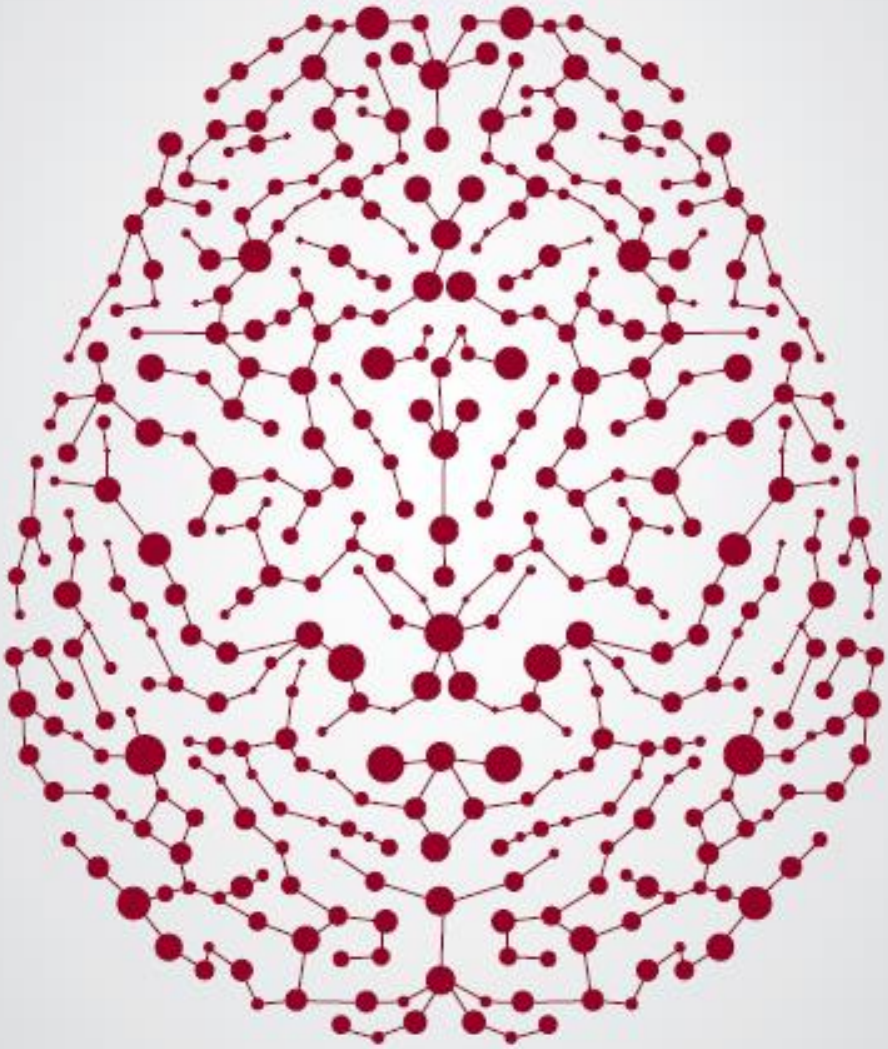
SENC
sociedad española de neurociencia

16 National Congress of the Spanish Society of Neuroscience

GRANADA | SPAIN 23 - 25 September 2015



Communications

Abstracts Book

16th National Congress of the Spanish Society of Neuroscience
Granada | Spain, 23 – 25 September 2015
ABSTRACTS BOOK
Vol 2 – Communication

Communications

Topics

Topic 1.

Developmental Neurobiology

DOWNREGULATION OF INSULIN RECEPTOR SUBSTRATE TYPE 1 IMPAIRS DENDRITIC ARBORIZATION OF HIPPOCAMPAL NEURONS IN CULTURE

M. Larrosa-Flor, N. Gomes, A.M. Sánchez-Pérez
Facultad de Medicina, Universitat Jaume I, Castellón de la Plana, Castellón, SPAIN.

Neurite outgrowth and arborization takes place during neuronal development, it declines with age and in certain neurodegenerative disorders. Dendrite and axon branching is influenced by a number of factors, both intrinsic or genetic and environmental or external factors. In particular, Insulin and insulin like growth factor type I (IGF-1) signaling had been shown to have a prominent role in these processes. Insulin/IGF1 signaling starts by the activation of the intracellular insulin receptor substrates (IRS). IRSs are engaged in directing Insulin and IGF actions. This work aims to provide further understanding in the IGF1 signaling pathway implicated in modulating dendritic patterning of cultured hippocampal neurons. To that end, we specifically knocked down IRS1 protein expression by shRNA transfection in hippocampal cultured neurons. We found that when knocking down IRS1 expression in our conditions there is a significant reduction in dendritic complexity as measured by Sholl analysis. Moreover, neurite length is reduced in neurons transfected with shRNA IRS1. Interestingly, we also observed that in rat brain the insulin receptor substrate type 1 (IRS1) displays an age-dependant expression profile being in old individuals near a 25% of the expression observed at embryonic ages. Taken together, our results lead us to propose a role for IRS1 as an important player in hippocampal neuron development. Additional studies are needed in order to determine the role of other IRS isoforms in dendritic arborization, such as IRS2, which is highly expressed in brain. Thus, our immediate goal is to carry out a comparative analysis of these two isoforms.

HUWE1-DEPENDENT DEGRADATION OF ASCL1 WORKS AS A BRAKE TO ADULT HIPPOCAMPAL STEM CELLS PROLIFERATION

Noelia Urbán, Debbie Van Den Berg, Jimena Andersen, François Guillemot
Division of Molecular Neurobiology, The Francis Crick Institute – Mill Hill Laboratory, The Ridgeway, NW71AA, London

Adult neurogenesis is regulated by a large number of extrinsic and intrinsic factors. The transcription factor Ascl1 is expressed by a subset of proliferating stem cells and early intermediate progenitors in the adult hippocampus. We have shown that Ascl1 is essential for stem cells to exit quiescence, divide and generate intermediate precursors during adult neurogenesis. We have also noted that in Ascl1 hypomorph mice, hippocampal stem cells proliferate at a lower rate, suggesting that the level of Ascl1 expression is an important parameter in the regulation of adult neurogenesis.
We have now identified the E3 ubiquitin ligase Huwe1 as a post-translational regulator of Ascl1 expression in neural stem cells. Huwe1 binds to and promotes the proteasomal degradation of Ascl1 protein in cultured neural stem cells. In the adult hippocampus, loss of Huwe1 function in stem cells increases the number of Ascl1-positive cells by more than 5-fold. Maintenance of Ascl1 protein in intermediate progenitor cells locks them in a proliferative state and blocks their ability to differentiate into neurons. Interestingly, loss of Huwe1 and maintenance of Ascl1 in activated stem cells prevents their return to a quiescent state, resulting eventually in the exhaustion of the active stem cell pool.
In conclusion, we have identified proteasomal degradation of Ascl1 by Huwe1 as an important and fine-tuned level of regulation of stem cell activity and neurogenesis in the adult brain.

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

THE PURKINJE CELL DEGENERATION MUTANT MOUSE AS A MODEL FOR NEURODEVELOPMENTAL DISORDER

R. Muñoz-Castañeda [1, 2], D. Díaz [1,2], J.M. Muñoz-Castañeda [4], C. Ávila [2,3], C. del Pilar [1], J.R. Alonso [1,2,5], E. Weruaga [1,2]

1. INCyL, Institute for Neuroscience of Castilla y León, Universidad de Salamanca, Salamanca, Spain
2. IBSAL, Institute of Biomedical Research of Salamanca, Salamanca, Spain.
3. Applied Statistics Group, Department of Statistics, Universidad de Salamanca, Salamanca, Spain.
4. ICMA, Materials Science Institute of Aragón. CSIC-Universidad de Zaragoza, Zaragoza, Spain.
5. Instituto de Alta Investigación. Universidad de Tarapacá, Arica, Chile.

Previous works have established a link between microtubules and neurodevelopmental disorders (NDs). Different researchers have proposed that NDs may have an important neurodegenerative component. In this sense, one of the most commonly reported finding in autism spectrum disorders is the reduction of the Purkinje cells (PCs) density. Thus, the PCD (*Purkinje Cell Degeneration*) mutant mouse is a good model to answer these questions. PCD mouse suffer of a degeneration of the PCs from P18 to P30 with no affectation of other structures at this time window. It is caused by the mutation of the CCP1 enzyme, a microtubule depolyglutamylase. To address these questions we performed different behavioural tests to analyse the effect of PCs alterations at different stages in memory, social preference and general behaviour. The morphological development of PCs was also analysed. Because of the implications of the endocannabinoid system in both development and degeneration, were also analysed the expression of the CB1, CB2 and PPARα receptors. Finally, to understand the effect of polyglutamylation, microtubules dynamics and curvature in culture fibroblasts were studied.

Results showed important alterations in PCs morphology beginning in the main dendrite, which precede their death. Concerning microtubules dynamics, alterations only on microtubules growth rate and catastrophe frequencies were demonstrated. Curvature was increased in PCD microtubules. Regarding the endocannabinoid receptors, PPARα receptors were increased from P15 onwards and CB2 from P22 to P30. Surprisingly, CB1 receptors were not affected by the *pcd* mutation. Morphological alterations seem to be sufficient to affect social behaviour from P15 onwards. However, object memory recognition was affected later, at P30. These, results demonstrate that polyglutamylation alters microtubules structure but its influence in the dynamics of the cytoskeleton is not remarkable. Besides, our results support the implication of the cerebellum in cognition and the relationship between the endocannabinoid system and neuronal death.

Full name: Rodrigo Muñoz Castañeda
E-mail: rodrigomc@usal.es; ewp@usal.es
Áreas temáticas
1. Neurobiología del Desarrollo
4. Neurociencia cognitiva y conductual

ALTERED METHYLATION PATTERN OF SPECIFIC IMPRINTING CONTROL REGIONS IN MOUSE TETRAPLOID CORTICAL NEURONS

I. Patiño-Parrado, A. Gómez-Jiménez, J.M. Frade
Instituto Cajal (CSIC), Madrid

Imprinted genes are expressed only from one of the two parental chromosomes. Most of them are clustered in specific genomic domains and co-regulated by cis-acting imprinting control elements that contain germline-derived differentially methylated regions (DMRs). These DMRs, required for maintaining monoallelic expression of the corresponding imprinted genes, are thought not to be altered in somatic tissues after fertilization. To explore the impact of neuronal tetraploidization on the methylation status of specific DMRs we have focused on the *Snrpn* and *Peg3* imprinted domains, all of them containing genes known to be active in neurons. To this aim we used FACS-isolated diploid and tetraploid neuronal nuclei obtained from mouse cerebral cortex. DNA was extracted from the isolated nuclei and then subjected to bisulfite modification followed by both Sanger sequencing and pyrosequencing. Here we show that, in cortical tetraploid neurons, the DMR of the *Peg3* imprinted domain becomes demethylated already at postnatal stages of development, thus suggesting that this genomic domain modifies its imprinted status in neurons containing four chromosomal copies. This effect is specific for this imprinted domain as the methylation pattern of the *Snrpn* DMR is not altered in cortical tetraploid neurons. Our results challenge the current view that imprinting-associated differential methylation cannot be altered in adult, non-transformed cells. We are currently studying the molecular mechanism regulating these alterations.

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

ONTOGENESIS OF PEPTIDERGIC NEURONS WITHIN THE GENOARCHITECTONIC MAP OF THE MOUSE HYPOTHALAMUS

N. Morales-Delgado[1], L. Puelles[1], C. Díaz[2].
1. School of Medicine. University of Murcia, Murcia, E30071, Spain.
2. School of Medicine, University of Castilla-La Mancha, Albacete, E02006, Spain.

Introduction: During early development, the hypothalamic primordium undergoes anteroposterior and dorsoventral regionalization into diverse progenitor domains, each characterized by a differential gene expression code. The types of neurons produced selectively in each of these distinct progenitor domains are still poorly understood. Recent analysis of the ontogeny of peptidergic neuronal populations expressing *Sst*, *Ghrh*, *Crh* and *Trh* mRNAs in the mouse hypothalamus showed that these cell types originate from particular dorsoventral domains, characterized by specific combinations of gene markers. Such analysis implies that the differentiation of diverse peptidergic cell populations depends on the molecular environment where they are born. Moreover, a number of these peptidergic neurons were observed to migrate radially and/or tangentially, invading different adult locations, often intermingled with other cell types. This suggests that a developmental approach is absolutely necessary for the understanding of their adult distribution.
Objectives and methodology: We examined the ontogenetic hypothalamic topography of twelve additional peptidergic populations documented in the *Allen Developmental Mouse Brain Atlas*, and compared shared *vs.* variant aspects in their apparent origins, migrations and final distribution, in the context of the respective genoarchitectonic backgrounds.
Results and conclusions: In summary, we observed that 1) peptidergic neurons (PN) originate independently in many areas; 2) the same PN type can originate in two or more separate areas; 3) Not all progenitor areas generate PN; 4) Most PN-producing areas generate several types sequentially; 5) One single progenitor area can generate simultaneously 2 or 3 types of PN; 6) No PN migrate from basal to alar; and 7) The sequential topography of most PN can be parsimoniously explained by the hypothesis of tangential migration. Our analysis should aid ulterior attempts to explain causally the development of neuronal diversity in the hypothalamus, and contribute to our understanding of its topographic complexity in the adult.

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

MIGRATION OF TELENCEPHALIC SOMATOSTATIN NEURONS ORIGINATED FROM THE MOUSE DIAGONAL AREA.

N. Morales-Delgado[1], P. Merchán[1], B. Castro-Robles[2], M. Martínez-de-la-Torre[1], C. Díaz[3], J. L. Ferran[1], Luis Puelles[1].
1. School of Medicine. University of Murcia, Murcia, E30071, Spain.
2. Institute of Biomedical Research of Lleida, University of Lleida, Lleida, Spain
3. School of Medicine, University of Castilla-La Mancha, Albacete, E02006, Spain.

Introduction: the telencephalic subpallium is the source of various GABAergic interneuron cohorts that invade the pallium via tangential migration. Based on genoarchitectonic studies, the subpallium has been subdivided into four major domains: striatum, pallidum, diagonal area and preoptic area (Puelles et al., 2013; Allen Developing Mouse Brain Atlas) and a larger set of molecularly distinct progenitor areas (Flames et al., 2007). In utero fate mapping and genetic lineage tracing studies have suggested that each subpallial subdivision produces specific sorts of inhibitory interneurons, distinguished by differential peptidic content, which are distributed tangentially to pallial and subpallial target territories (e.g., olfactory bulb, isocortex, hippocampus, pallial and subpallial amygdala, striatum, pallidum, septum). There has been so far no consensus about the origin of somatostatin-containing GABAergic neurons.
Material and methods: we mapped descriptively, by using in situ hybridization, the early differentiation and apparent migratory dispersion of mouse subpallial somatostatin-expressing (*Sst*) cells from E10.5 onwards, comparing their topography with the expression patterns of the genes *Dlx5*, *Gbx2*, *Lhx7-8*, *Nkx2.1*, *Nkx5.1* and *Shh*, which variously label parts of the subpallium.
Results and conclusions: whereas some previous experimental data suggest that *Sst* cells are pallidal, our data reveal that many, if not most, telencephalic *Sst* cells derive from de diagonal area (Dg). *Sst*-positive cells initially are only present at the embryonic Dg; later they selectively populate radially the medial part of the bed nucleus striae terminalis (from paraseptal to amygdaloid regions) and part of the central amygdala; they also invade tangentially the striatum, while eschewing the globus pallidum and the preoptic area, and integrate within most cortical and nuclear pallial areas between E10.5 and E16.5.

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

MULTIPLE GENE EXPRESSION PATTERN ANALYSIS OF THE PALLIAL DEVELOPMENT IN XENOPUS LAEVIS

A. González, S. Bandín, R. Morona, , J.M. López, N. Moreno.
Departamento de Biología Celular, Facultad de Biología, Universidad Complutense, Madrid, España.

The pallium in all vertebrates comprises the dorsal telencephalic division and is a very sophisticated and elaborated structure that shows large evolutionary differences, given its behavioral implications. Four pallial regions are recognized in all tetrapod vertebrates. In addition, in the amnote palliun cortical hem and anti-hem regions have been described as secondary organizer centers during development. In particular, the neuroepithelium that gives rise to the cerebral cortex is flanked by the hem medially and the anti-hem laterally. The aim of the present study focused on the development of the pallium in *Xenopus laevis*, selected as a reference model of amphibians, i.e. a crucial vetebrate group in phylogeny being the only anamniote tetrapods with special significance in the transition from water to land. We selected a set of important markers of the pallial territories described in other vertebrates to recognize the pallial subdivisions and boundaries, primarily on the basis of distinct gene expression patterns. In the case of the characterization of a cortical hem, we found in the medial territory of the *Xenopus* telencephalic hemispheres Wnt3a expression in a comparable region to the hem in amniotes, adjacent to the medial pallium discernible by Lhx2 expression. A possible anti-hem organizer was previously suggested in anurans in the region described as ventral pallium on the basis of its Lhx9 and Tbr1 expressions and the lack of Emx1, although typical anti-hem markers such as Dbx1 and Sfpr1 were not detected in this expected region. However, numerous reelin expressing cells occupied this region, like in mammals. Finally markers of the mammalian neocortical layers, also found in the pallium of other amniotes, such as Mef2C, FoxP1, ER81 and Fezf2 have been detected and analyzed in the pallium of *Xenopus*.

SEX DIFFERENCES IN THE NEURITOGENIC PROCESS OF PRIMARY MOUSE HIPPOCAMPAL NEURONS: INFLUENCE OF ENDOGENOUS AND EXOGENOUS ESTRADIOL

Isabel Ruiz-Palmero; Luis Miguel Garcia-Segura; Maria-Ángeles Arévalo Arévalo
Instituto Cajal, C.S.I.C., Madrid, Spain.

Estradiol is both an ovarian hormone and a neuroactive steroid synthesized in the brain. Previous studies have shown that estradiol promotes neuritogenesis in developing primary hippocampal neurons by a mechanism involving the upregulation of neurogenin 3 (Ngn3), a Notch-regulated transcription factor. These studies were conducted on neuronal cultures obtained from unsexed mouse embryos. In this study our aim was to determine whether the neuritogenic process and its regulation by estradiol are different in male and female neurons. Primary hippocampal neurons were obtained separately from male and female mouse embryos. Neurons from female embryos showed higher Ngn3 mRNA levels, higher axonal length, higher number of primary dendrites and higher values of dendritic arborization, as assessed by Sholl analysis, compared to male neurons. Estradiol (10^{-8} M) increased Ngn3 mRNA levels, the axonal length, the number of primary dendrites and the dendritic arborization in male neurons, but not in female neurons. To determine the possible influence of endogenous estradiol on neuritogenesis, female cultures were treated with letrozole, an inhibitor of the enzyme that synthesizes estradiol. Letrozole decreased the expression of Ngn3, the axonal length, the number of primary dendrites and the dendritic arborization of female neurons. Under these conditions exogenous estradiol increased Ngn3 mRNA levels over control values and restored the morphological parameters mentioned above to control levels in female neurons. These findings reveal sex differences in the neuritogenic process of hippocampal neurons and indicate that this process is under the regulation of endogenous and exogenous estradiol in female and male neurons, respectively.

1. Neurobiología del Desarrollo
2. Sistemas homeostáticos y neuroendocrino

e-mail: isabel.ruiz.palmero@gmail.com

PALLIAL NEUROGENESIS THROUGHOUT TELENCEPHALIC DEVELOPMENT IN XENOPUS LAEVIS

N. Moreno, S. Bandín, J.M. López, R. Morona, A. González
Departamento de Biología Celular, Facultad de Biología, Universidad Complutense, Madrid, España.

Most data about the pallial organization have been obtained in mammals, but during the last years the evolutionary interest has open the field to non-mammalian vertebrates. Notably, recent studies in amphibians (the sole group of anamniote tetrapods) have revealed strikingly conserved patterns in morphological units of the prosencephalon, as compared to amniotes. In mammals, the pallium primaryly shows a layered organization, with only minor nuclei formations, and is subdivided into functional areas and columns. In each area or column, most cells of all layers originate in the same ventricular sector, and migrate radially towards the mantle, producing the inside-out developmental ordering to the lamination. In addition, exclusively in mammals, there is a second germinative cell layer, the subventricular zone. By contrast, in other amniotes this germinative layer and the inside-out layer organization does not exist; the lamination of the pallium is reversed and it is subdivided into different areas, which radial glial disposition suggests that each subdivision constitutes a separate radial unit. In this context, the aim of the present study following the main developing pallial events described in amniotes, is to define the radial compartments that generate the different pallial regions in anuran amphibian *Xenopus*. We analyzed the proliferative zones and its developmental timing. In addition, we have studied the radial unit related to the histological organization and the proliferative rate in the developing pallium. Thus, glia is radially organied, as suggested by the observed cell column organization. The proliferation analysis (studying pH3 expression and BrdU date birthing assays), showed in the pallial neurogenesis that the newly generated neurons do not pass the previous ones, contrary to mammals (but similar to reptiles and birds), and it is not biased through the different pallial areas, thus the mitosis rate is comparable in the different zones analyzed at the different stages.

ROLE OF miRNAS IN OLFACTORY BULB FORMATION

V. Fernández-Martínez, M. A. Martínez-Martínez, U. Tomasello and V. Borrell.
Instituto de Neurociencias, CSIC – Universidad Miguel Hernández, Sant Joan d'Alacant, Alicante, Spain

During early telencephalic development a small domain of progenitors in the rostral pallium initiates a developmental program different from the nearby pallium causing a pronounced tissue growth and evagination, leading to the eventual formation of the olfactory bulb (OB). The cellular and molecular mechanisms regulating this specialization of rostral pallial progenitors into generating the OB, as opposed to neocortex, remain largely unknown. Here we tested the potential role of miRNAs in controlling the behavior of OB progenitor cells using a Dicer-flox/flox;Rx-Cre mouse, deficient in Dicer-dependent miRNAs from the onset of telencephalic development. We find that the absence of Dicer from very early stages of brain development causes deficits in OB formation and growth, which at E18.5 is much smaller than in WT littermates. $Dicer^{fl/fl}$;Rx-Dicer mutants display a significant reduction in progenitor cell proliferation and a high frequency of cell death at early stages (E10.5-E12.5), predominantly affecting Pax6+ progenitor cells and coincident with the peak of OB neurogenesis. At later stages (E13.5-E17.5) these deficits are gradually compensated by hyper-proliferation (increasing the number of S-phase progenitors and their cell cycle re-entry) and the formation of rosette-like structures in the basal telencephalon. An RNAseq analysis of differentially-expressed genes in Dicer mutant embryos points to a dysregulation of the p53 pathway as major responsible for the onset of this complex phenotype. Our results demonstrate an unprecedented crucial role for Dicer-dependent miRNAs during telencephalic development, both promoting progenitor cell survival and also limiting the proliferation of telencephalic progenitors.

Áreas Temáticas:
1ª: Neurobiología del Desarrollo

EFFECTS OF PHYSICAL EXERCISE ON ADULT HIPPOCAMPAL NEUROGENESIS: CHANGES IN DNA METHYLATION AND A FOCUS ON Smad2 GENE

S. Gradari[1]; A. Herrera[2]; A. Fontán-Lozano[1]; S. Pons[2]; J.L. Trejo[1]
1Instituto Cajal, CSIC, Madrid
2Instituto de Biología Molecular de Barcelona, CSIC, Barcelona

Physical activity is an important factor capable of inducing changes in the brain. This study aims to investigate if the expression of certain genes is regulated by changes in the DNA methylation in the hippocampus of the adult mouse, in response to exercise. In particular, the research has focused on the Smad2 gene, manipulating its expression in the dentate gyrus with lentiviral vectors, and studying its role during the process of neurogenesis.
First of all, the hippocampus regions from sedentary and runner animals were analyzed using a microarray detecting the DNA methylation status of 24 transcription factors, and then gene expression was assessed by means of a qPCR. Lentiviral vectors were therefore created to over-express Smad2, or to silence it by means of a shRNA. Afterwards, mice were subjected to bilateral stereotactic injections into the dentate gyrus to inoculate the different kind of lentivirus. Half of the animals from each group remained in their cage for the duration of the experiment, while the other half undertook physical exercise for two weeks. Finally, animals performed behavioural tests to assess activity, state of anxiety and ability in spatial learning and memory. Neurogenesis and modifications in synaptic plasticity were analyzed in the granule neurons with immunohistochemistry techniques.
Results show that exercise induces changes in the DNA methylation and it regulates the expression of many transcription factors, such as Smad2. The silencing and over-expression studies elucidate a role for this gene in the proliferation, survival and differentiation of certain subpopulations of granule cells. Moreover, Smad2 silencing compromises the spatial learning abilities in sedentary mice, while exercise is able to reverse the impairments due to the genetic manipulations. This study confirms that exercise alters DNA methylation, has a role in neuroprotection, in neural plasticity and, moreover, that Smad2 is involved in the modulation of neurogenesis.

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

CLONAL ANALYSIS OF ADULT NEWBORN INTERNEURONS IN THE OLFACTORY BULB

M. Figueres-Oñate and L. López-Mascaraque
Instituto Cajal-CSIC, Madrid.

During the adulthood new neurons are permanently added to the olfactory system. In particular, neuroblasts produced in the subventricular zone (SVZ) migrate along the rostral migratory stream to the olfactory bulb (OB), were they mature and integrate into the circuit. In order to explore the clonal progeny of postnatal SVZ neural precursors we used a novel clonal analysis approach, the UbC-*StarTrack*. This method is based in the stochastic incorporation of different fluorescent reporters into neural progenitors, generating unique and specific color-code in single cells and therefore in all their progeny. It was achieved by the co-electroporation of a mixture of 12 different fluorescent reporters (six expressed in the cytoplasm and six in the cell-nucleus) driven by ubiquitous promoter (UbiquitinC). To get heritable cell labeling, those fluorescent reporters were cloned into PiggyBac transposons, being integrated into the host cell genome. Cre/Lox system was additionally used to avoid the expression of non-integrated plasmids by the transposase, making the color code stable in all the progeny. Fluorescent reporters in addition of the PiggyBac transposase and the inducible Cre were co-electroporated, labeling progenitors lining the wall of the lateral ventricle in postnatal mice. Here, we perform an *in vivo* clonal analysis of those newly generated interneurons from postnatal progenitors. Our transposon-based genomic approach enabled us to track the entire progeny of single progenitors, which was not possible with previous methods due to the high number of cell divisions prior to their differentiation. Sibling neuroblasts migrated along rostral migratory stream and produced clones of interneurons widely dispersed within the OB after several weeks. Those clones were relatively smaller in number compared to glial clones labeled by UbC-*StarTrack*. Finally, this method allowed us to determine the cell distribution pattern and number of clonally related-cells in the population of newborn interneurons in the OB. (Supported by BFU2013-48807-R).

Áreas Temáticas:
1ª: Neurobiología del Desarrollo.
2ª: Nuevos métodos y tecnologías.

GENOARQUITECTONIC STUDY OF THE THALAMUS ALONG EMBRYONIC DEVELOPMENT OF *XENOPUS LAEVIS*

S. Bandín, R. Morona, N. Moreno, J.M. López, A. González.
Departamento de Biología Celular, Facultad de Biología, Universidad Complutense, Madrid, España.

