and striatum via the RAS, which is impaired by aging.

Áreas Temáticas:
1ª. Trastornos y reparación del sistema nervioso
2ª. Sistemas homeostáticos y neuroendocrino

CHRONIC BRAIN HYPOPERFUSION, BRAIN ANGIOTENSIN AND DOPAMINERGIC DEGENERATION: RELEVANCE TO VASCULAR PARKINSONISM, PARKINSON'S DISEASE AND AGING

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The possible interaction between brain hypoperfusion related to aging and/or vascular disease, vascular parkinsonism and Parkinson's disease (PD), and the contribution of aging-related chronic brain hypoperfusion (CBH) in the development of PD are unknown. Identification of factors and mechanisms linking hypoperfusion and neurodegeneration may lead to neuroprotective strategies. The brain renin-angiotensin system (RAS) may play a major role in linking vascular disease and dopaminergic degeneration. We have previously shown that brain Angiotensin II, via angiotensin type 1 (AT1) receptors, exacerbates dopaminergic cell death and that aging decreases nigral microvascularization and enhances nigral RAS activity.

In the present study, we used a rat model of CBH to study the long term effects of hypoperfusion on dopaminergic neurons (DN) and the possible synergistic effects between hypoperfusion and factors that are deleterious to DN (low doses of the dopaminergic neurotoxin 6-hydroxydopamine).Western blot, RT-PCR and enzymatic activity assays were used to study the involvement of the nigral RAS. Furthermore we investigated whether the blockage of AT1 receptors may inhibit the hypoperfusion-derived increased in dopaminergic vulnerability.

Using TH-immunohistochemistry we observed that CBH induced significant loss of DN and striatal dopaminergic terminals. Furthermore, intrastriatal administration of 6-hydroxydopamine in rats subjected to CBH induced a significantly greater loss of DN than in control rats. The DN loss was significantly reduced by treatment with the AT1 receptor antagonist candesartan. The levels of AT2 receptors were lower and the levels of AT1 receptors, interleukin-1β and NADPH-oxidase activity were higher in the substantia nigra of rats subjected to CBH than in control rats; this was significantly reduced by candesartan. The results suggest that early treatment of vascular disease should be considered in the treatment of aged PD patients and PD patients with cerebrovascular risk factors and that inhibition of brain RAS activity may be useful as a neuroprotective strategy.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso

LA DISMINUCIÓN DE LOS RECEPTORES A2A DE ADENOSINA EN ESTRIADO ASOCIADA A ALTERACIONES EN ESCALAS DE VALORACIÓN CLÍNICA DE ESQUIZOFRENIA SUGIERE UN POSIBLE SUBGRUPO MOLECULAR DE LA ENFERMEDAD

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La Esquizofrenia es una enfermedad mental de origen desconocido. Existen evidencias de que no es una única enfermedad puesto que hay pacientes con claras diferencias en cuanto a sintomatología, biomarcadores y curso de la enfermedad. Se presenta generalmente un estado hiperdopaminérgico con elevada actividad de los receptores D_2 de dopamina, aunque el estado hipodopaminérgico también ha sido propuesto. Sin embargo, existe una teoría reciente que implica la hipofunción de la señalización de la adenosina en su fisipatología. La adenosina es un nucleósido que actúa como neuromodulador y neuroprotector en el SNC. Sus efectos están mediados por la unión a diferentes receptores, fundamentalmente de tipo A_1 y A_{2A}. Los A_1 inhiben la adenilato ciclasa a través de proteínas $G_{i/o}$ y los A_{2A} activan dicha actividad enzimática, a través de proteínas G_s. En el presente trabajo se analiza el estado de ambos receptores en muestras postmortem de putamen de pacientes de SZ, mediante ensayos de unión de radioligando, Western-blot y PCR a tiempo real. Los resultados muestran que aproximadamente la mitad de los pacientes presentan unos niveles reducidos de A_{2A}, mientras que los A_1 se preservan. Por otro lado, se muestra cómo la metilación del DNA juega un papel en los niveles patológicos de A_{2A} puesto que se observó un incremento en 5-metilcitosina en la región 5' UTR del gen ADORA2A en los pacientes que presentaban bajos niveles de A_{2A}. Por último, se observa una importante correlación entre los niveles de expresión del gen A_{2A} y las alteraciones motoras analizadas con las escalas de valoración clínica PANSS, AIMS y SAS, sugiriendo la existencia de un subgrupo de pacientes de SZ con fenotipo motor alterado que presentan una mayor afectación de A_{2A}. Estos resultados implican a los receptores de adenosina en dicha patología y sugieren dichos receptores como una posible nueva diana terapéutica.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

MONOAMINERGIC AND METABOLIC POSITRON EMISSION TOMOGRAPHY OF UNILATERAL AND BILATERAL 6-OHDA RAT MODELS OF PARKINSON'S DISEASE: A LONGITUDINAL *IN-VIVO* STUDY

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Parkinson´s disease (PD) is characterized by nigro-striatal loss and dopaminergic striatal depletion. The aim of this work is to characterize *in-vivo* a time-course pattern of functional changes associated with dopaminergic striatal reduction in rat models of PD using Positron Emission Tomography (PET).

Forty-four male Sprague-Dawley rats (300-350gr) were used. PET imaging with monoaminergic (^{11}C-(+)-α-dihydrotetrabenazine; ^{11}C-DTBZ) and metabolic (^{18}F-fluorodeoxyglucose; ^{18}F-FDG) radiotracers were performed in a longitudinal study during 6 weeks of the following groups: a) unilaterally lesioned rats by injection of 4µg/4µl (low dose) and 8µg/4µl (high dose) of 6-hydroxydopamine (6-OHDA) in the left median forebrain bundle; and b) bilateral lesion model, in rats receiving intraventricular injection of 100µg/4µl/day of 6-OHDA during 7 days. At the 8th week, the glucose metabolism was also evaluated *ex vivo* by ^{18}F-FDG autoradiography. Bilaterally lesioned animals were not assessed with metabolic analyses.

^{11}C-DTBZ PET images showed a significant decrease of Striatal Binding (SB) values one week after the lesion (35% SB in the low and 20% SB in the high dose group of unilateral model, and 50% SB in the bilateral model). In the 6th week, no significant differences in these values were found in the unilaterally lesion rats, whereas animals with bilateral lesion showed a higher binding value (65% SB). Remarkably, the metabolic PET study in the unilateral model revealed hypometabolism in ipsilateral somatosensory cortex and hypermetabolism in contralateral entorhinal cortex since the 2nd week onwards. Additionally, the autoradiography analysis showed hypometabolism in bilateral somatosensory cortex and ipsilateral caudate-putamen, motor cortex and thalamus, and also hypermetabolism in the contralateral entorhinal cortex.

^{11}C-DTBZ PET is a sensitive method to ascertain dopaminergic depletion in both the bilateral and, unilateral 6-OHDA rat models. ^{18}F-FDG studies showed a dynamic metabolic pattern that can provide useful *in vivo* information to monitor brain changes (CIBERNED, UTE-FIMA).

ROLE OF APOLIPOPROTEIN D IN MACROPHAGE RECRUITMENT AND MYELIN PHAGOCYTOSIS UPON PERIPHERAL NERVE INJURY

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Apolipoprotein D (ApoD) is a Lipocalin expressed in the peripheral nervous system (PNS) by Schwann cells and its expression is strongly induced upon injury. Here we assess the function of ApoD in the molecular events that take place after a PNS injury. We have studied the process of Wallerian degeneration *in vivo* following sciatic nerve crush and we have assayed *ex vivo* the phagocytosis of labelled myelin from wt and ApoD-KO mice by flow cytometry.

The analysis of cellular processes and protein or mRNA expression of a group of signalling molecules induced by injury shows that the lack of ApoD results in an exacerbated MCP-1 and TNFa-dependent macrophage recruitment. At the injury site, free AA increases in the wt. Lack of ApoD results in higher basal levels and an injury-triggered decrease of AA. Control by ApoD of the availability of AA to produce the lipid mediators involved in macrophage recruitment is therefore a key mechanism that conditions Wallerian degeneration and injury resolution later on.

On the other hand, the phagocytosis of ApoD-KO CNS myelin is less efficient than the phagocytosis of wt myelin, therefore there are also genotype-dependent differences in myelin composition and/or in the interaction between myelin and macrophages. An electron microscopy analysis of myelin preparations reveals that the CNS myelin from ApoD-KO mice has abnormal periodicity and shows defective myelin compaction. Lipid analysis of ApoD-KO myelin shows an altered phospholipid composition, particularly in phosphoinositid species. These changes in lipids are paralleled changes in the expression of myelin proteins such as Mbp or Mag. A study of the function of ApoD in the myelination process is currently underway.

Our results demonstrate that ApoD function is relevant for both, myelin membrane properties that influence myelin-macrophage interactions, and the control of the lipid-mediated signalling events controlling the extent of macrophage recruitment.

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THE REGULATION OF THE MICRORNAS EXPRESSION INDUCES GENE EXPRESSION CHANGES OF THEIR TARGTES IN THE RAT VISUAL CORTEX UNDER EXPERIMENTAL CONDITIONS DIRECTED TO NEUROPLASTICITY

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MicroRNAs are key post-transcriptional regulators of gene expression. They have been demonstrated to play pivotal roles in neuroplasticity, but the mechanisms underlying this phenomenon still remain unexplored.

In our laboratory we detected changes in the expression of early-immediate genes (c-fos) and molecules implicated in neuroplasticity (BDNF) in the rat brain induced by application of magnetic field with a shift of 120° in the horizontal component and also by visual deprivation.

This study aims to analyze microRNAS involved in the plastic processes of visual system. First, a rat microarray was conducted to identify microRNAs whose expression pattern is altered in the visual cortex under the following experimental conditions: dark rearing, magnetic stimulation and a combination of both. Then RT-qPCR assays were performed to quantify the expression level of the microRNAs and of the corresponding targets. Moreover, preliminary in vivo experiments have been developed to inhibit specifically endogenous microRNAs to detect effects in the expression level of their targets.

Forty-seven microRNAs resulted to be altered in the visual cortex under the above mentioned experimental conditions. Five of them were significantly downregulated in animals subjected to dark rearing or eyelid suture and magnetic stimulation: let-7b*, miR-330, miR-338*, miR-376c and miR-542-5p. With the exception of miR-542-5p, the deregulated expression of these microRNAs triggered a statistically significant increase in the expression of their predicted targets: BDNF and Sncb; involved in regulation of synaptic plasticity; Gjb2, Tnr and Cntn4, implicated in regulation of axonal and dendritic projections. These results were supported by the administration of anti-miRNAs, showing an overexpression of the targets in the surrounding area of the injection.

Therefore, the regulation of expression of microRNAs by specifically-designed anti-miRNAs is proposed as a useful tool to develop therapeutic treatments for nervous illness due to their ability to regulate gene expression of molecules involved in plasticity processes.

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Áreas temáticas:
1ª: 3- Neurociencia de sistemas
2ª: 5- Transtornos y reparación del sistema nervioso

ESTUDIO ULTRAESTRUCTURAL DE LA RELACIÓN GLIA-PLACA EN UN MODELO APP/PS1 DE LA ENFERMEDAD DE ALZHEIMER

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La presencia de placas de beta-amiloide (Abeta) es una de las lesiones histopatológicas más características en el cerebro de los pacientes de Alzheimer. Estos depósitos extracelulares se encuentran íntimamente asociados con células gliales activadas, microglía y astroglía, que pueden producir diversos mediadores químicos, incluyendo citoquinas pro-inflamatorias. Además de un papel principal en la respuesta inflamatoria, se ha sugerido que estas células gliales pueden contribuir a la amiloidosis participando en la formación y/o fagocitosis de las placas. En el presente trabajo hemos estudiado la relación entre la astroglia/microglia y las placas en el hipocampo de ratones transgénicos APPSL/PS1M146L, mediante análisis ultraestructural y estudios inmunohistoquímicos a microscopía óptica. Los resultados muestran una estrecha relación entre ambos tipos de células gliales y las placas de Abeta, independientemente del tamaño de las placas o la localización de éstas en diferentes regiones del hipocampo. El análisis a microscopía electrónica reveló numerosas prolongaciones astrogliales que se interdigitaban de forma compleja con extensiones de material fibrilar de la placa. En las zonas de contacto con la placa, el citoplasma de los astrocitos carecía de filamentos gliales, muy abundantes en las prolongaciones de estos astrocitos reactivos. También, se observaron células microgliales en íntimo contacto con las placas, cuyas extensiones de material fibrilar se entremezclaba con las prolongaciones celulares. Aunque la mayoría de las placas presentaba astroglia y microglia alrededor, en algunas ocasiones se observaron placas de pequeño tamaño que estaban casi completamente rodeadas por las prolongaciones de una única célula microglial. Era frecuente observar cisternas de retículo endoplasmático rugoso en el citoplasma de la microglía que estaba en contacto con el material fibrilar de la placa. Nuestros resultados apoyan la idea de que las células gliales participan en la dinámica de formación/eliminación y/o aislamiento de las placas.
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5. Trastornos y reparación del sistema nervioso.
2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.

CB_2R ACTIVATION STIMULATES GLUCOSE UPTAKE IN THE RODENT BRAIN

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Brain glucose hypometabolism is a preclinical symptom of Alzheimer's disease (AD). Cannabinoid CB_1 and CB_2 receptors are widespread modulators of systemic glucose homeostasis. Since CB_2Rs are upregulated in AD patients and in animal models of AD we tested the putative involvement of this receptor in local cerebral glucoregulation. We have studied glucose uptake *in vivo* by positron emission tomography and in hippocampal slices *in vitro*. Here we report that *in vivo*, the selective CB_2R agonist, JWH133 acutely stimulates glucose uptake by 22-34% in different brain areas of aged WT C57bl/6j mice. *In vitro*, JWH133 (1 μM) stimulated glucose uptake in acute hippocampal slices of young CD-1 mice and Wistar rats, respectively, highlighting a novel general glucoregulator role for cerebral CB_2Rs. The non-selective cannabinoid agonist, WIN55212-2 (1 μM) as well as the cyclooxygenase-2 (COX-2) inhibitor, DuP697 (500 nM), also stimulated glucose uptake in acute hippocampal slices of the WT C57bl/6j mice, in a manner sensitive to AM630 (1 μM), the selective CB_2R antagonist. In the age-matched double Swedish mutant TgAPP mice, only WIN55212-2 and JWH133 had such effect while DuP697 not. The endocannabinoid anandamide is a COX-2 substrate, and we found that the hippocampal levels of anandamide are decreased by ~38% in the TgAPP mice. COX-2 inhibition also facilitated glucose uptake in the WT hippocampi likely via the inhibition of anandamide metabolism, leading to endogenous CB_2R activation. In the TgAPP mice, anandamide levels were apparently too low for COX-2 inhibition to induce CB_2R activation. Althogether, these indicate that the non-psychotropic CB_2R agonist may have nootropic potential to stimulate brain glucose uptake and further endorse its use in AD.

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SALUBRINAL REGULATES GLIAL REACTIVITY AND FIBROSIS AFTER A CORTICAL LESION

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The glial scar is a physical and functional barrier that prevents axonal regeneration. It is formed after CNS injury by mesenchymal cells (fibroblast and pericytes), astrocytes and microglia, that proliferate and secrete large amounts of cytokines, proteoglycans and extracellular matrix proteins (ECMs).

The synthesis of secreted proteins in the rough endoplasmatic reticulum (RER) is under the control of the initiation factor of translation eIF2alpha. Inhibiting the synthesis of secreted proteins by increasing the phosphorylation of eIF2alpha, might be a pharmacologically efficient way of modulating glial scar formation.

Salubrinal, a neuroprotective drug that decreases RER protein translation by maintaining eIF2alpha phosphorylated, reduced the synthesis of proteoglycans and extracellular matrix proteins (ECM) *in vitro*. However, Salubrinal increased the secretion of proinflamatory proteins and the vascular endothelial growth factor VEGF in cultures of reactive astrocytes.

A stab injury was inflicted in the parietal cerebral cortex of a group of mice and Salubrinal was administered by intraperitoneal injection. We found that the acute treatment of Salubrinal after injury reduced the glial scar markers, but increased fibronectin expression around the injury site. However, chronic Salubrinal treatment increased astroglial reactivity. Therefore, the effect of Salubrinal on glial reactivity and fibrosis depended on the postlesion time at which the animals were treated.

These results indicate that the state of eIF2alpha phosphorylation is key for its consideration as therapeutic target to modulate inflammation and fibrosis.

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Áreas temáticas:
1ª. Trastornos y reparación del sistema nervioso.
2ª. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.

CHANGES IN AUTOPHAGY MARKERS IN MOTONEURONS FROM AN *IN VITRO* MODEL OF SPINAL MUSCULAR ATROPHY

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Spinal muscular atrophy (SMA) is a genetic disorder characterized by degeneration of spinal cord motoneurons resulting in muscular atrophy and weakness. SMA is caused by mutations in the *Survival Motor Neuron 1* gene (*SMN1*) and decreased SMN protein. SMN is ubiquitously expressed and has a general role in the assembly of small nuclear ribonucleoproteins and pre-mRNA splicing requirements. SMN reduction causes neurite degeneration and cell death without classical apoptotic features, but the direct events leading to SMN degeneration in SMA are still unknown. Autophagy is a conserved lysosomal protein degradation pathway whose precise roles in neurodegenerative diseases remain largely unknown. In particular, it is unclear whether autophagosome accumulation is protective or destructive, but the accumulation of autophagosomes in the neuritic beadings observed in several neurite degeneration models suggests a close relationship between the autophagic process and neurite collapse. In the present work we describe an increase of the autophagy markers including autophagosomes, Beclin1 and LC3-II proteins in cultured mouse spinal cord motoneurons from two SMA cellular models, suggesting an increase of the autophagy process in Smn-reduced motoneurons. Over-expression of Bcl-x_L counteracts LC3-II increase, contributing to the hypothesis that the protective role of Bcl-x_L observed in some SMA models may be mediated by its role in autophagy inhibition. Our data indicate an increase in the autophagy process and autophagosome accumulation in the pathogenesis of SMA, providing a valuable clue in understanding the mechanisms of axonal degeneration and a possible therapeutic target in the treatment of SMA.