Previous developmental studies of the thalamus in birds and mammals have defined the molecular basis for the acquisition of the thalamic competence and the subsequent formation of the secondary organizer in the zona limitans intrathalamica (prepatterning); and its influence in the early specification of two anteroposterior domains (rostral and caudal progenitor domains) (patterning) within the thalamus. The aim of the present study was to analyze during the embryonic development the organization of the thalamus of the anuran amphibian *Xenopus laevis*, by studying the sequential expression patterns of a number of genoarquitectonic markers, correlative with those that are significant in the thalamus formation of other vertebrates. The expression patterns of 22 markers were analyzed from early embryonic stages to the beginning of the larval period by means of combined in situ hybridization and immunohistochemical techniques. Thus, early in development, the anteroposterior interactions between Fez, Wnt3a, Xiro1, demarcate the Zli as the p3/p2 boundary (prepatterning phase), and during the subsequent patterning phase, two distinct thalamus progenitor domains are formed, primarily in response to Shh gradient expression secreted from the Zli (and basal plate): a rostroventral part of the thalamus, close to Zli (rostral thalamus); and the caudodorsal part (caudal thalamus), distinguishable by molecular markers. The rostral progenitor cells express Lhx1, Nkx2.2, which finally leads to the GABA phenotype of thalamic neurons, and in the caudal thalamus, cells express Gli1/2, Ngn2, Lhx9, Dbx1, Gbx2, contributing to glutamatergic thalamic neurons. All these data, showed that the molecular characteristics observed during prepatterning and patterning in the thalamus of *Xenopus* (anamniote) share many features with those described during thalamic development in amniotes (common patterns in tetrapods), but also with zebrafish, reinforcing the idea of a basic plan in the organization of this diencephalic region in all vertebrates.

Areas temáticas:

1- Neurobiología del desarrollo

MULTIPLE GENE EXPRESSION PATTERN ANALYSIS OF THE PALLIAL DEVELOPMENT IN *XENOPUS LAEVIS*

A. González, S. Bandín, R. Morona, , J.M. López, N. Moreno.
Departamento de Biología Celular, Facultad de Biología, Universidad Complutense, Madrid, España.

The pallium in all vertebrates comprises the dorsal telencephalic division and is a very sophisticated and elaborated structure that shows large evolutionary differences, given its behavioral implications. Four pallial regions are recognized in all tetrapod vertebrates. In addition, in the amnote palliun cortical hem and anti-hem regions have been described as secondary organizer centers during development. In particular, the neuroepithelium that gives rise to the cerebral cortex is flanked by the hem medially and the anti-hem laterally. The aim of the present study focused on the development of the pallium in *Xenopus laevis*, selected as a reference model of amphibians, i.e. a crucial vetebrate group in phylogeny being the only anamniote tetrapods with special significance in the transition from water to land. We selected a set of important markers of the pallial territories described in other vertebrates to recognize the pallial subdivisions and boundaries, primarily on the basis of distinct gene expression patterns. In the case of the characterization of a cortical hem, we found in the medial territory of the *Xenopus* telencephalic hemispheres Wnt3a expression in a comparable region to the hem in amniotes, adjacent to the medial pallium discernible by Lhx2 expression. A possible anti-hem organizer was previously suggested in anurans in the region described as ventral pallium on the basis of its Lhx9 and Tbr1 expressions and the lack of Emx1, although typical anti-hem markers such as Dbx1 and Sfpr1 were not detected in this expected region. However, numerous reelin expressing cells occupied this region, like in mammals. Finally markers of the mammalian neocortical layers, also found in the pallium of other amniotes, such as Mef2C, FoxP1, ER81 and Fezf2 have been detected and analyzed in the pallium of *Xenopus*.

Areas temáticas:

1- Neurobiología del desarrollo

PALLIAL NEUROGENESIS THROUGHOUT TELENCEPHALIC DEVELOPMENT IN *XENOPUS LAEVIS*

N. Moreno, S. Bandín, J.M. López, R. Morona, A. González
Departamento de Biología Celular, Facultad de Biología, Universidad Complutense, Madrid, España.

Most data about the pallial organization have been obtained in mammals, but during the last years the evolutionary interest has open the field to non-mammalian vertebrates. Notably, recent studies in amphibians (the sole group of anamniote tetrapods) have revealed strikingly conserved patterns in morphological units of the prosencephalon, as compared to amniotes. In mammals, the pallium primaryly shows a layered organization, with only minor nuclei formations, and is subdivided into functional areas and columns. In each area or column, most cells of all layers originate in the same ventricular sector, and migrate radially towards the mantle, producing the inside-out developmental ordering to the lamination. In addition, exclusively in mammals, there is a second germinative cell layer, the subventricular zone. By contrast, in other amniotes this germinative layer and the inside-out layer organization does not exist; the lamination of the pallium is reversed and it is subdivided into different areas, which radial glial disposition suggests that each subdivision constitutes a separate radial unit. In this context, the aim of the present study following the main developing pallial events described in amniotes, is to define the radial compartments that generate the different pallial regions in anuran amphibian *Xenopus.* We analyzed the proliferative zones and its developmental timing. In addition, we have studied the radial unit related to the histological organization and the proliferative rate in the developing pallium. Thus, glia is radially organied, as suggested by the observed cell column organization. The proliferation analysis (studying pH3 expression and BrdU date birthing assays), showed in the pallial neurogenesis that the newly generated neurons do not pass the previous ones, contrary to mammals (but similar to reptiles and birds), and it is not biased through the different pallial areas, thus the mitosis rate is comparable in the different zones analyzed at the different stages.

BMP2/4 SIGNALLING DEFINES A WNT-RESPONSIVE STATE THAT FACILITATES NEURONAL DIFFERENTIATION OF ADULT HIPPOCAMPAL STEM CELLS

Armenteros T, Andreu Z, Hortigüela R, Mira H.
Laboratorio de Neurobiología Molecular. UFIEC. Instituto de Salud Carlos III, Madrid.

Neural stem cells generate new granule neurons in the hippocampus throughout the adult life in most animal species, including humans. These new neurons play a key role in spatial learning and pattern separation. However, the molecular mechanisms that control the neurogenic program in the adult hippocampus remain poorly understood. Local niche signals, such as the WNT ligands have been shown to play a major role, while the function of other signals, such as the Bone Morphogenetic Protein (BMP) family of ligands, in the regulation of adult hippocampal NSC differentiation has received less attention. We hereby report a novel function for BMP2 and BMP4 in regulating the neuronal fate commitment of adult hippocampal neural stem cells (AH-NSCs). We show that AH-NSCs from rat express different BMP type 1 and type 2 receptor patterns during proliferation and differentiation. Canonical BMP2/4 signalling through these receptors is sufficient to increase hippocampal neurogenesis *in vitro*, and this signalling specifies the neuronal fate within the first 24 hours. We also report that exposure to BMP2/4 increases the expression of key components of the WNT canonical pathway, such as the *Fzd8* membrane receptor and the transcription factor *Lef1*, pointing to a synergic effect between both the BMP and the WNT pathways. Indeed, our results demonstrate that combined activation of BMP2/4 signalling and WNT3A signalling greatly enhances neuronal differentiation of AH-NSCs *in vitro*. Conversely, inhibition of the WNT pathway impairs the pro-neurogenic BMP effect. These findings uncover a relevant crosstalk between the BMP and WNT pathways during adult hippocampal neurogenesis. We propose that BMP2/4 sets a WNT-responsive state that facilitates the neuronal differentiation program of AH-NSCs.

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

Tomás Armenteros Barrón
t.armenteros88@gmail.com

CORTICAL NEUROPLASTICITY IS CONTROLLED BY THE THALAMUS IN AN EXPERIENCE-INDEPENDENT MANNER

Verónica Moreno-Juan[1,*], Cecilia Mezzera[1,†,*], Noelia Antón-Bolaños[1,*], Luis Rodríguez-Malmierca[1], Rafael Susin[1], Oliver Schaad[2,‡], Michael Rutlin[3], Sacha Nelson[3], Miguel Valdeolmillos[1] and Guillermina López-Bendito[1,¶]

[1]Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-Consejo Superior de Investigaciones Científicas (UMH-CSIC), Sant Joan d'Alacant, Spain

[2]NCCR frontiers in Genetics, University of Geneva, Geneva, Switzerland

[3]Department of Biology and National Center for Behavioral Genomics, Brandeis University, Waltham, MA 02454, USA

[†]Present address: Champalimaud Neuroscience Programme, Champalimaud Centre for the Unknown, 1400-038 Lisbon, Portugal

[‡]Present address: Department of Biochemistry and Molecular Biophysics, HHMI, Columbia University Medical Center, New York, NY 10032, USA

[♯]Present address: Deptartment of Biochemistry, Sciences II, University of Geneva, Geneva, Switzerland

*These authors contributed equally to this work

The reorganizations of the brain in sensory deprived individuals affect both deprived and non-deprived cortical fields in a process known as cross-modal plasticity. These neuroplastic changes are believed derived from experience-dependent mechanisms through life. However, the stages at which cross-modal plastic changes appear and the role of experience in triggering cortical reorganization remains to be elucidated. Our aim is to determine the role of thalamic neurons in the processes of neuroplasticity upon sensory modification. To this end, we used two mouse models; first, we performed embryonic bilateral enucleations of the eyes in order to provoke significant changes in the somatosensory pathway development. Second, we used a transgenic animal where was genetically deleted a specific sensory-modality subset of thalamic neurons. Our results suggest intrinsic interthalamic nuclei communication that maintains homeostasis between the developmental programs of distinct sensory systems. This mechanism will unfold cross-modal neuroplasticity in the spared cortical areas following input loss before experience-dependent activity.

Areas

1. Neurobiología del Desarrollo
2. Neurociencia de sistemas

ELUCIDATING THE MOLECULAR PROGRAM UNDERLYING ADULT HIPPOCAMPAL NEURAL STEM CELL QUIESCENCE

L. Remacha[1], R. Hortigüela[1], M.C.Terrón[2], D. Luque[2], H. Mira[1]
[1]Laboratorio de Neurobiología Molecular. UFIEC. Instituto de Salud Carlos III, Madrid.
[2]Unidad de Microscopía Electrónica y Confocal. CNM. Instituto de Salud Carlos III, Madrid.

Neurogenesis occurs not only at embryonic stages, but also persists in the adult mammalian brain in the so-called neurogenic niches due to the existence of neural stem cells (NSCs). Most cells from the adult NSC pool are undifferentiated in a reversible quiescent state, largely dormant and away from the cell cycle. The balance between quiescence/proliferation of adult NSCs is regulated by different niche factors. Among them, the bone morphogenetic protein (BMP) and WNT signalling pathways are of main interest, given BMPs induce quiescence whereas WNTs enhance proliferation and neuronal specification. To elucidate the molecular program that characterizes the adult NSC quiescent state, we performed a wide gene expression analysis of adult NSCs isolated from the hippocampal niche employing microarrays. Cells were treated with FGF2+BMP4 to induce quiescence or with FGF2 only to promote proliferation, and their transcriptome was compared. Interestingly, many genes that trigger the WNT cascade response were upregulated under BMP4 stimulation *in vitro*, indicating a WNT signalling predisposition of the quiescent NSCs. Moreover, several ribosomal proteins, from the small (S) and large (L) subunits, were jointly downregulated in the BMP4 treated samples, possibly suggesting an overall reduction in protein synthesis. Previous studies have already pointed to a distinct translation state in proliferating murine embryonic stem cells *vs* differentiated cells (Sampath et al., 2008) that may be related to a post-transcriptional regulatory mechanism of gene expression. In order to explore this mechanism in adult NSC quiescence, we characterized the translational efficiency of quiescent and proliferating cells through cell fractionation via sucrose gradient centrifugation and we stablished a profile of polysome distribution. Additionally, certain genes were validated by qRT-PCR to compare the transcript abundance and the translation state in the quiescent and proliferative NSCs. Finally, the ultrastructure of the stem cells was analysed using transmission electron micrographs.

Reference

Sampath et al; Cell Stem Cell 2, 448–460 (2008).

Áreas temáticas:

1. Neurobiología del desarrollo
2. Trastornos y reparación del sistema nervioso

AGED NEURAL STEM CELLS IN THE HIPPOCAMPUS

Martín-Suárez S[1,2], Rebeca Cuesta Pou[1,2], Encinas JM [1,2,3.]
1. Achucarro Basque Center for Neuroscience, Zamudio, Spain.
2. University of the Basque Country (UPV/EHU), Leioa, Spain.
3. Ikerbasque Foundation, Bilbao, Spain

Adult hippocampal neurogenesis declines sharply with age, mainly due to a progressive and activation-coupled loss of the radial neural stem cells (rNSCs) that give rise to neurons in this neurogenic niche. There can be, however, other factors contributing to the impairment of neurogenesis associated with aging. We herein compare in the detail the dentate gyrus of young (3 month-old) and older (12 month-old) mice, with a special focus on NSCs.
We first describe how not only the population of NSC declines dramatically, but also there is an accumulation of NSC with a reactive-like multi-ramified phenotype (mNSCs) that get activated to enter the cell cycle with much lower frequency that their radial counterparts. Weather this phenotype denotes senescence of an intermediate state in astrocytic differentiation remains to be determined. Interestingly, the relative proportion of activated NSC, and the level of re-entry in cell cycle, in the aged mice remains similar to that of the young mice, suggesting the existence of important intrinsic mechanisms controlling the level of activation of the NSC population.
In addition, by measuring the distribution rNSCs in the young and aged mice we determined that their loss is not homogeneous or random, as the remaining NSCs are present in clusters with an internal distance between them similar to those of younger mice, again pointing out to intrinsic mechanisms governing NSC activation/depletion.
Finally, we observed in the aged mice changes in the number of connexin 43-positive gap junctions between NSCs and granule cells; a loss of mitotic potential of neuroblasts; and an accumulation of astrocytes, which present a reactive-like phenotype, with overexpression of GFAP and thicker processes.
Together, our results unveil new properties of adult hippocampal NSCs and provide new insight into the mechanisms of aging of the hippocampus and its adult neurogenic niche.

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

PRENATAL CANNABINOID ADMINISTRATION INDUCES LONG-LASTING ALTERATIONS IN THE OFFSPRING BY IMPAIRING CB_1 RECEPTOR-DEPENDENT REGULATION OF PROJECTION NEURON DEVELOPMENT

A. de Salas Quiroga[1,2*], J. Díaz Alonso[1,2*], D. García Rincón[1,2#], F. Remmers[3#], D. Vega[1,2], M. Gómez Cañas[1,4], J. Paraíso Luna[1,2], B. Lutz[3], M. Guzmán[1,2], I. Galve Roperh[1,2]
[1]. Center for Networked Biomedical Research in Neurodegenerative Diseases (CIBERNED), Institute Ramón y Cajal for Health Research (IRYCIS) and Institute of Neurochemistry (IUIN), Madrid, Spain
[2]. School of Biology, Complutense University, Madrid, Spain
[3]. Institute of Physiological Chemistry, Johannes Gutenberg University, Mainz, Germany
[4]. School of Medicine, Complutense University, Madrid, Spain
*# Equally-contributing authors

Objectives
Cannabis is the most widely consumed illegal drug during pregnancy. Here we try to elucidate the neurobiological substrates underlying structural and functional alterations caused by the disruption of cannabinoid CB_1 receptor fostered by embryonic Δ^9-tetrahydrocannabinol (THC) exposure.
Methods
We administered THC to pregnant mice during a restricted gestational time window and analyzed the consequences in the offspring by diverse histological and behavioral means. We then employed Cre-mediated, neuronal lineage-specific, CB_1-expressing mice in order to determine the neuronal identity of THC-exposure actions.
Results
Our approach produced a reduction in subcerebral projection neuron generation, altered corticospinal connectivity and caused long-lasting alterations in the fine motor performance of the offspring. Neuronal traits were reminiscent to those elicited by CB_1 receptor genetic ablation. CB_1-null mice were resistant to THC-induced alterations. Selective embryonic re-expression of CB_1 in dorsal telencephalic glutamatergic neurons rescued the deficits in corticospinal motor neuron development of CB_1-deficient mice and restored their susceptibility to THC-induced motor alterations. In addition, restricted embryonic THC administration induced an increase in seizure susceptibility which, in this case, was mediated by its ability to interfere with the CB_1-dependent regulation not only of glutamatergic neuron development but also inhibitory neuron development.
Conclusions
These findings show that some of the functional consequences of embryonic cannabinoid exposure that persist in the adulthood are solely mediated by the interference with the neurodevelopmental endogenous function of the CB_1 receptor, thus paving the way for identifying the precise neurobiological substrates that underlie the impact of cannabis abuse during pregnancy.

This work has been supported by FEDER and FIS (PI12-00919) funding.

1. Neurobiología del Desarrollo
2. Sistemas homeostáticos y neuroendocrino

PROPER MYELIN MATURATION DURING POSTNATAL DEVELOPMENT DEPENDS ON APOLIPOPROTEIN D FUNCTION

Nadia García-Mateo[1], Concepción Lillo[2], Miguel A. Gijón[3], Robert Murphy[4], Diego Sanchez[1], María D. Ganfornina[1]
[1]IBGM – Universidad de Valladolid / CSIC, Valladolid, Spain. [2]Instituto de Neurociencias de Castilla y León, Universidad de Salamanca, Salamanca, Spain. [3]Department of Pharmacology, University of Colorado Denver, Aurora, CO, USA

Apolipoprotein D (ApoD) is a Lipocalin expressed by glial cells during development, adulthood and aging of the vertebrate nervous system. In the peripheral nervous system (PNS) it is secreted by Schwann cells, while astrocytes and oligodendrocytes are the source of ApoD in the CNS. ApoD shows neuroprotective functions in response to oxidative stress, Wallerian degeneration and aging. We have demonstrated that ApoD controls the dynamics of post-injury myelin recognition and degradation in the PNS of adult and aged mice. Also, nerve conduction velocity is diminished in ApoD-KO mice. These effects are accompanied by genotype-dependent differences in myelin composition. Is ApoD altering myelin structure-function from early on in development, and thus, conditioning the response to injury in adult and aged mice?
To investigate this question we have performed a molecular profile and an electron microscopy analysis of ApoD-KO and wild-type mice from postnatal development to aging (3 days - 21 months). While early in development the initial phases of myelination take place correctly, both CNS and PNS myelin from ApoD-KO mice show abnormal periodicity and defective myelin compaction at 90 days. MBP and MAG protein profiles strongly support that lipid compaction is delayed in the absence of ApoD, and lipid analysis of ApoD-KO myelin shows altered phospholipid composition, particularly in phosphoinositid species, important for the interaction of MBP with myelin membranes in the process of compaction.
Our results demonstrate that ApoD function is relevant for an adequate myelin membrane compaction, a process where lipid-protein interactions have to be orchestrated in order to construct a proper electrically insulating layer. The presence of ApoD in myelin membranes from early periods of development is therefore required to construct nerves with adequate conduction properties and an adaptive response to injury.

Support: MICINN(BFU2011-23978), JCyL(VA180A11-2).

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

MESENCEPHALIC ORIGIN OF THE ROSTRAL SUBSTANTIA NIGRA PARS RETICULATA

M. P. Madrigal, J. A. Moreno-Bravo, J. E. Martínez-López, D. Echevarría, E. Puelles.
Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-CSIC, 03550 Sant Joan d'Alacant, Alicante, Spain

In embryonic development, the neurons that will constitute a heterogeneous nucleus may have distinct origins. The different components of these populations reach their final location by radial and tangential migrations. The Substantia nigra pars reticulata presents a high level of neuronal heterogeneity. It is composed by GABAergic neurons located in the mes-diencephalic basal plate. These inhibitory neurons usually display tangential migrations and it has been already described that the caudal Substantia nigra pars reticulata is colonized tangentially from rhombomere 1. Our aim is to unveil the origin of the rostral Substantia nigra pars reticulata. We have localized a Nkx6.2 positive ventricular domain located in the alar midbrain. Nkx6.2 derivatives fate map analysis showed mainly a rostral colonization of this GABAergic neuronal population. We confirmed the mesencephalic origin by the expression of Six3. Both transcription factors are sequentially expressed along the differentiation of these neurons. We demonstrated the origin of the rostral Substantia nigra pars reticulata, our data allowed us to postulate that this nucleus is composed by two neuronal populations distributed in opposite gradients with different origins, one from rhombomere 1, caudal to rostral, and the other from the midbrain, rostral to caudal. We can conclude that the Substantia nigra pars reticulata has multiple origins and follows complex mechanisms of specification and migration. Our results support vital information for the study of genetic modifications in these extremely complex processes that results in devastating behavioral alterations and predisposition to psychiatric diseases. Understanding the development, molecular identity and functional characteristics of these diverse neuronal populations might lead to better diagnostic and treatment of several forms of neurological and psychiatric disease. Work supported by ''Ministerio de Economía y Competitividad'' BFU2013-48230-P (FEDER Funds).

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

THE DARK SIDE OF NEURAL PLASTICITY. VISUAL DEPRIVATION INDUCES STRUCTURAL PLASTICITY OF INTERNEURONS IN THE VISUAL CORTEX OF ADULT MICE.

H. Carceller [1], C. Bueno-Fernandez[1], J. Nácher [1,2,3]
[1]. Neurobiology Unit, Cell Biology Dpt. Universitat de Valencia, Valencia
[2]. CIBERSAM: Spanish National Network for Research in Mental Health, Madrid
[3]. Fundación Investigación Hospital Clínico de Valencia, INCLIVA, Valencia

The study of neuronal structural plasticity has been classically focused on excitatory neurons, although interneurons play a key role in the control and synchronization of neural networks. Some studies have evaluated the recovery of plasticity in the adult visual cortex, obtaining promising results. Nevertheless, the role of interneurons in this recovery remains largely unknown. The aim of our study is to evaluate the effects of ten days of visual deprivation during adulthood on the structural plasticity of interneurons. We have developed a model of visual deprivation in transgenic mice, which express the green fluorescent protein (GFP) in an interneuronal subpopulation. In order to identify the specific subpopulation of the GFP-expressing interneurons, we have analyzed their neurochemical phenotype in the primary visual cortex. These interneurons have been identified mainly as Martinotti cells. To study whether visual deprivation had an impact on the structure of these interneurons we have quantified their dendritic arborization, dendritic spine density and typology, as well as the density of axonal of their *en passant* boutons. We found that the dendritic arborization and dendritic spine typology of primary visual cortex GFP-expressing interneurons were significantly altered after visual deprivation. In order to understand the impact of this sensory manipulation on other interneuronal populations and their plasticity, we have also analyzed the density of parvalbumin expressing interneurons and their colocalization with perineuronal nets, specialized extracellular matrix structures responsible for synaptic stabilization in the adult brain, finding a significant decrease in the number of parvalbumin expressing interneurons. In summary, our study shows that interneurons of the primary visual cortex are able to undergo structural remodelling in the adulthood in response to modifications in the visual experience. This restoration of cortical plasticity may improve our understanding of visual disorders like amblyopia and of the plastic capabilities of the adult central nervous system. Spanish Ministry of Economy and Competitiveness BFU2012-32512, Generalitat Valenciana Prometeo Excellence Program PROMETEO2013/069 and the Fundación Alicia Koplowitz.

1. Developmental neurobiology
2. Homeostatic and neuroendocrine systems

DEVELOPMENTAL MECHANISMS PATTERNING CEREBRAL CORTEX GYRIFICATION

C. De Juan Romero, E. Picó, MA. Martínez-Martínez and V. Borrell.
Instituto de Neurociencias, Consejo Superior de Investigaciones Científicas & Universidad Miguel Hernández, Sant Joan d'Alacant 03550, Spain

Malformations of cerebral cortical development include a wide range of disorders that are common causes of neurodevelopmental delay, intellectual disability, autism and epilepsy. The rapid recent evolution of molecular biology and genetics has increased our knowledge on the molecular regulation of cerebral cortex development and cortical malformations. Our recent work demonstrates the existence of genes with modular expression patterns along germinal layers of the developing cerebral cortex of gyrencephalic animals. Among these genes we have focused on FGG1 as an interesting candidate for defining the patterns of folds and fissures in the ferret cerebral cortex. We have found that FGG1 protein was largely absent from the VZ throughout development although this layer is a major site of FGG1 mRNA abundance, together with the SVZ. This suggests that FGG1 may be a priming factor for VZ progenitors to eventually commit to a neurogenic lineage at the SVZ. Importantly, we found that the modular expression patterns of FGG1 mRNA in the ferret VZ are remarkably well correlated with the prospective pattern of cortical convolutions, and hence these patterns might represent a map of progenitor cell priming to define cortical folds. Moreover, FGG1 is a transcription factor controlling the expression of multiple cortical patterning genes, and its mutation in humans causes severe cortical folding defects, again consistent with this gene playing a central role in patterning cortical folds. Our local electroporation of FGG1 in ferret embryos prior to the formation of the Outer Subventricular zone (OSVZ) lead to a very dramatic accumulation of progenitor cells specifically in the OSVZ, whereas in mouse this produced very different phenotypes. Our results demonstrate the striking differences in FFG1 expression patterns between lissencephalic and gyrencephalic species, and support the important role of FGG1in promoting the formation of OSVZ, and thus defining gyrification patterns in ferrets.

1ª: Neurobiología del Desarrollo
2ª: Nuevos métodos y tecnologías

DEVELOPMENT OF NEURONS DERIVED FROM THE SUPRAOPTO-PARAVENTRICULAR HYPOTHALAMIC DOMAIN IN RELATION TO THE FOREBRAIN RADIAL DIMENSION IN MOUSE

B. Castro-Robles, E. Desfilis, L. Medina
Universitat de Lleida, Institut de Recerca Biomèdica de Lleida (IRBLleida)

The supraopto-paraventricular domain (SPV) is a division of the alar hypothalamus that produces the neurons of the supraoptic and paraventricular hypothalamic nuclei, as well as a subpopulation of neurons for the medial extended amygdala. During embryonic development, this domain is characterized by expression of the transcription factor Otp (Orthopedia homolog), which is involved in the differentiation of neurons containing the neuropeptides arginine vasopressin and/or oxytocin. However, the radial or tangential migration of the neurons produced in this domain is unclear. The aim of this study was to investigate the spatio-temporal distribution of Otp-expressing cells in relation to radial glial fibers (using an antibody against RC2) and Islet1 (a transcription factor delineating the embryonic domains neighboring SPV) during mouse development. Our results show a high density of Otp cells in the radial SPV domain, at both terminal and peduncular prosomeric levels. These cells are restricted to the radial SPV domain at very early stages. The supraoptic nucleus appears to develop at the surface of the terminal SPV, while the main part of paraventricular hypothalamic nucleus develops in the medial region of the peduncular SPV. At E12.5, several subpopulations of Otp cells appear to invade tangentially neighboring domains, including the preoptic and pallidal domains (dorsally), as well as the suprachiasmatic domain and basal hypothalamus (ventrally). Some of the Otp cells in the preoptic and pallidal domains incorporate to the medial extended amygdala, mainly to the medial bed nucleus of the stria terminalis. In conclusion, the SPV appears to produce neurons for many structures, which if confirmed by migration assays opens new venues for studying their phenotypes and connections.

Supported by MINECO and FEDER (grant no. BFU2012-33029).

1ª: Neurobiología del desarrollo
2ª: Sistemas homeostáticos y neuroendocrino

ENDOCANNABINOID SIGNALLING IN THE BASOLATERAL AMYGDALA MODULATES THE ANXIOUS-LIKE BEHAVIOUR IN 3xTG-AD MICE

A. Llorente, M. Moreno, J. Martínez-Gardeazabal, E. González de San Román, L. Lombardero, I. Manuel, MT.Giralt, R. Rodríguez-Puertas.
Faculty of Medicine and Odontology. University of the Basque Country (UPV/EHU), Bizkaia, Spain.

Familial mutations have been identified in Alzheimer's disease (AD) patients affecting to the amyloid precursor and tau protein processing, which could be related to the cholinergic system impairment and therefore, to learning and memory. The endocannabinoid system is modulating these cholinergic pathways. We have used the 3xTg-AD mice model to study this interaction using both behavioural test and neurochemical analysis of CB_1 receptors. The effects of the direct CB_1 agonist WIN55,212-2 (1 mg/kg), and the indirect JZL-184 (8 mg/kg) were also studied.
Learning and memory latencies (sec) were evaluated with the passive avoidance test. CB_1 density (fmol/mg-prot) and activity (%) were measured by using [^{3}H]CP55,940 and functional [^{35}S]GTPγS autoradiography, respectively. The cellular localization of CB_1 was also performed by immunofluorescence.
An increase in the learning latency can be considered as anxious behaviour. We observed higher learning latencies in 3xTg-AD compared to WT (26.24 ± 1.8 vs 12.28 ± 1.9; $p<0,001$). CB_1 density in the basolateral amygdala (BLA) was increased in 3xTg-AD (497 ± 20 vs 386 ± 12; $p<0.001$). However, CB_1 activation by WIN55,212-2 or JZL-184 reduced both anxiety (15.7 ± 3.0 and 15.3 ± 2.4) and CB_1 density in the BLA (386 ± 32 and 247 ± 14) to the WT levels. CB_1 activity was also modulated in a similar way (168 ± 24 and 281 ± 41; $p<0.05$). The learning latencies correlated with the CB_1 density, (Pearson $r = 0.7139$; $p = 0.0091$) but not with changes in the CB_1 activity.
Histochemical studies revealed that the CB_1 were located on GABAergic terminals in BLA, where a significant decrease in the density of acetylcholinesterase-positive fiber (-12%) was measured in 3xTg-AD.
The present work shows anxious-like behaviour in 3xTg-AD, probably associated to the modulation of the cholinergic input in the BLA by the endocannabinoid signalling.

1ª: Neurociencia cognitiva y conductual
2ª: Trastornos y reparación del sistema nervios

Acknowledgements: This work was Supported by ISCIII (PI10/01202), co-funded by European Research Development Fund and Basque Government grant (IT584-13) to the "Neurochemistry and Neurodegeneration" Research group. A. LL. is the recipient of a fellowship from the Basque Government (BFI 2012-119), E. G. de S. R. has a postdoctoral contract from UPV/EHU.

NFATc3 REGULATES DIFFERENTIATION OF NEURAL PRECURSOR CELL

M. Fernández [1], M.C. Serrano-Pérez [1], F. Neria [2], M. Berjón-Otero [1], E. Doncel-Pérez [3], E. Cano [2] and P. Tranque [1].
[1]. Instituto de Investigación en Discapacidades Neurológicas (IDINE), Universidad de Castilla-La Mancha, Albacete
[2]. Instituto de Salud Carlos III, Majadahonda, Madrid
[3]. Hospital Nacional de Parapléjicos, Servicio de Salud de Castilla-La Mancha (SESCAM), Toledo

Neural precursor cells (NPCs) generate glial cells and neurons in the developing brain, but neurogenesis can also be triggered in the adult in response to injure or disease. Our previous work has focused on The Nuclear Factor of Activated T Cells (NFAT), a family of transcription factors that play active roles in cell survival, proliferation and differentiation. Our goal was to analyze the expression and actions of the NFAT system in NPCs, investigating its possible role in NPC survival and differentiation. NFAT activity was modulated through pharmacological inhibition and adenoviral overexpression. The results obtained support the presence of selective isoforms of the NFAT family in NPCs, and implicate NFAT proteins in the control of NPC survival and astrocyte and neuron differentiation. In addition, we identified NFATc3 as an isoform involved in these processes.
In summary, our work uncovers the active role of NFAT in NPC survival and differentiation, links NFAT to brain development and highlights its therapeutic potential for tissue regeneration.

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

NOVEL CONNECTION BETWEEN NEWBORN GRANULE NEURONS AND THE HIPPOCAMPAL CA2 FIELD.

M. Llorens-Martín[1,2]; J. Jurado-Arjona[1,2]; J. Ávila[1,2]; F. Hernández[1, 2].
[1] Centro de Biología Molecular Severo Ochoa (CSIC-UAM), c/ *Nicolás Cabrera 1, 28049 Madrid, Spain.*
[2] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED, ISCIII), c/ Valderrebollo 5. Madrid, Spain.

Objectives: Newborn neurons are continuously added to the hippocampal dentate gyrus (DG) throughout life. Mature and immature granule neurons were believed to send their axonal projections exclusively to the hippocampal CA3 field. However, recent data point to an alternative trisynaptic circuit, involving a direct axonal projection from mature granule neurons to the CA2 field (Kohara et al., 2013). Whether this circuit takes place only in mature granule neurons or, on the contrary, whether immature granule neurons also contribute to this novel connection is unknown. Here we aimed to analyze the potential contribution and time course of the connections between newborn granule neurons and the hippocampal CA2 region.
Material and Methods: To this end, we used various retroviral vectors (encoding for either GFP or Synaptophysin-GFP), and specific markers for the axons of newborn neurons (3R-Tau) and for the CA2 field (RGS14 and PCP4). In addition, we investigated whether deleterious (inflammation) and neuroprotective (physical exercise) stimuli can modulate these connections.
Results: By using various retroviral vectors, we show that immature granule neurons send axonal processes to and establish synaptic contacts with CA2 pyramidal neurons and that axonal growth follows a similar time course to that described for CA3 innervation. In addition, we provide experimental evidence demonstrating that the pathway connecting newborn granule neurons and the CA2 field can be modulated by physiological and deleterious stimuli.
Conclusions: Here we demonstrate the temporal pattern of innervation of the CA2 region by newborn granule neurons. Importantly, we have also shown that inflammation threatens the integrity of DG-CA2 connections, and that a neuroprotective stimulus such as physical exercise is able to reinforce the connection between newborn granule neurons and the CA2 field. Taken together, these data suggest the existence of a hitherto unknown mechanism of hippocampal plasticity which might be relevant for hippocampal functioning.

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

ROLE OF THALAMIC AXONS IN CORTICAL DEVELOPMENT AND CONNECTIVITY

N. Antón-Bolaños [1], E. Leyva-Díaz [2] and G. López-Bendito [1]
[1]. Instituto de Neurociencias de Alicante, UMH-CSIC, San Joan d'Alacant, Spain
[2]. Columbia University Medical Center, New York, USA

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 us area specification and cortical wiring, although these possibilities remain controversial. We will test the capacity of TCA 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 both area specification and identity. To this end, we have developed a genetic approach to selectively ablate the medial geniculate body (MGB, auditory thalamic complex) very early during embryonic life, before thalamic axons reach the cortical plate at E15.5. These mice develop normally, receiving sensory input but lacking a specific thalamic relay station. Our preliminary results show a rewired "auditory" pathway through the somatosensory thalamic nucleus. Moreover we are performing functional analysis to elucidate the auditory acuity in these animals. Beyond its relevance to thalamocortical projection, our study has important implications for understanding the brain plasticity through thalamic manipulation and for learning about its capacity to rewire and restore cortical function when an insult has occurred.

This work is supported by grants from the Spanish BFU2012-34298 and an ERC Grant ERC-2009-StG_20081210.

1ª: Neurobiología del Desarrollo

CB_1 RECEPTOR SIGNALING IN EMBRYONIC STEM CELL DIFFERENTIATION INTO CORTICAL NEURONS

J. Paraíso-Luna[1-2], J. Díaz-Alonso[1-2], A. de Salas-Quiroga[1-2], D. García-Rincón[1-2], M. Guzmán[1-2], I. Liste[3], I. Galve-Roperh[1-2]

[1]. Center for Networked Biomedical Research in Neurodegenerative Diseases (CIBERNED), Spain

[2]. School of Biology, Complutense University of Madrid, Institute Ramón y Cajal for Health Research (IRYCIS) and Institute of Neurochemistry (IUIN), Madrid, Spain

[3]. Unidad de Regeneración Neural, Unidad Funcional de Investigación de Enfermedades Crónicas (UFIEC), Instituto de Salud Carlos III, Madrid, Spain.

Pluripotent embryonic stem (ES) cell cultures constitute a powerful tool to investigate key aspects of nervous system development and particularly on the regulatory signaling mechanisms involved in neuronal generation and differentiation. The endocannabinoid system exerts a regulatory role of neurodevelopment that influences neural progenitor proliferation, identity and neuronal differentiation. In particular CB_1 receptor signalling controls the differentiation of corticofugal deep layer neurons owing to their ability to regulate the transcription factor switch Ctip2/Satb2. We have developed an ES default neuronal differentiation paradigm aimed to the generation of cortical projection neurons. ES-derived neuronal differentiation generates mainly excitatory glutamate-producing neurons that express distinctive markers characteristic of upper and deep layer cortical neurons. In addition, astroglial cells are also generated although at a lesser extent. Proliferating murine ES cells and their differentiated neuronal progeny express CB_1 receptors and therefore constitute a robust tool to investigate the role of CB_1 signaling in the transition of pluripotent ES to multipotent neural stem cells, and secondly in their neuronal differentiation program. Results derived from ongoing experiments using murine ES cells with floxed Cnr1 gene and a loss of function strategy will be presented. In addition, the impact in neuronal differentiation upon pharmacological regulation of CB1 receptor signaling in human and murine NSC lines will be shown. In summary, we have developed an ES-derived neuronal differentiation protocol allowing the efficient generation of cortical neurons that constitute a reliable indefinite source of cortical neurons suitable for the study of the neurodevelopmental role of the endocannabinoid system and for the assessment of the neuroprotective efficacy of new pharmacological drugs.

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

Financial support: Instituto de Salud Carlos III (PI12-00919) and FEDER.

DOUBLECORTIN SPATIO-TEMPORAL PATTERN OF EXPRESSION IN THE RETINA OF THE SEA LAMPREY

B. Fernández-López, D. Romaus-Sanjurjo, P. Senra-Martínez, R. Anadón, A. Barreiro-Iglesias, M.C. Rodicio
Facultad de Biología (CIBUS), Universidad de Santiago de Compostela, A Coruña.

Despite the importance of doublecortin (DCX) for the development of the nervous system, its expression in the retina of most vertebrates is still unknown. The key phylogenetic position of lampreys and their complex life cycle make them an interesting model to study retinal development. Here we studied the pattern of expression of DCX in the retina of the sea lamprey. The relationship between DCX-immunoreactive (-ir) structures and those expressing α-tubulin and cytokeratins was also analyzed in premetamorphic larvae. Tract-tracing methods were used to label ganglion cells. DCX immunoreactivity appears first in photoreceptors and ganglion cells of prolarvae. In the central retina of larvae smaller than 100 mm in body length, DCX expression was observed in photoreceptors, in fibers and cells located in the inner nuclear and plexiform layers and in the optic nerve. In premetamorphic larvae, DCX immunoreactivity was also observed in radially oriented cells and fibers, in a layer of cells located in the outer part of the inner neuroblastic layer and in ganglion cells of the lateral retina. In adults, DCX expression in the retina was observed in photoreceptors and in fibers ending in the outer limitant membrane. DCX and α-tubulin colocalization was observed in most DCX-ir structures. No structures were founded that could express both DCX and citokeratins. Tract-tracing experiments showed that the soma of ganglion cells with axons already reaching the optic nerve was DCX negative. These results suggest that, in lampreys, DCX could play roles in the migration of retinal cells that fully differentiate in the metamorphosis, in the establishment of the connections of horizontal and ganglion cells and in the differentiation of photoreceptors. Comparison of our observations with those reported in rodents, chick and sharks retinas reveals important similarities and also interesting differences probably due to the peculiar development of the sea lamprey retina.

1ª: Neurobiología del Desarrollo
2ª: Neurociencia de sistemas

RhoE DEFICIENCY ALTERS THE NUMBER OF CALBINDIN-EXPRESSING NEURONS IN THE OLFACTORY BULB OF MOUSE.

J. Terrado[1], B. Ballester-Lurbe[2], E. Poch[2], M. García-Manzanares[1], I. Pérez-Roger[2], R.M. Guasch[3]
[1]. Facultad de Veterinaria. Universidad CEU Cardenal Herrera. Valencia
[2]. Facultad de Ciencias de la Salud. Universidad CEU Cardenal Herrera. Valencia
[3]. Centro de Investigación Príncipe Felipe. Valencia

The subventricular zone represents an important reservoir of progenitor cells in the brain. Cells from the subventricular zone migrate along the rostral migratory stream and reach the olfactory bulb, where they originate two principal cell types: granule cells and periglomerular cells (PGC). PGC, in turn, can be divided in three non-overlapping populations based on their immunoreactivity to tyrosine hydroxylase, calbindin or calretinin. Rnd3/RhoE is a small GTPase that is in a constitutively active state. In this work we analyse the role of the small GTPase RhoE/Rnd3 in olfactory bulb development using mice lacking RhoE expression. Our results show that RhoE deletion alters, to some extent, the cell architecture of the OB: the thickness of the mitral layer was increased (43%) and, on the contrary, a slight but significant reduction (19%) was observed in the external plexiform layer thickness and a higher reduction (34%) was also observed in the granule cell layer thickness. However, the number of cells per area did not vary in these layers. No significant differences were observed in the thickness of the glomerular and inner plexiform layers. As a consequence, the overall thickness of the OB layers was reduced in RhoE null mice by 9%. Finally, the lack of RhoE expression affected the olfactory glomeruli inducing a severe reduction of calbindin expressing interneurons in the periglomerular layer. This was already evident in the newborns and even more pronounced 15 days later when RhoE null mice displayed 89% less cells than control mice. On the contrary, the number of tyrosine hydoxilase and calretinin expressing cells was not affected. Our results suggest that RhoE is involved in subventricular-derived cell differentiation affecting mainly the development of calbindin expressing cells in the olfactory bulb.

1. Neurobiología del Desarrollo

THE TBR1 TRANSCRIPTION FACTOR REGULATES CELL FATE AND MIGRATION IN THE DEVELOPING CEREBRAL NEOCORTEX

I. Crespo [1], J. Pignatelli [1,2], K. Veena[3], H.R. Méndez-Gómez[1,2], E. Díaz-Guerra[1,2], S. Tole [3,] C. Vicario-Abejón[1,2]

[1]Instituto Cajal, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain; [2]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED-ISCIII), Madrid, Spain.[3]Tata Institute of Fundamental Research, Mumbai, India.

The neocortex is composed of six layers of cells, each containing a unique subset of projection neurons with specific molecular profiles and axonal connectivity. Projection neurons originate from neural stem cells and progenitors located in the ventricular and subventricular zones and then migrate to the cortical plate in an “inside-outside” order crossing the intermediate zone (IZ). Lower IZ immature neurons become multipolar and then axogenesis is initiated. Finally, in the upper IZ neurons change the morphology from multipolar to bipolar and become radially orientated moving by locomotion along of radial glia.

T-box brain 1 (Tbr1) is a transcription factor expressed soon after cortical progenitors begin to differentiate and is associated with projection neuron differentiation, axon guidance, and regulation of neuron fate in the developing neocortex. Here we tested the actions of overexpressing Tbr1 with GFP-retroviral vectors and plasmids by intrautero injection followed by infection or electroporation. Tbr1 overexpression produced accumulation of GFP^+-cells in the IZ and deep layers (V and VI) at the expense of upper layer cells (II-IV). Indeed, the percentage of GFP^+-cells expressing Cux1 (a marker of layers II-IV) significantly decreased in the Tbr1 gain-of-function conditions. However, the percentage of cells expressing CTIP (a marker of layer V) was also reduced by Tbr1. Moreover, the length of the axons appeared shorter in the Tbr1 condition. Taken together, our findings suggest that increasing the levels of Tbr1 induces defects in radial cell migration in the developing cerebral neocortex, impairs axogenesis and thus the establishment of proper axonal projections.

Supported by grants from MINECO (Plan Nacional, Retos and CIBERNED) and Comunidad de Madrid.

FORMIN1 MEDIATES THE NEURITOGENIC ACTION OF ESTRADIOL IN MALE NEURONS AND THE GENERATION OF TRANSIENT SEX DIFFERENCES IN NEURITOGENESIS

A. Ortiz Rodríguez, I. Ruiz Palmero, L.M. García Segura, M.A. Arévalo Arévalo
Instituto Cajal, CSIC, Madrid

Estradiol has been demonstrated to exert neuroprotective and neurotrophic effects in the central nervous system, but its mechanisms of action are not completely known. Formins are proteins involved in the nucleation of actin filaments. Formin1 is associated with actin filaments and also with microtubules and regulates the process of neuritogenesis. The aim of this study was to determine if estradiol controls the expression of Formin1 and whether this molecule is involved in the neuritogenic actions of estradiol in cultured hippocampal neurons. Neurons were obtained separately from male and female E17 mouse embryos to determine the possible existence of sex differences in the mechanisms mediating the regulation of neuritogenesis by estradiol. At 2 days in vitro (DIV), neurons from female embryos showed higher Formin1 mRNA levels and higher number of primary neurites compared to neurons from male embryos. Downregulation of Formin1 by a specific siRNA resulted in a decreased number of primary neurites in neurons from both male and female embryos, abolishing sex differences in the number of primary neurites. In addition, estradiol (10^{-8} M) increased Formin1 mRNA levels and the number of primary neurites in neurons from male embryos but not in neurons from female embryos. Furthermore, Formin1 silencing prevented the neuritogenic effect of estradiol in male neurons. At 4 DIV, female neurons still showed higher mRNA levels of Formin1 but the difference was not statistically significant. In addition, at 4 DIV neurons from male and female embryos showed a similar number of primary neurites and the silencing of Formin1 did not affect the number of primary neurites. These findings suggest that Formin1 is involved in the generation of transient sex differences in neuritogenesis and mediates the neuritogenic action of estradiol in male neurons at 2 DIV.

1ª: Neurobiología del Desarrollo
2ª: Sistemas homeostáticos y neuroendocrino

POTENTIAL NOVEL FUNCTION OF CYCLIN D1 IN THE MOUSE DEVELOPING NERVOUS SYSTEM

Daniel Rocandio[1], Neus Pedraza[1], Ariadna Ortiz[1], Eloi Garí[1] and Joaquim Egea[1],
[1.] Medicine Department, Lleida University, Lleida (Spain)

The best known function of CyclinD1 within the cell is the integration of extracellular signals to cell division, in particular to the early to mid G_1 phase of cell cycle. Recent studies show that CyclinD1 may be located as well in the cytoplasm of the cells where it has been proposed to regulate cell adhesion of some cellular lines like macrophages or keratinocytes. However, the relevance of this function *in vivo* has not been investigated. Nervous system development is characterized by different aspects (migration, polarization, differentiation, etc ...) that require a thigh regulation of cell adhesion. In this context, we wanted to address the role of the cytoplasmic CyclinD1 *in vivo*. First, we performed an immunofluorescence analysis to check for CyclinD1 expression during several developmental stages in the brain. We observed that CyclinD1 is expressed, as expected, in the nucleus of the progenitor cells in the proliferative ventricular zone. Interestingly, we did observe a specific cytoplasmic localization of CyclinD1 within the cytoplasm of the radial glial process of radial glial cells (RGCs) in localized areas of the brain, in particular in the lateral ganglionic eminence (LGE) and in the thalamus, in a spatiotemporal pattern paralleling the initiation of neurogenesis and radial glial directed neuronal migration, which is known to commence and progress in a rostrolateral to caudomedial gradient. This cytoplasmic localization suggests a possible role of CyclinD1 unrelated to cell proliferation, like previous results in the regulation of keratinocyte adherence during differentiation have suggested. To test this idea and find a possible novel function of CyclinD1 in the developing nervous system besides regulation of cell cycle, we have started the analysis of CyclinD1 deficient mice throughout brain development. These mice display an abnormality in the leg-clasping reflex, suggesting the presence of neurological defects that might be related to developmental defects.

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

THE ENDOCANNABINOID SYSTEM PROMOTES MIGRATION OF PYRAMIDAL NEURONS IN THE DEVELOPING MOUSE CORTEX VIA RHOA DEGRADATION

Javier Díaz-Alonso[1,2 *], Adán de Salas-Quiroga[1,2 *], Maddy Parsons[3], Clara Andradas[1], Patricia Garcez[4], Juan Paraíso-Luna[1,2], Daniel García-Rincón[1,2], François Guillemot[4], Manuel Guzmán[1,2], Ismael Galve-Roperh[1,2]

[1]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain

[2]Department of Biochemistry and Molecular Biology I, School of Biology, Instituto de Investigación Sanitaria Ramón y Cajal (IRYCIS) and Instituto Universitario de Investigaciones Neuroquímicas (IUIN), Complutense University, 28040 Madrid, Spain

[3]Randall Division of Cell and Molecular Biophysics, King's College London, London SE1 1UL, UK

[4]Division of Molecular Neurobiology, MRC National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.

[*] Equally contributing authors.

Besides its well-known neuromodulatory role in adult brain synapses, the cannabinoid CB_1 receptor is an important regulator of mammalian brain development. CB_1 signaling has been proposed to regulate neuronal migration, but the precise molecular mechanisms involved remain obscure. In this study we demonstrate that the CB_1 receptor is required for proper radial migration of cortical pyramidal neurons in vivo. Transient CB_1 knockdown by *in utero* electroporation of a siRNA-CB_1 arrests radial migration of pyramidal neurons, resulting in long-lasting aberrant cortical layering and subcortical band heterotopias (SBH) that render the adult offspring more susceptible to pentylenetetrazol (PTZ)-induced seizures, pointing to an abnormal wiring. We found that a defined gradient of the endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA), acting through CB_1 receptors, direct migration of different classes of newborn pyramidal neurons in a postmitotic, cell-autonomous manner. We identified proteasomal degradation of the small G-protein RhoA as a key downstream event in the pro-migratory cascade triggered by CB_1 activation. Hence, simultaneous RhoA knockdown rescued the migration arrest induced by CB_1 loss of function and restores latency to PTZ-induced seizures. Our study demonstrates that the CB_1 receptor drives radial migration of pyramidal neurons in the developing mouse cortex and indicates that abnormal endocannabinoid function during development might underlie some types of malformations of cortical development.

This work has been supported by FEDER and FIS (PI12-00919) funding.

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

MiR-17~92 CLUSTER, A CRITICAL FACTOR IN ADULT NEURAL STEM CELL QUIESCENCE.

L. Robado de Lope[1], S. Fra-Bidó[1], P. González-Gómez[1], R. Hortigüela[1], H. Mira[1].
[1]. Laboratorio de Neurobiología Molecular. UFIEC. Instituto de Salud Carlos III, Madrid.

Small regulatory eukaryotic RNAs, including microRNAs (miRNAs), mediate sequence-specific post-transcriptional control of gene expression. Their role in adult neural stem cell regulation and neurogenesis remains largely unexplored. Here, we identify the miRNA cluster miR-17~92 as a key modulator of stem cell activity in the adult hippocampal neurogenic niche. We analysed the effect of miR-17~92 in the biology of neural stem cells (NSCs) isolated from the adult hippocampal dentate gyrus, one of the mature brain neurogenic niches. Employing both *in silico* analysis and gain and loss-of-function approaches, we searched for possible gene targets to better understand its function. Our results showed that the miR-17~92 cluster is overexpressed during NSC proliferation, decreasing when NSCs enter into a quiescent state. We also identified the bone morphogenetic protein type 2 receptor (BMPR2) as a target of two members of the miR-17~92 cluster, namely miR-17-5p and miR-20a. Luciferase reporter assays further confirmed these data. Given that BMP signalling act as a gatekeeper for NSC quiescence in the hippocampus, our findings suggest that the miR-17~92 cluster is essential for the switch between active proliferation and quiescence of adult NSCs.

1. Neurobiología del desarrollo.
2. Trastornos y reparación del sistema nervioso.

THE THYROID HORMONE TRANSPORTER MONOCARBOXYLATE TRANSPORTER 8 IS REQUIRED FOR NORMAL CNS MYELINATION IN THE HUMAN BRAIN

D. López-Espíndola[1,2], J. Bernal[1,3], A. Guadaño-Ferraz[1,3]
[1]. Instituto de Investigaciones Biomédicas Alberto Sols, CSIC-UAM, Madrid, España
[2]. Universidad de Valparaíso, Viña del Mar, Chile
[3]. Centre for Biomedical Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, España

Mutations in the gene encoding the highly specific thyroid hormone (TH) transporter Monocarboxylate transporter 8 (MCT8) produce an X-linked disease known as Allan-Herndon-Dudley Syndrome (AHDS), consisting of cognitive disability and motor disorders with abnormal serum TH levels. MRI shows delayed myelination in these patients but our recent neurohistopathological findings in the brain of two MCT8-deficient subjects indicate a myelination delay at 30 gestational weeks (gwk) and persistent hypomyelination (lower myelin lipid, protein content and density of myelinated axons) at 11 years of age in different brain regions.
We aim to better understand the myelination defect in MCT8-deficient subjects during development and childhood. Given the relationship between axon diameter and myelination, we first analyzed the morphology and diameter of myelinated axons in several brainstem axonal tracts from autopsy tissue of the MCT8-deficient 11-year-old boy. For this we used the luxol fast blue technique (LFB) and immunohistochemistry for neurofilament-70kD (NEFL). As TH regulate oligodendrocyte generation we also characterized the normal cellular expression of MCT8 in the white matter at several fetal stages and the MCT8 expression in the MCT8-deficient fetal brain.
LFB showed mainly low caliber myelinated axons with a lower proportion of large diameter myelinated axons in the MCT8-deficient boy ($P< 0.0001$). NEFL immunohistochemistry confirmed these results.
We found that white matter MCT8 expression begins around 25 gwk in a large number of glial cells, and interestingly, a drastic increase was observed at 30 gwk. This high level was maintained at 38 gwk. MCT8 expression was confined to GFAP+ and Olig2+ cells, which correspond to astrocytes and oligodendrocytes precursors, and myelinating oligodendrocytes. However, the MCT8-deficient fetus did not express MCT8 in white matter glial cells.

These results highlight the importance of TH local availability regulation by MCT8 in axonal growth and glial cells differentiation in the human brain.

1. Neurobiología del Desarrollo
2. Sistemas homeostáticos y neuroendocrino

CLONAL CELL ANALYSIS OF NG2 CELLS

A. Bribian[1], R. Sanchez-Gonzalez[1], M. Figueres-Oñate[1], L. López-Mascaraque[1].
1 Instituto Cajal-CSIC, Madrid, Spain.

Diverse neural types are generated during development throughout sequential events of cell division. Neural stem cells give rise to a progeny that is gradually specified towards distinct cell fates to differentiate into defined neural types. Thus, progenitor cells become gradually more restricted to differentiate into neurons and/or glial cells. Focus on glial cells, they are classically classified into: astrocytes, oligodendrocytes and microglia, although recent studies revealed NG2 cells as the fourth type of glial cells. The increasing knowledge of the diversity and complexity of NG2 cells function, argues for a heterogenic cell population. NG2 cells constitute 5-8% of all brain cells and they also comprise most of the proliferating cell population outside the neurogenic niches in the adult mouse brain. Using the StarTrack method we revealed that NG2 cells form clones comprised by various hundreds of cells in adult brain (García-Marqués et al., 2014), evidenced temporal differences in proliferation rates. Thus, this genetic tool allows the analysis of astrocytes and NG2 cells lineage. In order to explore the complete clonal cell progeny of NG2 cells, we also used a novel *in vivo* clonal analysis approach, UbC-StarTrack. This method allows to track the entire progeny of single progenitors, based in the co-electroporation of a mixture of 12 different fluorescent reporters (six expressed in the cytoplasm and six in the cell nucleus) driven the ubiquitous promoter, Ubiquitin C. Complementary we have also developed new constructs under the NG2 promoter. In this work we aim to determine the origin, clonal dispersion and cell fate potentials of NG2 cells in the adult cortex.

(Supported by research Grant BFU2013-48807-R from the Spanish Ministry of Economy and Competitiveness).

1. Neurobiología del Desarrollo
2. Nuevos métodos y tecnologías

PRESYNAPTIC MUSCARINIC ACETYLCHOLINE AUTORECEPTORS (M1, M2 AND M4 SUBTYPES) MODULATE THE DEVELOPMENTAL SYNAPSE ELIMINATION PROCESS ON THE NEUROMUSCULAR JUNCTION

L. Nadal, N. Garcia, E. Hurtado, A. Simón, M. Tomàs, M.A. Lanuza, M. Santafé, and J. Tomàs
Unidad de Histología y Neurobiología (UHN). Facultad de Medicina y Ciencias de la Salud. Universidad Rovira i Virgili. C/Sant Llorenç 21, 43201 Reus. Spain.

Individual skeletal muscle fibres in newborn vertebrates are innervated at a single endplate by several motor axons. During the first postnatal weeks, the polyneuronal innervation decreases in an activity-dependent process of synaptic elimination by axonal competition. The muscarinic ACh autoreceptors (mAChR) may allow the direct competitive interaction between axons through a differential activity-dependent ACh release in the synaptic cleft. Then, the more active ending may directly punish those less active. Here we investigate by quantitative immunohistochemistry the involvement of the individual M1-, M2- and M4-subtypes of mAChRs in the control of the axonal elimination in developing neuromuscular junction (NMJ).