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Áreas temáticas: Transtornos y Reparación del Sistema Nervioso

EARLY DOWN-REGULATION OF PKCΔ IN HUNTINGTON'S DISEASE BRAIN: ROLE IN CELL SURVIVAL

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Cell death is a common point in neurodegenerative diseases, and its study is of high interest in order to improve actual treatments. Here, we show a dys-regulation in some members of the PKC family in the brain of R6/1 mouse model of Huntington's disease (HD). We observed that the pro-apoptotic protein PKCδ and the pro-survival PKCα and βII were down-regulated along the progression of the disease in all the brain regions analyzed (striatum, cortex and hippocampus). As PKCδ showed the highest reduction we further analyzed the role of that kinase in HD pathology. We checked PKCδ protein levels in the striatum and cortex of other HD mouse models (R6/2 and $Hdh^{Q111/Q111}$) as well as in the putamen of HD patients finding also a significant reduction. Nuclear translocation of PKCδ is required for the induction of apoptotsis. However we detected a reduction in both cytoplasmic and nuclear enriched fractions of R6/1 mouse striatum, cortex and hippocampus, and no co-localization of the kinase with mutant huntingtin aggregates. These results suggest a protective role of PKCδ down-regulation in response to mutant huntingtin-induced toxicity. To further explore this hypothesis, we performed *in vitro* experiments showing that over-expression of PKCδ together with mutant huntingtin increases cell death whereas over-expression of a PKCδ negative dominant form or knock-down of PKCδ, significantly reduces mutant huntingtin-induced cell death. In addition, we show a good correlation between lamin B, one of the PKCδ targets, and PKCδ protein levels *in vitro* and *in vivo* thus confirming changes in PKCδ activity in cells expressing mutant huntingtin. In conclusion, our results support the hypothesis that the reduction of PKCδ protein levels acts as a pro-survival mechanism in response to mutant huntingtin-induced toxicity, which could explain why cell death is delayed in the disease.

Areas Temáticas:
Trastornos y reparación del sistema nervioso

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L-DOPA TREATMENT SELECTIVELY RESTORES SPINE DENSITY IN D2R-EXPRESSING PROJECTION NEURONS IN DYSKINETIC MICE

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Background: L-DOPA-induced dyskinesia is an incapacitating complication of L-DOPA therapy which affects most patients with Parkinson's disease. Previous work indicating that molecular sensitization to D1 dopamine receptor (D1R) stimulation is involved in dyskinesias prompted us to perform electrophysiological recordings of striatal projection "medium spiny neurons" (MSN). Moreover, because enhanced D1R signaling in drug abuse induces changes in spine density in striatum, we investigated whether the dyskinesia is related to morphological changes in MSNs.

Methods: Wild type and BAC transgenic mice (D1R-tomato and D2R-GFP) mice were lesioned with 6-hydroxydopamine and subsequently treated with L-DOPA to induce dyskinesia. Functional, molecular and structural changes were assessed in corticostriatal slices. Individual MSNs injected with Lucifer-Yellow were used for DAB-derived 3-D reconstructions with Neurolucida software. Intracellular current-clamp recordings with high-resistance micropipettes were used to characterize electrophysiological parameters.

Results: Both D1R-MSNs and D2R-MSNs showed diminished spine density in totally denervated striatal regions in parkinsonian mice. Chronic L-DOPA treatment, which induced dyskinesia and aberrant FosB expression, restored spine density in D2R-MSNs but not in D1R-MSNs. In basal conditions, MSN are more excitable in parkinsonian than in sham mice, and excitability decreases towards normal values following L-DOPA treatment. Despite this normalization of basal excitability, in dyskinetic mice, the selective D1R agonist SKF38393 increased the number of evoked action potentials in MSNs, compared to sham animals.

Conclusions: Chronic L-DOPA induces abnormal spine re-growth exclusively in D2R-MSNs and robust supersensitization to D1R-activated excitability in denervated striatal MSNs. These changes might constitute the anatomical and electrophysiological substrates of dyskinesia.

Áreas Temáticas:
Trastornos y reparación del sistema nervioso
Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

ANÁLISIS DE LA REGULACIÓN DE LA EXPRESIÓN DE APOLIPOPROTEÍNA D EN EL SISTEMA NERVIOSO EN CONDICIONES DE ESTRÉS OXIDATIVO

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Apolipoproteína D (ApoD) es una Lipocalina principalmente glial, sobre-expresada con el envejecimiento y la neurodegeneración. En organismos modelo (Drosophila y ratón) hemos demostrado que ApoD tiene un efecto protector ante el estrés oxidativo. En astrocitos, el estrés oxidativo produce una inducción temprana y transitoria de ApoD regulada por la MAP-quinasa JNK. Sin embargo, se desconocen los mecanismos moleculares que controlan el patrón espacio-temporal específico de la expresión de ApoD en el sistema nervioso, y los parámetros que controlan su expresión ante el daño celular.

El análisis bioinformático de las regiones 5' y 3'-UTR del gen de ApoD nos revela variantes de *splicing* alternativo que podrían condicionar la traducción de su mRNA. La presencia de estas variantes en diferentes especies sugiere un mecanismo conservado en el control de la traducción de ApoD tanto en tejidos específicos como ante determinados estados fisiológicos.

Mediante RT-PCR estándar y a tiempo real hemos analizado la expresión de estas variantes en diversos tejidos, tiempos de desarrollo y situaciones de estrés oxidativo. Estudiamos la expresión diferencial de algunas variantes en el sistema nervioso, tanto adulto como durante el desarrollo, y demostramos que una variante del 5'-UTR (5'Var-E) se induce específicamente en situaciones de estrés oxidativo. Para definir el papel de 5'Var-E, estamos estudiando tanto esta variante como la canónica (5'Var-A) con ensayos de reportero (luciferasa) en una línea celular astrocitaria sometida a distintos tipos de estrés.

Asimismo, estudiando *in silico* la región *upstream* del transcrito del gen de ApoD en humanos y ratón identificamos una región que podría actuar como promotor alternativo para la variante de *splicing* 5'Var-E que responde al estrés oxidativo. Debido al interés de este hallazgo, hemos clonado las regiones del posible promotor alternativo y del promotor canónico de ApoD de ratón para estudiar su efecto en la regulación transcripcional de ApoD.

Support: MICINN(BFU2011-23978); JCyL(VA180A11-2); Fund. Rodríguez-Pascual

1ª: Transtornos y reparación del sistema nervioso.
2ª: Desarrollo

ADULT SPINAL CORD STEM CELL NICHE: AN INSIGHT INTO THE ACTUAL HUMAN SITUATION

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During the last few years, several groups have described the existence of neural stem/precursor cells around the central canal of the adult rodent spinal cord. This has uncovered an endogenous potential to replace damaged cells after injury, but it is unknown if it is also the case for humans. Indeed, there are reports claiming that human central canal collapses in the adult life and may be different from that found in rodents.

Objective:
To study the patency and morphology of the adult human central canal.

Material And Methods:
We assessed the frequency of central canal patency in healthy volunteers by Magnetic Resonance Imaging, to test if central canal collapse is a postmortem artifact and to establish the normal levels of canal patency in the whole non-lesioned spinal cord. We have also used histochemistry and immunohistochemistry on fixed postmortem human tissue (control individuals deceased without a specific spinal cord damage) to study the structure of the central canal at different stages of obliteration.

Results:
We show that the absence of a patent canal is a common feature in the general population older than 18 years (less than 20% of individuals show patency at any level of the cord). We also report that human spinal cord central canal is notably different than its equivalent in rats and mice both in structure and in cytological composition.

Conclusion:
There are notable differences between human and rodent central canal patency, morphology and cytoarchitecture, that may require a reappraisal of human ependyma as a neurogenic niche and its role in reparative strategies after spinal cord pathologies.

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Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Desarrollo

PATHOLOGICAL ALTERATIONS IN MOTONEURONS, GLIA AND CENTRAL SYNAPSES IN THE SMNΔ7 MOUSE MODEL OF SMA

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Spinal muscular atrophy (SMA) is an autosomal recessive disease that affects α-motoneurons in the spinal cord and causes muscular weakness and atrophy of limb and trunk muscles. SMA is caused by the homozygous deletion or specific mutations in the Survival Motor Neuron-1 gene, codifying for SMN protein. Although SMN is a ubiquitous protein, deficient levels of SMN selectively damage lower motoneurons. We hypothesized that some other factor besides motoneuron degeneration must play a role in the pathogenesis of SMA. The SMNΔ7 mouse model of SMA was selected for the study. These mice have a life span of approximately two weeks. In this model, we studied the loss of lumbar spinal cord motoneuron in association with a possible astroglial and microglial activation, and also the loss of excitatory and inhibitory synapses on motoneuron somata in relation with the activation of neuronal NOS (nNOS) in motoneurons.

Moderate but significant loss of motoneurons was observed in the ventral horn of the lumbar spinal cord in the SMNΔ7 mouse model of SMA. Motoneuron loss already started at P4-5, before becoming symptomatic. Astroglial activation was also evident at P4-5, and microglial activation was manifest at P7-8 around degenerating motoneurons. Moreover, we found a decrease in both glutamatergic excitatory and gabaergic inhibitory synapses on motoneuron somata. It has been proposed that nitric oxide, trough the RhoA/ROCK pathway may cause the retraction of synaptic boutons. In these sense, we found an increase in nNOS positive motoneurons, a decrease in synaptophysin-positive puncta around motoneurons, as well as a dramatic increase in the puncta immunoreative for P-MLC, the final effector of the RhoA/ROCK pathway.

Together these results suggest that neuronal nitric oxide, together with the RhoA/ROCK pathway, could play a role in the loss of synapses on spinal cord motoneurons in SMNΔ7 mice.

THE SPECIFICITY PROTEIN FACTOR SP1 MEDIATES TRANSCRIPTIONAL REGULATION OF P2X7 RECEPTORS IN THE NERVOUS SYSTEM

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Aims: P2X7 receptors are involved not only in physiological functions but also in pathological processes, regulating proliferation, differentiation, and cell death in both CNS and non-CNS tissues. Although an increasing number of findings indicate that altered receptor expression has a causative role in neurodegenerative diseases and cancer, little is known about how expression of *P2rx7* gene is controlled. Here we report the first molecular and functional evidence that Specificity protein 1 (Sp1) transcription factor plays a pivotal role in the transcriptional regulation of P2X7 receptor in neural cells.

Methods And Results: We delimited a minimal region in the murine *P2rx7* promoter containing four SP1 sites, two of them being highly conserved in mammals. The functionality of this SP1 sites was confirmed by site-directed mutagenesis, and Sp1 overexpression/downregulation in neuroblastoma cells. Inhibition of Sp1-mediated transcriptional activation by mithramycin A reduced endogenous P2X7 receptor levels in primary cultures of cortical neurons and astrocytes. Using *P2rx7*-EGFP transgenic mice that express enhanced green fluorescent protein under the control of *P2rx7* promoter, we found a high correlation between reporter expression and Sp1 levels in the brain, demonstrating that Sp1 is a key element in the transcriptional regulation of P2X7 receptor in the nervous system. Finally, we found that Sp1 mediates P2X7 receptor upregulation in neuroblastoma cells cultured in the absence of serum, a condition that enhances chromatin accessibility and facilitates the exposure of SP1 binding sites.

CONCLUSSION: Transcriptional expression of P2X7 receptor is upregulated by Sp1 factor in neuroblastoma cells, neurons and astrocytes.

Áreas Temáticas: Seleccione las **2** áreas temáticas que más se ajusten a su trabajo en orden de prioridad:
1ª: Trastornos y reparación del sistema nervioso
2ª: Desarrollo

SHORT-TERM BRAIN METABOLIC IMPAIRMENT IN THE LITHIUM-PILOCARPINE MODEL OF EPILEPSY AND ITS MODULATION BY THE CENTRAL SEROTONERGIC SYSTEM

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Introduction

It is accepted that a neurochemical imbalance between glutamatergic and GABAergic systems causes epileptic hyperexcitability. A reduction in cerebral metabolic activity is often observed during the interictal stages of epilepsy. Nevertheless, the role of other neurotransmitter systems is not fully characterized.

Objectives

The aim of this study consisted of evaluating the involvement of the central serotonergic system in an experimental model of temporal lobe epilepsy. The induction of epileptogenesis was performed in conditions of elevated and reduced serotonergic tone, measuring different neurochemical markers and brain metabolic activity.

Materials and Methods

The lithium-pilocarpine model of temporal lobe epilepsy was used in adult male SD rats. Two different conditions of serotonergic tone were established by subchronic administration of the selective serotonin reuptake inhibitor fluoxetine (10 mg/kg/day, ip) or the tryptophan hydroxylase inhibitor p-chlorophenylalanine (150 mg/kg, ip).
Positron emission tomography (PET) was used to evaluate the cerebral metabolic activity before (baseline) and 3 days after induction of epilepsy. Fluoro-Jade C, GAFP and DAPI histological techniques were employed to investigate the neuronal damage due to pharmacological manipulations. GABAergic, serotonergic and glutamatergic neurotransmission were also evaluated using autoradiographic techniques.

Results

Three days post administration; pilocarpine caused a significant reduction in metabolic activity of brain areas involved in epileptogenesis (hippocampus; SUV 2.26±0.19 vs 3.00±0.14). Pilocarpine-induced hypometabolism was completely inhibited by previous administration of FLX (hippocampus; SUV 2.95±0.14; 98.2% as compared with the control group), but not affected by PCPA (hippocampus; SUV 2.27±0.29).

Pilocarpine induced neurodegeneration in the hippocampal dentate gyrus, as revealed by both Fluoro-Jade C and GFAP histochemical studies. In addition, neurodegeneration was prevented by FLX in accordance to the PET results.

Conclusions

According to these results, the serotonergic system plays a protective role in epileptogenesis induced by this experimental model.

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ROLES OF ANOSMIN-1 AND FGF-2 IN THE BIOLOGY OF ADULT MURINE AND HUMAN OLIGODENDROCYTE PRECURSOR CELLS

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During development, oligodendrocyte precursor cells (OPCs) generated in germinative regions migrate until their final destination and then differentiate to myelin-forming oligodendrocytes. OPCs exist in mature rodent and human central nervous system (CNS; around 5-7% of the total cells) and constitute an interesting source for regenerative therapies in demyelinating diseases, like multiple sclerosis (MS). There is an increasing bulk of evidences showing that embryonic OPCs and those isolated from adult CNS are not functionally identical. Furthermore, different molecules are involved in OPC morphofunctional development during embryogenesis and postnatal stages, and some of them are upregulated in MS lesions, suggesting their involvement in the pathogenesis of the disease.

This is the case of anosmin-1, an extracellular matrix glycoprotein coded by the *KAL1* gene and responsible for the X-linked form of Kallmann syndrome. The best known mechanism of action of anosmin-1 seems to be mediated through the interaction of this protein with fibroblast growth factor receptor 1 (FGFR1) and the modulation of the activation of this receptor by FGF2. This protein participates in the adhesion, migration and differentiation of various cell types in the CNS. In addition, previous results of our group also suggest a role of anosmin-1 in demyelinated lesions from MS patients and point to the feasible pharmacological and genetic manipulation of the FGF2/FGFR-1/anosmin-1 system on endogenous and/or exogenous OPCs in demyelinating lesions.
In the present work we studied the functional implications of anosmin-1 and FGF-2 on neurobiology (cell death, proliferation, motility, migration) of postnatal/adult murine OPCs isolated from cerebral cortex (P0, P15, P60) and of OPCs isolated from adult human biopsies, using both in vivo and in vitro techniques. These results would be useful for the design of effective neuroreparative therapies in MS and other demyelinating diseases.

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Áreas Temáticas:

1ºTrastornos y reparación del sistema nervioso

2º Desarrollo

DISRUPTION OF TYROSINE HYDROXYLASE IN PC12 AND ITS EFFECT ON DOPAMINE SYNTHESIS: A MODEL FOR THE STUDY OF TYROSINE HYDROXYLASE DEFICIENCIES

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Background: Tyrosine Hydroxylase is the enzyme responsible for converting the amino acid tyrosine to L-DOPA. This reaction is the rate limiting step in the production of catecholamines like dopamine, norepinephrine or epinephrine. These catecholamines are a group of neurotransmitters that play a crucial role in neurological processes like motor control, movement, sympathetic nervous system response, motivation, attention or learning. Tyrosine Hydroxylase Deficiency is a rare metabolic disorder, recessively inherited, that is caused by mutations in Tyrosine Hydroxylase; these mutations can nullify Tyrosine Hydroxylase expression or codify a protein with low enzymatic activity which is unable to produce the levels of L-DOPA and derived cathecolamines necessary for correct organism function and development.

Objectives: Developing a cell culture model for the study of Tyrosine Hydroxylase Deficiencies by disruption of Tyrosine Hydroxylase synthesis.

Materials and methods: PC12, the established cathecolaminergic cell line derived from rat pheochromocytoma (ATCC: CRL-1721) are cultured under the standard conditions. Tyrosine Hydroxylase expression is interfered using commercial ShRNAs (OriGene) specifically designed for this target. Dopamine levels are determined by HPLC and immunocytochemistry using specific antibodies. Protein levels are analyzed by western-blot.

Results: The effect of disruption of Tyrosine Hydroxylase expression on dopamine synthesis is studied in relation to the basal levels present in PC12 cells. The impact of Tyrosine Hydroxylase ablation on different proteins of the pathway of cathecolamine synthesis is also analyzed.