We found that in the initial phase of synapse elimination (P7) the unselective block (atropine) and stimulation (oxotremorine) of all subtypes reveal a mAChR-mediated acceleration of the two-to-one transition of the dual junctions. It seems that NMJ with distinct maturation levels have at this phase different sensitivities to the muscarinic regulation. However, some days later, at P9, it is fully manifested a constitutive, tonic, muscarinic mechanism, mediated by the subtypes M1 and M2, committed to promote axonal disconnection and synapse elimination of the supernumerary nerve terminals in all NMJ. This mechanism may operate near their maximum rate and thus, may be not forced to increase their efficacy, beyond P7 with exogenous muscarinic agonists. In spite of the continued presence of selective mAChR inhibitors, the elimination process come to the normal conclusion at the end of the second postnatal week suggesting that other signalling cooperate in a multifactorial process. By blocking TrkB receptor pathway (with TrkB-Ig) and the full set of the adenosine receptors (AR, with 8SPT), we found that these receptors similarly to the mAChRs, contribute to hasten axonal elimination. Thus, the three receptor sets promote axonal disconnection at the beginning of the second postnatal week.

This work was supported by 2014SGR0344 and 2014PFR-URV-B2-83.

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

BEX3 DIMERIZATION MODULATES NGF-DEPENDENT NEURONAL SURVIVAL AND DIFFERENTIATION BY REGULATING TRKA GENE TRANSCRIPTION.

L. Calvo[1], B. Anta[1], S. López-Benito[1], C. Martín-Rodríguez[1], F.S. Lee[2], P. Pérez[3], D. Martín-Zanca[3] and J.C. Arévalo[1]

1 Instituto de Neurociencias de Castilla y León (INCyL) and Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca.

2 Weill Medical College of Cornell University, New York, NY 10065.

3 Instituto de Biología Funcional y Genómica, CSIC/Universidad de Salamanca, Salamanca.

The development of the nervous system is a temporally and spatially coordinated process that relies on the proper regulation of the genes involved. Neurotrophins and their receptors are directly responsible for the survival and differentiation of sensory and sympathetic neurons. However, it is not fully understood how genes encoding Trk neurotrophin receptors are regulated. Here, we show that Bex3 protein specifically regulates TrkA expression by acting at the *trkA* gene promoter level. Bex3 dimerization and shuttling to the nucleus regulates the transcription of the *trkA* promoter under basal conditions and also enhance NGF-mediated *trkA* promoter activation. Moreover, qChIP assays indicate that Bex3 associates with the *trkA* promoter within a 150 bp sequence, immediately upstream from the transcription start site, which is sufficient to mediate the effects of Bex3. Consequently, the down-regulation of Bex3 using shRNA increases neuronal apoptosis in NGF-dependent sensory neurons deprived of NGF and compromises PC12 cell differentiation in response to NGF. Our results support an important role for Bex3 in the regulation of TrkA expression and in NGF-mediated functions through modulation of the *trkA* promoter.

1. Neurobiología del desarrollo.
2. Trastornos y reparación del sistema nervioso

TANGENTIAL NEURONAL MIGRATIONS DURING DEVELOPMENT OF THE ROSTRAL HINDBRAIN

B. Lorente-Cánovas[1,2], R. Corral-San-Miguel[1], F. Marín[1], L. Puelles[1], P. Aroca[1]
[1]. University of Murcia, Murcia, Spain
[2]. King's College London, London, United Kingdom

Objectives

Migratory neuronal populations can be identified by the expression of a specific combinatory of transcription factors, signaling molecules, and receptors/mediators of attractive or repulsive molecules and morphogens. In this work we aimed to analyze tangential migrations of neuronal populations in the rostral hindbrain, in particular for the interpeduncular (IP) and locus ceruleus (LoC) nuclei. We were interested as well in the molecular characterization of the subdivisions of the IP nucleus by the expression pattern of specific transcription factors in mouse and chick embryonic brains.

Methods

We analysed the expression pattern of diverse transcription factors, signaling moleculaes and neurotransmitters, by in situ hybridization and/o immunohistochemistry, as well as mining of online gene expression databases, combined with fate-mapping experiments consisting of quail-to-chick chimeric grafts.

Results

We have demonstrated that different progenitor domains of the first rhombomere (r1) neuroepitelium generate diverse neuronal populations that follow specific and independent patterns of tangential migrations to form the LoC and the various populations of the IP. These populations were traced across successive developmental stages by the expression of specific transcription factors (Phox2a, Pax7, Nkx2.1, Otp, Otx2). The results of quail-chick grafts corroborated a dorsal-to-ventral tangential migration of these neuronal populations in r1. Additionally, our experiments showed a novel internal regionalization of the IP nucleus in chick and mouse. One of the most interesting molecular markers for IP subpopulations is DCC, involved in axon guidance and neuronal migration.

Conclusions

The IP and LoC nuclei display a similar pattern of migration and internal regionalization in the avian and mammalian brains. The expression of DCC in the IP nucleus suggests an involvement of the DCC/Netrin signaling in the migration of specific populations of this nucleus and/or their connectivity pattern. Similarly, the gene expression profiles of each population point to the respective involvement of other molecular networks in their migration and/or specification.

TOWARDS RECREATING THE STRIATAL NICHE *IN VITRO*

P. Sanders [1], A. Lagunas [2,3], M. Straccia [1], G. Bombau [1], P.J. Kemp [5], N.D. Allen [5], J. Samitier [2,3,4], J.M. Canals [1].

[1]. Department of Cell Biology, Immunology and Neuroscience, Faculty of Medicine, CIBERNED, IDIBAPS, University of Barcelona, Barcelona, Spain.

[2]. Networking Biomedical Research Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.

[3]. Institute for Bioengineering of Catalonia (IBEC), Baldiri-Reixac 15-21, Barcelona 08028, Spain.

[4]. Electronics Department, University of Barcelona, Martí i Franquès 1-11, Barcelona 08028, Spain.

[5]. Divisions of Pathophysiology & Repair and Neuroscience, School of Biosciences, Cardiff University, Cardiff, United Kingdom.

Cell therapy is a key strategy in regenerative medicine to replace damaged tissues, and a viable approach for modeling and/or treating Huntington's disease (HD) where striatal GABAergic medium spiny neuron (MSN) degeneration occurs. Implementation of cell replacement strategies, and recapitulating striatal development and HD using pluripotent stem cells (PSCs), requires the development and characterization of new differentiation protocols that recreate the striatal niche as previous protocols to produce MSNs are long and inefficient.

In this study we use a recently developed *in vitro* neuronal differentiation protocol that recapitulates *in vivo* developmental stages to generate a significant level of MSN-like neurons from human PSCs. We characterise the protocol by analysing gene expression during differentiation from PSCs using a customised quantitative PCR platform that monitors expression of 112 genes specific for neural developmental stages and/or encephalic areas. To evaluate the protocol we benchmark the gene expression data using human foetal and adult striatal tissue expression profiles generated using the same technology.

Subsequently we use the protocol as a baseline to study striatal development and enhance MSN specification and maturation. We aim to improve differentiation by recreating the striatal niche using approaches such as the controlled ectopic expression of transcription factors, and the characterization of extracellular proteins involved in striatal development using a microprinted cellular array format.

We show that this differentiation protocol generates MSN-like neurons at a level comparable to or better than previous protocols in a shorter time.

After 16 days of differentiation PSC-derived neural progenitors display gene expression similar to human foetal whole ganglionic eminence (WGE), often a source of progenitors for transplantation. Subsequently, PSC-derived neurons cluster between WGE and adult striatum, indicating progression towards mature MSNs.

To enhance differentiation and efficiently obtain mature MSNs *in vitro*, integration of a range of approaches is essential to recreate the striatal niche.

This study was supported by grants from the Ministerio de Economia y Competitividad and from the ISCIII-Subdirección General de Evaluación and European Regional Development Fund (ERDF) [RETICS and CIBERNED], Spain; and CHDI Foundation, USA.

1ª: Neurobiología del Desarrollo

2ª: Trastornos y reparación del sistema nervioso

DEIODINASE 2 EXPRESSION THROUGHOUT PERINATAL STAGES OF DEVELOPMENT IN RODENTS

S. Bárez-López [1,2], J. Bernal[1,2], A. Guadaño-Ferraz[1,2]
1. Instituto de Investigaciones Biomédicas Alberto Sols, CSIC-UAM, Madrid
2. Centre for Biomedical Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid

Thyroid hormones (TH) play an essential role both in the developing and the adult central nervous system (CNS). The thyroid gland produces mainly thyroxine (T4) but the active hormone at a transcriptional level is the 3,5,3'-triiodothyronine (T3). In the adult CNS 80% of the intracellular concentrations of T3 are modulated by the activity of type 2 deiodinase (Dio2) in astrocytes that converts T4 into T3. However, studies in rodents suggest that during embryonic development the only pathway for T3 availability in the brain is the one mediated by Dio2. Nevertheless, the timing and localization of the expression of Dio2 at perinatal stages of development in rodents still remains unknown. This is probably due to technical limitations because Dio2 has a very short half-life as it undergoes selective ubiquitination and proteasomal degradation. It is crucial to determine its location to fully understand T3 action.
Here we have developed the necessary tools to study the mRNA and protein expression of Dio2 *in vivo* at a regional and cellular level. We have chosen several perinatal stages of development (Embryonic day15 (E15), E18, Postnatal day 1 (P1), P3 and P5) and we have successfully optimised immunohistochemistry and *in situ* hybridisation techniques. We have characterised *Dio2* expression pattern that changes throughout development especially from E15 to E18. Among other regions, Dio2 appears to be located at the Blood-Brain-Barrier and the Cerebrospinal-Fluid-Barrier. We have also obtained preliminary results of Dio2 protein expression with a commercial antibody that has provided additional information and better resolution. We are currently working on verifying the specificity of this antibody.
In conclusion, Dio2 presents a dynamic expression throughout perinatal brain ontogeny, possibly adapting to meet the different cellular T3 requirements during development. The main role of Dio2 at early stages seems to be converting T4 into T3 at the brain barriers.

1. Neurobiología del Desarrollo
2. Sistemas homeostáticos y neuroendocrino

RHOE REGULATES THE PROCESS OF NEUROGENESIS THROUGH NOTCH SIGNALING.

F. Ruso-Julve,[1], L. Garcia-España[1], M.A .Pérez-Aragó[1], B. Ballester-Lurbe[2], J. Terrado[2], I. Pérez-Roger[3], R.M. Guasch[1]
[1]. Centro de Investigación Príncipe Felipe. Valencia.
[2]. Facultad de Veterinaria. Universidad CEU-Cardenal Herrera. Valencia.
[3]. Facultad de Ciencias de la Salud. Universidad CEU-Cardenal Herrera. Valencia.

Objectives: RhoE protein is an atypical member of the Rho family, initially characterized as a regulator of cytoskeleton dynamics and lately involved in the development of the central nervous system. We have demonstrated that RhoE deficiency induces important alterations such as an early postnatal mortality, neuromotor and neuromuscular alterations and a delay in the process of neuronal polarization. Moreover, we have recently demonstrated an accumulation of neural progenitor cells (NPC) in the rostral migratory stream. Our main objective is to study the process of neurogenesis in the absence of RhoE.
Material and Methods: We have used neurosphere cultures from mice lacking RhoE expression as a model to study the process of neurogenesis in the subventricular zone (SVZ). Both proliferation and differentiation of NPC have been studied by different techniques such as EdU incorporation, immunofluorescence staining and growth curves. To investigate the molecular mechanism by which RhoE participates in the development of the nervous system we have used western blotting and RT-PCR techniques.
Results: Our findings show that NPC from a mice lacking RhoE expression exhibit an increase in their proliferation due to an increase in the rate of DNA synthesis. They also show a decrease in survival as a result of the reduction in apoptosis cell death. Finally, we have observed that the Notch signaling pathway is involved those alterations.
Conclusions: Our results suggest that RhoE participates in the regulation of the NPC proliferation from the SVZ during early postnatal stages. RhoE deficiency seems to alter the Notch pathway, which is essential in the neurogenesis process. The ultimate goal of this research is to reveal the molecular mechanisms involved in neurodegeneration due to the absence of RhoE protein as well as to investigate its modulation to develop new therapeutic strategies in the treatment of neurodegenerative diseases.

1.- Neurobiología del Desarrollo.

POTENTIAL NOVEL FUNCTION OF CYCLIN D1 IN THE MOUSE DEVELOPING NERVOUS SYSTEM

Daniel Rocandio[1], Neus Pedraza[1], Ariadna Ortiz[1], Eloi Garí[1] and Joaquim Egea[1],
[1] Medicine Department, Lleida University, Lleida (Spain)

The best known function of CyclinD1 within the cell is the integration of extracellular signals to cell division, in particular to the early to mid G_1 phase of cell cycle. Recent studies show that CyclinD1 may be located as well in the cytoplasm of the cells where it has been proposed to regulate cell adhesion of some cellular lines like macrophages or keratinocytes. However, the relevance of this function *in vivo* has not been investigated. Nervous system development is characterized by different aspects (migration, polarization, differentiation, etc ...) that require a thigh regulation of cell adhesion. In this context, we wanted to address the role of the cytoplasmic CyclinD1 *in vivo*. First, we performed an immunofluorescence analysis to check for CyclinD1 expression during several developmental stages in the brain. We observed that CyclinD1 is expressed, as expected, in the nucleus of the progenitor cells in the proliferative ventricular zone. Interestingly, we did observe a specific cytoplasmic localization of CyclinD1 within the cytoplasm of the radial glial process of radial glial cells (RGCs) in localized areas of the brain, in particular in the lateral ganglionic eminence (LGE) and in the thalamus, in a spatiotemporal pattern paralleling the initiation of neurogenesis and radial glial directed neuronal migration, which is known to commence and progress in a rostrolateral to caudomedial gradient. This cytoplasmic localization suggests a possible role of CyclinD1 unrelated to cell proliferation, like previous results in the regulation of keratinocyte adherence during differentiation have suggested. To test this idea and find a possible novel function of CyclinD1 in the developing nervous system besides regulation of cell cycle, we have started the analysis of CyclinD1 deficient mice throughout brain development. These mice display an abnormality in the leg-clasping reflex, suggesting the presence of neurological defects that might be related to developmental defects.

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

ANALYSIS OF MICE DEFICIENT FOR *RHOE* REVEALS A KEY ROLE OF STRIATAL AXONS IN GUIDING THALAMOCORTICAL PROJECTIONS AT THE DIENCEPHALON-THELENCEPHALON BOUNDARY

P. Marfull[1], E. Sanz[1], I. Pérez-Roger[2], J. Egea[1].
[1] IRB-LLEIDA, University of Lleida, Lleida
[2] Facultad de Ciencias de la Salud, Universidad CEU Cardenal Herrera, Moncada, Valencia

Axon guidance regulation in the developing brain often requires previous migration of some neurons or other axons. Ultimately, this is mediated by axon guidance cues that act through specific receptors to remodel axon cytoskeleton and trigger attractive or repulsive responses. In thalamocortical projections (TCAs), migration of the so-called "corridor cells" is necessary for TCAs to cross the subpallium. Later in development, corticofugal axons have been proposed to help TCAs to reach the pallium in what is known the "hand-shake" hypothesis. Other axon-axon interactions, such as the striatal-thalamic interaxonal interactions have been proposed to help TCAs. Rho GTPases are well-known transducers of the effect of axon guidance cues on cytoskeleton dynamics. RhoE is an atypical RhoGTPase involved with axon growth and neuron migration. *RhoE* knock-out mice (*RhoE* gene-trapping allele or *gt*) show postnatal lethality, neurodevelopmental delay and impairment of olfactory bulb development. We used this mouse model to study brain axonal connectivity during development. Immunofluorescence and DiI/DiA tracing analysis revealed that $RhoE^{gt/gt}$ mice show severe axonal projection defects: TCAs are unable to cross the diencephalon-telencephalon boundary (DTB), striatonigral axons (SNAs) are misguided ventrally and corticothalamic axons are disorganized. Surprisingly, Islet1 staining shows a properly formed corridor at rostral levels, although it is wider at caudal ones. RhoE is expressed in subventricular regions and in striatum mantle and thalamus. However, we propose that TCAs missdevelopment is indeed secondary to SNAs misguidance for which RhoE, through an unknown upstream receptor, is a key signaling regulator. We are currently working on the hypothesis that SNAs exert an attractive/permissive effect on TCAs to cross the DTB. In summary, our results indicate a function of RhoE in the correct development of brain connectivity and an important role of SNAs in TCAs guiding through the DTB.

1. Developmental Neurobiology
2. Neuronal excitability, synapses and glia: cellular mechanisms

EFFECTS OF T-CELL ACTIVATION OVER THE BIOLOGY OF OLIGODENDROCYTE PRECURSOR CELLS

C. Melero-Jerez, D. Clemente, F. de Castro
Grupo de Neurobiología del Desarrollo-GNDe, Hospital Nacional de Parapléjicos, Toledo.

çMultiple Sclerosis (MS) is the most frequent neuroimmune demyelinating disease of the central nervous system (CNS). T lymphocytes previously "mis-sensitized" against myelin antigens in the peripheral compartments invade the CNS parenchyma and contribute to the loss of oligodendroctyes, the myelin-forming cells in the CNS, which consequently affectsthe myelin sheath. As a consequence, tissue damage and axonal dysfunction occur, giving rise to neurological symptoms. Oligodendrocytes precursor cells (OPCs) are the source of mature oligodendrocytes during CNS embryonic and postnatal development. In the adult CNS, this population represents around 5-8% over total cells and retains its capacity to proliferate, migrate and differentiate into myelinatingmature oligodendrocytes. The total or partial exclusion of these cells from the demyelinated plaque of chronic lesions (where spontaneous remyelination does not occur) constitutes a main histopathological hallmark of the MS scenario, although their recruitment to the plaque of active or the periplaque of chronic-active lesions (where, besides active inflammation,remyelination simultaneously success) are indicative of immune system participation not only in myelin destruction but also in its spontaneous repairing processes. It is in this sense that the study of the direct influence of the immune component on OPC biology represents a major goal in the field. Although there are diverse attempts to understand the role of the innate immune system components on these processes, i.e. M1/M2 macrophages, there is a clear lack of knowledge about the direct role of T cell effect on the biology of OPCs and on maturating re-myelinating oligodendrocytes. In the current work, we describe by first time the effects of T cell activation on OPC survival, proliferation, migration and differentiation towards myelin-forming oligodendrocytes. Our hypothesis looks to shed more light on the pathophysiologycal relationship between the immune and the nervous system in order to design pharmacological and/or cell-based therapies to promote effective myelin preservation and repair for MS and eventually other demyelinating diseases.

This work was supported by the Spanish *Ministerio de Economía y Competitividad (*SAF2012-40023; RD07-0060-2007 and RD12-0032/0012, and partially cofinanced by F.E.D.E.R., European Union, *"Una manera de hacer Europa")* to FdC and ARSEP Foundation (France) to DC. DC and FdC are hired by SESCAM. CM-J holds a Research Training fellowship by the Spanish *Ministerio de Economía y Competitividad* (BES-2013-062630).

1ª: Neurobiología del Desarrollo
2ª: Trastornos y Reparación del Sistema Nervioso

TAU REGULATES EB1/3 LOCALIZATION AND FUNCTION IN DEVELOPING NEURONAL CELLS

C. L. Sayas[1,2,3], E. Tortosa[2,3], F. Bollati [4], S. Ramírez-Ríos [4], I. Arnal[4] and J. Avila[2.3]
[1] Centro de Investigaciones Biomédicas de Canarias (CIBICAN), Instituto de Tecnologías Biomédicas (ITB), Universidad de La Laguna (ULL), Tenerife
[2]Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Madrid
[2]Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Madrid, [4]Grenoble Institut des Neurosciences, [4]INSERM U836, Grenoble, France

Background: Reorganization of microtubular cytoskeleton underlies most of the morphological changes that occur during neuronal differentiation. Classical microtubule-associated proteins (MAPs) and microtubule plus-end tracking proteins (+TIPs), participate in the regulation of microtubule dynamics and stability. However, no much is known about the crosstalk between MAPs and +TIPs during neuronal development.
Objectives: Here we addressed the putative interplay between tau, an axonal classical MAP, and End-binding proteins 1 and 3 (EB1/3), the core +TIPs, in differentiating neuronal cells.
Material and methods: Studies were performed in mouse neuroblastoma cells (N1E-115) and wildtype and tau-knockout primary hippocampal neurons. Tau-deficient stable neuroblastoma cell lines were generated by shRNA lentiviral transduction. Confocal immunofluorescence was performed to show protein localization. Immunoprecipitation, pull-down, and FRAP assays were used to show protein-protein interaction. Quantification and analyses of EB comets number and length was performed using ImageJ free software.
Results: Tau and EBs partially colocalize at extending neurites of N1E-115 cells and axons of hippocampal neurons. Tau downregulation leads to a reduction of EB1/3 comet length whereas tau overexpression at high levels induces EBs relocalization to microtubule bundles at extending neurites. In differentiating primary neurons, tau is required for the proper accumulation of EBs at stretches of microtubule bundles at the medial and distal regions of the axon. Our data indicate that tau and EB proteins interact directly, as shown in non-neuronal and neuronal cells as well as in whole brain lysates. In summary, we show here that EB1/3 cellular mobility and localization, in extending neurites and axons, are modulated by tau levels and localization.
Conclusion. We describe a novel function for tau as a direct regulator of End binding (EB) proteins in differentiating neuronal cells. Our data provide new evidence of the coordinated action of classical MAPs and "core" +TIPs during neuronal development.

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

STUDY OF CORTICAL PROGENITORS USING A DOUBLE-ELECTROPORATION METHODOLOGY

M.A. Albert-Maestro, Ll. Fortes-Marco and J.de Carlos.
Instituto Cajal, CSIC, Madrid.

Neocortex is organized into six layers, which develop from some neuroepithelial precursor populations that cover the lateral ventricles. This laminar organization is a consequence of the inside-out generation and migration pattern, where neurons of deeper layers are born first, and late born neurons populate the upper layers. During early development it can be distinguished two germinative zones: the ventricular zone (VZ) and subventricular zone (SVZ). The last one is populated by intermediate progenitor cells that do not contact the ventricle.
To establish the proliferative zone which gives rise to each layer, and reveal if there is a common progenitor for the generation of all pyramidal neurons, or different progenitor lineages that specifically generate each subset of projection cells types, several double electroporations were performed in utero at different cortical developmental stages. We inject a GFP encoding plasmid mix with another that codifies for cherry protein. Mice were analyzed at birth time (P0).
The results show high percentages of individual GFP+ and mCherry+ cells, which display a variable distribution pattern depending on the electroporation stage: electroporations performed at E12-E13 were correlated with infragranular layers labeling, while those made between E14-E17 labeled the supragranular layers. A well-defined separation between the two generation waves was found. Further, a low percentage of double-labelled cells were also observed. These are of interest to analyze the existence of common progenitors between different stages and layers.

1. Neurobiología del Desarrollo

LPAR1-EGFP TRANSGENIC MICE TO TRACE THE DEVELOPMENTAL ORIGIN OF ADULT HIPPOCAMPAL RADIAL NEURAL STEM CELLS

O. Pastor-Alonso[1,2], R. Valcárcel-Martín[1,2], S. Martín-Suárez[1,2] J. M. Encinas[1,2,3.]
[1] Achucarro Basque Center for Neuroscience, Bizkaia Science and Technology Park, Zamudio, Spain.
[2] University of the Basque Country (UPV/EHU), Leioa, Spain.
[3] Ikerbasque Foundation, Bilbao, Spain.

A population of radial Neural Stem Cells (rNSCs) persists in the subgranular zone (SGZ) of the dentate gyrus (DG) of most mammals, and is able to generate neurons through adulthood, through a process known as adult neurogenesis. Despite the large amount of ongoing research in the field, the origin of the rNSCs remains unknown, and understanding their early behavior and development could be a key point to explain some of their later intrinsic properties.
Thus, in order to shed light into the origin of adult rNSCs we resorted to a transgenic mouse in which lysophosphatidic acid receptor 1 (LPAR1) drives the expression of the enhanced green fluorescent protein (EGFP). Because this transgenic line has never been used before for the study of neurogenesis, we first demonstrated that LPAR-EGFP expression selectively labels rNSCs in the hippocampus, being a valuable tool for these kind of studies. We have traced rNSCs and their mitotic activity during the early postnatal period, up to 3 weeks, using different cellular and proliferation markers and proving that LPAR1-EGFP mice is an adequate model to study the early postnatal and adult neurogenesis.
Our results point at postnatal day 7, as a key time window for the establishment of the rNSCs population in the DG, as clear migratory patterns of LPAR1-EGFP expressing cells can be distinguished. On the other hand, postnatal rNSCs appear to aquire their typical radial morphology and expression of markers by PD13. However, there are differences in the expression of LPAR1-EGP compared to other rNSC markers such as nestin and GFAP, suggesting that the LPAR signaling pathway might have a role in regulating early postnatal and adult neurogenesis.

1ª. Neurobiología del Desarrollo.
2ª. Nuevos métodos y tecnologías.

CYCLIND2 CONTROLS THE GENERATION OF A SUBPOPULATION OF RETINAL GANGLION CELLS BORN IN THE CILIARY MARGINAL ZONE OF THE MAMMALIAN RETINA

F. Marcucci[1*], V. Murcia-Belmonte[2*], Qing Wang[1], T. Kuwajima[1], Celia Soares[1], Sania Khalid[1], S. Ferreiro-Galve[2], E. Herrera[2] and CA. Mason[1]

[1]. Department of Pathology and Cell Biology. College of Physicians and Surgeons. Columbia University. New York (USA).

[2]. Instituto de Neurociencias de Alicante (Consejo Superior de Investigaciones Científicas-Universidad Miguel Hernández, CSIC-UMH). San Juan de Alicante, Alicante (Spain).

As the eye-cup emerges and expands in size during retinal development, there is a wave of neurogenesis progressing from the central retina to the periphery. In lower vertebrates, after the onset of this central-to peripheral neurogenesis, neurons are added to the eye from a population of proliferating progenitors located at the retinal periphery in a stem cell niche termed the ciliary marginal zone (CMZ) that continues proliferating throughout adult life. In the adult mammalian retina CMZ cells lack this regenerative capacity of adding new cells to the periphery and it is not clear whether a number of retinal neurons derive from the developing CMZ. To test the capacity of CMZ to produce differentiated retinal neurons during development we have used a transgenic mouse reporter line that expresses GFP in the CMZ cells and have tracked them by time-lapse imaging. A number of CMZ cells move laterally to more central positions to finally locate into the layer of differentiated retinal ganglion cells (RGCs). In albinos, fewer ipsilaterally-projecting RGCs are produced, and the number of mitotic cells is also reduced in the ventral CMZ retina. These features suggested that a subpopulation of ipsilateral RGCs may derive from the ventral CMZ. We found that CyclinD2, a protein that regulates progression through the cell cycle, is highly expressed in the ventral CMZ of pigmented retina but its expression is significantly reduced in the albino retina. CyclinD2 deficient mice show a decrease in the number of ipsilateral RGCs and reduced ipsilateral projections. Together, these results reveal that the ventral CMZ may give rise to a distinct subpopulation of RGCs and that their generation is CyclinD2-dependent.

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

OLIGODENDROCYTE PRECURSOR CELLS ARE PHYSIOLOGICALLY HETEROGENEOUS: THE ESSENTIAL ONTOGENETIC LESSONS FOR BRAIN REPAIR

A. Bribián [1], EM. Medina-Rodríguez [1], C. Melero-Jerez [1], Machín I[1], V. Murcia-Belmonte [1,2], R. García de Sola[3], J. Pastor [3], F. de Castro [1].
[1] Grupo de Neurobiología del Desarrollo-GNDe, Hospital Nacional de Parapléjicos, Finca "La Peraleda" s/n, E-45071 Toledo, Spain.
[2] Laboratory of Development and assembly of bilateral neural circuits, Instituto de Neurociencias, Univ. Miguel Hernández-CSIC, 03550-San Juan (Alicante), Spain
[3] Servicio de Neurofisiología Clínica, Hospital Universitario La Princesa, Madrid, Spain

Oligodendrocyte precursor cells (OPCs) are the source of mature oligodendrocytes during CNS embryonic and postnatal development and, consequently, myelination. After oligodendroglionesesis, OPCs migrate towards their final destinations in an ordered migration regulated by growth factors, adhesive molecules and chemotropic cues.In the adult CNS, an important contingent of OPCs remains quiescent, playing relevant functions in physiological and pathological circumstances.The loss of myelin-forming oligodendrocytes is the main histopathological event in primary demyelinating diseases, like Multiple Sclerosis (MS), as well as in demyelination secondary to normal ageing, neurodegenerative diseases, craneoencephalic traumatism and spinal cord injury, for example. In these pathological scenarios, molecules involved in oligodendroglionenesis, OPC migration and myelination become up-regulated. The potential of adult OPCs and these up-regulated signals is evident for neural repair and singularly to develop repairing therapies in MS by potentiating spontaneous remyelination. While most of the studies in literature have been performed on OPCs isolated from neonatal or early postnatal cerebral cortex of rodents, there is an increasing bulk of evidences reporting the heterogeneity of OPCs when isolated form different species, CNS regions and ages. In the current work we systematically study the effects of known factors on the physiology of OPCs isolated from brain cortex at different stages of development and maturity, as well as from different mammal species (rat, mouse, human). We therefore demonstrate the OPCs heterogeneous physiology and how carefully it should be studied the type and stage of OPCs to test potential cell and supplementary therapies for MS and other diseases showing clinically significant loss of myelin in order to get effective myelin restoration and symptoms recovery.

This work was supported by the Spanish*Ministerio de Economía y Competitividad*(SAF2009-07845; SAF2012-40023; ADE10-0010; RD07-0060-2007 and RD12-0032/0012, and partially cofinanced by F.E.D.E.R., European Union, *"Unamanera de hacer Europa"*) and *Association pour la Recherche en Sclerose en Plaques-ARSEP* (France), all to FdC. IM and FdC are employees of SESCAM. AB and VM-B were employed under ADE10-0010. EM-R was supported by BES-2010-042593 and CM-J by BES-2013-062630 predoctoral contracts (associated to SAF2009-07845 and SAF2012-40023, respectively).

1ª: Neurobiología del Desarrollo
2ª: Trastornos y Reparación del Sistema Nervioso

ARMS/KIDINS220 TEMPORALLY COORDINATES NEUROTROPHIN-MEDIATED DIFFERENTIATION AND REGULATED SECRETION

S. López-Benito[1,3], J. Sánchez-Sánchez[1,3], L. Calvo[1,3], Á. Hernández[2], C. Lillo[1,3], S. Fernández-Fernández[4], J.P. Bolaños[4], P. Pérez[4], L. Tessarollo[5], M.V. Chao[6] and J.C. Arévalo[1,3]
[1:] Instituto de Neurociencias de Castilla y León (Salamanca, Spain)
[2:] Universidad de Salamanca (Salamanca, Spain)
[3:] Instituto de Investigación Biomédica (Salamanca, Spain)
[4:] Instituto de Biología Funcional y Genómica (Salamanca, Spain)
[5:] National Cancer Institute, Frederick (MD, USA)
[6:] Skirball Institute of Biomolecular Medicine (NY, USA)

During nervous system development secretion must be tightly controlled meanwhile neurons differentiate projecting axons and dendrites to their specific targets. Neurotrophins regulate, among other functions, differentiation and regulated secretion in the nervous system. However, the molecular mechanisms underlying the temporal coordination of differentiation and secretion by neurotrophins are unknown. Here we describe the involvement of ARMS/Kidins220, a downstream protein of Trk neurotrophin receptors, acting through Synembryn-B and Trio in the regulated secretion mediated by neurotrophins. We observed that PC12 differentiation and regulated secretion are temporally controlled since non-differentiated or differentiated neurons display a poor or strong regulated secretion in response to neurotrophins, respectively. Interestingly, high levels of ARMS/Kidins220 and Synembryn-B are required for differentiation, at a time when regulated secretion is minimal, whereas a strong downregulation of both proteins occurred once the cells are differentiated, at a time that regulated secretion is maximal. Manipulation of ARMS/Kidins220 and Synembryn-B levels in non-differentiated PC12 cells supports previous observations since overexpression or downregulation of both proteins blocks or potentiates NGF-mediated secretion, respectively. Similarly, regulated secretion of BDNF in response to NT-3 or NT-4 in cortical neurons augments with a concomitant downregulation of ARMS/Kidins220 and Synembryn-B protein levels. In addition, knockdown of ARMS/Kidins220 and Synembryn-B potentiated further BDNF regulated secretion. Finally, using an ARMS/Kidins220 conditional mouse line to downregulate ARMS/Kidins220 protein *in vivo*, we observed an accumulation of BDNF in the striatum coming from the cortex and hippocampus suggesting that more BDNF is released in the mutant animals.

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

DOPAMINERGIC GENETIC PROGRAM IS CONSERVED FROM NEMATODES TO MAMMALS

L. Remesal Gómez, L. Chirivella Clemente, Angela Jimeno, N. Flames Bonilla
Instituto de Biomedicina de Valencia. CSIC Valencia. Department of neurobiology and development.

Dopamine (DA) is one of the main neurotransmitters in the brain and regulates a variety of complex behaviors. All DA neurons, from any organism, express a battery of phylogenetically conserved genes which are involved in the synthesis, release and re-uptake of dopamine, termed "DA pathway genes". Using the model organism *C. elegans* our lab has found that a combination of three transcription factors (TFs) from the ETS, DLL and PBX families are required for DA pathway genes expression. Considering the evolutionary conservation of dopaminergic neurons we have started to explore the possibility that DA regulatory logic is conserved as well.

Interestingly, both ETS (Etv1/Er81) and DLL (Dlx2) TFs are already known to be required for mouse olfactory bulb (OB) DA differentiation. Thus, in this work we analyzed if worm orthologs of other mouse TF required in OB DA differentiation are also required in *C.elegans* DA differentiation. Surprisingly, RNAi against Pax6, Meis2 and CouptfI worm orthologs (*vab-3, unc-62* and *unc-55* respectively) impedes DA differentiation in *C.elegans*.

Thus, as the TFs required for OB DA differentiation seem to be the same in *C.elegans* and mouse, we next tested if, homologous to what we found in the worm, a PBX TF is also required for mouse OB differentiation.

Here we show that Pbx1 and Pbx2 are expressed in the OB DA neurons. Pbx2 mutants show normal numbers of DA neurons in the OB, whereas conditional Pbx1 mutants show a reduction in the number of DA neurons in the OB. Finally, ectopic expression of Pbx1 in the mouse OB is sufficient to induce dopaminergic differentiation.

Our results show an unprecedented degree of evolutionary conservation in the DA differentiation genetic program between mammals and nematodes. Moreover, this conservation has allowed us identify a new TFs (Pbx1) as a key determinant in OB DA differentiation.

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

SOXD GENES IN THE CONTROL OF DEVELOPMENTAL AND ADULT NEUROGENESIS

Alejandra C. Quiroga[1], Marco A. Cañizares[1], Fausto Vélez[1], M. Mar Muñiz[1], Ruth Diez del Corral[1], Silvia Nicolis[3], Véronique Lefebvre[2] and Aixa V. Morales[1].
[1]Instituto Cajal, (C.S.I.C), Madrid, Spain.
[2]Cleveland Clinic Lerner Research Institute, Cleveland, Ohio, USA
[3]University of Milano-Bicocca, Milano, Italy.

During the development of the nervous system, the generation of hundreds of subtypes of neurons and glial cells relies upon the relatively fast production, amplification, specification and differentiation of a pool of neural progenitors and neural stem cells (NSCs). Surprisingly, this strategy is retained to some extent in niches in the adult nervous system throughout lifetime under physiological conditions. Nevertheless, adult neurogenesis is very restricted, both in number and subtypes of neural cells generated. Although the molecular mechanisms involved in both embryonic and adult neurogenesis are conserved, it is not clear how the differences in the cell production rates and in the temporal extent of neurogenesis can be attained. One of the longer lasting neurogenic niches, both in mice and humans, is the subgranular zone (SGZ) of the dentate gyrus of the hippocampus that generates granule neurons involved in learning and memory processes.

Genes of the Sox family of transcription factors are essential during neurogenesis. In the developing spinal cord, we have determined that Sox5 controls cell cycle exit of neural progenitors and the specification of subtypes of dorsal interneurons, counteracting the Wnt signalling pathway (Martínez-Morales et al., 2010; Quiroga et al., 2014). Recent reports, in forebrain embryonic NSCs, point to a role of Sox6 in the maintenance of stemness of neural stem/progenitor populations (Ohta et al., 2013). More recently, we have characterized that both Sox5 and Sox6 are expressed in the majority of NSCs in the SGZ of the dentate gyrus of adult mouse hippocampus.

We are currently analysing the hypothesis that Sox5 and Sox6 control the activation, proliferation and/or stemness of NSCs during adult hippocampal neurogenesis activating similar genetic programs to those of embryonic neurogenesis and specific programs that could be at the core of the singularities of adult neurogenesis. For that purpose, we are using inducible targeted deletions: Sox5fl+/fl+ and Sox6fl+/fl+ mice crossed to a transgenic Sox2-cre-ERT2 line inducible by tamoxifen.

1. Developmental Neurobiology.

GENOARQUITECTURE OF THE EMBRYONIC REGION SURROUNDING THE PALLIO-SUBPALLIAL BOUNDARY

A. Abellán, L Medina
Facultat de Medicina, Universitat de Lleida, Institut de Recerca Biomèdica de Lleida (IRBLleida)

The pallio-subpallial boundary (psb) represents the most important limit of the telencephalon, acting like a barrier for the expression of transcription factors during early development. The embryonic progenitor domains neighboring the psb show different gene expression patterns. In mammals, the ventral pallium is found on the pallial side and shows early expression of Dbx1 and ER81, while the dorsal lateral ganglionic eminence (LGEd) is found on the subpallial side and expresses ER81 but not Dbx1. The genetic profile of the domains neighboring the psb seems partially different in non-mammals given the lack of Dbx1 expression in the ventral pallium in chicken (Bielle et al., 2005). To better understand the genetic profile and extension of the divisions neighboring the psb in mammals and non-mammals, we carried out a comparative study of the mRNA expression of Jagg1, Sfrp2, Dbx1, ER81, Sp8 and Dbx2 in the telencephalon of mouse and chicken. In mouse, Jagg1 and Sfrp2 are expressed in the vz of the ventral pallium, but neither in the dorsal nor lateral pallium. In contrast, the expression of these two genes in chicken is different, since Jagg1 is absent from the ventral pallium at least at early stages, and Sfrp2 is present in the vz throughout most of the pallium. On the subpallial side, Sp8 and ER81 are expressed in the dorsal striatal division (LGEd-like) of mouse and chicken. In contrast, Dbx2 is expressed in this striatal division in chicken, but not mouse. Thus, the Dbx1/2, Jagg1 and Sfrp2 genes have changed their expression around the psb in evolution, and data in reptiles are needed in order to know what was the ancestral condition. This also opens new venues for studying the funcions of such genes during forebrain development, and the evolutionary consequences of their expression change.

Sponsor: Spanish MINECO, grant no. BFU2012-33029

1. Neurobiología del Desarrollo

SOXD GENES IN THE CONTROL OF PATTERNING AND PROLIFERATION OF THE SPINAL CORD

Alejandra C. Quiroga[1], C. Claus Stolt[2], Ruth Diez del Corral[1], Spas Dimitrov[1], Elisabeth Sock[2], Michael Wegner[2] and Aixa V. Morales[1]
[1]Instituto Cajal, CSIC, Madrid, 28002, Spain.
[2]Institut für Biochemie, Universität Erlangen-Nürnberg, Erlangen, D-91054, Germany.

The basic organization of somatosensory circuits in the spinal cord is set up during the initial patterning of the dorsal neural tube. Extrinsic signals, such as Wnt and TGF-β pathways, activate combinatorial codes of transcription factors that are responsible for generating a pattern of discrete domains of dorsal progenitors (dp) and the dorsal interneurons (dI) that they generate. The Wnt/ βcatenin signaling pathway, acting in a graded fashion, controls specification of dp/dI1-3 progenitors and interneurons. However, it is not clear how this activity gradient is controlled and how the dI1-3 identities are differentially regulated. We have determined that two SoxD transcription factors, Sox5 and Sox6, are expressed in restricted domains of dorsal progenitors in the neural tube. Using gain- and loss-of function approaches in chicken embryos, we have established that Sox5 controls cell fate specification of progenitors dp2 and dp3 and, as a result, controls the correct number of the corresponding dorsal interneurons. Furthermore, Sox5 exerts its function by restricting dorsally Wnt signaling activity via direct transcriptional induction of the negative Wnt pathway regulator *Axin2*. By that way, Sox5 acts as a Wnt pathway modulator that contributes to sharpen the dorsal gradient of Wnt/βcatenin activity to control the distinction of two functionally distinct types of interneurons, dI2 and dI3 involved in the somatosensory relay. Finally, as Sox5 also controls cell cycle exit in neural progenitors, Sox5 emerges as an essential node in the transcriptional network that coordinates proliferation and dorsal patterning in the spinal cord.

1. Developmental Neurobiology.

UNDERSTANDING THE PATHOMECHANISMS OF PVNH RELATED TO FlnA DYSFUNCTION

C. Llinares Benadero, A. Cárdenas, M. Martínez-Martínez, C. de Juan Romero, V. Borrell
Instituto de Neurociencias de Alicante, CSIC - Universidad Miguel Hernández, Sant Joan d'Alacant, Alicante, Spain.

During embryogenesis, the developing cerebral cortex undergoes a dramatic expansion and folding. Alterations in cortical folding cause intellectual impairment and epileptogenic disorders, demonstrating its significant functional relevance. These pathologies are commonly related to defects in neuronal migration, such as periventricular nodular heterotopia (PVNH) where neurons accumulate ectopically in the vicinity of the telencephalic ventricles forming nodules, which act as epileptic foci. PVNH has been associated to mutations in the *FLNA* gene. Here, we first analyzed the expression patterns of *FlnA* in the cortical development of mouse and ferret, and observed the highest levels of expression in germinal layers in both animal models. Importantly, *FlnA* expression in ferret was heterogeneous along the Outer Subventricular Zone (OSVZ), being significantly higher in the prospective gyri compared to sulci. Next we performed gain- and loss-of-function experiments by in utero electroporation of full length *FlnA* and *FlnA*-shRNA, respectively, in E14.5 mouse embryos. Overexpression of FlnA seemed to have little effect on neuronal migration at early stages (E18.5), but then by P21 it caused the retention of some neurons in the white matter, unable to reach their final destination in the cortical plate. In contrast, loss-of-function of FlnA dramatically impaired radial migration at embryonic stages, but this seemed to have mild consequences postnatally. Interestingly, in both conditions we observed a reduction in the thickness of upper layers, suggesting a defect in neurogenesis or neuron survival. Similar phenotype was observed in electroporated ferrets. To investigate the dynamics of these defects in neuronal migration and in progenitor cell proliferation, we have set-up time-lapse videomicroscopy of cortical progenitor cell proliferation and radial neuron migration in living brain slices. We will use this system to interrogate the role of FlnA in the maintenance of radial glia cell integrity and in the neuron migration phenotypes.

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

EARLY DEVELOPMENT OF THE MESENCEPHALIC TRIGEMINAL NUCLEUS IN A BASAL GNATHOSTOME, THE SHARK *SCYLIORHINUS CANICULA*

S. Cardeña-Núñez[1], I. Rodríguez-Moldes Rey[1] and S. Pose-Méndez[2]
[1]Department of Cell Biology and Ecology. CIBUS-Faculty of Biology, University of Santiago de Compostela Santiago de Compostela A Coruña
[2]Department of Biology, College of Arts and Sciences, Univ. of Miami, Miami, Florida, USA

The mesencephalic nucleus of the trigeminal nerve (MesV) is located in the dorsal mesencephalon of jawed vertebrates (gnathostomes). It contains primary afferent neurons that innervate muscles of the jaw being the only primary sensory neurons located within the central nervous system. Studies in zebrafish, frog, chick and mouse have revealed that MesV neurons are the earliest ones born in the dorsal mesencephalon, and that their axons form part of the early scaffolding of the brain. These neurons ~~s~~hare embryonic lineage with neural crest cells, since they originate from the mesencephalic neural crest. The evolution of MesV appears related to that of jaws, but studies about its origin are lacking in basal vertebrates as cartilaginous fishes, the most ancient radiation of gnathostomes and thus closer to the ancestral condition.

To gain knowledge on embryonic development of MesV in basal gnathostomes, we have studied the optic tectum in embryos of the catshark *Scyliorhinus canicula* (representative model of cartilaginous fishes) using doublecortin (DCX) to reveal migrating neuroblasts. In early embryos (stage 25-28). DCX immunoreactive (ir) cells were exclusively observed just adjacent to the pial surface extending their processes longitudinally while, later on, labelled cells showing radial process were also abundantly observed in the tectum walls.

On the basis of their migratory pattern, time of origin and lack of processes to the ventricular layer, the early peripheral DCX-ir cells of the catshark optic tectum may be considered as neuroblasts precursors of the MesV. The early development of these cells and their peripheral location support their possible origin from mesencephalic neural crest. Because in the adult shark the MesV is characteristically located medially on the ventricular surface, the embryonic neurons of MesV are crucial to study out-inside migratory processes in the vertebrate brain.

Supported by Ministerio de Economía y Competitividad-FEDER (BFU2014-5863)

1. Neurobiología del Desarrollo
2. Neurociencia de sistemas

FUNCTIONAL ACTIVITY OF CORNEAL COLD SENSORY NERVE TERMINALS IN YOUNG AND AGED MICE AND BASAL TEARING RATE VARIATION

González-González, Omar[1]; Alcalde, Nacho[1]; Íñigo-Portugués, Almudena[1]; Bech, Federico[1]; Gallar, Juana[2]; Belmonte, Carlos[1,2]; Merayo-Lloves, Jesus[1]
[1].Instituto Universitario Fernández-Vega. Universidad de Oviedo. Spain.
[2].Instituto de Neurociencias. Universidad Miguel Hernández-CSIC. San Juan de Alicante. Spain.

Objectives: To determine the differences, in function electrophysiological properties, in mice corneal cold sensory nerve terminals with aging and their possible relation with variation in mice basal tearing rate.
Material & Methods: C57BL6/J mice of 3 and 24 months were studied. Mice were sacrificed by CO2 exposure. The excised eye was drawn in a solution similar to tear and bubbled with carbogen gas. A peltier device was used to control the temperature. Extracellular electrical activity of single sensory nerve endings of the corneal surface was recorded with an Ag/AgCl borosilicate micropipette electrode filled with the same solution and placed with slight suction into the cornea surface with a micromanipulator. All data obtained were filtered and analyzed with Spike2 software Basal tearing was measured in anesthetized animals using phenol red threads.
Results: Two different types of cold-sensitive nerve terminals were found in mice's cornea: Low- (LTC) ad High- (HTC) Threshold Cold fibers. LTC presented a low cooling threshold, high background activity, vigorous respond to cooling and were menthol activated. Their proportion was reduced from 37% in 3-months mice to 26% in 24-months old mice. On the other hand, HTC presented a high cooling threshold, very low background activity and weak response to cooling, being menthol activated too. Contrarily to LTC, HTC proportion increased from 17% in 3-months mice to 40% I 24-months old mice. Other cold population was found only in 24-months old mice, sharing characteristics with both groups: mixed-type cold endings (MTC). Basal tearing rate increased significantly in old mice.
Conclusions: The changes in percentage in LTC and MTC fibers, together with the apparition of MTC fibers in old mice, may suggest a change in the functional properties of at least one group of cold terminals, and may be related with the changes in basal tearing rate developed with aging.

1º Neurobiología del desarrollo.
2º Excitabilidad neuronal.

CHARACTERIZATION OF NEURAL POPOULATIONS EARLY GENERATED IN RMTW

Ll. Fortes-Marco, G. Requena-Vaquero, M.A. Albert-Maestro, M. Figueres-Oñate, J.A. de Carlos
Instituto Cajal - CSIC, Madrid

Brain development is a continuous regulated space-temporal process where specific brain regions achieved a neurogenic role during different developmental stages. Newborn cells are able to migrate radially to near destinations or tangentially for longer distances. A case of tangential migration are the neural cells produced in the rostromedial telencephalic wall (RMTW) that migrate rostro-caudally to populate the cortical preplate and established, during the development of this structure, in the subplate layer, as well as, in minor extent, in layers V and VI. RMTW is a pallial but extracortical structure that, in embryonic stages, produces several heterogeneous neuronal populations.

In this work we are characterizing the different cell populations generated in RMTW and checking whether there is a clonal relationship among different populations. To do so, we performed CFDA injections into the RMTW of embryonic (E10-E11) C57BL/J6 mice. Injections were executed in utero, guided by an ecographic device and the embryos allowed to born. After fixing the brains and cutting them in serial sections we did immunohistochemistry against some typical markers of interneurons and study if the labelled cells co-express the tracer CFDA.

To establish clonal relations we used the UbC-Star Track approach. It is based in the stochastic combination of 6 fluorescent proteins expressed both in cytoplasm and in cell nucleus, under a ubiquitous promoter (Ubiquitin C, UbC). With the incorporation of the Piggyback and the inducible Cre-Lox system we generated a stable and heritable color-code in those electroporated progenitors.

(This work is funded by grant BFU2013-49114 from Spanish Ministerio de Economía y Competitividad).

1ª: Neurobiología del Desarrollo
2ª: Nuevos Métodos y Tecnologías

POSTNATAL CHRONIC CLONIDINE TREATMENT EFFECTS ON OXYGEN CONSUMPTION AND ELECTROPHYSIOLOGICAL RECORDINGS (EMG, EKG, EEG) IN MICE

SY Prieto-Soler, JM Delgado-García, E Domínguez-del-Toro.
División de Neurociencias, Universidad Pablo de Olavide, Sevilla, Spain.

During postnatal development, a critical period for the neuroendocrine and behavioral development, α_2-adrenergic receptors raise their maximum expression values at the level of the brainstem, and have been proposed as possible regulators of different processes during development. Chronic treatment with clonidine (an agonist of α_2-adrenergic receptors) during postnatal development has been proposed as a model of REM sleep deprivation in rats. Neonatal manipulation of these receptors in rats has been demonstrated to have long lasting consequences in the adult, affecting reflex responses such as startle and pre-pulse inhibition.
During postnatal development in rats, the majorities of the studies sought after sleep/wake and REM/noREM cycles and were performed during the immediate effect of the drug. In a recent work, we have demonstrated that mice treated postnatally with clonidine show a general delay in psychomotor development, affecting execution of motor tasks, vestibular reflexes and general locomotor activity.
In this research, we have compared the short-term effects of treatment with clonidine in newborn mice (after application of the drug) with long term effects (24 hours) on various physiological parameters. We have shown that from P6, clonidine administration reduces immediately oxygen consumption by 50% and depresses the heart rate and percentage of REM sleep. 24 hours later, looking for long term effects, an increase in respiratory rate and percentage of REM sleep is detected from P10, with no effect on heart rate.

Keywords: EKG, EEG, sleep-wake cycle, heart rate, oxygen consumption, clonidine, postnatal, mice

1ª: Neurobiología del Desarrollo
2ª: Neurociencia de sistemas

STUDY OF GENE EXPRESSION PATTERNS IN THE DEVELOPING INNER EAR OF THE SHARK Scyliorhinus canicula.

S. Pereira-Guldrís[1], I. Rodríguez-Moldes[1], S. Mazan[2], S. Pose-Méndez[3]
[1]Department of Cell Biology and Ecology. CIBUS-Faculty of Biology, University of Santiago de Compostela
[2]CNRS, Development and Evolution of Vertebrates Group, Station Biologique, Roscoff, France
[3]Department of Biology, College of Arts and Sciences, University of Miami, US

The inner ear is an elaborated structure responsible for the detection of sound, balance and acceleration. Despite its complexity, the entire inner ear is formed from the otic placode, a thickened epithelium adjacent to the hindbrain that invaginates (in sharks, amphibians, birds and mammals) or cavitates (in teleosts and reptiles) to form the otic vesicle.

Our current knowledge about the development of inner ear mainly comes from studies in bony fishes (zebrafish), amphibians (Xenopus), birds (chick) and mammals (mouse) but similar studies are lacking in cartilaginous fish, the most ancient radiation of jawed vertebrates (gnathostomes). Because cartilaginous fish are closer to the ancestral condition of gnathostomes, studies in this group may shed light on the development of the gnathostome inner ear.

We have analyzed with in situ hybridization on whole embryos of S. canicula, the expression pattern of a variety of genes related to the inner ear formation, as Wnt5a, BF-1, Dlx2/5, Emx2, Emx3 and BMP4. We have found rather similar expression patterns to that described in other gnathostomes. ScWnt5a gene was expressed in the whole otic cup, especially next to the ganglion, where labeling was also observed. ScBF-1 appeared expressed only in the statoacoustic ganglion. In the otic vesicle, ScDlx2/5 were expressed in the dorsal part and ScBMP4 in the anterior and posterior ventral parts. Expression of ScEmx2 appeared all along the endolymphatic duct and that of ScEmx3 only appeared at its dorsal end.

These results revealed many similarities among cartilaginous fish, birds and mammals that reveal the conservation of the early developmental processes that take place during the inner ear development. Moreover, it highlights the usefulness of genoarchitectonic studies in this model species to gain knowledge about vertebrate ear evolution.

Supported by Ministerio de Economía y Competitividad-FEDER (BFU2014-5863).

1. Neurobiología del Desarrollo
2. Neurociencia de sistemas

GFAP IN THE RETINA OF SHARKS: A STUDY OF RADIAL GLIA DEVELOPMENT.

N. Sánchez Farías, I. Rodríguez-Moldes, E. Candal
Grupo BRAINSHARK, Centro de Investigación en Biología (CIBUS), Universidad de Santiago de Compostela (USC), Santiago de Compostela, A Coruña (nuria.sanchez@usc.es)

Radial glia (RG) is a class of undifferentiated progenitor cells originated from neuroepithelial cells. While these cells had been first considered as guiding cables for migrating neurons, they are now accepted to be the major source of neurons in several regions of the central nervous system (CNS) and later, after neurogenesis is complete, they shift towards differentiation into glial cells. The molecular mechanisms directing the switch from radial glial neurogenesis to gliogenesis are not completely understood.
The retina of *Scyliorhinus canicula* offers a unique model to analyze the processes underlying RG differentiation because of several reasons: (1) we have a good knowledge of neuronal proliferation and differentiation patterns based on previous studies; (2) neurogenesis occurs under an stereotyped pattern that give rise to a highly organized structure that contains five classes of neurons and one type of glial cell (Müller cells); (3) Müller glia shows properties typical of RG cells, including the expression of the glial fibrillary acidic protein (GFAP) and their late function as guide for neurons; (3) as in other fishes, it has an active stem cell zone, the ciliary marginal zone, wherein both neurons and Müller cells are added throughout the entire life of the animal; (4) *S. canicula* is a slow-growing species which allows a detailed monitoring of the changes that occur from neuroepithelial stem cells to undifferentiated RG and to differentiated Müller cells. We have analyzed the immunohistochemical pattern of GFAP and we have explored its emergence and localization with respect to the proliferation marker proliferating nuclear antigen (PCNA), the neuronal migration marker doublecortin (DCX), and the neuronal differentiation marker HuC/D. GFAP was expressed in both proliferating (PCNA-immunoreactive) cells in the early retina and in Müller cells that serve as guide for migrating (DCX-immunoreactive) cells within the mature retina. GFAP was never observed in mature (HuC/D-immunoreactive) neurons.

Supported Ministerio de Ciencia e Innovación (BFU2010-15816), European Community-Research Infrastructure Action under the FP7 “Capacities” Specific Programme (ASSEMBLE 227799), and Ministerio de Ciencia e Innovación (BFU2014-5863).