Conclusions: Interference of Tyrosine Hydroxylase alters the dopamine synthesis in PC12 cells. The possibility of using these Tyrosine Hydroxylase-disrupted PC12 as a model for the study of Tyrosine Hydroxylase Deficiency and preclinical assays of potential treatments of this syndrome is also discussed.

Áreas Temáticas:

1ª: 5 - Trastornos y reparación del sistema nervioso
2ª: 2 - Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

NEURAL STEM CELL AND NEUROGENESIS DYNAMICS DURING THE TRANSITION FROM HEALTHY TO ALZHEIMER'S DISEASE-LIKE AGING IN THE SENESCENCE ACCELERATED MOUSE MODEL SAMP8

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In the adult mammalian Central Nervous System (CNS), new neurons are generated from multipotent neural stem cells (NSCs) located in discrete brain regions such as the Subventricular zone (SVZ) of the lateral ventricles and the Subgranular zone (SGZ) of the Dentate Gyrus (DG) in the Hippocampus. Although this process is maintained throughout animal lifespan, the rate of neurogenesis decreases dramatically during ageing and in Alzheimer's Disease (AD) due to little-known causes that remain to be deciphered. In this study, we describe NSC proliferation and neurogenesis dynamics in SAMP8, a non-transgenic mouse strain that recapitulates the transition from normal ageing to AD. *In vivo* studies show a transient increase in the proliferation rate of NSCs in young animals and a steep decrease at later stages, once AD sympthoms come forth. Moreover, *in vitro* studies point to soluble and monomeric Aß(1-42) peptide as the signaling molecule which is inducing proliferation and self-renewal of NSCs through activation of PI3K/AKT pathway. This over proliferation of NSCs prior to the appearance of AD pathology may compromise NSC function in the long run and may underlie neurogenic failure during age-related progression of Alzheimer Disease.

Áreas Temáticas: Seleccione las **2** áreas temáticas que más se ajusten a su trabajo en orden de prioridad:

1ª: Trastornos y reparación del sistema nervioso

2ª: Desarrollo

UNIVERSAL DYNAMICS OF ELECTROCLINICAL PROGRESSION OF SUBTLE GENERALIZED CONVULSIVE STATUS EPILEPTICUS

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Status epilepticus (SE) represents a major neurological emergency. Characterizing the electroclinical evolution is essential in order to delineate pharmacological interventions aimed to reduce subsequent brain and cognitive damage. Previously, it was suggested that SE proceeds through different stages in animal models using systemic injections of lithium-pilocarpine. However, there is still poor evidence of similar progression dynamics in human. Here, we describe the electroclinical evolution of an elderly patient developing an episode of subtle generalized convulsive SE (SGCSE) in which we recorded four of the five EEG patterns previously defined in animal models.
A single episode of subtle SGCSE started as a simple partial motor secondarily generalized tonic-clonic seizure that ceased with intravenous diazepam. Seven hours later, the patient suffered from a second similar motor seizure that lasted 10 minutes and subsequently remained comatose with occasional myoclonic jerks involving the right arm. Electroencephalographic (EEG) evaluation showed a discontinuous pattern of rhythmic asymmetrical generalized burst of high amplitude epileptiform discharges with superimposed fast low voltage rhythms on the right side, similarly as described in animal models (stage 1 phase). This neurological condition remained unchanged for three days in spite of administration of phenytoin (300 mg/24 h) and levetiracetam (1500 mg/24h). Subsequent EEG recordings revealed a pattern of continuous high amplitude irregular spike-wave complexes, similar to stage 3 described in animal models. At the end of this EEG recording, we observed a progression to SE stage 4. On day 10 after the onset, generalized periodic epileptiform discharges occurring at intervals of 1.0 to 1.5 seconds (stage 5) were seen. We propose that SGCSE follows a universal dynamics though it may occur at a slower time scale in human as compared with experimental models. Further studies of SE experimental models can help to define decision trees regarding the diagnosis and treatment of this condition.

Áreas temáticas:
1ª Neurociencia de sistemas
2ª Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

DIETARY RESVERATROL REDUCES AMYLOID PLAQUE PATHOLOGY AND PREVENTS MEMORY LOSS IN APP/PS1 TRANSGENIC MICE.

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Alzheimer's disease (AD) is a common, progressive and incurable neurodegenerative disorder characterized by memory loss and accumulation of amyloid plaques and neurofibrillary tangles in the brain.

Resveratrol (3,5,4′-trihydroxystilbene) is natural non-flavonoid polyphenolic compound mainly found in grapes and red wine. Resveratrol has been extensively investigated, and many studies address its beneficial effects on cancer and heart disease, longevity, inflammation, neuroprotection, obesity and metabolism. Moreover, several studies have indicated that most of these effects are driven through Sirtuin 1 (SIRT1) regulation.

The aim of this study was to investigate the effect of early long-term treatment with resveratrol in an AD mice model, and to elucidate the mechanisms and molecular pathways involved.

For this purpose, 2 months-old APPswe/PS1dE9 (APP/PS1) transgenic mice which presents senile plaque formation, increase of tau phosphorylation and memory loss, were fed with a standard diet or resveratrol diet (1 g/kg) for 7 months. At 9 months of age, Novel Object Recognition Test (NORT) was performed to evaluate any improvement in learning and memory. Samples were obtained afterwards for western-blot, immunohistochemistry and real-time PCR analysis.

Results showed that resveratrol reduces amyloid plaque pathology and prevents memory loss in APP/PS1 mice, although no changes were observed in tau phosphorylation. Neither ADAM10, BACE1 and PS1 subunits (corresponding to α-, β- and γ-secretase complexes), nor SIRT1 pathway, were modified by resveratrol treatment, and its beneficial effects in this model could be related to its antioxidant properties.

In summary, dietary resveratrol could be a possible strategy to prevent or delay some aspects of Alzheimer’s disease, even though more studies are needed to elucidate its mechanisms of action.

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Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso.
2ª: Neurociencia cognitiva y conductual.

EL TRATAMIENTO CON AM80 IMPIDE LA RECUPERACIÓN SINTOMÁTICA EN UN MODELO ANIMAL DE ESCLEROSIS MÚLTIPLE MEDIANTE LA DIFERENCIACIÓN DE LAS CÉLULAS MIELOIDES SUPRESORAS

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La variante clínica más frecuente de la esclerosis múltiple (EM) es la forma recurrente-remitente (EM-RR), la cual presenta una etapa de exacerbación de los síntomas seguida de una fase de remisión parcial de los mismos. La transición entre ambas fases viene determinada por procesos de inmunomodulación en los que participan distintos tipos celulares, entre los que se encuentran las células mieloides supresoras (MDSCs). Las MDSCs son una población heterogénea formada por células mieloides inmaduras capaces de suprimir la respuesta inflamatoria. Uno de los mecanismos empleados para lograr dicha inmunosupresión se basa en la actividad de la enzima Arginasa-I (Arg-I). Nuestro grupo ha descrito que las MDSCs expresan Arg-I y entran de forma transitoria en la médula espinal de ratones con EAE, controlando la respuesta inmune mediante la aceleración de la apoptosis de las células T, especialmente en el momento de mayor discapacidad. Así, los cambios en la población de las MDSCs durante el curso clínico de la EAE afectan a su capacidad inmunosupresora, alterando la evolución de la enfermedad.
Las moléculas de la familia del ácido retinoico se usan para el tratamiento de diferentes tipos de leucemia, actuando como factores de diferenciación de las MDSCs y eliminando su capacidad inmunosupresora. El Am80 es un análogo retinoide sintético que, dependiendo del momento en que se administre, presenta diferentes efectos sobre el curso clínico de la EAE. En este trabajo, administramos Am80 poco antes del periodo de mayor discapacidad (momento en que ocurre la inmunomodulación), lo que afectó específicamente a la población de las MDSCs y empeoró el curso clínico de la EAE debido a la desaparición de la fase de remisión sintomática. Estos resultados confirman a la población de las MDSCs endógenas como nueva diana terapéutica para tratar la EM, acelerando la transición desde la fase recurrente a la remitente.

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Área temática
1ª opción: Trastornos y reparación del sistema nervioso

REGULACIÓN DEL PROCESAMIENTO DE APP MEDIADA POR RECEPTORES PURINÉRGICOS P2X7 Y P2Y2.

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La producción de péptido amiloideo (Aβ) a partir de la proteína precursora de amiloide (APP) es esencial en la formación de las placas seniles características de la enfermedad de Alzheimer. Sin embargo, las señales extracelulares que mantienen el balance entre los procesamientos proteolíticos amiloidogénicos y no-amiloidogénicos de la APP, mediados por α-secretasa y β-secretasa respectivamente, permanecen poco conocidos. En el presente trabajo, describimos la regulación del procesamiento de APP mediada por el receptor P2X7.
En líneas celulares la inhibición del receptor P2X7, tanto nativo como sobre-expresado, incrementa la actividad α-secretasa, efecto que parece estar mediado a través de la inhibición de la Glucógeno sintasa quinasa 3β (GSK3β). Por el contrario, la activación de P2X7 conduce a una reducción de la actividad α-secretasa en estos modelos. Por otro lado la activación del receptor P2Y2 presente en estas celulas, incrementa la actividad α-secretasa, indicando una estrecha regulación del procesamiento de APP a través de los receptores purinérgicos.
La inhibición *in vivo* del receptor P2X7 en el ratón J20, un modelo murino de la variante familiar de la enfermedad de Alzhéimer, el cual expresa la APP humana con las mutaciones Swe/Ind, induce un descenso significativo del número de placas amiloideas localizadas en el hipocampo. Esta disminución en el número de placas en los ratones tratados con el inhibidor de P2X7 coincide con un descenso en la actividad de GSK3β incrementándose el procesamiento no-amiloidogénico de APP debido a un incremento en la actividad α-secretasa.
Las evidencias *in vivo* mostradas en este trabajo demuestran el potencial terapéutico de la modulación de los receptores purinérgicos en el tratamiento del Alzheimer familiar.

Áreas Temáticas
1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia cognitiva y conductual

TIME–COURSE OF CHANGES ON SIGNALLING PATHWAYS ASSOCIATED TO HIPPOCAMPAL NEUROGENESIS AND PLASTICITY IN THE OLFACTORY BULBECTOMIZED RAT MODEL OF DEPRESSION

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Bilateral olfactory bulbectomy (OB) is a validated animal model of depression displaying changes similar to human depression reversed by chronic antidepressants. Recently, Wnt/β-catenin pathway, involved in hippocampal neurotegenesis, and mammalian target of rapamycin (mTOR) pathway related to synaptic plasticity, have been described to be downregulated in depressed individuals, and up-regulated following antidepressant treatment.

We have evaluated by western blot in bulbectomized rats the time course (30, 45 and 90 days post-surgery) of the activation of components of the Wnt/β-catenin and mTOR pathways. In addition, the modulation by the serotonin reuptake inhibitor (SSRI) fluoxetine (10 mg/kg/day, 14 days; s.c.) was assessed in a group of rats treated after 15 days post-OB.

Our results demonstrate a significant decrease in β-catenin ($p<0.01$) and AKT ($p<0.05$) protein expression in OB rats 45 days post-surgery, returning to sham expression levels 90 days post-surgery. At day 30 post-OB, ratio pmTOR/mTOR was significantly increased ($p<0.01$) and reached normal levels 45 and 90 days post-OB. The activation of the downstream component p70S6 kinase (p70S6K) was also increased ($p<0.001$) after 30 days post-OB but not changes were detected after 45 and 90 days post-OB. In contrast, other downstream protein, the eukaryotic initiation factor 4E binding protein 1 (4E-BP1), was unaltered after 30 days, but displayed a clear tendency to the increase after 45 and 90 days ($p<0.05$). Administration of fluoxetine induced a significant increase in p-4EBP1 in sham and OB rats, while mTOR activation was promoted only in sham rats. No changes appeared for p70S6K.

These results suggest a differential regulation for p70S6K (translation activator) *vs* 4EBP1 (translation inhibitor). Shortly after bulbectomy, the exaggerated activation of mTOR and p70S6K may reflect their implication in the inflammatory reaction associated to bulbectomy. This increase may hide the decreased mTOR activity linked to depression, therefore dampening the results observed after antidepressant treatment.

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Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia cognitiva y conductual

ANALYSIS OF A MLC1 KO MOUSE MODELS PROVIDE NEW INSIGHTS IN THE PATHOPHYSIOLOGY OF THE LEUKODYSTROPHY MLC: CHLORIDE CHANNELS ARE NOT WORKING PROPERLY

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Cell volume regulation is pivotal to ensure normal brain function. Its alteration can represent a serious challenge for neuronal survival due to space constrictions within the skull. Thus, brain edema is a major problem in neurology, leading to death in most cases, and it is caused by many defects such as stroke or brain cancer, among others. Our research approaches the study of cell volume regulation in the context of the human genetic disease Megalencephalic Leukoencephalopathy with subcortical Cysts (MLC), as a working model to study brain ionic transport pathophysiology.
MLC brains are affected by chronic white matter oedema suggesting a disruption in water and ion homeostasis in astrocytes, which in turn may alter their cell volume regulation abilities. MLC pathology was shown to be primarily caused by a defect in a highly conserved oligomeric plasma membrane protein named MLC1. MLC1 is mostly expressed in astroglial processes and presents low homology to ion channels.
However, both the pathophysiological mechanism of MLC disease as well as MLC1 function remained unknown until today, although MLC1 has been related with the activation of volume-regulated chloride channels. Recently, we have identified the second gene responsible for MLC pathology (i.e., GLIALCAM) (1) and described its biochemical role as a MLC1 beta subunit (2). Moreover, we have shown that GlialCAM protein functions as an accessory subunit of the chloride channel ClC-2 (Jeworutzki *et al.*, Neuron (2012)). In this meeting, we will provide new studies with a KO model of MLC1, indicating that dysfunction of chloride channels is a common physiopathological mechanism in MLC disease.

Áreas Temáticas:

1ª: Trastornos y reparación del sistema nervioso
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

NEW EXPERIMENTAL MODEL FOR ATTENTION DEFICIT HYPERACTIVITY DISORDER

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Objectives: The characterization of a conditional knockout mouse model lacking CNS Adrenomedullin peptide, which has hyperactive phenotype, focused to Attention Deficit Hyperactivity Disorder (ADHD), would define a new target for the treatment of prevalent ADHD, and establish a model for further study of this disorder. We have initially developed two objectives using the modified mouse above mentioned. One of them is the characterization in the prefrontal cortical and striatal regions of dopamine, and noradrenaline, and serotonin systems, which are impaired in patients. The other one is to evaluate the volume of these cortical and striatal structures.

Method: We have employed Immunohistochemistry, and Western blotting techniques to analyze the tyrosine hydroxylase, dopamine, and serotonin expression, in order to develop the first objective. The cortical and striatal volumes were determined by Nuclear Magnetic Resonance technique.

Results: The striatum and *substantia nigra pars compacta* exhibit lowered expression for tyrosine hydroxylase in the modified mice when compared with their wild type littermates. The serotonin transporter expression in the *dorsal raphe nucleus*, as well as the dopamine D4 and D2 receptors expression in the *striatum* are also disrupted. In relation to *striatum* and cortex volumes, our results indicate that the modified mice *cingulate* cortex presents a smaller volume than *wild type* littermates ones.

Conclusion: The conditional knockout mouse model lacking brain Adrenomedullin peptide has some of the features than patients with ADHD, and thus could be used like experimental model to study this psychiatric disorder, as well as adrenomedullin peptide like possible therapeutic target.

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METHYLTHIOADENOSINE ENHANCES MYELINATION AND PROMOTES REPAIR IN DEMYELINATION MODELS

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Methylthioadenosine (MTA) is a metabolite of the polyamine pathway with previosuly described anti-oxidant, anti-proliferative and immunomodulatory properties. Here, we report that MTA also displays a wide array of neuroprotective activities against different insults. As such, MTA is able to protect neurons, oligodendrocytes and myelin-forming cells from toxicity and inflammation in vitro, promoting remyelination and protecting against demyelination. In vivo, MTA was able to protect against cuprizone demyelination of the corpus callosum in C57BL/6 mice but also was able to promote repair in animals in which treatment started after demyelination.
MTA mechanisms of action are still not very clear but here we show that specifically, MTA accelerated oligodendrocyte maturation in vitro without affecting oligodendrocyte survival or proliferation by increasing the phosphorylation of transcription factors (STAT3, ATF2 and NR4A) and promoted the release of the cilliary neurotrophic factor (CNTF). When STAT-3 phosphorylation was blocked by a chemical specific inhibitor (LLL12), MTA capacity to enhance myelination and promote repair was lost supporting the strategy of STAT-3 increase phosphorylation as a major neuropprotective mechanism of action of MTA.
Our findings suggest that MTA could be useful as a new neuroprotective therapy of demyelinating diseases.

A GENOME-WIDE APPROACH TO STUDY TRANSCRIPTIONAL DYSREGULATION IN A POLYGLUTAMINE DISORDER MODEL

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Disruption of multiple processes (such as mitochondrial respiration, vesicle trafficking, protein degradation and transcription) is a common feature in neurological disorders. Molecular systems biology has the potential to explain complex biological phenomena, like the etiology and progression of neurodegenerative diseases, by establishing interrelations between several cellular and molecular mechanisms from a global point of view. Within this research field, disease transcriptomics can provide novel therapeutical targets, biomarkers and quantitative outputs for ameliorative strategies.
Transcriptional dysregulation is a very early event in polyglutamine (polyQ) pathologies. The mouse model used in this study, the transgenic N171-HD82Q strain, expresses a fragment of the human huntingtin protein with an aberrant expansion of the polyQ stretch, which is sufficient to cause phenotypical traits resembling Huntington's disease symptomatology. Histone deaceylation has been observed in a number of models for neurodegenerative conditions, not only polyQ diseases but also Alzheimer's and Parkinson's diseases among others, and it is hypothesized to be one of the causative forces for transcriptional dysregulation. Supporting this observation, pharmacological inhibition of histone deacetylation by HDACi has been proved to be beneficial in these models. To investigate the genome-wide correlation between gene expression and histone acetylation changes we combined for the first time microarray and ChIP-seq analyses in our mouse model.
Our analyses revealed extensive changes at the level of transcription and histone acetylation. However, both phenomena were poorly correlated, except for a small subset of neuronal genes (10% of the altered transcripts) which showed a specific deacetylation of the lysines 9 and 14 of histone H3 at their transcription start sites. This result challenges the proposed mechanism of HDACi action in ameliorative strategies. Currently we are exploring additional histone modifications (such methylation) and the role of transcription factors that were predicted in our polyQ gene profiling.
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Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Nuevos métodos y tecnologías

EFFECT OF PROLONGED TREATMENT WITH PRAMIPEXOLE ON THE DOPAMINE TRANSPORTER

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The dopamine transporter (DAT) is a membrane glycoprotein expressed in dopaminergic neurons, which is responsible for the clearing of dopamine from the extracellular space, and is regulated by different presynaptic proteins, including DA D_2 and D_3 autoreceptors. DAT dysfunction is involved in neurological and psychiatric conditions, such as Parkinson's disease, depression and attention deficit hyperactivity disorder, in whose treatment D_3 receptor (D_3R) ligands are increasingly being used. However, the interaction between D_3R and DAT is little known.

Subject: This work focuses on the effects, and mechanistic aspects, of prolonged treatment with the D_3R preferential agonist pramipexole on DAT in the mouse mesostriatal system.

Materal and methods: C57BL/6J and D2-/- mice were injected with the preferential D_3R agonist pramipexole (0.1mg/kg/d for 6 days) and with either the D_3R antagonist NGB2904 or the D_2R antagonist L741,626. Brain samples were processed for DA uptake, western-blot, immunohistochemistry, cross-linking, A11 dot-blot, immunoprecipitation and *in situ* proximity ligation assay.

Results: Pramipexole induces: 1. D_3R-mediated D_2R-independent DA uptake decrease with reduced DAT affinity but no quantitative changes in DAT expression and active uptake sites, 2. Physical interaction between DAT and D_3R , and 3. Formation of DAT oligomeric complexes, ranging between 150 kDa and 250 kDa, which accumulate DAT in its homomeric form interacting with D_2R and α-synuclein. Similar to the DA uptake decrease, modifications in DAT interactions were prevented by co-treatment with the D_3R antagonist NGB2904, and disappeared after PPX withdrawal.

Conclusion: Prolonged treatment with pramipexole induces changes in DAT interactions with its proteome-interactome partners and DA uptake decrease. These changes may be involved in the neuroprotective and antidepressant effect of pramipexole.

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Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia de sistemas

RESVERATROL-ENRICHED DIET IMPROVES MOTONEURON FUNCTION AND EXTENDS LIFESPAN IN SOD1^{G93A} ALS MICE

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Objectives: Amyotrophic lateral sclerosis (ALS) is a fatal adult onset neurodegenerative disorder that causes progressive paralysis and death due to the degeneration of motor neurons in the spinal cord, brainstem and motor cortex. Nowadays, therapy is mainly symptomatic and patients die 2-5 years after diagnosis. Resveratrol (trans-3,4′,5-trihydroxystilbene) is a naturally produced polyphenol mainly found in grapes. Several *in vitro* and *in vivo* studies support the hypothesis that resveratrol may be a promising neuroprotective agent since it induces the expression and activation of Sirtuin 1, a deacetylase enzyme that promotes neuronal survival. In fact, it was recently reported a protective effect of resveratrol administration on ALS in *in vitro* models. The main goal of the present work was to assess the role of resveratrol as a therapy in SOD1^{G93A} ALS mice.

Methods: Mice were fed with a standard with or without resveratrol supplementation. We determined the onset of symptoms by rotarod means and evaluated upper and lower motoneuron function using electrophysiological tests. We assessed the survival of the animals and determined the number of spinal motoneurons. Finally, we further investigated resveratrol mechanism of action by means of western blot and immunohistochemical analysis.

Results: Resveratrol treatment from 8 weeks of age significantly delayed disease onset in both female and male animals. This was accompanied by a marked preservation of lower and upper motoneuron function evidenced by the maintenance of the amplitude of muscle action potentials and motor evoked potentials, respectively. Resveratrol treatment significantly extended animal's lifespan and promoted neuroprotection of spinal motoneurons. Delayed resveratrol administration from 12 weeks of age also improved spinal motoneuron function. Further analysis revealed that this effect was mediated by an increased expression and activation of Sirtuin 1 in the ventral spinal cord. Resveratrol administration also reestablished autophagic flux and improved mitochondrial function.

Conclusions: Resveratrol seems to be a promising candidate as a therapeutic strategy for ALS.

Áreas temáticas:

1ª: Trastornos y reparación del sistema nervioso.

EFFECTS OF LYSOPHOSPHATIDYLCHOLINE IN THE PATHOPHYSIOLOGY OF THE INJURED SPINAL

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Macrophages are essential for clearance of tissue debris, tissue remodeling and repair after injury or infection of most host tissues. After spinal cord injury, however, macrophage activation leads to secondary damage and functional impairment. In contrast to the harmful properties of activated macrophages at the site of SCI, macrophages activated as a result of intraspinal injection of lysophophatidylcholine (LPC) do not cause secondary damage. We have done an Affymetrix GeneChip analysis of macrophages purified from the spinal cord after contusion injury and intraspinal LPC injection in mice. These analyses revealed that both types of macrophages are phenotypically distinct, and led to the identification of several genes which could be new potential markers for cytotoxic macrophages. Interestingly, injection of LPC into the injured spinal cord resulted in functional recovery and histological outcomes. The protective responses of LPC in SCI were accompanied with changes in cytokines and chemokines expression but not with altered recruitment of macrophages. These results suggest that LPC may drive macrophages activation towards a non-cytotoxic phenotype when injected into the contused spinal cord.

Áreas Temáticas:
1ª:Trastornos y reparación del sistema nervioso
2ª: Neurociencia de sistemas

ARGINASE-I$^+$-MYELOID-DERIVED SUPPRESSOR CELL DISTRIBUTION IN THE MULTIPHASIC MURINE MODEL OF MULTIPLE SCLEROSIS

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The Relapsing-Remitting is the most frequent clinical variant of Multiple Sclerosis (MS), characterized by a relapsing phase with increasing neurological symptoms and a remitting period, where patients totally or partially recover. During the course of the disease, immune cells infiltrating the central nervous system (CNS) contribute to the relapsing-to-remitting transition. In the last years, the role of a population of immature myeloid cells, the Arginase-I$^+$-myeloid-derived suppressor cells (MDSCs), has become more relevant. It has been shown that they are capable of the immune response modulation through the induction of T cell apoptosis not only in the primary lymphoid organs but also within the CNS parenchyma.

The most useful animal model to study the neuroimmune component of MS is Experimental Autoimmune Encephalomyelitis (EAE). The disease can present either a monophasic (one relapse) or multiphasic (several relapses) course depending on the mouse strain (C57/BL6 or SJL/J, respectively) and the peptide used for the immunization (MOG or PLP, respectively). In a previous work, we described the MDSC spatial-temporal distribution of MDSCs within the spinal cord of the monophasic MOG-immunized model, demonstrating a clear parallelism with the suppressive activity and the clinical course of the disease. In the present work, we explore the presence of this specific cell type in different stages of the multiphasic PLP-immunized murine model (onset, relapsing and remission of the two first relapses) in order to check whether: i) MDSC presence/activity may be extrapolated to this other EAE variant; ii) MDSCs are equally present and active along the different relapses. Together with the previous data, our present work points to MDSCs as a promising endogenous target for MS treatment.

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Topic:
1º: Trastornos y reparación del sistema nervioso
2º: Desarrollo

GENETIC INDUCIBLE FATE MAPPING TO TRACE THE DIFFERENTIATION OF NEURAL STEM CELLS INTO REACTIVE ASTROCYTES IN A RODENT MODEL OF EPILEPSY

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Objectives: To analyze the differentiation of adult hippocampal neural stem cells into reactive astrocytes in a rodent model of mesial temporal lobe epilepsy by genetic inducible mapping.

Materials and methods: Expression of YFP in nestin-expressing cells in transgenic Nestin-Cre-ERT2-Rosa26-YFP was induced by intraperitoneal injection of tamoxifen. Two days later the animals were subjected to intrahippocampal stereotaxical injection of 50 nL of saline or of either 0.75 nM (a sub-pathological and non-epileptogenic dose); or 20nM (a seizure- inducing dose) kainic acid (KA). The animals were sacrificed 3, 14 or 30 days after the KA injection. Brain sliced were processed for multilabeling for specific cell-markers. Analysis and quantification were performed using design-based stereology and confocal microscopy.

Results: After induction with tamoxifen, most of the YFP-expressing cells (80-85%) were rNCS: GFAP-immunopositive cells with the soma located in the subgranular zone and a radial process that crossed the granule cell layer and arborized in the molecular layer.
No difference in the total number of YFP-expressing cells or in the relative proportion of cell populations was found 3 days after KA injection. After 14 and 30 days, there was a significant increase in the total number of YFP-expressing cells, especially with the high dose of KA. In both the control and the low-KA mice the majority of the YFP-expressing cells (70 and 80% respectively) were NeuN-immunopositive neurons . In the high-KA mice the majority of YFP-expressing cells were reactive astrocytes (80-85%) characterized by morphology, size and expression of GFAP and S100ß.

Conclusions: The low KA dose increased the generation of neurons from rNSCs without major changes in the relative proportions of neurons and astrocytes respect to the controls. The high-KA dose, mimicking epilepsy, induced the transformation of rNSCs into reactive astrocytes, a property never before described.

Áreas temáticas:
1. Trastornos y reparación del sistema nervioso.
2. Desarrollo.

SUSTAINED RELEASE OF NGF WITH PLGA MICROSPHERES INCREASES DRG NEURITE OUTGROWTH AND ENHANCES NERVE REGENERATION

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Introduction: Nerve guide conduits (NGCs) have been widely used to repair transected nerves. Furthermore, filling NGCs with neurotrophic factors or biocompatible scaffolds have been reported to enhance regeneration. However free neurotrophic factors can be rapidly degraded, can diffuse outside the internal lumen or get diluted after liquid infiltration resulting in sub-optimal concentrations and poor regeneration outcomes. Therefore, a local and sustained release of neurotrophic factors would enhance peripheral nerve regeneration in comparison with single bridging.

Objectives: To evaluate whether NGF encapsulated in Poly-Lactic Co-Glycolic acid (PLGA) microspheres (MP) can improve both in vitro neurite outgrowth and in vivo nerve regeneration.

Material and methods: A Water-in-oil-in-water (WoW) protocol was followed to synthesize PLGA (50:50, mw 40kDa-75kDa) MPs to encapsulate aqueous solutions of NGF, fluorescent-BSA and PBS as negative control. DRG were cultured in a 3D collagen matrix under pre-conditioned medium during 0, 1 or 2 weeks with MP with PBS (MPPBS), free NGF and MP with NGF (MPNGF). Neurites were labeled with immunohistochemistry and quantified using the Neurite-J plugin for Image-J. In vivo testing of MPNGF was conducted in rats with complete transection of the sciatic nerve bridged with a silicone NGC filled with collagen with MPPBS, with NGF or with MPNGF leaving a 6mm interstump gap. Retrograde labeling with Fluoro-Gold and nociceptive sensitivity with a plantar algesimeter were used to evaluate nerve regeneration.

Results: MP by themselves does not interfere with nerve regeneration. On the other hand, NGF encapsulated in MP increases neurite growth after 1 and 2 weeks of pre-conditioning in comparison with free NGF. Furthermore, MPNGF rats showed an increase of regenerated DRG neurons and a decreased pain threshold in comparison with free NGF and MPPBS treated animals.

Conclusion: NGF encapsulation in microspheres boosts neurite outgrowth of DRG slices in vitro and enhances axon regeneration after nerve transection.

Áreas Temáticas:
1. Trastornos y reparación del sistema nervioso
2. Nuevos métodos y tecnologías

ACTIVATION OF HIPPOCAMPAL RADIAL NEURAL STEM CELLS IN RODENT MODELS OF TEMPORAL EPILEPSY AND ELECTROCONVULSIVE SHOCK

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Objectives: We aim to compare the effect of two models of neuronal hyperexcitation on activation of adult hippocampal radial neural stem cells (rNSCs). We will compare the effect of on rNSC activation (entry in the cell cycle) of electroconvulsive shock (ECS), the experimental model of electroconvulsive therapy which is employed as a treatment for drug-resistant depression patients, to those of intrahippocampal injection of kainic acid (KA), a model of temporal lobe epilepsy.
Materials and methods: Nestin-GFP transgenic mice were subjected to intrahippocampal stereotaxical injection of 50 nl of saline or of either 0.75 nM (a subpathological and non-epileptogenic dose); or 20 nM (a seizure-inducing dose) kainic acid (KA). Separately, another group of Nestin-GFP mice were subjected to ECS or sham manipulation. The animals were sacrificed shortly after the procedures. Brain sliced were processed for multilabeling for specific cell-type and proliferation markers. Analysis and quantification were performed using design-based stereology and confocal microscopy.
Results: Both the low (90%), but specially the high dose of KA (300%) significantly increased the number of rNSCs entering mitosis. ECS also induced an increase of the number of activated rNSCs (60%). rNSCS were identified as GFAP-immunopositive cells with the soma located in the subgranular zone and a radial process that crossed the granule cell layer and arborized in the molecular layer. Both the low-KA dose (100%) and ECS (125%) , but not the high-KA dose, increased also the number of dividing secondary progenitors.
Conclusions: Neuronal hyperexcitation induced by different methods recruits and activates adult hippocampal rNSCs in higher numbers. We have previously demonstrated that the rNSC population declines due to activation-coupled astrocytic differentiation. Therefore, although transient increases in neurogenesis can be found in temporal epilepsy and after ECS, an accelerated decline of the rNSC, and consequent chronic impairment of neurogenesis may occur.

Áreas temáticas:
1. Trastornos y reparación del sistema nervioso.
2. Desarrollo.

ULTRAESTRUCTURA DE LOS GRÁNULOS DEGENERATIVOS PRESENTES EN EL HIPOCAMPO DE LOS RATONES SENESCENTES SAMP8.

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La cepa de ratones senescence-accelerated mouse prone 8 (SAMP8) es un modelo murino experimental de envejecimiento que desarrolla una senescencia acelerada. Estos animales presentan gránulos patológicos en el hipocampo que se agrupan en forma de clusters y que aumentan en número y tamaño con la edad. El contenido de los gránulos y su origen celular no se han esclarecido aún. El objetivo del presente trabajo es estudiar la ultraestructura de los gránulos hipocampales de los ratones SAMP8, así como determinar su origen celular y su proceso de formación. Con ese fin, se determinaron el número y localización de los gránulos en cortes cerebrales por técnicas de inmunohistoquímica, y las secciones consecutivas fueron procesadas por técnicas de microscopía electrónica. La observación de los cortes ultrafinos del hipocampo mostró que los gránulos son depósitos de material fibrilar con un espacio periférico alrededor, en el que se encuentran ocasionalmente orgánulos degenerados. Todos los gránulos observados contenían una membrana discontinua alrededor, hecho que indicaba su origen intracelular. La extensión de los gránulos era de hasta 3 μm de diámetro, mientras que el núcleo denso tenía un tamaño de 0,5-2 μm. Se identificaron además algunos gránulos en los primeros estadíos de formación. Estos gránulos presentaban un tamaño menor y carecían de un núcleo compacto, puesto que estaban formados sólo por estructuras membranosas. La identificación preliminar del origen celular de los gránulos indicaría que se originan probablemente en los astrocitos.

1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia de sistemas

PRESENCIA DE UN NUEVO NEO-EPÍTOPO GLICOSÍDICO Y AUSENCIA DE BETA AMILOIDE Y PROTEÍNA TAU EN LOS GRÁNULOS DEGENERATIVOS DEL HIPOCAMPO DE LOS RATONES SENESCENTES

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Algunos gránulos patológicos relacionados con procesos degenerativos aparecen y aumentan progresivamente con la edad en el hipocampo de numerosas cepas de ratones. En este trabajo describimos la presencia de un neo-epítopo de tipo glicosídico situado en los gránulos que no se encuentra en otras áreas cerebrales, siendo así un nuevo marcador de estas estructuras degenerativas. Se describe también en este trabajo el reconocimiento de este epítopo por parte de anticuerpos IgM presentes en anticuerpos obtenidos en ascites de ratón o en suero de ratón o conejo. Estos resultados evidencian la necesidad de revisar los componentes constituyentes descritos en los gránulos, como es el caso de los péptidos β-amiloides, descritos en los gránulos de los animales con senescencia acelerada SAMP8 (*senescence-accelerated mouse prone-8*). La caracterización de la composición de los gránulos del hipocampo en los animales SAMP8 teniendo en cuenta la presencia de este neo-epítopo y de la IgM contaminante ha demostrado que los gránulos no contienen péptidos β-amiloide o proteína tau. La presencia de un neo-epítopo en los gránulos pero no en otras áreas cerebrales abre nuevas posibilidades hacia el estudio de procesos neurodegenerativos relacionados con la edad, y la cepa de ratones SAMP8, donde la progresión de los gránulos es mayor puede ser un buen modelo para ello.

1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia de sistemas

IMMUNOHISTOCHEMICAL LOCALIZATION OF ADRENOMEDULLIN IN ASTROCYTES SURROUNDING BETA-AMYLOID PLAQUES IN APP/PS1 MICE.