1. Neurobiología del Desarrollo

AMYLOID PRECURSOR PROTEIN (APP) AFFECTS CELL FATE SPECIFICATION OF HUMAN NEURAL STEM CELLS

R. Coronel[1], M. Dominguez[1], A. Revilla[1], I. Ocaña[1], P. Sainz[1], A. Borges[1], C. González[1], A. Martínez-Serrano[2], E. Cano[3], I. Liste[1]

[1].Unidad de Regeneración Neural, Unidad Funcional de Investigación de Enfermedades Crónicas. Instituto de Salud Carlos III (ISCIII), Majadahonda, Madrid

[2]. Center of Molecular Biology "Severo Ochoa" (CBMSO), Universidad Autónoma de Madrid-Consejo Superior de Investigaciones Científicas (UAM-CSIC), Madrid, Spain.

[3].Unidad de Neuroinflamación. Unidad Funcional de Investigación de Enfermedades Crónicas. Instituto de Salud Carlos III (ISCIII), Majadahonda, Madrid

The involvement of the Amyloid Precursor Protein (APP) in Alzheimer Disease (AD) is well known nowadays; although its normal biological function remains still unclear. Some studies have shown that APP stimulates the proliferation and neuronal differentiation of Neural Stem Cells (NSCs), while other works have reported an increased gliogenesis in these cells.

We have found abundant expression of APP in human NSCs (hNSCs), both under division and along the differentiation time; suggesting a possible role of APP in these processes. At cellular level, APP expression is more abundant in neural precursor- and glial cells, while it is lower in neurons as these differentiate and mature.

To investigate the potential role that APP plays in hNSCs fate decisions and differentiation, we transiently overexpressed APP in these cells, analyzing the cell intrinsic effects.

We have found that hNSCs over expressing APP exit the cell cycle earlier than the controls, differentiating mainly to glial phenotype, while significantly decrease neuronal differentiation. These effects could be mediated, at least in part, by the C-terminal domine of APP (AICD), by its molecular interaction with different target genes.

These results indicate an action of APP modulating hNSCs differentiation, and may be thus important for the future development of stem cell therapy strategies for the diseased mammalian brain. And may have implications in the pathophysiology of diseases where APP abundance or metabolism is altered such as Down syndrome or AD.

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

THE PRETHALAMIC EMINENCE IS A SOURCE OF GLUTAMATERGIC NEURONS THAT POPULATE PARTS OF THE TELENCEPHALIC COMMISSURAL PLATE AND PREOPTIC AREA.

Alonso A.[1], Puelles L[1] and Trujillo C.M.[2]
[1.] School of Medicine. University of Murcia and IMIB. Murcia. Spain
[2.] School of Biology. University of La Laguna. Canary Island. Spain.

It is becoming increasingly clear that many sorts of neuroblasts reach their final locations in the brain wall following tangential migration pathways. Various tangential migrations take place between the subpallium and the pallium. We report here experimental data obtained with quail/chick grafts on tangential migrations involving as a source the prethalamic eminence (PThE), a caudal neighbor of the telencephalon, and various telencephalic sites as targets. Some earlier studies mentioned such migrations in mammalian embryos; for instance, Puelles and collaborators (2000) suggested that cells from the PThE migrate to the POA in the mouse.
The aim of our work was to examine whether the avian PThE is a source of migratory neurons targeting the subpallium. To this aim, we performed homotopic and homochronic PThE transplants between quail and chick embryos, at early developmental stages HH10/HH11, and we sacrificed the chimeras at different intermediate developmental stages, mapping the grafted quail tissue by means of QCPN immunochemistry. Some chimeric brains were processed by *in situ* hybridization, using mRNA probes to detect *Tbr1* (a characteristic PThE marker, largely absent in the telencephalic subpallium). The analysis of chimeric brains in which the presumptive PThE territory was grafted demonstrated that numerous eminential cells migrate tangentially out of the PThE, and enter the caudal subpallium. *Tbr1*/QCPN double-positive subpallial cells appeared in the preoptic area (POA), the diagonal area (Dg; e.g., the basal nucleus of Meynert), various septal areas such as the commissural septal nucleus, the triangular septal nucleus, the nucleus of the hippocampal commissure (HiC), and the nucleus of the anterior commissure. We conclude that the PThE is a source of glutamatergic neurons that populate parts of the POA, Dg and the telencephalic septal commissural plate nuclei.

1ª: Neurobiología del Desarrollo

CRB2 CONCLUDES THE POLARIZATION PROCESS IN RETINAL PIGMENT EPITHELIAL CELLS

A. Escudero Paniagua, J. Fernández. Dolón, V. Valle Álvarez, A. Velasco Arranz, C. Lillo Delgado
Instituto de Neurociencias de Castilla y León, Universidad de Salamanca, IBSAL, Salamanca

The retinal pigment epithelium (RPE) is a cellular monolayer embracing the outer segments of the photoreceptor cells with many important roles, such as controlling the retinal homeostasis. Most of its functions are performed thanks to its cell polarity. Two protein complexes provide the apical polarity to polarized cells, the Crumbs and PAR complexes. To date, all members of these complexes except for the transmembrane proteins CRB have been detected in the RPE, and little is known about their role in this tissue. Our aim is to characterize the expression and location of these protein complexes, including the CRB proteins, a hitherto observation, in the mature RPE, as well as during the polarization process. To do that, we have first generated and characterized two new antibodies to distinguish CRB2 and CRB3 proteins. We have then determined the expression and location of these proteins as well as those of the PAR complex in the adult mouse RPE. We have also developed and characterized a human RPE polarized cell culture showing molecular and physiological features of a highly polarized epithelium. This culture model allowed us to characterize the expression and localization pattern of the apical polarity complexes and formation of cell-cell junctions at different polarization stages. All the proteins of the PAR and Crumbs complexes were found at the tight junctions in the adult mouse RPE as well as in fully polarized RPE cells in culture. However, during the polarization process, the PAR complex proteins are the first to be expressed together with the adherens junctions proteins followed by the expression of some of the Crumbs complex and tight junctions proteins. Finally, at the latest stages when cells acquire fully polarized features, CRB2 is located at the tight junctions, together with the rest of the apical complexes proteins. Therefore, the RPE expresses all the members of the apical polarity complexes, Crumbs and PAR, including the CRB2 and CRB3 proteins. These proteins are sequentially located at the cell junctions during the polarization process concluding with the positioning of CRB2 at the RPE tight junctions.

1ª: Neurociencia de sistemas
2ª: Neurobiología del Desarrollo

CPT1C and Atlastin-1 are required for ER morphology and axonal development.

M.Palomo Guerrero[1], MC Grijota Martínez[1], N.Casals Farré[1]
[1]Basic Sciences Department, Faculty of Medicine and Health Sciences, Universitat Internacional de Catalunya, Sant Cugat del Vallés, Barcelona, Spain

Introduction: Hereditary spastic paraplegias (HSPs) are a group of neurological disorders which appears as a result of axonopathy in the corticospinal tract. Genetic analysis has identified more than 50 different loci involved in HSP. Mutations in SPAST, Protrudin, Atlastin-1 (ATL-1) and REEP are responsible for almost 60% of HSP cases. These four genes encode proteins that have a crucial role in shaping the endoplasmic reticulum (ER). As a result of this, any alteration in them could produce abnormalities in the ER function.
It has recently been shown the interaction between ATL-1 and the brain specific isoform carnitine palmitoyl-transferase 1C (CPT1C). CPT1C is widely expressed through the central nervous system including hypothalamus, hippocampus and cortex, and localizes to the ER. Our group has demonstrated that CPT1C KO mice have impaired motor coordination, and reduced locomotor activity and muscle strength.
Recently, it has also been identified a CPT1C mutation in a family with pure HSP. This mutation induces structural perturbations in the protein.
Our hypothesis is that CPT1C interacts with ATL-1 to assist in the formation and maintenance of long cellular processes, suggesting that CPT1C plays a role in axonal development.
Objetives: To study the role of CPT1C in axonal development.
Methods: Primary cultures of mouse cortical neurons were prepared from E-16 WT or KO CPT1C embryos to study axonal morphology. Immunoprecipitation studies in brain samples were used to confirm the endogenous interactions between CPT1C and ATL-1. The HEK293T cell line was used for ER morphology analysis.
Results: We show that CPT1C KO axons have morphological alterations and exhibit a development delay compared to WT axons. In addition, we demonstrate the endogenous interaction between CPT1C and ATL-1.
Conclusion: CPT1C binds ATL-1 in the brain and is involved in axonal development.

1. Developmental Neurobiology
2. Disorders and nervous system repair

ROLE OF REELIN IN SYNAPTOGENESIS, SYNAPTIC STABILIZATION AND ASTROCYTIC ENSHEATHMENT IN THE ADULT BRAIN

C. Bosch [1], D. Expósito-Alonso [1], A. Martínez [1], N. Masachs [1], C. M. Teixeira [1], I. Fernaud [2,3], F. Ulloa [1], E. Pérez-Martínez [1], C. Lois [4], J. X. Comella [5], J. DeFelipe [2,3], A. Merchán-Pérez [2,6], E. Soriano [1]

[1] Developmental Neurobiology and Regeneration Unit, Department of Cell Biology and Parc Científic de Barcelona, University of Barcelona, Barcelona, Spain

[2] Laboratorio Cajal de Circuitos Corticales, Centro de Tecnología Biomédica, Universidad Politécnica de Madrid, Madrid, Spain

[3] Instituto Cajal (Consejo Superior de Investigaciones Científicas), Madrid, Spain

[4] Department of Neurobiology, University of Massachusetts Medical School, Worcester, Massachusetts, USA

[5] Institut de Neurociències, Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Barcelona, Spain

[6] Departamento de Arquitectura y Tecnología de Sistemas Informáticos, Escuela Técnica Superior de Ingenieros Informáticos, Universidad Politécnica de Madrid, Madrid, Spain

Adult neurogenesis in the dentate gyrus is essential for learning and memory, but the mechanisms controlling synaptogenesis of adult-born granule cells (GCs) and their integration in neural networks remain poorly understood. To decipher the logic behind neural assembly, the complex 3D architecture of neuronal samples calls for their reconstruction from serial sections. Here we show that focused ion beam/scanning electron microscopy (FIB/SEM) allows efficient, complete, and automatic 3D reconstruction of identified dendritic spines from GFP/DAB-labeled neurons, their presynaptic partners and perisynaptic astrocytic processes. We applied this technology to analyze the synapse formation and stabilization of labeled adult-generated GCs in control mice and up- and down-regulation of the Reelin signaling pathway. Connectomics analysis and 3D reconstructions of dendritic spines on new-born GCs revealed two different stages of spine development and unexpected features of synapse formation (vacant and branched spines, and presynaptic terminals establishing synapses with up to 10 spines), as well as varied morphological alterations in the synapse development associated with Reelin dysregulation. Intensive perimeter quantifications of neuronal elements enwrapped by astrocytes showed variations in the perisynaptic ensheathment depending on neuronal age and Reelin signaling pathway, which suggests a role of Reelin protein in astrocytes, hence synaptic stabilization. Together, these findings point to FIB/SEM representing a fundamental step in the field of ultrastructural analysis due to its reliability, efficiency and high resolution, and the wide use of DAB in conventional EM. This technology allows the characterization of identified synaptic circuits in neurons in a high-throughput manner, which is crucial for the study of underlying molecular and cellular mechanisms of brain wiring.

1: Developmental neurobiology.
2: New methods and technologies.

NEUROGENESIS OF GLUTAMATERGIC CELLS DURING DEVELOPMENT OF THE TELENCEPHALON OF *SCYLIORHINUS CANICULA*. AN EVOLUTIONARY APPROACH.

A.Docampo-Seara, MA. Rodríguez, I. Rodríguez-Moldes
Departament of Cell Biology and Ecology. University of Santiago de Compostela. A Coruña.

Neurogenesis is the transformation of progenitor cells into terminally differentiated neurons which takes place during the development of nervous system and adulthood. The dorsal part of the telencephalon (pallium) presents neurogenesis during the lifespan in all vertebrates. Previous studies of our research group have revealed that the pallium of sharks, as in mammals, contains GABAergic (inhibitory) neurons that originate in the ventral telencephalon (subpallium), which revealed that the neurogenesis of pallial GABAergic is rather conserved. However there are not studies in sharks about the origin, development and organization of the system of glutamatergic (excitatory) neurons of the pallium.

In the present study we study the origin and distribution of glutamatergic cells in the developing and adult pallium of the shark *Scyliorhinus canicula* (a basal vertebrate whose phylogenic position makes it essential in assessing the ancestral organization of the vertebrate brain) by using antibodies against glutamate and Tbr1 as marker of glutamatergic differentiation. As it is well established that in the mammalian telencephalon glutamatergic neurogenesis follows a multistep processes (cell proliferation, migration and differentiation) in which specific molecules operate [Hodge et al., Cell. Mol. Life Sci. (2012) 69:2125], we have comparatively analyzed the spatiotemporal distribution of immunomarkers of cell proliferation, migration and neural differentiation (PCNA, Pax6, DCX, NeuroD and calretinin) in embryos at key stages in the morphogenesis of the telencephalon: stage 31, when the basic regionalization occurs, and stage 32, when the basic mature structure of the telencephalon is achieved.

Our results reveal the pallial origin of glutamatergic cells of the dorsal telencephalon of *S. canicula*, and that the transformation of pallial progenitors of glutamatergic cells into differentiated neurons could progress following a similar sequence to that described in other vertebrates, thus suggesting that molecular mechanisms underlying glutamatergic neurogenesis are rather conserved and present in basal vertebrates.

Supported by Ministerio de Economía y Competitividad-FEDER (BFU2014-5863)

1. Neurobiología del Desarrollo
2. Neurociencia de sistemas

FGF8 CONTRIBUTION IN THE SPECIFICATION AND DIFFERENTATION OF THE HYPOTHALAMUS

C. Estornut [1], A. Andreu [2], Y. Sabuco [1], S. Martínez [1,3], E. Puelles[1], D. Echevarría[1]
[1]. Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-CSIC, 03550, Alicante, Spain
[2]. CNRS UMR7622, Université Pierre et Marie Curie, 9 Quai Saint-Bernard, 75252 Paris Cedex 05, France.
[3]. IMIB-Arrixaca, University of Murcia, 30071 Murcia, Spain.

Secreted molecules produced by patterning centers (secondary organizers) regulate neural tube morphogenesis. In the forebrain multiple patterning centers are juxtaposed in developing tissues to provide qualitatively distinct signals that regulate regional identity and growth. Among these signals Fibroblast growth factor 8 (FGF8) functions to coordinate these anterior secondary organizers at which modifications of relative strength of FGF signaling can have profound effects in regional identity and growth.
We have examined in detailed from E11.5 to E17.5 mouse embryos the role of FGF8 during the development of the hypothalamic region by genetically known down *Fgf*8 expression in the territory of *Nkx2.1* transcription factor (a homeobox-containing gene member of the vertebrate Nkx family, expressed in proliferative and postmitotic zones of the forebrain that give rise to a large region of the subpallium and to the hypothalamus).
Conditional deficiency of *Fgf8* in these mutant mice ($Nkx2.1^{Cre/+}/Fgf8^{flox/-}$) resulted in a disruption of ventro-caudal midline region of the basal hypothalamic region (acroterminal and terminal hypothalamic subdivision). This disruption consisted in the loss of midline nuclei such as median eminence, infundibular region and neurohypophisis pouch and a fusion of ventral arquate nuclei and mammillary tubercle.
This Work is supported by ‘‘Ministerio de Economía y Competitividad’’ BFU2013-48230-P (FEDER Funds).

1ª: Neurobiología del Desarrollo
2ª: Neurobiología del Desarrollo

ENVIRONMENTAL ENRICHMENT REVERTS NEUROVASCULAR AND COGNITIVE EFFECTS PRODUCED AFTER VEGFR-2 BLOCKADE THROUGHOUT BDNF-TRKB PATHWAY

H. Bengoetxea [1], I. Rico-Barrio [1], N. Ortuzar [1], JV. Lafuente [1]
[1]. Department of Neuroscience, University of the Basque Country (UPV/EHU), Leioa, Bizkaia

Enriched environment improves recovery from brain injury due to, among other, increased neurotrophic factors expression, such as VEGF or BDNF. Through these neurotrophins, important cortical and hippocampal cellular changes occur. Vandetanib is a tyrosine kinase inhibitor that targets vascular endothelial growth factor receptor 2 (VEGFR-2) signalling, which apart from angiogenesis processes is involved in neuroprotective, neurotrophic and neurogenic pathways. Our aim was to investigate the effectiveness of the enriched environment counteracting cognitive and cellular effects after VEGFR-2 blockade during the critical period of the rat visual cortex.
Long Evans rats were reared under two different conditions: standard laboratory condition and enriched environment, both groups received vandetanib or vehicle from P21 to P28. Visuospatial learning was tested with Morris Water Maze. Neuronal, interneuronal and vascular densities were measured by inmunohistochemistry and histochemistry techniques. Quantifications were performed in the hippocampus and in the visual cortex. Akt, Erk, BDNF and TrkB were measured by western blot technique.
Results showed that vandetanib produces a significant decrease in vascular and neuronal densities in the primary visual cortex as well as in dentate gyrus. And in addition a reduction in phospho-Akt/Akt and phospho-Erk/Erk expression levels, molecules involved in survival and proliferation processes respectively. These results leaded to a cognitive impairment in visuospatial test. On the other hand, animals reared in an enriched environment are able to revert the negative effects induced by VEGFR-2 blockade, activating PI3K-AKT and MAP kinase pathways mediated by BDNF-TrkB binding.
Present results provide novel and consistent evidences about the usefulness of living in enriched environment as a strategy to improve deleterious effects generated by VEGFR-2 blockade and the notable role of the BDNF-TrkB pathway to balance the neurovascular unit and cognitive effects.

1ª: Neurobiología del Desarrollo
2ª: Neurociencia de sistemas

AUTONOMIC SYSTEM REGULATION IS ALTERED IN CHILDREN WITH CEREBRAL PALSY DURING DAILY LIFE ACTIVITIES: A 24-HOURS ECOLOGICAL STUDY

Riquelme[1], S. Belmonte[1], B. Sáez[1], J. Rechach[1], R. Silva do Rosario[1], J. López[1], A. Vicens[1], MC. Abraham[2], K. Vehmaskoski[3], P. Natunen[3], P. Montoya[1]
[1] University of the Balearic Islands, Palma de Mallorca, Spain
[3] ASPACE-Baleares, Palma de Mallorca, Spain
[4] University of Applied Sciences, Jyvaskyla, Finland

Objetives. Although cerebral palsy has been described mainly as a motor disability disorder, this pathology is usually associated with concomitant disorders such as the dysfunction of the Autonomous Nervous System. Individuals with cerebral palsy have shown disturbed sympathovagal balance compared with healthy controls. Moreover, physical activity, which has been associated to a parasympathetic predominance, is less intense in children with cerebral palsy than in their healthy peers. The assessment of autonomic function is usually based on controlled laboratory examination, that may not be relevant for activities that occur in daily life, resulting in poor ecological validity and difficulties in interpretation of the results. The aim of the present study was comparing autonomic regulation and physical activity between children with cerebral palsy and healthy age-paired children during their daily life activities. **Methods.** 22 children with spastic cerebral palsy and 24 healthy peers undertook a 24-hour measure of heart rates and physical movement by using a cardiac measure device (Fistbeat) and an accelerometer (Actiwatch). Entropy analyses of heart rate variability, as an indirect measure of the autononomic nervous system regulation, and accelerometry signals were performed. **Results.** Entropy of heart rate variability and accelerometer signals were higher in children with cerebral palsy than in healthy children. Most of the accelerometry scale differences were displayed during the sleep and during activities in the afternoon. **Conclusions.** Autonomic nervous system regulation seems altered in children with cerebral palsy. Further research is warranted to deepen into the comorbidities that may affect autonomic regulation in this population. **Funds.** This research has been funded by the Regional Council of Education, Culture and Universities of the Goverment of the Balearic Islands and European Regional Development Funds (AAEE23/2014).

1. Neurobiología del desarrollo
2. Trastornos y reparación del sistema nervioso.

$GABA_A$ RECEPTOR EXPRESSION IN OLIGODENDROCYTES IS REGULATED BY AXON-TO-GLIA INTERACTION

M.V. Sánchez-Gómez [1], R.O.Arellano [1,2], E. Alberdi [1], M. Canedo-Antelo [1], A. Palomino [1], A. Pérez-Samartín [1], C. Matute [1]

[1] Achucarro Basque Center for Neuroscience, CIBERNED y Facultad de Medicina y Odontología, Universidad del País Vasco (UPV/EHU), Leioa, Bizkaia

[2] Instituto de Neurobiología, Universidad Nacional Autónoma de México, Juriquilla, Querétaro

Axon myelination is the main function of oligodendrocytes (OLG) in the central nervous system. This highly concerted phenomenon requires of glia-neuron communication, and both chemical transmitters and contact interactions are essential for myelination. OLG are endowed with neurotransmitter receptors whose levels and properties vary during maturation and myelination. However, knowledge about how OLG-neuron interactions regulate those changes is scant. Here, we studied the expression and function of neurotransmitter receptors in oligodendrocyte progenitors (OPC) and OLG from the optic nerve in cells cultured alone or in the presence of dorsal root ganglion (DRG) neurons. Cell responses to GABA, glutamate (Glut), and ATP were recorded by patch-clamp at different days in vitro. Both OPCs and OLGs cultured alone in differentiation medium showed inward currents to these transmitters. Intriguingly, sensitivity to GABA drastically diminished to less than 10% in both cell types after 2 days in culture, while that of Glut and ATP remained constant. In contrast, the amplitude of the GABA responses was unalterably high in either OPC or OLG co-cultured with DRG neurons. Immunochemistry and electrical properties indicated that OLGs that were responsive to GABA in co-cultures with neurons were engaged in axon myelination, whereas OLGs in the same culture but without axonal contact lost GABA sensitivity. Response was mediated by $GABA_A$ receptors with a distinctive pharmacology (likely formed by α3, β2/3 and γ1/3 subunits) and its activation elicited an increase in intracellular Ca^{2+} concentration, according with previous studies. The results strongly suggested that GABA receptor regulation may be relevant to OLG maturation and to OLG-neuron signalling.

Supported by CIBERNED, MINECO, Gobierno Vasco, Bizkaia Talent and PASPA-UNAM.

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

NEW BRAIN TARGETS FOR TREATING ADDICTION: IT'S NOT ONLY DOPAMINE

Jose Miñarro López. Department Of Psychobiology, University of Valencia

Drug addiction remains as one of the most relevant public health problems in the world. Despite our deep knowledge in reward-related mechanisms, few effective therapies are authorized for the treatment of addicted patients. Research oriented exclusively to dopamine transmission-related events have limited the possibilities of understanding alternative processes that in the brain might modulate drug seeking, drug self-administration or associative learning leading to context-related relapse. In the present symposium we will present four alternative targets that modulates drug addiction-related processes. They range from peptide regulating feeding to nuclear receptors (PPARy), bioactive lipids derived from membrane phospholipids (Lysophospholipids, endocannabinoids and acylethanolamides), or their receptors (CB2 or LPA1 receptors). Interestingly, the research developed around these brain targets has been demonstrated to be efficient in preclinical models. Some of these new targets are already under experimental research in humans. Overall, this new look to addiction processes stress the importance of developing integrative neuroscience strategies to unveil the utility of targeting molecular processes apparently located far away of motivational circuits regulating drug consumption.

Topic 2.

Neuronal excitability, synapses and glia

BIDIRECTIONAL MODULATION OF SOCIABILITY BY PIP_3 PATHWAY

C. Sánchez-Puelles[1], C. Venero[2], M. Serrano[3], R. Luján[4], J. A. Esteban[1], S. Knafo[5]
1. Centro de Biología Molecular "Severo Ochoa", Consejo Superior de Investigaciones Científicas (CSIC) / Universidad Autónoma de Madrid, Madrid, Spain.
2. Facultad de Psicología, Universidad Nacional de Educación a Distancia, Madrid, Spain.
3. Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain.
4. Facultad de Medicina, Instituto de Investigación en Discapacidades Neurológicas (IDINE), Universidad de Castilla-La Mancha, Albacete, Spain.
5. Unidad de Biofísica CSIC-UPV/EHU, Universidad del País Vasco, Bilbao, Basque Country, Spain.

Sociability is a complex behavior controlled by multiple brain regions, like the amygdala and the hippocampus. Alterations in this behavior are related to autistic spectrum disorders. In particular, high rates of Pten (phosphatase and tensin homologue deleted on chromosome 10) mutations have been found within the human autistic population. Additionally, mice carrying a Pten deletion show autistic-like behavior. PTEN is a negative regulator of the PIP_3 pathway and is highly expressed in the brain where it controls growth, proliferation, synaptogenesis and synaptic plasticity. Nevertheless, it is still unclear how PTEN influences social behavior. To further explore this we have employed a transgenic mouse presenting moderate overexpression of PTEN (*Pten*^{tg} mice, M. Serrano's laboratory). The PIP_3 pathway is downregulated in these mice, leading to a reduced growth, with smaller brain and reduced cell number in hippocampus and amygdala. At the structural level, *Pten*^{tg} mice display decreased synapse density in both structures. In addition, synapses are smaller in the amygdala. Anatomical changes in *Pten*^{tg} mice were correlated with alterations in synaptic function using electrophysiological recordings. Thus, *Pten*^{tg} mice present depressed excitatory basal synaptic transmission in hippocampus and altered both excitatory and inhibitory transmission in the amygdala. From a behavioral point of view, *Pten*^{tg} mice display lower anxiety, higher sociability, and impaired amygdala-dependent fear memory. In contrast, hippocampal dependent-memory is normal. To conclude, this study strengthens the role of PTEN in synapse organization and function and leads us to hypothesize a bidirectional modulation of social behavior by PIP_3 pathway.

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: Neurociencia cognitiva y conductual.

First author: Cristina Sánchez-Puelles, e-mail: csanchezpuelles@cbm.csic.es

REGULATION OF THE ENDOSOMAL PROTEIN RAB11-FIP2 DURING SYNAPTIC PLASTICITY PROCESSES IN THE HIPPOCAMPUS

Y. Gutiérrez Gonzalo[1], M. Royo Cantabrana[1,2], J. A. Esteban García[1]
[1] Centro de Biología Molecular "Severo Ochoa". Consejo Superior de Investigaciones Científicas (CSIC)-Universidad Autónoma de Madrid (UAM). Madrid. Spain.
[2] Champalimaud Neuroscience Programme, Champalimaud Centre for the Unknown. Lisbon. Portugal.

Synaptic plasticity mechanisms are widely studied as the cellular and molecular basis for learning and memory. A fundamental mechanism is the transport of AMPA-type glutamate receptors to and from the synapse in response to changes in neuronal activity. This continuous turnover of membrane proteins is mediated by Rab11-driven endosomal trafficking. We have investigated the role of the Rab11 interacting protein FIP2 as a modulator of endosomal trafficking during synaptic plasticity.
We have shown that FIP2 retains receptors in extrasynaptic compartments different from the recycling endosomes and independently from its interaction with Rab11. This retention is mediated by an interaction between FIP2 and the GluA1 subunit of the AMPA receptors in basal conditions. This complex is then dissociated after the induction of long-term potentiation (LTP). Using biochemical tools we have demonstrated that, in hippocampal neurons, FIP2 is endogenously phosphorylated and this phosphorylation is regulated during synaptic plasticity processes. Because this phosphorylation could modulate the action of FIP2 during synaptic plasticity, we have performed biochemical experiments in the presence of pharmacological inhibitors to explore the intracellular signaling pathways that mediate this regulation. Furthermore, by performing electrophysiological recordings from hippocampal neurons expressing FIP2 phosphorylation mutants or an shRNA against FIP2, we have also studied the functional consequences of FIP2 modulation during synaptic plasticity processes, both LTP and long-term depression (LTD).

Overall, these experiments have allowed us to propose a model in which FIP2 is subject to regulation during plasticity induction and is a key component of an active retention-release mechanism of AMPA receptors.