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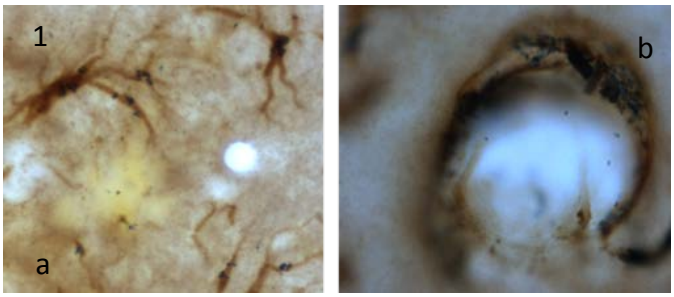
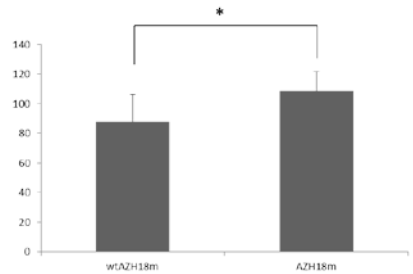
Adrenomedullin (AM) is a potent vasodilator peptide highly expressed throughout the brain.



In addition to angiogenic properties, AM is considered a neuromodulator that possess antiapoptotic and antioxidant properties which suggests that this peptide can protect organs from damage. In a previous study we have found that AM exerts a neuroprotective action in the brain and that this protection may be mediated by regulation of nitric oxide synthases, matrix metalloproteases, and inflammatory mediators. In this research we have investigated the immunohistochemical localization of AM in a mouse model of Alzheimer disease bearing human mutations associated with early-onset Alzheimer's disease (APPswe,PSEN1dE9). This study was carried out under both light (Fig. 1) and electron microscopes (Fig. 2) to detect the expression of AM and correlation analysis of AM expression in neural cells and beta-amyloid plaques. Results of this study reveal characteristic higher AM expression in astrocytes from APP/PS1 mice than in wild type littermates. Astrocytes showing AM immunostaining were particularly associated to beta-amyloid plaques in both the cerebral cortex and hippocampus. The presence of immunoreactive elements surrounding beta-amyloid deposits in APP/PS1 mice are consistent with the fact that AM may be involved in the pathophysiology of the disease. Supporting this, we have found by RT-PCR significant increase in AM gene expression in double transgenic animals compared to wild-type littermates (* $p < 0.05$) (Fig. 3).

Fig. 1.- Double-antigen staining for AM (blue, granular) and GFAP (brown and diffuse). A. shows astrocytes exhibiting both AM and GFAP immunoreaction products surrounding a beta-amyloid plaque (A: 40x; B: 100x). B. illustrates AM immunoreaction product in a capillary near a beta-amyloid plaque. Fig. 2. Electron micrograph showing an AM-positive astrocyte near a degenerated axon (15000x). Fig. 3. Illustrates AM gene expression in wt (wtAZH) and APP/PS1 (AZH) mice. (Supported by MICINN SAF2010-15173)



Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Nuevos métodos y tecnologías

REGROWTH OF TRANSECTED RETINAL GANGLION CELL AXONS DESPITE PERSISTENT ASTROGLIOSIS IN THE LIZARD

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We analysed the astroglia response that is concurrent with spontaneous axonal regrowth after optic nerve (ON) transection in the lizard *Gallotia galloti*. At different post-lesional time points (0.5 to 12 months), we used conventional electron microscopy and specific markers for astrocytes [GFAP, vimentin (Vim), Sox9, Pax2] and for proliferating cells (PCNA). The experimental retina showed a limited glial response since the increase of gliofilaments was not significant if compared to controls, and proliferating cells were undetectable. Conversely, PCNA+ cells populated the regenerating ON, optic tract (OTr). Subpopulations of these PCNA+ cells were identified as GFAP+ and Vim+ reactive astrocytes. Reactive astrocytes up-regulated Vim at 1 month post-lesion, and both Vim and GFAP at 12 months post-lesion in the ON-OTr, indicating long-term astrogliosis. They also expressed Pax2, Sox9 in the ON, and Sox9 in the OTr. Concomitantly, persistent tissue cavities and disorganised regrowing fibre bundles reaching the OT were observed. Our ultrastructural data confirm abundant gliofilaments in reactive astrocytes joined by desmosomes. Remarkably, they also accumulated myelin debris and lipid droplets until late stages, indicating their participation in myelin removal. These data suggest that persistent mammalian-like astrogliosis in the adult lizard ON contributes to a permissive structural scaffold for long-term axonal regeneration and provides a useful model to study the molecular mechanisms involved in these beneficial neuron-glia interactions.

This work was supported by the Spanish Ministry of Education (Research Project BFU2007-67139), the Regional Canary Island Government (ACIISI, Research Projects SolSub200801000281 and ULPAPD-08/012-4).

ROLE OF THE RNA-BINDING PROTEIN HUR IN NEUROFIBROMAS & MALIGNANT PERIPHERAL NERVE SHEATH TUMORS

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Malignant peripheral nerve sheath tumors (MPNST) are aggressive soft tissue sarcomas that arise within the peripheral nerve with very poor prognosis. Schwann cells are the crucial pathogenic cell type in MPNST. The aim of this study was to examine the role of the RNA-binding protein HuR in human Neurofibromas and MPNST.

HuR expression was examined by immunohistochemistry (IHC) in human tissue arrays containing normal nerves (n=7), neurofibromas (n=105) and MPNST (n=34). In addition, HuR protein and mRNA levels were examined by Western blotting (WB) and qPCR respectively in independent frozen human control nerve samples (n=5), solid human tumors [dermal (n=5), plexiform (n=7), NF1-related MPNST (n=8), sporadic MPNST (n=7)] and MPNST derived cell lines (n=4). To identify HuR mRNA targets, RNA immunoprecipitation coupled with microarray analysis (RIP-CHIP) were performed in the same frozen tissue samples. The functional role of HuR was examined by lentiviral-mediated silencing in MPNST derived cell lines and features such as proliferation, apoptosis, migration, invasion, colony formation and anchorage-independent growth.

We found that HuR expression was significantly increased in NF and MPNST samples compared to normal nerves, with a strong correlation between HuR expression and degree of malignancy, both by IHC, and qPCR analysis and WB. RIP-CHIP showed that the number of mRNAs bound to HuR increased as malignancy progresses. Amongst them, several ones with well-defined roles in oncogenesis were identified. HuR silencing *in vitro* using MPNST cell lines significantly reduced the expression of these genes and proliferation, migration, colony formation and invasion and also made these cells more sensitive to apoptotic death by UV irradiation.

In summary, we propose that HuR plays a key role in the control of expression of critical cancer-associated genes that regulate oncogenic functions such as proliferation, cell survival and metastasis.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

INVOLVEMENT OF THE NEUROVASCULAR UNIT IN ALZHEIMER´S DISEASE AFTER STROKE. DONOR LA419: POSSIBLE STRATEGY FOR TREATMENT

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Neurovascular unit dysfunction contributes to ischemia, cognitive disorders and neurodegeneration, including progression of chronic disorders of the CNS such as Alzheimer's disease (AD). When the neurovascular unit function is affected, in addition to a decreased oxygen and metabolite supplies, the amyloid beta protein clearance is compromised which favors the progression of the disease. Thus, the search for effective therapies for the prevention and / or treatment of neurovascular unit dysfunctions is a matter of basic and practical interest.

In this study we have performed permanent focal ischemia by occluding the middle cerebral artery (pMCAO) in a mouse model for AD: double transgenic bearing human mutations associated with early-onset AD (APPswe-PS1dE9). So, we have tested a nitric oxide donor developed by LACER SA laboratories, the compound LA419, as a potential candidate for the neurovascular unit dysfunction.

The product LA419 is a new generation organic nitrate which acts through the eNOS signaling pathway. Its antioxidant, anti-ischemic, antithrombotic, and antiatherosclerotic properties at doses that did not induce hemodynamic changes that alter blood pressure, have been well described. The drug was administered intraperitoneal in APP/PS1 mice 15 minutes after infarction.

Our results indicate that LA419 decreases the infarct volume in double transgenic mouse 3 months years old submitted to MCAO compared to the untreated one. This decrease not occurred in the same experimental model with 12 months years old.

We conclude that administration of LA419 can be considered a possible clinical strategy for treatment of diseases that course with neurovascular affectation.

Thematic Areas:
1st: Disorders of the nervous system and repair
2nd: New methods and technologies

Financial support: **LACER, SA**, MCINN (SAF 2010-15173)

AB OLIGOMERS ACTIVATE INTEGRINS TO PRODUCE OXIDATIVE STRESS THROUGH PI3K/PKC/RAC1/NADPH OXIDASES IN ASTROCYTES

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Cognitive impairment in Alzheimer disease (AD) is strongly associated with both extensive levels of amyloid beta peptide (A□)and oxidative stress, but the exact role of these indices in the development of dementia is not clear. NADPH oxidase (NOX) is involved in AD pathogenesis as A□ activates NOX in astrocytes generating oxidative stress that ultimately causes neuronal death. Here, we describe the molecular signaling events mediating NOX activation by A□ oligomers in cultured astrocytes. First, we found that astrocytes express mRNAs encoding for the regulatory subunit p47phox, NOX1, 2, and 4, and the dual oxidases (DUOX) 1 and 2, but not NOX3 subunits. Then, we observed that A□ oligomers (5 μM) induced a rapid (30-120 min) ROS production which was Ca^{2+}-dependent. A□-induced ROS generation was prevented by NOX inhibitors apocynin, DPI and gp91ds-tat peptide. To further investigate the pathway underlying A□-mediated ROS generation, we analyzed the activation of NOX-interacting protein Rac by Rac-GTP affinity precipitation and PAK1 phosphorylation assay. We found that A□ oligomers triggered a sustained Rac activation that was blocked by inhibition of the classic but not by the novel PKC activities. In addition, ROS generation in A□-treated astrocytes was reduced by inhibition of integrins as well as of PI3K and PDK activities which in turn blocked the levels of PKC and PDK phosphorylation. Our results demonstrate that A□ oligomers activate integrins to produce ROS via PI3K/PKC/Rac/NADPH oxidase pathway in astrocytes. These mechanisms may be relevant to AD pathophysiology.

Supported by CIBERNED, Gobierno Vasco and MINECO.

ADVANCED OXIDIZED PROTEIN PRODUCTS (AOPP) COULD SERVE AS A PROGNOSTIC MARKER FOR SEVERITY OF PARKINSON'S DISEASE

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Objectives: Protein and amine halogenation is a type of oxidative stress induced by phagocytic over-stimulation, and its role in Parkinson's disease has not been discerned.
Methods: Halogenative stress-induced protein changes in serum and CSF were analyzed in PD patients (n=60) and control subjects without any neurological disorder (n=45), by using ELISA, western-blotting and mass spectrometry.
Results: We have detected that advanced oxidized protein products, markers of protein halogenation, are reliably enhanced in serum of patients with Parkinson's disease (n=60) relative to control subjects (n=45, $p<0.012$), and to a lesser extent in cerebrospinal fluid. Amine halogenation, as evaluated through 3-chlorotyrosine, is not affected. Mieloperoxidase and hydrogen peroxide levels, halogenative factors of phagocytes, are devoid of changes. Levels of advanced oxidized protein products are progressively reduced over time, and duration of Parkinson's disease is larger in Hoehn-Yahr stage 2/3 patients (n=34) with low serum levels ($R^2= 0.0145$, $p<0.003$). Levodopa treatment contributes to this reduction ($R^2= 0.259$, $p<0.001$).
Conclusions: AOPP are not cytotoxic, unlike 3-chlorotyrosine, but they are known to form inflammatory mediators after conjugation with serum albumin. Our observations lead to the hypothesis that serum level of advanced oxidized protein products is a prognostic marker of Parkinson's disease duration, and these oxidized proteins could participate in the development of Parkinsonian neuroinflammation.
Supported by grants to EFE by Junta de Andalucia (BIO127), and Spanish Ministerio de Sanidad (RETICS, RD06/001/002; RD06/010/1007; Instituto Carlos III, co-financing with FEDER, European Fund for Regional Development).

1. Trastornos y reparación del sistema nervioso
2. Nuevos métodos y tecnologías

ACTIVATION OF NFAT TRANSCRIPTION FACTORS IN NEURAL PRECURSOR CELLS INDUCES ASTROCYTE DIFFERENTIATION

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The nuclear factor of activated T cells (NFAT) was initially described as a family of transcription factors with key functions for lymphocyte maturation and activation, so that NFAT is implicated in the progression of inflammation. However, NFAT factors are also expressed in other tissues including the nervous system, where they control mechanisms related to cell survival, proliferation, differentiation, migration, adhesion and activation. Our previous work described the presence of the NFAT system in astrocytes, and found a link between NFAT activation and metalloproteinase expression as well as glial reactivity during ischemia, and traumatic and excitotoxic brain injury. In addition, we have recently detected NFAT expression in neural precursor cells (NPCs) leading us to investigate NFAT roles in NPCs. The present work analyzes possible NFAT connections with proliferation and differentiation of NPCs cultured as neurospheres from neonatal mouse subventricular zone (SVZ). Adenoviral infection to overexpress a constitutively-active form of isoform NFATc3 induced a phenotypic change in NPCs, so that adhesion and migration was stimulated while cell cycle was arrested. Meanwhile, cells extended thin and branched processes, and GFAP was upregulated. All together, our observations indicate that NFAT promotes astrocytic differentiation in NPCs. Funded by SAF2009-12869 (P. Tranque) and PI09/0218, PI12/0238 (E. Cano).

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Desarrollo

A MORPHOLOGICAL APPROACH TO EVALUATE THE DEGENERATION DEGREE OF SUBSTANTIA NIGRA IN A PARTIAL MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is characterized by loss of dopaminergic neurons in Substantia Nigra (SN). Nigrostriatal projections are topographically organized, therefore our aim was to quantify and compare changes in TH+ neuron density of whole SN and inside a delimited region under different treatment options.

Rats underwent intrastriatal injections of 6-OHDA and 3 weeks later implantation of PLGA-nanospheres (NS). Parkinsonized rats were randomly divided in experimental groups: vehicle, empty-NS and VEGF+GDNF-NS (1.25 µg + 1.25 µg). In vivo effects were assessed using amphetamine induced rotational behavior test. After twelve weeks a single dose of BrdU (200 mg/kg) was intraperitoneally injected. Following fixation brains were removed one week after and samples processed for immunohistochemistry against TH, BrdU and doublecortin (DCX). TH-positive neuronal density was measured in SN delimiting two regions: the whole SN and a delimited lateral region named "external SN" including SN-Lateral, one third of SN-reticulata and the lateral half of SN-compacta.

Partial model of PD showed a lesion mainly in postero-lateral caudoputamen complex. VEGF+GDNF-NS treated group significantly reduced the number of rotations induced by amphetamine. Stereological study showed changes in whole SN neuron density, which were statistical significant for the VEGF+GDNF-NS group. These changes were significantly higher among groups when the "external SN" was considered. The lesioned hemisphere displayed a 43.52±6.45 % of neurons, whereas empty-NS and only vehicle administered showed 23.51±4.44 % and 11.64±1.66 % respectively. A high amount of cells expressing BrdU positivity were found in subventricular zone of VEGF+GDNF-NS group. Some of these cells co-express DCX.

In conclusion, these findings support the neurorestorative role of VEGF+GDNF on the dopaminergic system, where functional improvement was accompanied with a morphological restoration. Measurements focused on the "external SN", which are topographically related to lesioned area of striatum, achieve more specific and significant results than in whole SN.

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Áreas temáticas:

1ª: Trastornos y reparación del sistema nervioso

2ª: Nuevos métodos y tecnologías

CELL DEATH INDUCED BY METHYLMERCURY IS ASSOCIATED WITH CELL TYPE-DEPENDENT COFILIN AND/OR ACTIN TRANSLOCATION TO MITOCHONDRIA

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Objectives: Methylmercury (MeHg) is a neurotoxic environmental contaminant with high selectivity for cerebellar granule cells in mammals. One of the main intracellular targets for MeHg neurotoxicity is the mitochondria. In many cell models, mitochondria are the major organelles in the signal transduction and biochemical execution of apoptosis. Cofilin is a cytosolic protein belonging to the family of the actin depolimerizting factors (ADF) which regulate the process of polimerization/depolimerization of actin filaments and participates in processes leading to apoptotis. The aim of this work was to deep on the effects of prolonged exposure to methylmercury on cofilin phosphorylation and translocation to the mitochondria in cortical neurons(CCN) and in cerebellar granule cells (CGC) under differentiation.

Materials and Methods: Primary cultures of CCN and of CGC were exposed to 0 – 300 nM MeHg for 4 – 8 DIV. Cofilin and actin levels were determined by Western Blot in cytosolic and mitochondrial extracts. Protein oxidation was determined by ELISA assay and cell viability by propidium iodide uptake.

Results: Exposure to MeHg reduced the amount of phosphorylated cofilin, increased the carbonylation of proteins and induced cell death in both neuronal types. Non-phosphorylated cofilin was translocated from cytosol to mitochondria in CGC, but not in CCN,whereas actin was in both cell types. CGC also showed increased lysosomal cathepsin D activity in the cytosol. All these effects were prevented by probucol. On the other hand, the cathepsin D inhibitor pepstatin A did not avoid MeHg-induced actin and cofilin translocation into mitochondria in CGC.

Conclusions: Oxidative stress is the key upstream signal in the cofilin and/or actin-related cell death mechanism of MeHg. The translocation of cofilin from cytosol to mitochondria may be essential for the differential sensitivity of cell types against MeHg.

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Áreas Temáticas:
1ª: 5 (Trastornos y reparación del sistema nervioso)

TRAUMATIC INJURY INDUCES CHANGES IN THE EXPRESSION OF THE SEROTONIN 1A RECEPTOR IN THE SPINAL CORD OF LAMPREYS

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In contrast to mammals, lampreys recover locomotion after a complete spinal cord transection. After spinal cord injury in mammals, the loss of serotonin coming from the brainstem reduces the excitability of motor neurons and leads to a compensatory overexpression of serotonin receptors. In this study, we adapted a semiautomatic quantification method to quantify the changes in the expression of the 5-HT_{1A} in the spinal cord of larval sea lampreys after a complete spinal cord transection as seen by *in situ* hybridization. Our results showed that there is a marked increase in the expression of the 5-HT_{1A} acutely (1 wpl) after the transection both rostrally and caudally to the injury site. The expression returns to levels similar to those found in unlesioned animals a few weeks (3-7 wpl) after the injury. Interestingly, overexpression of the 5-HT_{1A} in rostral levels after spinal cord injury has not been reported in mammals, suggesting that this could be part of the plastic events that lead to the recovery of function in a regenerating vertebrate like the sea lamprey. The analysis of changes in 5-HT_{1A} expression by zones (periventricular region and horizontally extended grey matter) showed that they followed the same pattern of changes detected in the spinal cord as a whole, with the exception of the caudal periventricular layer, where no significant differences were observed between control and experimental animals at any time post lesion. This suggests that different molecular signals act on the periventricular cells of the rostral and caudal spinal cord after the lesion. Our observations in the sea lamprey and previous data in other vertebrates showing that the activation of this receptor is beneficial after spinal cord injury, suggest that a higher availability of the 5-HT_{1A} could be beneficial for behavioural recovery to compensate for a reduced availability of the agonist.

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Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

REACTIVE ASTROCYTE UPTAKE OF NEUROTRANSMITTERS AFTER A COMPLETE SPINAL CORD TRANSECTION IN THE SEA LAMPREY

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In contrast to the situation in mammals, the spinal cord of lampreys recovers spontaneously from an injury. Special properties of the astrocytes of lampreys appear to contribute to the success of spinal cord regeneration. The main aim of this study is show the astrocyte uptake of neurotransmitters and the changes in glutamate, GABA and glycine expression in neurons during the first week following a complete spinal cord transection in the sea lamprey. Although it is known that neurotransmitter levels change after spinal injury and that glutamate is uptaken by astrocytes in the first hours after the lesion, this is the first time that the astrocyte response and the neurotransmitter release from neurons are observed by immunohistochemistry. Complete spinal cord transections were done at the level of the 5th gill and glutamate, GABA, glycine and cytokeratin immunoreactivities were studied from 0 to 7 days after the injury. Spinal injury resulted in the immediate loss of glutamate, GABA and glycine expression in neurons in the adjacent region to the lesion site (except for the cerebrospinal fluid-contacting GABA immunoreactive cells). In addition, glutamate, GABA and glycine expression was observed in astrocytes at different time points after the lesion. Moreover, there was a massive accumulation of the inhibitory neurotransmitters around some descending axons. This accumulation could be related to the regeneration ability of the axons since it was observed around good regenerator axons. Lamprey astrocytes have high capacity of glutamate uptake and maintained over time, unlike what is observed in mammals. The behaviour of the central nervous system (CNS) cells during the first week following an injury may be critical for the future recovery. Thus, the features observed in lamprey CNS after an injury could provide some clues about what is necessary to achieve a satisfactory regeneration.

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Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

EFFECT OF DNA POLYMERASE λ KNOCKOUT ON CAG SOMATIC EXPANSION AND ON PHENOTYPE OF HUNTINGTON´S DISEASE MICE

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Somatic expansion of CAG has been postulated to contribute to polyglutamine-induced toxicity and selective vulnerability of certain brain regions in Huntington´s disease (HD). Hence, somatic repeat instability was postulated to contribute to polyglutamine toxicity.
Patterns of CAG repeat distribution are remarkably reproducible between individuals, implying that the mutation behaves in a predetermined manner. Since neurons are postmitotic, somatic expansion is believed to occur during DNA repair, due to oxidative damage.

Family X of polymerases in mammals is formed by structurally related enzymes specialized in repair pathways involving gap and double strand breaks (DSB). Several DNA repair mechanisms have been proposed to be involved in trinucleotide repeat somatic expansion. DNA Polimerase λ (Polλ) has been implicated in gap filling during base excision repair (BER) and during repair of DSB in DNA by nonhomologous DNA end joining (NHEJ). In addition, Polλ has been identified to play an important role in oxidative damage repair.
Given the susceptibility to oxidation of CAG repeats, we reasoned that, in the context of HD, this DNA polymerase might be implicated in the promotion of CAG repeat expansion in somatic cells.

Here, we aim to take advantage of mouse genetics to explore this. Accordingly, we first generate Polλ knockout mice. Then, we combine R6/1 N-mutant huntingtin mice with Polλ knockout mice. Analysis of somatic expansion reveals that Polλ is implicated in this process, since R6/1; Pol λ-/- mice showed lower CAG repeat somatic expansion. At the histopathological level, striatal N-mutant huntingtin inclusion size were smaller in R6/1;Polλ-/- mice, although motor impairment was more severe in this group of animals.
Our results suggest a possible role for Polλ in somatic expansion, although this process does not play an important role in polyglutamine-mediated toxicity, leading us to conclude that somatic expansion is not a therapeutic target.

Áreas Temáticas:
1ª: Trastornos y reparación del Sistema Nervioso
2ª: Neurociencia de sistemas

MECHANISMS OF REGULATION OF MITOCHONDRIAL LENGTH BY NEURONAL ACTIVITY

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In neurons, signals that have opposite effects on cell survival cause a comparable fragmentation of the mitochondrial network. On the one hand, apoptotic stimuli such as nitrosative stress, Amyloid Beta or low neuronal activity all produce mitochondrial fragmentation before causing damage to cytoplasmic membranes. On the other hand, high neuronal activity or activation of membrane receptors linked with neuronal survival also induces mitochondrial fragmentation. In previous studies, we reported that Neuronal Pentraxin 1 contributes to the mitochondrial fragmentation evoked by low neuronal activity during apoptosis. We have now investigated the role of cytoplasmic membrane receptors and of Neuronal Pentraxin 1 (NP1) in the fragmentation of the mitochondrial network evoked by high neuronal activity or by oligomeric Amyloid Beta (Ab) (2uM). We found that treatment of cultured cortical neurons with a depolarizing concentration of K+ (50mM) or with Ab both reduce mitochondrial length. The mitochondrial fragmentation caused by Ab was prevented with either AMPA or NMDA glutamate receptor antagonists but not with Nimodipine (5uM) an L-type Ca2+ channel blocker. In contrast, mitochondrial fragmentation evoked by depolarization with high K+ did not depend on glutamate receptor activation. The reduction of mitochondrial length evoked by both depolarization and Ab are blocked by a DRP1 inhibitor and are associated with translocation of DRP1 from cytoplasm to mitochondria. Deletion or Knockdown of NP1 inhibited the translocation of DRP1 to mitochondria and mitochondrial fragmentation evoked by both treatments, indicating that NP1 is a key mechanism where different signal transduction pathways converge for the regulation of neuronal activity dependent mitochondrial dynamics.

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Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

SFRP1 ACTS AS A PRO-INFLAMMATORY CYTOKINE DURING BRAIN RESPONSE TO NEURO-DEGENERATION

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Secreted-Frizzled-Related-Protein 1 (Sfrp1) is a multifunctional regulator of cell-to-cell communication. Its function modulates Wnt signaling and regulates the activity of different metalloproteases, such as ADAM10. ADAM10 is a α-secretase with multiple substrates, including Amyloid Precursor Protein (APP), a protein involved in the onset and development of Alzheimer Disease (AD) and inflammatory cytokines (i.e. fractalkine, TNFa) and their receptors (i.e. TNFR, IL1R, IL6R). By crossing a mouse model of AD (APP;PS1) with a Sfrp1$^{-/-}$ mouse line, we generated an APP;PS1;*Sfrp1*$^{-/-}$ line, in which amyloid deposits and brain inflammation, otherwise characteristic of APP;PS1 mice, are nearly absent. This suggests that Sfrp1 could contribute to both pathological traits. We have thus undertaken a study to determine whether Sfrp1 might directly modulate brain inflammation or if decreased inflammation observed in APP;PS1;*Sfrp1*$^{-/-}$ is simply a consequence of the absence of amyloid deposits. To this end, we treated cultures from postnatal wild type cortex with pro-inflammatory compounds such as LPS and IL6, demonstrating that in their presence both astrocytes and microglial cells up-regulate the levels of *Sfrp1* expression. Similar results were observed after intra-cortical or intra-ventricular infusion of LPS in wild type. Notably, lentiviral mediated *Sfrp1* gene addition in the brain of wild type mice activates a strong inflammatory response characterized by the presence of activated microglial cells that persist at least over a month. Altogether these data suggests that Sfrp1 act as pro-inflammatory molecule in the brain, which may be relevant in many neurodegenerative diseases. *Supported by MINECO BFU2010-16031; Fundaluce and Fundacion ONCE.*

1. Trastornos y reparación del sistema nervioso
2. Desarrollo

EFFECTS OF INTRAVENOUS ADMINISTRATION OF HUMAN UMBILICAL CORD BLOOD STEM CELLS IN 3-ACETYPYRIDINE LESIONED RATS

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Cerebellar ataxias include a heterogeneous group of infrequent diseases characterized by lack of motor coordination caused by disturbances in the cerebellum and its associated circuits. Current therapies are based on the use of drugs that correct some of the molecular processes involved in their pathogenesis. Although these treatments yielded promising results, there is not yet an effective therapy for these diseases. Cell replacement strategies using human umbilical cord blood mononuclear cells (HuUCBMCs) have emerged as a promising approach for restoration of function in neurodegenerative diseases. The aim of this work was to investigate the potential therapeutic activity of HuUCBMCs in the 3-acetylpyridine (3-AP) rat model of cerebellar ataxia. Intravenous administered HuUCBMCs reached the cerebellum and brain stem of 3-AP ataxic rats. Grafted cells reduced 3-AP-induced neuronal loss and promoted activation of microglia in the brain stem, and prevented the over-expression of GFAP elicited by 3-AP in the cerebellum. In addition, HuUCBMCs up-regulated the expression of proteins that are critical for cell survival, such as phospho-Akt and Bcl-2, in the cerebellum and brain stem of 3-AP ataxic rats. As all these effects were accompanied by a temporal but significant improvement in motor coordination, HuUCBMCs grafts can be considered as an effective cell replacement therapy for cerebellar disorders.

1ª: Trastornos y reparación del sistema nervioso
2ª: Nuevos métodos y tecnologías

CEREBROSPINAL FLUID SYNAPTIC PROTEINS AS USEFUL BIOMARKERS IN TYROSINE HYDROXYLASE DEFICIENCY

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Tyrosine hydroxylase (TH) deficiency is an inborn error of dopamine biosynthesis with variable clinical presentation and a variable response to treatment with L-Dopa. Two broad clinical phenotypes have been described. Type “B”: early onset severe complex encephalopathy, and type “A”: later onset, and is in general less severe with better response to L-Dopa.

Objectives: To study the expression of some key synaptic dopaminergic and gabaergic proteins in the cerebrospinal fluid of a series of patients with TH deficiency and their possible relation with the clinical phenotype and response to L-Dopa.

Materials and Methods: We measured dopamine transporter (DAT), D2-receptor and vesicular monoamine transporter type 2 (VMAT2) in the CSF of 10 subjects with TH deficiency by Western blot analysis. In 3 patients, data of pre- and post-treatment with L-Dopa were available, and in one of them the GABA vesicular transporter was determined. Results were compared to an age-matched control population. SPSS 20.0 was used for statistical analysis.

Results: The concentration of D2-receptor in CSF was significantly higher in patients with TH deficiency than in controls. Similarly, DAT was up-regulated. No differences were found in the vesicular monoamine transporter type 2. Finally, the studies performed before L-Dopa therapy and on L-Dopa therapy showed that in patients with A phenotype D2 receptor expression decreases with treatment, while in one patient with B phenotype a paradoxical response was noted with increase of D2 receptor expression together with increase of GABAVT expression as L-Dopa doses and homovanillic concentration gradually raise.

Conclusions: These results suggest the GABA-Dopa interaction or co-release, which is also discussed.

RTP801 IN THE MAMMALIAN RETINAL GANGLION CELLS

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Purpose: Rtp801 (also known as Redd1, and encoded by Ddit4) is a stress-related protein, triggered by adverse environmental conditions to inhibit the mammalian target of rapamycin (mTOR) and so enhance oxidative stress-dependent cell death. Glaucoma is characterized by the progressive death of retinal ganglion cells (RGCs) and all evidence strongly suggests that oxidative stress is a major player in the death process. New strategies to delay or halt RGC loss are urgently required because present methods of treatment benefit only a limited number of glaucoma patients. We therefore wondered whether RTP801 is located to RGCs and as a consequence might act as a potential amplifying switch that can be manipulated with drugs like rapamycin as a means for preserving their functions in glaucoma.

Methods: Protein and mRNA expression in RGC cultures and rat retinas were analysed by western blot and PCR, as well as immunohistochemistry and immunocytochemistry assays.

Results and conclusions: Our findings to date support such a supposition. RTP801 immunoreactivity is highly expressed in the retinal ganglion cell layer of mouse and rat retinas with only traces of immunoreactivity located to other retinal areas. Moreover, double labelling experiments show that the RGC marker Brn3a often co-localises with RTP-801 immunoreactivity. In addition, western blot and real-time PCR data unequivocally reveal that the protein and mRNA RTP801 are present in retinal tissues. We therefore tentatively conclude that retinal RTP801 is primarily associated with RGCs. Experiments are now in progress to investigate whether pharmacological damage to RGCs *in situ* (e.g. ischemia, NMDA toxicity) can be positively manipulated by targeting RTP801 with rapamycin.

Áreas temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Neurociencia de sistemas

IMPLICACIÓN DEL RECEPTOR MEGALINA EN LA PATOGÉNESIS DE LA ESCLEROSIS MÚLTIPLE Y EN LA ALTERACIÓN DE LA BARRERA HEMATOENCEFÁLICA

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La megalina (también conocida como LRP-2) es un receptor multiligando perteneciente a la familia de los receptores de lipoproteínas de baja densidad, que es indispensable para el correcto desarrollo del sistema nervioso central y está implicado en el transporte de moléculas a través de la barrera hematoencefálica (BHE). Además, hemos descrito con anterioriad su participación en el efecto quimioatrayente y mitogénico de Sonic hedgehog sobre los precursores de oligodendrocitos durante el desarrollo. Se ha descrito previamente la presencia de LRPs en lesiones activas de pacientes de esclerosis múltiple (EM), pero no existen datos acerca de la expresión del receptor megalina en particular. En este trabajo, analizamos la distribución y la caracterización celular de megalina en muestras *post-mortem* de pacientes de EM para estudiar su papel en la patogénesis de esta enfermedad. Los cambios observados en la distribución de megalina son paralelos a la histopatología de las diferentes lesiones de EM: así, observamos megalina en macrófagos de lesiones activas y de la periplaca de lesiones crónico-activas, áreas donde la remielinización puede ocurrir de forma espontánea, y en una subpoblación de astrocitos perivasculares de la sustancia gris aparentemente normal. La presencia de megalina en vasos sanguíneos nos llevó a analizar el estado de la BHE para determinar alteraciones estructurales y/o funcionales. En conjunto, la expresión de megalina en las muestras de pacientes de EM podría reflejar diferentes aspectos relacionados con el desarrollo de la enfermedad: i) supone un potencial objetivo a considerar para mejorar la remielinización; ii) es un indicador funcional, más que estructural, de la alteración de la BHE. Así, este receptor se convierte en un nuevo candidato a tener en cuenta para el diseño de futuras estrategias terapéuticas basadas en la modulación de un LRP específico.

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Área temática

1ª opción: Trastornos y reparación del sistema nervioso

IN VIVO OVEREXPRESSION OF GSK3 IN NEURAL PRECURSOR CELLS

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Glycogen Synthase Kinase 3 (GSK3) is a kinase ubiquitously expressed with particularly high levels in brain. GSK3 deregulation has been implicated in several neurological disorders such as Alzheimer´s disease (AD), bipolar disorder or schizophrenia. Deficiencies in neural precursor cells and neurogenesis have been postulated as an etiology of some of these diseases. Many evidences support the key role of GSK3 in fundamental processes during neurodevelopment. In addition, pharmacological data has strongly implicated GSK3 in the regulation of self-renewal and differentiation of neural progenitors.

In AD, GSK3 plays a central role in the pathogenesis of the disease, since it is the kinase that phosphorylates most tau protein epitopes. Moreover, it acts downstream of Aβ and interacts with PS1. In transgenic mouse model of AD, generated in our lab, which overexpress GSK3β under CamkII promoter, several dentate gyrus neurogenesis alterations have been published. Between others the depletion of neurogenic niches, decrease in the number of mature neurons, aberrant expression of neurogenic markers or altered postsynaptic densities and dendritic morphology (Fuster-Matanzo A. *et al.* Hippocampus 2011) (Fuster-Matanzo A. *et al.* Hum Mol Genet. 2013) (Llorens-Martin M. *et al.*Mol Psychiatry 2013).

To deep inside in the role of GSK3 in adult neurogenesis, we are interested in further investigate its implications in the physiology and regulation of neural precursor cells. To analyses this, we have generated a transgenic mouse model which overexpress GSK3β under GFAP promoter, a neural stem cell marker. Preliminary data show that *in vivo* overexpression of GSK3 in neural precursor cells produces an increase of this population pool. An increase in the total dentate gyrus volume and mature neurons was observed. Finally, differences in behavioral test were also checked.