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

ROLE OF OCULOCEREBRORENAL LOWE SYNDROME PROTEIN (OCRL) IN SYNAPTIC FUNCTION

N. Mohammed El Demerdash, MónicaFernández-Monreal
Instituto de Investigación Puerta de Hierro de Majadahonda, Hospital Universitario Puerta de Hierro de Majadahonda, 28222, Madrid, Spain

Keywords: OCRL, Phosphoinositides, Protein trafficking

The enzyme OCRL is one of the main Inositol-5-phosphatases that regulate the levels of cellular Phosphatidylinositol-(4,5)-biphosphate. Mutations in OCRL gene cause Lowe Syndrome, an X-linked rare disease characterised by eye alterations, renal dysfunction and mental disease. Research in cell lines and fibroblasts from Lowe syndrome patients have shown that OCRL is mainly localized in Golgi, and have evidenced its participation in protein trafficking between plasma membrane and intracellular compartments. However, its function in the Central Nervous System is mostly unknown.

We aim to characterized OCRL distribution and function in brain using molecular biology, biochemistry and fluorescent microscopy. We have found that OCRL expression increases during development in the hippocampus in parallel with synaptic proteins, which suggest a role in synaptic function regulation. In addition, we have localized OCRL protein in synapses of hippocampal neurons *in vitro*.

On the other hand, we have investigated the cellular defects in neurons depleted on OCRL by using lentivirus driving the expression of shRNA for OCRL. We have found that young neurons deficient in OCRL presented alterations on actin cytoskeleton. Interestingly, OCRL depletion did not affect synaptic density in cultured hippocampal neurons. Furthermore, electrophysiological recordings onorganotypic slices infected with the shRNA lentivirus showed that basal transmission was not affected by OCRL depletion. Finally, we have found that OCRL was involved in several forms of synaptic plasticity. These results are in agreement with the role of phosphoinositide metabolism in synaptic function, and could explain some of the neuropathological features of Lowe syndrome.

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

2. 2ª: Neurociencia de sistemas

Financial Support
1- Public funds (Spanish Ministry of Economy and CompetitivenessSAF2013-40802-R)
2- Private funds (Fundación de Investigación Biomédica Puerta de Hierro 2014-2017)

ALTERATION IN THE BIDIRECTIONAL COMMUNICATION BETWEEN ASTROCYTES AND NEURONS IN ALZHEIMER'S DISEASE

M. Navarrete[1], J.A. Esteban[1].
Centro de Biología Molecular "Severo Ochoa", CSIC, Madrid

Recent evidence indicates the existence of bidirectional communication between astrocytes and neurons, and suggests a role for astrocytes in the physiology of the nervous system. Astrocytes respond with intracellular Ca^{2+} elevations to neurotransmitters released from synaptic terminals, and modulate neuronal activity and synaptic transmission through the release of gliotransmitters. Alzheimer's disease (AD) is a complex neurodegenerative disorder of the central nervous system manifested by cognitive impairment and behavioral disorders. It is the most common form of dementia. However, the involvement of neuron-glia communication in AD is poorly known.

One unexplored neuronal signal that could potentially impact astrocyte-to-neuron communication is AD toxicant amyloid-beta (Aβ), since it has been demonstrated that synaptic activity leads to increased interstitial Aβ under normal physiological conditions

Using electrophysiological and Ca^{2+} imaging techniques in rat hippocampal slices, we have found that bath application of Aβ, the toxic trigger for AD, induces calcium elevations in *stratum-radiatum* astrocytes and evoked NMDAR-mediated slow inward currents (SICs) in CA1 neurons.

Moreover, we quantified the synaptic transmission properties at the tripartite synapsis, using the minimal stimulation method. We found that bath application of Aβ decreases the synaptic efficacy at single CA3-CA1 synapses.

Therefore, these results indicate that Aβ modulates astrocytic activity, which may have important consequences for synaptic physiopathology. They also indicate that hippocampal astrocytes represent a particularly vulnerable target in AD prior to overt deficits in hippocampal plasticity and cognition.

Supported by: *"I Convocatoria De Ayudas Fundación BBVA a Investigadores, Innovadores y Creadores Culturales"*

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: Neurociencia de sistemas

INVOLVEMENT OF KAINATE RECEPTORS IN CEREBELLAR CLIMBING FIBER TO PURKINJE CELL SYNAPTIC TRANSMISSION AND MATURATION

A.V. Paternain, M. I. Aller and J. Lerma
Instituto de Neurociencias, CSIC-UMH, 03550 San Juan de Alicante, Spain.

Precise control of the development of synapses and neuronal connectivity is critical for proper formation of neural network and normal brain function. Cerebellar Purkinje cells (PC) receive two types of excitatory inputs, one through parallel fibers and the other through climbing fibers. By stimulating granular cell layer, responses showing pair pulse facilitation can be recorded, which correspond to the activation of multiple 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 that present significant pair pulse depression. During the last decade besides the role of Kainate receptors (KARs) in synaptic transmission, participation of KARs in synaptic maturation and refinement has started to be explored.

We have seen that part of the synaptic component at CF-PC synapses is mediated by heteromeric GluK1/GluK4 KARs, and that removal of one of these subunits results in the absence of KAR-mediated responses. Unexpectedly, GluK4 or GluK1 KO mice (i.e. lacking functional KARs) showed a significant reduction of the AMPA receptors mediated component of CF-PC EPSC (EPSC: WT=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 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). While N and p values did not vary significantly in the GluK4 $^{(-/-)}$ compared to the WT mice, q was >30% lower in GluK4 $^{(-/-)}$ (q_{wt}=10$\pm$1.3 pA; q_{GluK4}=6.2$\pm$0.5 pA). We also evaluate possible defects in motor performance by two different behavioral tests, the rotarod and treadmill. While WT animals were able to run and reach 40 rpm speed in the treadmill, GluK4 $^{(-/-)}$ animals performed this task poorly, presenting some defects in the rotarod performance.

These results indicate that GluK1/GluK4 KARs are not only involved in synaptic transfer in CF-PC synapses but also modulate their maturation, playing a significant role in cerebellar function in adult mice.

Supported by the Ministry of Science and Innovation (BFU2011-24084). The IN is a Center of Excellence “Severo Ochoa”.

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

SPARC TRIGGERS A CELL-AUTONOMOUS PROGRAM OF SYNAPSE ELIMINATION

F. J. López Murcia[1], B. Terni[1], A. Llobet[1,2]
[1]. Bellvitge Biomedical Research Institute, L'Hospitalet de Llobregat
[2]. Facultat de Medicina - Campus Bellvitge, Universitat de Barcelona, L'Hospitalet de Llobregat

Elimination of the excess of synaptic contacts established in the early stages of neuronal development is required for refining the function of neuronal circuits. Here we investigate whether SPARC, a molecule secreted by glia, is involved in synapse removal. SPARC production peaks when innervation of the rat superior cervical ganglion and the tail of *Xenopus tropicalis* tadpoles is remodeled. Formation of new cholinergic synapses in autaptic single cell microcultures is inhibited by SPARC. The effect resides on the EC domain, which is also responsible for triggering a concentration and time dependent disassembly of stable cholinergic synapses. The loss of synaptic contacts is associated to the formation of retracted axon terminals, containing multivesicular bodies and secondary lysosomes. The biological relevance of *in vitro* results is supported by injecting the tail of Xenopus tropicalis tadpoles with peptide 4.2, a 20 amino acid sequence derived from SPARC that mimics full-length protein effects. Swimming is severely impaired ~5 hours after peptide application, caused by the massive elimination of neuromuscular junctions and pruning of thin axonal branches. Effects revert 3 to 6 days after injection, as cholinergic innervation re-forms. In conclusion, SPARC triggers a cell-autonomous program of synapse elimination in cholinergic neurons that likely occurs when protein production peaks during normal development.

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

DYE COUPLING BETWEEN CELLS FROM SUBVENTRICULAR ZONE NEUROSPHERES AND GLIA

Rocío Talaverón[1], Paola Fernández[2], Rosalba Escamilla[2], Ángel M. Pastor[1], Juan Carlos Sáez[2] and Esperanza R. Matarredona[1]
[1]Departamento de Fisiología, Universidad de Sevilla, Spain
[2]Departamento de Fisiología, Pontificia Universidad Católica de Chile, Chile

The postnatal subventricular zone lining the lateral ventricles contains neural progenitor cells (NPCs) that generate new olfactory bulb interneurons. Communication via gap junctions between NPC-derived neuroblasts and between neuroblasts and niche astrocytes modulates neuroblast proliferation and migration towards the olfactory bulb. Subventricular zone NPCs can be isolated and expanded *in vitro* in the form of neurospheres. Neurosphere-derived NPCs have been widely used for transplantation purposes in different types of brain lesions. We have previously reported that NPCs establish gap junctions with host glial cells when they are implanted after mechanical injury (Talaverón et al., Glia 2014). In order to analyze whether NPC-glial cell gap junctions are functional we performed dye coupling experiments in co-cultures of subventricular zone neurosphere-derived cells and primary astrocytes or microglia. Neurosphere-derived cells expressed mRNA for the hemichannel/gap junction channel proteins connexin 43 (Cx43), Cx45, Cx26 and pannexin 1. Hemichannel activity was also observed in neurosphere cells in time-lapse measurements of ethidium bromide uptake. Dye coupling experiments revealed that cell-cell coupling occurred among cells in neurospheres (incidence of coupling: 100%; index of coupling: 3.0 ± 0.3). A strong NPC-astrocyte cell coupling was also detected (incidence of coupling: 91.0 ± 4.7%; index of coupling: 2.4 ± 0.3) between neurosphere-derived cells and astrocytes maintained in co-culture. Heterocellular coupling between neurosphere-derived cells and microglia was also evident in co-culture experiments (incidence of coupling: 71.9 ± 6.7%; index of coupling: 2.1 ± 0.4). Altogether, these results propose the existence of functional cell-cell coupling among cells within postnatal subventricular zone neurospheres. In addition, they demonstrate that neurosphere-derived cells can establish gap junctional communication with astrocytes or microglia. Therefore, gap junctional communication with host glial cells might be involved in the integration, survival and functionality of NPCs after implantation in the lesioned brain.

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

DHHC15-MEDIATED PALMITOYLATION MODULATES STRIATAL DOPAMINE LEVELS AND SPONTANEOUS LOCOMOTION

R. Mejias Estévez[1,2], A. Adamczyk[3], M. Niwa[4], I.N. Krasnova[5], G.M. Thomas[6], M. Han[2], J. Wang[2], Z.-X. Xi[5], R.L. Huganir[7], M.V. Pletnikov[8], J.L. Cadet[5], A. Sawa[4], and T. Wang[2].
[1]. Facultad de Biología, Universidad de Sevilla, Sevilla.
[2]. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore.
[3]. Liaison Librarian for Life Sciences, Drexel University, Philadelphia.
[4]. Schizophrenia Center, Johns Hopkins University, Baltimore.
[5]. National Institute on Drug Abuse, NIH, Baltimore.
[6]. School of Medicine, Temple University, Philadelphia.
[7]. Department of Neuroscience, Johns Hopkins University, Baltimore.
[8]. Department of Psychiatry and Behavioral Sciences, Johns Hopkins University, Baltimore.

Dopamine (DA) is a major neurotransmitter in striatum and plays an important role in the modulation of activity, movement, rewards, motivation, and executive function. Regulation of striatal DA metabolism and signaling are extremely complex. Palmitoylation, a reversible lipid post-translational modification, is a newly recognized mechanism in the regulation of DA function. Several DA signaling proteins including dopamine transporter (DAT) and DA receptors (D1/D2L) are palmitoylated but their specific palmitoyl-acyl-transferases (PATs) have not been full characterized. *Dat*-knockout mice show extreme hyperactivity, reduced tissue DA levels and increased extracellular DA levels in striatum. We investigated a line of knockout mice of *dhhc15*, a neural-enriched PAT. Mutant mice exhibit an increase in the novelty-induced ambulatory activity in open field. Neurochemistry studies of monoamine levels in olfactory bulb, brain cortex, striatum, ventral mesencephalon, and hippocampal tissues in *dhhc15*-KO mice identified a significant and specific reduction of tissue dopamine and its metabolite, DOPAC in striatum. Interestingly, basal extracellular DA levels in ventral striatum of *dhhc15*-KO mice are increased during the habituation time to a new environment using *in vivo* microdialysis methods. This profile suggests a partial DA reuptake or dopamine release defect in striatal dopaminergic neurons. Using an acyl-biotin exchange (ABE) assay, we found no significant difference in the steady-state palmitoylation levels of known DHHC15 substrates including postsynaptic density protein 95 (PSD95), growth associated protein 43 (GAP43), cysteine string protein (CSP), sortilin, and stathmin 2/3, in striatal tissues of *dhhc15*-KO mice. Characterization of palmitoylation levels of proteins involved in DA metabolism and signaling, including DAT and DA receptors, in the striatum of *dhhc15*-KO mice are in progress. Data from our studies implicate an important role of dhhc15-mediated pamitoylation in the regulation and release of striatal DA in mice.

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

1. Neurobiología del Desarrollo
2. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
3. Neurociencia de sistemas
4. Neurociencia cognitiva y conductual
5. Trastornos y reparación del sistema nervioso
6. Sistemas homeostáticos y neuroendocrino
7. Nuevos métodos y tecnologías
8. Historia, Docencia, Divulgación y Ética

EXOCYTOSIS ACCELERATES ENDOCYTOSIS

Cano R and Tabares L
Department of Medical Physiology and Biophysics
School of Medicine, University of Seville, Seville

We have generated a transgenic mouse line that expresses Synaptophysin-pHluorin (SypHy) under the specific neuronal promoter Thy 1.2 in order to study exo- and endocytosis in real time at mature motor nerve terminals. The time course of the SypHy signal decay after stimulation is a good measure of endocytic rate when exocytosis ends. During stimulation, however, the situation is more complex, as both exo- and endocytosis are occurring simultaneously. In order to separate both components, we combined fluorescent measurements with electrophysiological recordings of synaptic potentials for calculating total quanta release.
We found that endocytosis rates *during* and *after* high frequency repetitive stimulation not only depend on calcium but also on the amount of synaptic vesicles (SVs) incorporate to the plasma membrane. Quantification of the rates of endocytosis *during* stimulation showed an initial acceleration phase followed by a plateau of about 2700 SVs per second. *After* stimulation, however, endocytic speed decline to about 700 - 1200 SVs/s. To check the participation of dynamin 1 *during* and *after* stimulation we used several dynamin inhibitors: dynasore, dyngo4a, and a dynamin 1-inhibitor peptide. Dynasore and dyngo block the GTPase activity of dynamin, while the peptide competitively blocks binding of dynamin to amphiphysin. After checking by immunofluorescence that dynamin 1 was expressed in motor nerve terminals, we found that all three dynamin-1inhibitors blocked endocytosis, both *during* and *after* high frequency stimulation. In addition, these compounds also decreased exocytosis in a dose dependent manner. Our results suggest that both calcium and the amount of vesicle to be retrieved from the plasma membrane at a given moment determine the rate of dynamin 1-dependent endocytosis.

Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Nuevos métodos y tecnologías

Ministerio de Economía y Competitividad (BFU2013-43763-P).

EPHRIN-B2 ABROGATES NMDAR ANTIBODY EFFECTS IN A MURINE MODEL OF ANTI-NMDAR ENCEPHALITIS

M. Petit-Pedrol[1], J. Planagumà[1,2], F. Mannara[1,4], H. Haselmann[3], B. Grünewald[3], E. Aguilar[1], E. Martin-Garcia[4], R. Maldonado[4], M. Lakadamyali[2], C. Geis[3], J. Dalmau[1,5,7]

[1]. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clínic, Universitat de Barcelona, Barcelona, Spain
[2]. ICFO-Institut de Ciències Fotòniques, Barcelona, Spain
[3]. Hans-Berger Department of Neurology, Jena University Hospital, Jena, Germany
[4]. Departament de Ciencies Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain
[5]. Department of Neurology, University of Pennsylvania, PA, USA
[6]. Servei de Neurologia, Hospital Clínic, Universitat de Barcelona, Barcelona, Spain
[7]. Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

Anti-NMDAR encephalitis is a severe neuropsychiatric disorder that results in alteration of memory, behavior and cognition, mediated by autoantibodies against the GluN1 subunit of the NMDA receptor (NMDAR). We recently reported a mouse model of cerebroventricular passive transfer of patients' antibodies demonstrating a dramatic decrease of memory (novel object recognition test) along with anhedonia and depressive-like behaviors together with an antibody-mediated reduction of synaptic NMDAR.

Taking advantage of this murine model, we aimed to determine if ephrin-B2 ligand abrogated the antibody effects using behavioral assessments and analysis of the density of synaptic NMDAR and field potential recording in CA1 region of hippocampus. For 14 days C57BL6/J male mice received continuous cerebroventricular infusion of CSF from patients with NMDAR antibodies with or without ephrin-B2; animals receiving control CSF with or without ephrin-B2 ligand served as controls. Behavioral tests were regularly performed until day 26, and sets of animals were sacrificed on days 5, 18 and 26 to determine the hippocampal density of synaptic NMDAR and synaptic plasticity (long-term potentiation, LTP). The findings showed that animals treated with patients' antibodies with ephrin-B2 ligand did not develop memory loss and depressive-like behaviors compared with animals treated with patients' antibodies which showed prominent memory-behavioral deficits. Moreover, animals treated with patients' antibodies with ephrin-B2 showed a significant preservation of the density of NMDAR clusters and limited alteration of synaptic plasticity compared with animals that did not receive ephrin-B2. These animals showed a significant decrease of the density of NMDAR and alteration of synaptic plasticity as revealed by a significant impairment of LTP. Taken together, these findings indicate that ephrin-B2 ligand prevents the effects of patients' antibodies in anti-NMDAR encephalitis, and suggest a potential therapeutic approach (e.g., ephrin-B2-like molecules) for disorders related to a depletion of synaptic NMDAR.

DYRK1A, A NOVEL REGULATOR OF GLUN1/GLUN2A RECEPTORS: IMPLICATIONS FOR DOWN SYNDROME AND ALZHEIMER'S DISEASE

M. Gómez de Salazar Honrubia [1], C. Grau [1], K. Arató [2,3], J.M. Fernández-Fernández [4], P. Sanchís [1], D. Soto [1], A. Valderrama [1], C. Sindreu [5], C. Fillat [6], I. Ferrer [1], S. de la Luna [2,3,7] y X. Altafaj [1]
[1].Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat Barcelona, Spain.
[2] .Gene Regulation, Stem Cell and Cancer Programme, UPF and Center for Genomic Regulation (CRG), Barcelona, Spain
[3].CIBER de Enfermedades Raras (CIBERER), Barcelona, Spain
[4].Laboratory of Molecular Physiology and Channelopathies, Department of Experimental and Health Sciences, Pompeu Fabra University, Barcelona, Spain
[5] .Department of Anatomical Pathology, Pharmacology and Microbiology, University of Barcelona, Barcelona, Spain
[6] .Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
[7] .Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

N-Methyl-D-Aspartate (NMDA) glutamate receptors play a pivotal role in synaptic plasticity processes, but under certain conditions their activation can induce neuronal dysfunction and excitotoxicity, which are associated to synaptic dysfunctions present in Alzheimer's disease (AD) and Down syndrome (DS). Regarding the latter condition, we previously shown that DYRK1A kinase overexpression promoted an increased surface expression of GluN1/GluN2A receptors, together with a prolonged decay of NMDA-induced calcium currents. Considering the strong regulatory effects of phosphorylation events on NMDAR amounts, subcellular distribution (synaptic, *vs*. extrasynaptic) and biophysical properties, we hypothesized that DYRK1A might be regulating NMDAR.
In this study, we show that endogenous DYRK1A is recruited to GluN2A-containing NMDARs in the adult mouse brain, and we identify a DYRK1A phosphorylation site at Ser (1048) of GluN2A, within its intracellular C-terminal domain. Mechanistically, the DYRK1A-dependent phosphorylation of GluN2A at Ser (1048) hinders the internalization of GluN1/GluN2A, causing an increase of surface GluN1/GluN2A in heterologous systems, as well as in primary cortical neurons. Furthermore, GluN2A phosphorylation at Ser (1048) increases the current density and potentiates the gating of GluN1/GluN2A receptors, whereas GluN1/GluN2B receptors are not modulated by the presence of DYRK1A. We conclude that DYRK1A is a direct and specific regulator of GluN1/GluN2A subtype of NMDA receptors and we propose a novel mechanism for the control of NMDAR activity in neurons. Interestingly, we have detected an increase of the DYRK1A-mediated phosphorylation of GluN2A at Ser (1048) in adult brain of DS murine models and we are currently exploring whether these changes are also present in AD mouse models. In both models, our efforts are currently focused to determine, by a proteomics-based approach, the subcellular phosphorylation pattern of NMDARs. These data will contribute to understand the role of NMDAR phosphorylation in the initial stages of these related synaptopathies, with potential therapeutic applications.

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

Macarena Gómez de Salazar Honrubia. Predoctoral student IDIBELL.
Email: mgomezdesalazar@idibell.cat

ENHANCEMENT OF SYNAPTIC TRANSMISSION IN A MOUSE MODEL OF SPINAL MUSCULAR ATROPHY

R. Tejero, L. Tabares
Department of Medical Physiology and Biophysics, School of Medicine, University of Seville, Seville.

Spinal Muscular Atrophy (SMA), the most frequent genetic cause of infant mortality, is an autosomal recessive neurodegenerative motor neuron disease caused by a defect in the Survival Motoneuron1 (*SMN1*) gene. In a mouse model of the disease (SMNΔ7 mice) important structural and functional alterations at the neuromuscular junction (NMJ) have been found.

Here we investigated whether the limitation in secretion found in SMN-deficient motor nerve terminals were due to a change in the voltage-dependent calcium channel (VDCC) subtype mediating neurotransmission, to a defect on the calcium sensitivity of the secretory apparatus, a deficit in synaptic vesicles availability, or to a defect in the number of functional release sites. In addition, we explored the capability of SMNΔ7 mice to modulate neurotransmission by short-term plasticity, and by drugs.

Synaptic potentials were recorded in the *ex vivo* Transversus abdominis muscle, one of the most affected in this disease, in wild-type and SMN-deficient mice at a postnatal age of 9-11 days. Expression and distribution of VDCC at the NMJ were studied by quantitative confocal fluorescent microscopy.

We found that, as in wild-type, synaptic transmission in SMN-deficient terminals is mainly mediated by P/Q-type VDCCs, without either change in the spatial distribution pattern of the channels. Secretion at different extracellular calcium concentrations showed that the calcium sensitivity of the release machinery is not altered in SMNΔ7 mice. Statistical binomial analysis of release, however, revealed that while these terminals were unable to recruit new release sites (*n*) when calcium influx increases, an ester of phorbol and nifedipine significantly increased *n*. In addition, mutant terminals respond with a large increase in facilitation when stimulated at 20 Hz.

Together these results revealedthatSMN-deficient motor nerve terminals, despite their structural and functional alterations, are able to positively regulate synaptic transmission through different pathways.

Financial support by MINECO(BFU2013-43763-P and BES-2011-048901) and by the Tatiana Pérez de Guzman el Bueno Foundation.

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

LONG TERM SYNAPTIC PLASTICITY DEPENDENT ON CANNABINOID CB_1 RECEPTORS ACTIVATION IS ALTERED IN THE DENTATE GYRUS OF ADULT MICE EXPOSED TO ETHANOL DURING ADOLESCENCE

S. Peñasco[1,2], N. Puente[1,2], A. Ramos[1,2], N. Royo[1,2], A. Gutiérrez[1,2], I. Bonilla[1,2], L. Reguero[1,2], M.J. Canduela[1,2], J. Mendizabal-Zubiaga[1,2], F. Rodríguez de Fonseca[3], J. Suárez[3], I. Elezgarai[1,2], P.Grandes[1,2]

[1] Department of Neurosciences, Faculty of Medicine and Dentistry, University of the Basque Country UPV/EHU, E-48940 Leioa

[2]Achucarro Basque Center for Neuroscience, Bizkaia Science and Technology Park, Zamudio

[3] Unidad de Gestión Clínica y Salud Mental, Hospital Regional Universitario de Málaga, Instituto de Investigación Biomédica de Málaga (IBIMA), Málaga

Alcohol drinking, especially among adolescents and young adults, is a serious public health concern. Ethanol interacts with the endocannabinoid system (ECS) whose function may be altered in ethanol dependence. Here, we investigated the effect of ethanol consumption on excitatory synaptic transmission and plasticity mediated by the cannabinoid CB_1 receptor in dentate gyrus (DG).

Male C57BL6 mice were exposed to intermittent ethanol intake (20% (v/v) in tap water) using a 4 days drinking-in-the-dark procedure during adolescence (PD 30±2 to 54±2). Animals were given access to ethanol (or water) for 2h sessions during 3 days, and 4h session on the 4th day. At 18-21 days withdrawal from ethanol, adult mice were sacrificed. Electrophysiological, immunohistochemical, and molecular techniques were applied.

Excitatory postsynaptic potentials (fEPSPs) were evoked after stimulation of the medial perforant path and recorded in the supragranular zone of the dentate molecular layer (ML) in the presence of the $GABA_A$ antagonist picrotoxin. CB_1 activation by CP55,940 (10μM) inhibited fEPSPs in controls (26.43±2.77% of baseline) as already shown. However, this effect was not observed in ethanol-exposed mice (4.9±7.47% of baseline). Furthermore, ML synaptic stimulation (10min, 10Hz) triggered a long term depression (LTD) of the excitatory transmission that was absent in adult mice after ethanol consumption during adolescence (2.7±3.12% of inhibition; $p<0.0001^{***}$). This plasticity was CB_1 dependent as the AM251 antagonist (4μM) abolished LTD (8±6.6% of inhibition).

CB_1 immunoreactivity decreased in ML of ethanol-exposed (87.47±0.58%) vs control (100±0.77%) mice. Also, the relative mRNA and CB_1 protein significantly decreased, while a significant increase in MAGL (mRNA and protein) was detected.

Altogether, repetitive exposure to ethanol during adolescence leads to a deficit of endocannabinoid-dependent LTD in adult DG excitatory synapses, probably due to a down-regulation of CB_1 receptors and a reduction of the endocannabinoid tone by an increase of MAGL.

Funded by RETICS, ISCIII (RD12/0028/0001; RD12/0028/0004); MINECO (BFU2012-33334); Basque Government (IT764-13); UPV/EHU (UFI11/41); ISCIII (MINECO) (CP12/03109, PI13/02261); JA (SAS111224); JA UE/ERDF (PI45403, CTS-8221); SP is in receipt of a PhD contract from MINECO (BES-2013-065057).

Key words: endocannabinoid system, electrophysiology, pre-embedding immunogold, electron microscopy, hippocampus, rodent.