Áreas tematicas:

1- Trastornos y reparación del sistema nervioso
2- Desarrollo

CHANGES IN GABA AND GLYCINE IMMUNOREACTIVITY AFTER SPINAL CORD INJURY IN THE SEA LAMPREY

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GABA and glycine are the main inhibitory neurotransmitters in the central nervous system (CNS) and inhibitory inputs are key elements in the networks controlling locomotion behaviour. Lampreys have become an animal model in studies of spinal cord regeneration because they are capable of spontaneously recover locomotion after a complete spinal cord transection. So, understanding how the locomotor networks adapt after the injury in lampreys could provide new clues to develop therapies for mammalian models. The aim of this study was to investigate the changes in the number and organization of GABA and glycine immunoreactive (-ir) cells and fibres of the spinal cord 2, 4, 10 and 24 weeks after a complete spinal cord transection. Numbers of fibres were also quantified using the Fiji software. The complete spinal cord transection was done at the level of the fifth gill and the animals were let to recover for the chosen time points. Our results showed that the normal pattern of glycine- and GABA-ir cells and fibres of the lamprey spinal cord was altered after the injury. An acute loss of GABA-ir and glycine-ir cells was observed initially after the injury. Then, the first cells that recovered their immunoreactivity were the cerebrospinal fluid contacting cells (CFSc). This was followed by the recovery of dorsal and lateral cells and then by the recovery of glycinergic edge cells. There was also a loss in fibres immunoreactivity two weeks after lesion and a progressive recovery. Our results suggest that phenomena of plasticity and regeneration occur in the spinal cord inhibitory systems of lampreys to achieve functional recovery after a complete spinal cord injury. We can now use this model to understand the signalling pathways that control the regeneration and plastic changes of the GABAergic and glycinergic spinal cord systems of vertebrates after injury.

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Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

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15º Congreso Nacional de la Sociedad Española de NeuroCiencia

Tema 6

Sistemas homeostáticos y neuroendocrino

Posters

ADMINISTRATION OF A LEPTIN ANTAGONIST DURING THE PHYSIOLOGICAL NEONATAL LEPTIN SURGE INDUCES LONG-TERM SEX DEPENDENT ALTERATIONS IN THE HIPPOCAMPUS AND PREFRONTAL CORTEX OF ADOLESCENT RATS

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Objetive: To assess whether interference with the physiological neonatal surge of leptin impairs the development of extrahypothalamic brain regions.

Material and methods: Neonatal female and male Wistar rats were treated with rat "mono-pegylated super active leptin antagonist" (mutant D23L/L39A/D40A/F41A), from PND 5 to 9 (5mg/kg/day s.c.; Leptin Antagonist: Lep Ant) or with the corresponding vehicle (Control: Co). Animals were sacrificed at PND43 (males) and PND33 (females) and their brains were removed. Levels of neuronal, glial and neuroplasticity-related proteins were measured in hippocampus (HC) and prefrontal cortex (PCx) by Western Blot.

Results: A baseline sexual dimorphism was found in the levels of glial fibrillary acidic protein (GFAP) and neuronal nuclei (NeuN) , with Co females exhibiting a significant lower content of both proteins when compared with Co males in PCx ($p<0.05$). The Lep Ant treatment induced the following significant ($p<0.05$) effects. Treated males: Decreased levels of synaptophysin and CB2 cannabinoid receptors in HC and PCx and of neural cell adhesion molecule (NCAM) in PCx, and increased levels of Reelin in PCx and of CB1 cannabinoid receptors in HC. Treated females: Decreased levels of NCAM, Reelin and CB1 receptor in PCx and of NG2 (a marker of oligodendrocytes progenitor cells) in HC and PCx.

Conclusions: The present results indicate that disruption of leptin actions by administration of a leptin antagonist during the neonatal leptin surge induces long-lasting sex-dependent changes in the developing hippocampus and prefrontal cortex.

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Áreas Temáticas:
1ª: Sistemas homeostáticos y neuroendocrino
2ª: Desarrollo

CENTRAL ACTIONS OF THE MATERNAL HORMONE: PATTERNS OF PROLACTIN-INDUCED BRAIN ACTIVATION IN MALE AND FEMALE MICE

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Besides inducing milk production, prolactin (PRL) promotes maternal behaviours, thus deserving the name of "maternal hormone", even if it is also present in males. Being a hypophyseal hormone, to modulate behaviour prolactin must enter the brain. In fact, it is actively transported to the ventricles in the choroid plexuses from where it should diffuse through the brain, a process somewhat limited by its large size (22 kDa).

The aim of this work was to evaluate the PRL-induced activation pattern in the mouse brain, by means of the immunohistochemical detection of PRL-induced phosphorylation of STAT5 (pSTAT5). To do so, we have compared the pattern of pSTAT5 immunostaining in male and female mice that received i.p. injections of ovine PRL, versus controls.

Control mice displayed labeling only in the choroid plexuses, circumventricular organs and arcuate nucleus. In contrast, PRL-treated animals showed abundant labeling in: a) septofimbrial nucleus; b) anteroventral and periventricular preoptic nuclei; c) arcuate and ventromedial nuclei of the tuberal hypothalamus; and d) dorso-medial, central amygdala and intra-amygdaloid BST. This labeling was common to males and females, whereas other nuclei showed labeling only in females: a) lateral septum; b) BST-preoptic area; c) anterior and tuberal hypothalamic nuclei, subthalamus and zona incerta; d) ventro-medial and basomedial amygdala; and e) periaqueductal grey and dorsal tegmentum.

Circulating PRL levels seem a limiting factor for its access to the brain: controls (physiological circulating PRL) show virtually no pSTAT5 immunostaining, whereas exogenous administration of prolactin (assuring high circulating levels) results in a broad pattern of immunoreactivity. This suggests that PRL action on the brain would be virtually limited to those physiological events associated with high PRL secretion, such as lactation or acute stress. Only in these situations prolactin would be able to modulate socio-sexual behaviours, like mating, aggression or parental care.

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PHARMACOLOGICAL ADMINISTRATION OF THE ISOFLAVONE DAIDZEIN ENHANCES CELL PROLIFERATION AND REDUCES HIGH FAT DIET-INDUCED APOPTOSIS AND GLIOSIS IN THE RAT HIPPOCAMPUS

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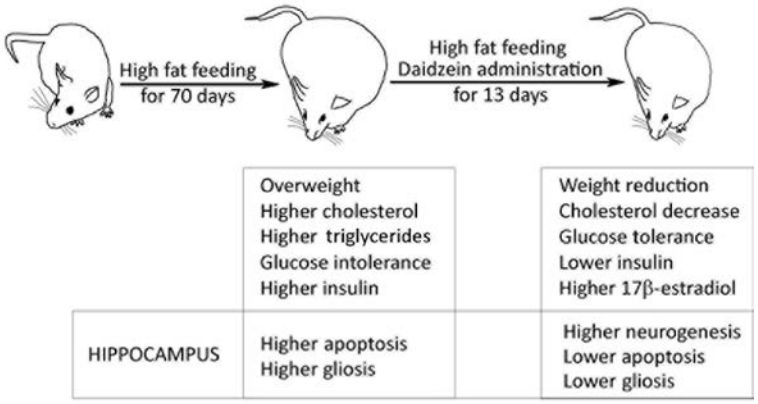
Soy extracts have been claimed to be neuroprotective against brain insults, an effect related to the estrogenic properties of isoflavones. However, the effects of individual isoflavones on obesity-induced disruption of adult neurogenesis have not yet been analyzed. In the present study we explore the effects of pharmacological administration of daidzein, a main soy isoflavone, in cell proliferation, cell apoptosis and gliosis in the adult hippocampus of animals exposed to a very high-fat diet. Rats made obese after 12-week exposure to a standard or high-fat (HFD, 60%) diets were treated with daidzein (50 mg kg^{-1}) for 13 days. Then, plasma levels of metabolites and metabolic hormones, cell proliferation in the subgranular zone of the dentate gyrus (SGZ), and immunohistochemical markers of hippocampal cell apoptosis (caspase-3), gliosis (GFAP and Iba-1), food reward factor FosB and estrogen receptor alpha (ERα) were analyzed. Treatment with daidzein reduced food/caloric intake and body weight gain in obese rats. This was associated with glucose tolerance, low levels of HDL-cholesterol, insulin, adiponectin and testosterone, and high levels of leptin and 17β-estradiol. Daidzein increased the number of phospho-histone H3 and 5-bromo-2-deoxyuridine (BrdU)-ir cells detected in the SGZ of standard diet and HFD-fed rats. Daidzein reversed the HFD-associated enhanced immunohistochemical expression of caspase-3, FosB, GFAP, Iba-1 and ERα in the hippocampus, being more prominent in the dentate gyrus. These results suggest that pharmacological treatment with isoflavones regulates metabolic alterations associated with enhancement of cell proliferation and reduction of apoptosis and gliosis in response to high-fat diet.



Áreas Temáticas: 1ª: Sistemas homeostáticos y neuroendocrino. 2ª: Neurociencia de sistemas

ANATOMICAL ORGANIZATION OF MAGNOCELLULAR ACCESSORY NUCLEI ALONG HYPOTHALAMIC BLOOD VESSELS

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Whereas the biological significance of magnocellular (MGC) neurons in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) is well established, their role in the accessory nuclei (AN) of the anterior hypothalamus is less understood. The present study reveals that there is an actual arterial network interconnecting anatomically most of these AN with the SON and, at least some of them, with the PVN. Accessory MGC neurons form chains in the perivascular space and their projections course parallel and close to the vessels intersecting the AN. These projections include axonal bundles and long stretches of neurosecretory dendrites. In addition, our results show that perivascular astrocytes closely encircle accessory MGC neurons through their processes and express vascular endothelial growth factor A to which the MGC neurons are sensible. These astrocytes are also the source of various connexins (Cx), which in combination with Cx26 expressed by MGC neurons, might be important for the function of these neurons. In conclusion, we propose that the arterial scaffolding interconnecting different MGC nuclei might facilitate functional coordination between MGC nuclei in response to specific physiological situations. A possible transmission of nervous stimuli mediated by dendritic release of vasopressin is further discussed.

1ª: Sistemas homeostáticos y neuroendocrino
2ª: Neurociencia de sistemas

INTERLEUKIN-6 REGULATES BODY WEIGHT AND BODY FAT INFLUENCING CENTRAL HYPOTHALAMIC NEUROPEPTIDES

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Objective: In the absence of inflammation interleukin-6 (IL-6) is synthesized in muscle and adipose tissue, playing a role in body weight regulation, fat mass and metabolic functions. Nevertheless, the mechanisms underlying these effects are not completely understood because central and peripheral actions of IL-6 are plausible. The main objective of this study was to gain further insight into the central effects of IL-6 on energy homeostasis.

Material and Methods: We used different animal models: IL-6 deficient mice (total IL-6 knockout mice, and muscle and adipose-specific IL-6 knockout mice), as well as mice expressing the IL-6 gene under the control of the GFAP promoter (GFAP-IL-6 mice), therefore with central nervous system-restricted over-expression of IL-6. In these animals we evaluated body weight, food intake, different biochemical and hormonal parameters and the expression of the main hypothalamic neuropeptides involved in the control of energy homeostasis (NPY, AgRP, TRH, preproOx, POMC and CRH). We used animals of both sexes, different ages and different feeding conditions (fasting, high and low fat diet).

Results and Conclusion: We demonstrated that IL-6 has a role in energy homeostasis acting mainly at the central level by regulating the expression of NPY, AgRP, POMC and, CRH, although peripheral effects of IL-6 can not be discarded. The effect of IL-6 is dependent on sex and age and on the tissue where IL-6 is synthesized, reflecting the complicated regulatory functions of this protein.

Áreas Temáticas:
1ª: Sistemas homeostáticos y neuroendocrino
2ª: Neurociencia de sistemas

SENC

15º Congreso Nacional de la Sociedad Española de NeuroCiencia

Tema 7

Nuevos métodos y tecnologías

Posters

ASSOCIATIVE LEARNING BASED ON tACS-INDUCED TACTILE SENSATIONS SUCCESFULLY PREPARE THE RABBIT FOR NATURAL TACTILE STIMULUS

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The transcranial current stimulation (tCS) is a non-invasive brain stimulation technique that has been successfully applied in both basic and clinical researchs. Whereas transcranial direct-current stimulation (tDCS) is capable of inducing changes in neuronal membrane potentials in a polarity-dependent way, transcranial alternating-current stimulation (tACS) has been proposed to interact with ongoing cortical oscillations enhancing or diminishing specific frequencies of cortical activity. In this study, we investigate the direct effects of tACS on the activity of the somatosensory cortex (SS) and its implications in tactile perception phenomena. Rabbits were prepared for the chronic recording of local field potentials (LFPs) in the SS in response to whisker stimulation and for classical eyeblink conditioning during simultaneous tACS. In a first series of experiments, we determined whether slow tACS applied to the SS could modify the characteristics of LFPs evoked in the vibrissal SI area of behaving alert rabbits by air-puff stimulation of the contralateral whisker pad. The amplitude of the N1 component of the LFP was amplified by the simultaneous presence of an anodal tACS peak and reduced by a cathodal tACS trough. In a second series of experiments, we checked whether short (100 ms) high-frequency (10, 30 and 100 Hz) tACS pulses applied over the SI cortex were able of inducing a tactile sensation. Specifically, we checked whether sinusoidal wave current could substitute for the whisker conditioned stimulus (CS) during an associative learning task. tACS-CS induced conditioned responses (CR) similar to those observed when direct stimulation of the whisker pad was carried out. In addition, animals conditioned by using tACS stimulus as CS responded in a conditioned way when natural whisker stimuli were presented. In summary, we show that tACS applied to the SS is capable of modifying sensory potentials at low frequencies and, at higher ones, of inducing artificial tactile perception resembling natural ones.

1. Nuevos métodos y tecnologías
2. Neurociencia cognitiva y conductual

Acknowledgements
Supported by grants BFU2011-29089, P07-CVI-02487 to J.M.D.-G., and BFU2011-29286, P07-CVI-02686 to A.G., from Spain. In addition, this work was funded by the EU FP7 FET-Open HIVE (222079) project.

EFFECTS OF tDCS ON CLASSICAL EYEBLINK CONDITIONINGAND OPERANT CONDITIONING IN BEHAVING RABBITS

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Transcranial current stimulation (tCS) is a non-invasive brain stimulation technique that has been successfully applied in both basic and clinical research. Transcranial direct-current stimulation (tDCS) induces changes in neuronal membrane potentials in a polarity-dependent way. Nevertheless, little is known about tCS effects on cortical circuits. In a previous work, we demonstrated that pairs of pulses applied to the thalamic VPM nucleus during tDCS modify thalamocortical synapses at presynaptic sites. In addition, blocking the activation of adenosine A1 receptors prevented the long-term depression evoked in the somatosensory cortex following cathodal tDCS. Here, we studied the effects of tDCS on two different learning paradigms. A first set of rabbits were prepared for classical eyeblink conditioning using a tone as conditioned stimulus and an airpuff as unconditioned stimulus. tDCS was presented to the prefrontal cortex on conditioning days 2 and 8. The acquisition of this classical eyeblink conditioning was potentiated or depressed by the application of cathodal or anodal tDCS, respectively, on conditioning day 2. A second set of animals were prepared for operant conditioning in a rabbit Skinner box, using a fixed (5:1) ratio paradigm. Animals were presented with tDCS applied over the prefrontal cortex on conditioning days 2 and 8. Here, the performance of the conditioned lever responses was also modulated by the application of cathodal or anodal tDCS, either on conditioning day 2 or 8. In conclusion, results reported here confirm earlier studies in humans regarding the effects of tDCS cerebral cortex activities, highlighting the potential of this technique for modulating associative learning using either classical or operant conditioning paradigms.

1. Nuevos métodos y tecnologías
2. Neurociencia cognitiva y conductual

Acknowledgements
Supported by grants BFU2011-29089, P07-CVI-02487 to J.M.D.-G., and BFU2011-29286, P07-CVI-02686 to A.G., from Spain. In addition, this work was funded by the EU FP7 FET-Open HIVE (222079) project.

EFFECTS OF FOCAL STATIC MAGNETIC FIELDS ON THE HUMAN CORTEX

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The non-invasive modulation of motor cortex excitability by the application of static magnetic fields through the scalp was investgated in healthy humans. Static magnetic fields were obtained by using cylindrical NdFeB magnets. (tSMS) in conscious subjects. We observed an average reduction of motor cortex excitability of up to 25%, as revealed by TMS, which lasted for several minutes after the end of 10 minutes of static magnetic field stimulation (tSMS). The effect of tSMS was dose-dependent (intensity of the magnetic field) and duration dependent, but not polarity-dependent. We used transcranial electric stimulation (TES) to establish that the tSMS-induced reduction of motor cortex excitability was not due to corticospinal axon and/or spinal excitability, but specifically involved intracortical networks.

We further explored the tSMS effects on EEG oscillations in the visual cortex and during visual attentional performance in healthy humans. We specifically examined the hypothesis that these effects could be related to an increase of alpha band activity, and therefore, associated to an "inhibitory" effect. During real but not sham tSMS over the visual cortex, there was a significant increase of the alpha band power. Moreover, we observed a similar reaction time (RTs) pattern during real and sham tSMS for most of trials. However, a significant slowing of RTs emerged across those trials with a higher difficulty levels during real in comparison to sham tSMS.

Further studies using tSMS are required to extend the knowledge of the functional significance of cortical excitability changes and brain oscillations changes induced by the application of small magnets over the scalp. These results suggest that tSMS using small static magnets may be a promising tool to modulate cerebral excitability in a non-invasive, painless, and reversible way.

A LONGITUDINAL MICROSCOPE-BASED METHODOLOGY TO ASSESS THE EFFECT OF ALPHA-SYNUCLEIN PATHOLOGICAL MUTATIONS AND POST-TRANSLATIONAL MODIFICATIONS ON STABILITY AND SURVIVAL ON NEURONS *IN SITU*

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Abnormal accumulation of alpha-synuclein (α-syn) into Lewy Bodies is a key neuropathological feature of a heterogeneous group of disorders (synucleinopathies) including Parkinson´s disease (PD) and dementia with Lewy Bodies (DLB) among others. Although 90% of PD cases are sporadic, mutations on the gene encoding α-syn (SNCA) cause autosomal dominant PD. Indeed, an abnormal increase in α-syn levels is sufficient to cause Parkinsonism with prominent dementia, as observed in familial cases with genomic duplications and triplications of SNCA gene. Therefore, α-syn levels constitute a predictive factor of neuronal death. Yet, the mechanisms that determine α-syn steady-state levels on sporadic and familial PD cases with point mutations on the SNCA coding region remain unclear. It has been hypothesized that point mutations and/or posttranslational modifications on α-syn alter the stability of the protein leading to an increase of its steady state levels and eventually to neuronal death. To test this hypothesis we used a microscope-based methodology to longitudinally track individual neurons and determine the risk of neuronal death induced by wild-type (wt) and mutant versions of α-syn. In order to determine the stability of wt and mutant versions of α-syn in living neurons we applied a novel optical pulse-chase methodology based on the photoswitchable protein Dendra2 and longitudinal analysis to measure its half-life on neurons *in situ*. Among the pathological α-syn mutations, the E46K mutation increases significantly the risk of neuronal death on primary cortical neurons. Post-translational modifications modulate α-syn dependent neuronal death risk. We are currently analyzing whether these effects are associated with a change on the stability of the protein.

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AREAS TEMATICAS

1. Nuevos métodos y tecnologías
2. Transtornos y reparación del sistema nervioso

SHORT AND LONG-TERM EFFECTS IN CORTICOMOTOR RESPONSES AFTER A VIRTUAL REALITY THERAPY IN PARKINSON DISEASE: A DOUBLE-BLIND RANDOMIZED CONTROLLED PILOT STUDY

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Abstract: Parkinson´s Disease (PD) is characterized by the progression of motor and non-motor dysfunctions. Clinically, hypometry is a key sign in PD; neurophysiologically, there are large alterations in the control of inhibitory intracortical circuits. Amongst the new neurorehabilitation strategies in motor disorders, movement imitation seems to be a promising option. Virtual Reality (VR) facilitates imitation approaches allowing visual feedback to be customized. Here, we present a double-blind randomized controlled pilot study on the potential effect of imitation therapy in Virtual Reality environment to treat PD motor deficits.

Objective: To evaluate an imitation therapy into a Virtual Reality System on motor function in Parkinson´s Disease patients.

Methods: 16 PD participated in this study. Participants were randomly assigned in two groups: 8 subjects were included in the Experimental Group and 8 into the active placebo-controlled group. Both groups underwent 4-weeks of VR training of finger-tapping with the dominant hand (three days per week, 30-45 minutes/session). While experimental group imitated large-amplitude -avatar´s movements, the control group observed their own movements displayed on real-time, as a 1st person´s VR-avatar.

Movement features and functioning of corticoespinal networks were evaluated before, and immediately after the VR training; then again after two-weeks of washing-off. Transcranial Magnetic Stimulation was used to evaluate cortical silent periods and input-output curves on both hemispheres. Finger-tapping execution was evaluated by the study of movement amplitude (goniometer) and cycle duration (event detector) of the trained and the untrained hand.

Results: Movement amplitude significantly increased after therapy. Importantly, this was not different for the trained and un-trained hand. This effect was only observed in the Experimental Group. In this group, the intracortical inhibitory control was increased towards physiological conditions after therapy, returning to baseline levels after two weeks of follow-up with no training.

Conclusion: Virtual Reality Therapy based on imitation might be useful to improve motor execution in PD.

Áreas Temáticas:
1ª Nuevos métodos y tecnologías
2ª Trastornos y reparación del sistema nervioso

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ELECTROPORACIÓN LOCALIZADA CON RNA VIRAL DE NEURONAS INDIVIDUALES EN EL CEREBRO DEL RATÓN ADULTO

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Las técnicas de marcado individual del axón de neuronas de proyección mediante vectores virales están permitiendo, por vez primera, analizar cuantitativamente y con resolución celular los circuitos cerebrales. El procedimiento más potente publicado hasta la fecha es la transfección mediante el vector viral SINDBIS-pal-GFP. Sin embargo, dicha técnica presenta importantes limitaciones intrínsecas, dada su escasa precisión espacial y en cuanto al tipo celular transfectado. Las técnicas de electroporación en células individuales podrían en principio solventar estos problemas. Sin embargo, dichas técnicas se habían aplicado con éxito hasta ahora sólo en cerebros en desarrollo o en estudios "in vitro", mientras que su aplicación in vivo en adulto resulta muy compleja y tiene baja eficacia.

En el presente trabajo hemos examinado la viabilidad de electroporar RNA viral SINDBIS-pal-GFP "in vivo" en el cerebro de ratones adultos utilizando para ello diversos protocolos de pulsos eléctricos y de soluciones hiperosmolares, aplicados bien aisladamente o en combinación. Nuestros datos muestran que el mejor rendimiento (90%) se obtiene mediante un protocolo que combina ambas técnicas. Dicho protocolo permite una transfección con alto rendimiento y espacialmente precisa de neuronas o micropoblaciones neuronales adultas. Además, el método es rápido y relativamente sencillo en cuanto a la instrumentación necesaria.

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1ª: Nuevos métodos y tecnologías
2ª: Neurociencia de sistemas

NEURITE-J: AN IMAGE-J PLUGIN FOR AXONAL GROWTH ANALISYS IN ORGANOTYPIC CULTURES

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Introduction: Organotypic cultures are multicellular *in vitro* models that preserve both cytoarchitecture and cell interactions that form the tissue, providing a closer approximation of *in vivo* processes in comparison with dissociated cell cultures. Previous studies in our lab proposed a method of dorsal root ganglia (DRG) and spinal cord slice (SC) organotypic 3D cultures to study motor and sensory axonal regeneration (Allodi et al., 2011). Although these models are useful to test how some molecules can affect axonal growth, sample analysis could be tiresome and high time-consuming.

Objectives: To design and set-up a numerical method and algorithm to quantify axonal growth in 3D organotypic cultures of DRG and SC

Material and methods: DRG and SC were cultured in a 3D collagen matrix. Explants were cultured under culture medium (control condition) and culture medium plus neurotrophins (NGF and GDNF for DRG, FGF and GDNF for SC). Neurites were labeled by immunohistochemistry against RT-97 and pictures were obtained using an epifluorescence microscope. To quantify axonal growth we adapted the Sholl method of concentric rings for dendrite arbor analysis to our cultures and the algorithm was implemented as an ImageJ plugin.

Results: Neurite-J plugin gives a good description of neurite growth providing with counts of neurite number and neurite area at different distances from the explant. Moreover, this plugin follows semi-logarithmic analysis of the concentric Sholl method, bringing a numerical value of the neurite number decrease ratio. The most adjusted less time-consuming analysis was obtained with measures taken every 25μm from the slice.

Conclusion: Neurite-J plugin provides a semi-automated quantification method of neurite arbours in 3D organotypic cultures that gives the researcher an easy, fast and reliable tool to study axonal growth.

Áreas Temáticas: Nuevos métodos y tecnologías, Trastornos y reparación del sistema nervioso

CALCIUM IMAGING OF SINGLE CELLS IN DEEP BRAIN REGIONS OF FREE-MOVING MICE

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Fiber optic-based confocal fluorescent microscopy allows to visualize individual cells in deep brain regions of live animals with high spatial (3µm) and temporal (5 ms) resolution, filling the gap between traditional neuroimaging techniques and 2-photon microscopy. The Cellvizio® system uses a miniature fiber-optic probe to transport the excitation (488 nm) and emission light. Recent effort in protein engineering has improved the sensitivity of genetically encoded calcium indicators (GECIs), being now comparable to calcium sensitive dyes. We employed viral vectors carrying GECIs to visualize and record individual cells in deep brain regions of free-moving mice (hippocampus, striatum and olfactory bulb). After stereotactic injection, the images were acquired and analyzed with the Cellvizio® microscope to perform *in vivo* calcium imaging. In conclusion, the combination of viral vector technology with this new *in vivo* optical imaging technique allows us to record the activity of individual cells in the brain of free-moving animals.

Áreas Temáticas:
1ª: Nuevos métodos y tecnologías
2ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

ALTERED MITOCHONDRIAL DYNAMICS AND MITOPHAGY ARE FOUND IN SPORADIC ALZHEIMER DISEASE FIBROBLASTS

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Increasing evidence is showing that neurodegenerative disorders, including Alzheimer disease (AD), are not merely neurological illnesses and cells from other tissues such as fibroblasts exhibit altered properties correlating with the brain pathology. We have characterized fibroblasts from sporadic AD (sAD) patients in comparison with age-matched non demented controls. Higher oxidized and polyubiquitinated protein levels were found in sAD. Mitochondrial dynamics was also altered showing delayed recovery of their filamentous morphology and membrane potential after an insult, correlating with diminished levels of the fission protein DLP1. This was accompanied by a defect in mitochondrial recycling suggested by the increased levels of the mitochondrial constitutive protein TOM20 as well as the accumulation of autophagic vacuoles and diminished autophagy flow. In addition, sAD fibroblasts exhibited lysosomal alterations such as smaller amount of lysosomes with more acidic pH associated with diminished cathepsin B protein levels but showing significantly increased activity. Additionally, levels of Parkin, an ubiquitin ligase involved in mitophagy, were decreased in both total and mitochondrial fractions in sAD cells. This was linked with a deregulation of PINK1 levels. All these results suggest that a defect in mitophagy might take place. We finally studied the overexpression of Parkin with lentivectors as a therapeutic strategy to rescue mitochondrial impairment. We could achieve a significant improvement of the membrane potential recovery and a decrease of the ubiquitination levels in sAD samples. Parkin overexpression also restored the induction of autophagic vacuoles and increased the autophagy flux. In addition, Parkin transduction diminished the differences in the amount of mitochondrial Parkin, PINK1 levels and TOM20 accumulation in sAD fibroblasts. Hence, the impairment of mitophagy was able to be restored by Parkin overexpression. Our results demonstrate that some of the alterations described for AD brains and familiar AD animal models can be found in fibroblast from sporadic patients.

1ª: Nuevos métodos y tecnologías
2ª: Trastornos y reparación del sistema nervioso

NUCLEUSJ: DESARROLLO DE UN PLUGIN EN FIJI PARA EL ANÁLISIS DE MODELOS DE MUERTE NEURONAL

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La técnica de High Content Screening (HCS) consiste en la adquisición de imágenes mediante microscopía digital automatizada, combinada con software de análisis masivo de datos. Esta técnica permite adquirir información espacial o temporal de una muestra y su cuantificación automática. La complejidad de la estructura neuronal, y la alta densidad de neuronas en el sistema nervioso, han impedido la utilización del HCS para el análisis morfológico y funcional del sistema nervioso.

Nuestro objetivo es el desarrollo de software que basado en algoritmos topogeométricos permita la identificación de la estructura neuronal y el análisis automatizado de imágenes de neurofisiología. En este trabajo presentamos un plugin para Fiji/ImageJ para la cuantificación de neuronas de hipocampo de rata, marcadas con: MAP2B (neuronal) y DAPI (nuclear). El problema biológico abordado es la muerte neuronal por excitoxidad. Es éste un proceso patológico que daña las neuronas por una actividad descontrolada de los receptores de glutamato. La activación de la vía de PI3K controla la expresión de genes antiapotóticos y promueve la supervivencia neuronal. En el proyecto hemos empleado cultivos de neuronas de hipocampo tratados con NMDA y con diversas concentraciones de un péptido activador de PI3K(PTD4-PI3K-Ac).

Las imágenes HCS se han obtenido con el módulo MatrixScreening usando un microscopio Confocal TCS-SP5 (Leica Microsystems). Las imágenes mosaico cubren un área promedio de 4x2 mm. El plugin es semiautomático e identifica los núcleos de neuronas vivas, y el número de células totales basándose en diferentes criterios encadenados, que parten de la umbralización de la imagen y de la comparación entre las estructuras entre canales. Al final del proceso, el programa permite la corrección de datos por parte del usuario.

Los resultados muestran un efecto concentración dependiente de la muerte neuronal por NMDA (50%, 10μM), en estas condiciones, el PTD4-PI3K-Ac (21μM) rescata un 20% de la población neuronal.

Áreas Temáticas:
1ª: Nuevos métodos y tecnologías
2ª: Trastornos y reparación del sistema nervioso

IMAGING OF Ca^{2+}-DYNAMICS IN THE ENDOPLASMIC RETICULUM OF NEURAL TISSUES MONITORED WITH A NEW FLUORESCENT Ca^{2+} SENSOR

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Genetically encoded calcium sensors are essential tools to monitor localized Ca^{2+} signals in organelles. There are a number of Ca^{2+} sensors available that have been used and provided information on Ca^{2+} signalling. However, their application in transgenic mammals has proven to be problematic. We have recently developed a new family of fluorescent Ca^{2+} indicators, dubbed GAP, for GFP Aequorin Protein based on the fusion of the two *Aequora victoria* proteins. This new Ca^{2+} sensor has been optimized to be targeted to different organelles, including the endoplasmic reticulum (ER). Here we report the application of GAP to visualize Ca^{2+} dynamics in the ER of different neural tissues such as dorsal root ganglia (DRG), hippocampus or spinal cord motor neurons. First, we produced herpes virus amplicons carrying erGAP and infected primary cultures of rat DRG neurons. Transduced neurons showed correct erGAP localization to the ER with high levels of expression. Application of caffeine resulted in a dose-response decrease in the $[Ca^{2+}]_{ER}$. Using Sr^{2+} as a Ca^{2+} surrogate, we showed that depolarizing high K^+ pulses provoked Sr^{2+} release from the ER, indicating the presence of a calcium-induced calcium release (CICR) mechanism in DRG neurons.

We next generated transgenic mice expressing erGAP under a ubiquitous promoter. We found wide orthogonal expression in a variety of neural tissues, mostly in hippocampus, cerebral cortex, cerebellum and spinal cord. We measured robust and reproducible $[Ca^{2+}]_{ER}$ changes after caffeine application or by inhibition of the sarco-endoplasmic Ca^{2+}-ATPase (SERCA) in cultured neurons dissociated from transgenic tissues. Moreover, the new erGAP enabled visualization of ER Ca^{2+} changes in transgenic acute organotypic hippocampal slices, with a high effective dynamic range. To our knowledge, this is the first report of Ca^{2+} dynamics in the lumen of the ER in intact neural tissues.

GENERATION OF INDUCED PLURIPOTENT STEM CELLS (IPS) USING NON VIRAL POLYCISTRONIC VECTORS

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Somatic cells can be reprogrammed to induced pluripotent stem (iPS) cells by ectopic expression of specific sets of transcription factors. The delivery of these transcription factors has mostly entailed the use of integrating viral vectors (retroviruses or lentiviruses), carrying the risk of both insertional mutagenesis and oncogenesis due to misexpression of these exogenous factors.
The main goal of this work was the generation of iPS cells from different sources of somatic cells using non viral vectors, and the characterization of the generated iPS cells. For that we have used polycistronic vectors expressing three or four transcription factors (Oct4, Sox2, Klf4 with/without c-myc). The vectors were introduced by nucleofection. In order to increase reprogramming efficiency, several enhancer factors have also been tested.
We have successfully generated and established iPS cells from mouse embryonic fibroblasts (MEFs) and human fibroblasts. These cells have been characterized morphologically, by the expression of endogenous pluripotency transcription factors, and the potential to differentiate into specialized cells from the three germ layers, including dopaminergic neurons. Systematic comparison with Embryonic Stem Cells (ES cells) and already established viral iPS cells was also performed.
Our results demonstrate that although the reprogramming efficiency is extremely low, it is possible to generate “bona fide” iPS cells using non-viral and non-integrative vectors. This opens up a whole range of biomedical applications such as cell therapy, drug development and disease modeling.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Nuevos métodos y tecnologías

NEURAL GROWTH AND FUNCTIONAL DEVELOPMENT ON NEW BIOCOMPATIBLE HYBRID MATERIALS

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Objectives: The application of electrical current to injured tissue is considered a potential treatment to promote healing in spinal cord injury and other neurodegenerative diseases. Electric field therapy requires electrodes that are inert towards the biological environment. We have developed new biocompatible materials, based on Iridium oxide (IrOx) and the electroactive polymers PPy and PEDOT, that will allow the development and proper physiology of neural cells.

Materials and Methods: Synthesis by electrodeposition of new hybrid material films based on: i) Polymers dopped with proven biocompatible counterions such as amino acids and
ii) Combination of different materials like IrOx, PEDOT and Carbon nanotubes (CNT) to improve independent components.

Primary cortical neurons were cultured on the surface of the newly generated electroactive materials to assess their biocompatibility. Viability and morphology of neurons were analyzed by immunostaining and cellular count. Functional parameters such as the ability to release glutamate and GABA were determined by HPLC to analyze the levels of neurotransmitters after a depolarizing pulse. The expression of relevant receptors for these neurotransmitters was assessed by immunocytochemistry.

Results: We have generated new amino acid-doped polymers that highly enhance neuronal viability. The presence of lysine or glutamine in PEDOT-PPY bilayers enable the correct growth and development of cortical neurons in comparison to other common non permissive polymer. Neurons cultured on top of the new IrOx-CNT and IrOx-PEDOT-CNT hybrids remain viable after several days in vitro. These IrOx-based hybrids didn't affect the expression of NMDA and GABA A receptor neither the release of glutamate and GABA in response to depolarization in comparison with the common borosilicate glass substrate.

Conclusions: We present new electroactive-enhanced materials with high biocompatible features toward neuronal cells that could be used as safer electrodes for electric-field stimulation therapy.

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Áreas Temáticas:
1ª: 7 (Nuevos métodos y tecnologías)
2ª: 5 (Trastornos y reparación del sistema nervioso)

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