Áreas Temáticas:
1º Excitabilidad neuronal, sinapsis y glía: mecanismos celulares.
2º Neurociencia cognitiva y conductual.
e-mail: sara.penasco@ehu.eu

CHRONIC STRESS IMPAIRS CANNABINOID 1 (CB_1) RECEPTOR-MEDIATED CONTROL OF GLUTAMATERGIC TRANSMISSION AND PLASTICITY IN YOUNG ADULT MICE DENTATE GYRUS

N. Royo[1,2], N. Puente[1,2], S. Peñasco[1,2], L. Reguero[1,2], A. Gutierrez[1,2], I. Bonilla[1,2], M.J. Canduela[1,2], J.L. Mendizabal-Zubiaga[1,2], I. Elezgarai[1,2], A. Ramos[1,2] and P. Grandes[1,2]
[1]Department of Neurosciences, Faculty of Medicine and Dentistry, University of the Basque Country UPV/EHU, E-48940 Leioa, Spain.
[2]Achucarro Basque Center for Neuroscience, Bizkaia Science and Technology Park, Zamudio, Spain

The endocannabinoid (eCB) system plays a role in stress responses, also hippocampal CB_1 receptor expression and binding capacity were shown to be altered under chronic stress. However, anatomical and physiological changes of CB_1 at synapses of the stress-involved dentate gyrus (DG) are poorly reported.
We have performed *ex vivo* electrophysiological and anatomical techniques in DG of young adult male mice subjected to either acute or chronic restraint stress. Upon dentate medial perforant path (MPP) stimulation, the CB_1R agonist CP55,940 (10 µM) reduced field excitatory postsynaptic potentials (fEPSP) amplitude to 20.51 ± 5.881% of baseline ($p < 0.001$; n=7) in the presence of the $GABA_A$ antagonist picrotoxin (100 µM) in non-stressed mice. After an acute restraint stress the effectiveness of CP55,940 was only slightly reduced (fEPSP amplitude: 15.68 ± 3.40% of baseline; $p < 0.001$, n=5). However, after repetitive stress exposure fEPSP amplitude was completely impaired (3.46 ± 5.91% of baseline; $p > 0.05$, n = 5). Furthermore, synaptic stimulation of the dentate molecular layer (10 min, 10 Hz) triggered a long term depression of the excitatory synaptic transmission (12.19 ± 1.31% inhibition; $p < 0.001$, n=3) in non-stressed mice, that was absent after chronic stress (0.1 ± 1.19% of inhibition, $p < 0.001$, n=4).
Anatomically, there was a slight significant decrease of CB_1 immunoparticle density in DG excitatory terminals after chronic stress (1.02 ± 0.08) versus non-stressed mice (1.21 ± 0.06 $p < 0.05$, n=2), in contrast with the high significant CB_1 increase in inhibitory terminals (stress: 23.71% ± 0.72%; non stress: 16.09% ± 0.89% $p < 0.001$, n=2).
In summary, chronic stress causes a neuronal excitatory/inhibitory imbalance with small CB_1 changes in excitatory synapses but a remarkable impairment of excitatory synaptic transmission and plasticity in the dentate molecular layer.

Funding: Dr. Pedro Grandes´ laboratory is supported by the Ministerio de Economía y Competitividad (BFU2012-33334), The Basque Government (IT764-13), Red de Trastornos Adictivos, RETICS, Instituto de Salud Carlos III (RD12/0028/0004), The Basque Country University (UFI11/41) and NR is in receipt a PhD contract from The Basque Country University (PIF //13/107).

Key Words: limbic system, endocannabinoid system, perforant path, synaptic transmission.

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

UNDERSTANDING APOD NEUROPROTECTIVE FUNCTION: APOD DISTRIBUTION IN PH-DEPENDENT SUB-DOMAINS OF THE ASTROGLIAL LYSOSOMAL COMPARTMENT UPON METABOLIC AND OXIDATIVE STRESS

R. Pascua-Maestro[1], M. D. Ganfornina[1], D. Sánchez[12]
[1]. IBGM – Universidad de Valladolid / CSIC, Valladolid

Apolipoprotein D (ApoD) is expressed in the nervous system, increases with aging and neurodegeneration, and is induced in response to serum deprivation or oxidative stress. To understand how ApoD traffic through different subcellular compartments affects glial cells protecting mechanisms, we analyze the time course of ApoD subcellular traffic upon exposure to low-serum and paraquat stimuli in a human astroglioma cell line.
Confocal microscopy analysis of ApoD localization indicates that secretion is followed by interaction with the plasma membrane, endocytosis via both the caveolin and clathrin-mediated pathways, and location in endosomes. No ApoD is immunodetected in mitochondria or nuclei.
Interestingly, an important fraction of ApoD is targeted to lysosomes. Large LAMP2-ApoD-positive organelles indicate ApoD presence in autophagolysosomes as well, which is confirmed by co-localization with LC-3. ApoD presence inside lysosomes and autophagolysosomes is stable over time, suggesting an active role in lysosomal-autophagy function.
We have developed a method to analyze ApoD distribution differences in lysosomes after in vivo measurement of individual lysosomes pH, and find that metabolic or oxidative stress treatments, not only change lysosomal pH, but also the pattern of ApoD distribution within lysosomal populations. This close relationship between lysosomal pH and distribution of ApoD is currently being analyzed under conditions of pharmacological induction or inhibition of autophagy, bringing light on the role of this classically extracellular apolipoprotein in lysosomal performance.
In the light of this new findings, previous hypotheses on ApoD neuroprotective roles in glial cells must be refined: control of plasma membrane and of the lysosome/autophagosome function must be key elements in the function of this lipid-binding protein.

Support: MICINN(BFU2011-23978).

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

Raquel Pascua Maestro E-mail: raquel.pascua@alumnos.uva.es

ROLE OF MICROGLIA IN ORGANOTYPIC CULTURES OF POSTNATAL MOUSE RETINAL EXPLANTS

Rosa M. FERRER-MARTÍN, David MARTÍN-OLIVA, Ana SIERRA, Maria-Carmen CARRASCO, María MARTÍN-ESTEBANÉ, Ruth CALVENTE, Sandra M. MARTÍN-GUERRERO, José L. MARÍN-TEVA, Julio NAVASCUÉS, Miguel A. CUADROS.
Facultad de Ciencias, Universidad de Granada, Spain

The role of microglia during neurodegeneration remains controversial as they have been attributed with both negative (neurotoxic) and positive (neurotrophic) actions. We investigated the role of microglial cells in the retina by using organotypic cultures of retinal explants. This system allowed us to study the microglial function while excluding any influences from outside the retina.
Retinal explants from 10-day-old mice received one of three *in vitro* treatments: (1) with minocycline to block microglial activation, (2) with LPS to increase microglial activation, or (3) with liposomes loaded with clodronate (Lip-Clo) to deplete microglial cells. Flow cytometry was used to assess the viability of retinal cells in the explants and the TUNEL method to localize the distribution of dead cells. Immunophenotypic and morphological features of microglia and their distribution were analyzed using flow cytometry and immunocytochemistry with anti-CD11b, anti-CD45, and anti-CD68 antibodies.
Treatment of retinal explants with minocycline was effective to reduce microglial activation while simultaneously inducing a significant decrease in cell viability and increase in TUNEL-labeled cell profiles in comparison to non-treated controls. The minocycline treatment also prevented migration of microglial cells towards the outer nuclear layer, where cell death was most abundant. The LPS treatment appeared to increase microglial activation but to have no effect on cell viability or microglial distribution with respect to controls. Finally, microglial depletion with Lip-Clo decreased cell viability in the explant, showing a similar effect to that of minocycline.
In conclusion, cell viability is diminished in retinal explants cultured *in vitro* when microglial cells are removed or their activation is blocked, indicating a neurotrophic role for microglia in this system.

Financial support: Grant P07-CVI-03008 from Junta de Andalucía, Spain, and Grant BFU2010-19981 from Ministerio de Economía y Competitividad, Spain.

Presenting author: Miguel A. Cuadros. Biología Celular. Universidad de Granada (macuadro@ugr.es)

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

DISTRIBUTION OF SYNAPSES AND MITOCHONDRIA IN THE NEUROPIL OF THE RODENT SOMATOSENSORY CORTEX

A. Santuy[1], J.R. Rodríguez[1, 2], J. DeFelipe[1, 2], L. Alonso-Nanclares[1, 2], A. Merchán-Pérez[1, 3]
[1]Laboratorio Cajal de Circuitos Corticales, Centro de Tecnología Biomédica, Universidad Politécnica de Madrid. Pozuelo de Alarcón, 28223 Madrid, Spain. [2]Instituto Cajal, Consejo Superior de Investigaciones Científicas, 28002, Madrid, Spain.[3]Departamento de Arquitectura y Tecnología de Sistemas Informáticos, Universidad Politécnica de Madrid. Boadilla del Monte, 28223, Madrid, Spain.

Most cortical synapses are established in the neuropil. There are two main morphological types of synapses, asymmetric (excitatory) and symmetric (inhibitory). Synapses can be established on dendritic spines or on dendritic shafts. Mitochondria generate most of the energy required by neurons, and their dysfunction is involved in some of the most prevalent degenerative diseases. Therefore, knowing the density and distribution of synapses as well as the density of mitochondria in the neuropil is critical for understanding the design of cortical circuits. For this purpose, we have used FIB/SEM microscopy to obtain stacks of serial sections from the neuropil of the rat somatosensory cortex. Using a specifically developed software (Espina), we have three-dimensionally reconstructed more than 6100 synaptic junctions in these samples. Asymmetric synapses were 87-93% of the total number of synapses, depending on the cortical layer. Approximately 80–90% of asymmetric synapses were located on spines, while the rest were on dendritic shafts. By contrast 62–85% of symmetric synapses were established on dendritic shafts. We further analyzed the occurrence of multiple synapses on dendritic spines, and the presence of perforated synapses. The volume occupied by mitochondria in the neuropil, estimated by stereological techniques, was approximately 5%. This volume increased from layer I to layer IV, and then decreased to its minimum in layer VI. We found a moderate correlation between the volume fraction of mitochondria and the density of synapses in the different cortical layers. Mitochondria located in dendrites outnumbered those located in axons in a 3:1 proportion. The proportion between mitochondria in excitatory axons and inhibitory axons was also 3:1. This study provides new quantitative data that may contribute to the knowledge of the ultrastructure of the cortex and develops a methodology that permits the accurate quantification of synapses and mitochondria, allowing their distribution to be examined in the brain.

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

ASSESSMENT OF TYPE-I CANNABINOID RECEPTORS IN ASTROCYTES OF MUTANT MICE DENTATE GYRUS

A. Gutiérrez Rodríguez[1,2,3,4], N. Puente Bustinza[1,2], G. Marsicano[3,4], P. Grandes Moreno[1,2]
[1] Faculty of Medicine and Dentistry, University of the Basque Country UPV/EHU, E-48940 Leioa, Spain. [2]Achucarro Basque Center for Neuroscience, Bizkaia Science and Technology Park, Building 205, Zamudio, Spain. [3]INSERM, Neurocentre Magendie, Physiopathologie de la Plasticité Neuronale, Endocannabinoids and Neuroadaptation, U862, Bordeaux, France. [4] University of Bordeaux, F-33076, Bordeaux, France

Type-1 cannabinoid (CB_1) receptor is widely expressed in the brain mediating the effects of (endo)cannabinoids. Evidences have shown its activation in astrocytes might be playing an important role in neuronal modulation of synaptic transmission and plasticity. To identify low levels of CB_1, avoiding their misinterpretation as background staining; "rescue" strategies are needed. However, it is necessary to evaluate if genetic re-expression maintains normal CB_1 expression in specific cell types of the "rescue" mutants.
We analyzed the subcellular CB_1 distribution in astrocytes of the dentate molecular layer. We used conditional "CB_1 rescue mice" which re-express CB_1 only in astrocytes (GFAP-*CB_1*-RS mice) and transgenic mice carrying GFP under the control of the GFAP promoter (GFAP-GFP mice). Specific CB_1, GFAP and GFP antibodies combined with a preembedding immunogold and an immunoperoxidase method for electron microscopy were applied to hippocampal sections of the mutants, as well as of *CB_1*-WT and of *CB_1*-KO mice that were used as controls.
The results showed that 40.69% ± 3.69% of astrocytic sections were CB_1 immunopositive in GFAP-*CB_1*-RS. No significant differences were observed comparing with *CB_1*-WT (44.81% ± 3.62%). Sparse unspecific particles were detected in a few astrocytic elements of *CB_1*-KO (1.77% ± 0.72%) and GFAP-*CB_1*-KO mice (3.08% ± 1.03%). In GFAP-GFP mice, 51.68% ± 2.70% of the GFP immunoreactive astrocytic processes were CB_1 positive (significant difference compared to *CB_1*-WT, *; $p< 0.05$).
To summarize, the proportion of CB_1 immunopositive astrocytic processes in *CB_1*-WT is maintained in GFAP-*CB_1*-RS mice, showing the great potential of these transgenic mice to study CB_1 in brain cell types where the CB_1 expression is low. Besides, more CB_1 positive astrocytic processes were observed in GFAP-GFP mice, suggesting that a better CB_1 detection in astrocytes can be achieved in these reporter mice. The expression of CB_1 in astrocytes might be higher than what was expected.

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

Ana Gutiérrez Rodríguez: **ana.gutierrezr@ehu.es**

Key words: astroglia, cannabinoid system, immuno-electron microscopy, mutant mice.
Funding: P. Grandes´ laboratory is supported by Ministerio de Economía y Competitividad BFU2012-33334, The Basque Government IT764-13, Red de Trastornos Adictivos, RETICS, Instituto de Salud Carlos III RD12/0028/0004 and The University of the Basque Country UFI11/41. A. Gutiérrez is supported by a Predoctoral Cotutelle Contract between UPV/EHU and University of Bordeaux.

ROLE OF GLIA IN INFLAMMATION AND ALZHEIMER'S DISEASE

A. Jordá[1], Z. Barrasa[1]. M. Aldasoro[1], A. Iradi[1] and SL. Vallés[1]
[1]. Facultad de Medicina, Universitat de Valencia, Valencia.

During decades glia cells have been considered such as protective and nutrient cells taking care neurons in the brain. In this decade many scientific have published different roles for astrocytes, oligodendroglia, microglia an endothelial cells. The main of this study was to show the role of glia in Alzheimer's disease using transgenic APP/Preseniline 1 and comparing with Wild type mice. We detect increase in inflammatory genes in wild type mice compared with transgenic one, demonstrating a chronic inflammation in that mice. Also we noted increase in CCL3 and CCL4 genes involved in brain demyelination compared with wild type mice, which can explain the cleansing job of astrocytes in transgenic mice trying to eliminate $A\beta_{1-42}$ plates. By microarray CCR8, CX3CL1 and CXCR3 genes were significantly high expressed in wild type compared with transgenic mice, showing us the proper positioning of activated T cells with adhesive, trafficking and migratory functions in wild type. In fact we detect also a significant increase of IL-3 in transgenic mice compared to wild type indicating us activation of T cells and induction of proliferation and differentiation of T cells in transgenic mice. Integrin activation, cytoskeletal changes and chemotactic migration was altered in transgenic mice compared to wild type in our study. For instants, astrocytes play important roles such as protector of neurons in front of inflammation imbalance and regenerating damage intake. Further we detect presence of tumour resistant gene in transgenic mice, ABCF 1, without any expression of this gene in wild type and on the contrary expression of CCL12, cancer gene, in wild type without any expression in transgenic mice. These last data indicate a resistance of transgenic mice to cancer compared to wild type mice. In the future the study of the communication between all brain cells will be necessary to understand many neurodegenerative illness and the protection of stem natural cells of our young brain will be the next frontier.

Áreas Temáticas:

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

A ROLE FOR GSK3 IN THE MAINTENANCE OF BASAL SYNAPTIC TRANSMISSION

Jonny Draffin[1], Shira Knafo[1], Jose A. Esteban[1]
[1] Centro de Biología Molecular Severo Ochoa. Universidad Autónoma de Madrid, Madrid

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. In support of the role of GSK3 in this depression of synaptic responses, we found that activation of Akt, a negative regulator of GSK3 activity, induces a similar run-down of AMPAR-mediated currents. Using electrophysiologically-tagged recombinant AMPA receptors, we identified GluA2 as the AMPA receptor subunit removed from the synapse during inhibition of GSK3. Previous work has shown that activity of GSK3 is required for the induction of long-term depression (LTD) of synaptic responses. Accordingly, we have found that overexpression of predominantly active GSK3 is sufficient to induce depression of AMPAR-mediated synaptic responses. Therefore, we propose a dual role for GSK3: in the maintenance of basal synaptic transmission, and in the removal of AMPA receptors during LTD.

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

IMMUNOLOCALIZATION OF MITOCHONDRIAL CB_1 IN CA1 HIPPOCAMPUS OF CHRONIC STRESSED MICE

I.Bonilla del Río[1, 2], A. Gutiérrez Rodríguez[1, 2, 3, 4], N. Puente Bustinza[1, 2], N. Royo Zubillaga[1, 2], S. Peñasco Iglesias[1, 2], G. Marsicano[3, 4], P. Grandes Moreno[1, 2]

[1]Department of Neurosciences, University of the Basque Country UPV/EHU, E-48940 Leioa, Spain. [2]Achucarro Basque Center for Neuroscience Bizkaia, Science and Technology Park, Building 205, Zamudio, Spain. [3]INSERM, Neurocentre Magendie, Physiopathologie de la Plasticité Neuronale, Endocannabinoids and Neuroadaptation, U862, Bordeaux, France. [4]Universite Bordeaux, Bordeaux, F-33076, France

The seven-transmembrane G protein coupled cannabinoid receptor type-1 (CB_1) forms part of the brain endocannabinoid (eCB) system. This system is involved in many neural functions ranging from food intake to cognition. Because of the functional activity, mammalian brain is one of the organs with the highest energy demands and mitochondria are key determinants of its functions.

Stress responses are crucial for survival and normal functioning of the organism. However, an inadequate or long-lasting response causes physiological alterations. Increasing evidences suggest that the eCB system participates in stress. We have recently demonstrated in the hippocampus that CB_1 receptors are at neuronal mitochondria ($mtCB_1$) membranes, where they directly control cellular respiration and energy production and modulate synaptic plasticity. As hippocampal CB_1 expression and its binding capacity have been reported to be altered due to chronic stress, we wondered whether $mtCB_1$ is affected under stress conditions.

The aim of this study was to investigate the subcellular distribution of $mtCB_1$ in the CA1 hippocampal region of chronic stressed mice. For this purpose, young adult Swiss mice were subjected to chronic restraint stress for 1 hour/day during ten days. Thereafter, mice were sacrificed and perfusion-fixed through the heart. A preembeddding immunogold method for high resolution electron microscopy was applied to hippocampal sections of control and stressed mice. After analyzing the CB_1 distribution pattern, 29.34 ± 0.91% of total mitochondria were CB_1 immunopositive in chronic stressed mice while in controls about 17.49 ± 0.67% localized $mtCB_1$. The difference between both groups was statistically significant ($p < 0{,}0001^{***}$, Mann Whitney test).

These results are indicating that exposure of young adult mice to chronic stress significantly increases CB_1 inmunopositive mitochondria, possibly reflecting a link between chronic stress and the modulation of brain mitochondria activity through CB_1.

Áreas Temáticas

1º Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

2º Neurociencia cognitiva y conductual

Itziar Bonilla del Río: **ibonilla003@ikasle.ehu.es**

Key words: mitochondria, inmuno electron-microscopy, endocannabinoid system, stress.

Funding: P. Grandes´ laboratory is supported by the Ministerio de Economía y Competitividad BFU2012-33334, The Basque Government IT764-13, Red de Trastornos Adictivos, RETICS, Instituto de Salud Carlos III RD12/0028/0004 and The University of the Basque Country UFI11/41.

THE LOCAL ANESTHETIC TETRACAINE ENHANCES NICOTINIC RECEPTOR DESENSITIZATION

R. Cobo, A. Alberola-Die, I. Ivorra, A. Morales
División Fisiología. Facultad de Ciencias. Universidad de Alicante. Alicante (Spain)

Tetracaine (Ttc) is a local anesthetic that inhibits muscle-type nicotinic acetylcholine (ACh) receptors (nAChRs). However, the mechanisms of nAChR blockade by Ttc are yet poorly understood. The aim of this study was to explore Ttc effects on the desensitization rate of nAChRs, since other local anesthetics bearing tertiary amine groups, like lidocaine, accelerate nAChR desensitization.

Torpedo nAChRs were transplanted to *Xenopus* oocytes and currents elicited by ACh (I_{ACh}s), either alone or co-applied with Ttc, were recorded at a holding potential of -60 mV, unless otherwise noted. In some cells, Ttc was pre-applied for 12 s before challenging the cell with ACh. nAChR desensitization was measured by fitting the I_{ACh} decay to the best fit of either a single- or two-exponential function. Ttc reversibly blocked I_{ACh}s with an IC_{50} close to 0,7 µM. Ttc effects, at its IC_{50}, on nAChR desensitization were: i) co-application of Ttc with ACh induced a faster I_{ACh} desensitization, increasing when raising ACh dose; ii) pre-application of Ttc, before superfusing the oocyte with ACh, caused a smaller I_{ACh} blockade than when co-applying both drugs and did not enhance nAChR desensitization; and iii) at positive holding potentials (+40 mV), co-application of Ttc and ACh elicited a similar I_{ACh} blockade to that mediated by just Ttc pre-application and, again, without changing the desensitization rate.

It is concluded that Ttc increases nAChR desensitization, being this effect mediated by its interaction within the channel pore.

This work was supported by grants CSD2008-00005 and BFU2012-31359 from the MINECO (Spain).
Raúl Cobo Velacoracho (raul.cobo@ua.es)

HIPPOCAMPAL SYNAPTIC PLASTICITY AND ENDOPLASMIC RETICULUM DYNAMICS ARE CORRELATED

Alberto Perez-Alvarez[1], Shuting Yin[1], Christian Schulze[1], John A. Hammer III[2], Wolfgang Wagner[1] and Thomas G. Oertner1[1].
[1]Center for Molecular Neurobiology Hamburg (ZMNH), Hamburg, Germany;
[2]National Health, Lung and Blood Institute, NIH, Bethesda, USA.

The presence of endoplasmic reticulum (ER) in dendritic spines allows mGluR-dependent depression and modifies local calcium signaling in a subset of synapses. However, knocking out synaptopodin, a protein that is essential for ER organization into a 'spine apparatus', has modest effects on synaptic plasticity. Therefore, we looked for synapse-specific plastic properties monitoring over time the volume of dendritic spines and movements of GFP-labelled ER. ER movements in and out of spines were much more dynamic than previously thought, occurring on a time scale of minutes rather than days. We could distinguish 2 classes of ER dynamics: In some spines (~10%), the ER remained present for hours. The majority of ER intrusions, however, were short-lasting (< 20 min). About 20% of CA1 hippocampal spines possess ER at any given time point, but more than 50% were visited within 2 hours, and progressively more in longer time periods. Interestingly, the volume of dendritic spines reached its maximum at the time of ER insertion and decayed after this point, pointing to a tight correlation between ER and structural plasticity. Inducing spine structural plasticity (sLTP) by repetitive two photon glutamate uncaging, we observed that spines were typically invaded by ER immediately after sLTP induction. Spines that contained stable ER before sLTP induction did not grow further. We found that fast ER dynamics depended on myosin Va activity and were positively modulated by glutamate receptors. Expressing in single CA1 neurons a globular tail domain of myosin Va (GTD) not only abolished the aforementioned fast ER dynamics and reduced the fraction of the stable ER-positive spines, but also blocked further LTP induction. Strikingly, we observed AMPA- and NMDA-receptor-enriched synapses in CA1 neurons expressing GTD. These findings are consistent with the concept that spine ER acts as a 'brake' on spine growth and synaptic potentiation.

Áreas Temáticas:
1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Nuevos métodos y tecnologías

DIFFERENT VULNERABILITY OF DEEP AND SUPERFICIAL PYRAMIDAL CELLS ALONG THE CORNU AMMONIS IN AN EXPERIMENTAL MODEL OF TEMPORAL LOBE EPILEPSY

L. Ballesteros-Esteban[1], E.Cid[1], D. Gomez-Dominguez[1], B. Gal[2] and L. Menendez de la Prida[1]
[1].Instituto Cajal, CSIC, Ave Doctor Arce 37, Madrid Spain
[2].Universidad Europea de Madrid, Villaviciosa de Odon, Madrid Spain

Temporal lobe epilepsy (TLE) with a prevalence of 5,4 per 1000 inhabitants in EU alone represents a common form of pharmacoresistant epilepsies. A major obstacle in developing novel therapies is that our understanding of the rules governing epileptic microcircuits still remains rudimentary. Recent genetic evidence suggests an exquisite organization of hippocampal cell identity and connectivity along the deep-superficial and proximo-distal axes of the *cornu ammonis*, but their reorganization in TLE remains ignored. Here, using immunohistochemical and histological techniques that exploit cell-type specific markers of pyramidal cell populations such as calbindin, alpha-actinin and PCP4, we evaluated cell injury in some neuronal subpopulation in experimental models of TLE. We found that the population of pyramidal cells immunoreactive to PCP4 at the CA2 region remained relatively intact in TLE rats as compared to controls. In contrast, CA1 pyramidal cell loss exhibited striking gradients along the structure. Superficial CA1 pyramidal cells immunoreactive to calbindin were proportionately more affected than calbindin-negative deep cells, and this pattern was more strongly concentrated at the proximal than at distal regions. The study of these alterations shed light on the functional reorganization of hippocampal microcircuits in TLE.

Áreas Temáticas:

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

GALANIN RECEPTOR 2 MODIFIES NEUROPEPTIDE Y Y1 RECEPTOR INTERNALIZATION AND β-ARRESTIN RECRUITMENT

Manuel Narváez[1], Dasiel O. Borroto-Escuela[2], Carmelo Millón[1], Antonio Flores-Burgess[1], Belén Gago[3&],Luis Santín[4], Kjell Fuxe[2], José Angel Narváez[1] & Zaida Díaz-Cabiale[1]

1. Universidad de Málaga, Instituto de Investigación Biomédica de Málaga, Facultad de Medicina, Campus de Teatinos s/n, 29071 Málaga, España

2. Department of Neuroscience, Karolinska Institute, Stockholm, Sweden

3. Universidad de Málaga, Instituto de Investigación Biomédica de Málaga, Facultad de Ciencias, Campus de Teatinos s/n, 29071 Málaga, España. [&]current address: Instituto Biodonostia, 20014, San Sebastián, Spain

4. Universidad de Málaga, Instituto de Investigación Biomédica de Málaga, Facultad de Psicología, Campus de Teatinos s/n, 29071 Málaga, España

We have recently described a Galanin receptor 2(GALR2) and Neuropeptide Y Y1 receptor(NPYY1R) interaction at behavioural, cellular and receptor levels through GALR2/NPYY1R heterodimers. The aim of this work was to study if GALR2 and NPYY1R costimulation modified NPYY1R internalization and β-Arrestin recruitment after in HEK293T cells.

HEK293T cells were transfected with $NPYY1R^{EGFP}$or $\beta\text{-Arrestin2}^{GFP2}$ cloned with standard molecular biology techniques employing PCR and fragment replacement strategies. $NPYY1R^{EGFP}$/GALR2 and NPYY1R/GALR2 with $\beta\text{-Arrestin2}^{GFP2}$ HEK293T coexpressing cells were incubated with NPY 1μM and/or GAL1μM, at different times. Antagonist studies were performed 15 min prior to the addition of agonist with NPYY1R antagonist BIBP3226 10μM or GALR2 antagonist M871 10 μM. Timed-interval images of $NPYY1R^{EGFP}$ or $\beta\text{-Arrestin2}^{GFP2}$ endosomes in different cell groups were acquired using a confocal microscope following agonist addition. Percentage of internalization was determined by Leica software analysis of total membrane fluorescence compared to total internal compartment fluorescence at the various time points.

We observed that addition of NPY induced a rapid decrease in the cell surface expression of $NPYY1R^{EGFP}$ and a redistribution of $\beta\text{-Arrestin2}^{GFP2}$. In fact, we observed a maximum of internalization of 80% three minutes after the NPY stimulation. However, combined treatment with GAL and NPY induced a delay in the internalization of $NPYY1R^{EGFP}$, with a maximum of internalization thirty minutes after the co-stimulation. Moreover, a delay in the $\beta\text{-Arrestin2}^{GFP2}$ redistribution was observed. The specific GALR2 antagonist M871 abolished these delays in internalization of $NPYY1R^{EGFP}$ and $\beta\text{-Arrestin2}^{GFP2}$ redistribution, suggesting that this effect was mediated through the coactivation of GALR2 and NPYY1R. These results demonstrate that costimulation with GAL and NPY delays the internalization of $NPYY1R^{EGFP}$ by decreasing recruitment of $\beta\text{-Arrestin2}^{GFP2}$ and probably could change intracellular signaling. This study was supported by Junta de Andalucia CVI6476.

Áreas Temáticas:

1ª: Neurociencia cognitiva y conductual

SENSORY INPUT- DEPENDENT CHANGES IN GLUTAMATERGIC NEUROTRANSMISSION-RELATED MOLECULES IN THE ADULT RAT TRIGEMINAL GANGLION

Fernández-Montoya, J.[1], Egea, J[2]. Martín, Y.B.[3], González Montero, R.[1], Avendaño, C., [1] Negredo, P. [1]
[1] Dept. Anatomy, Histology & Neuroscience, Medical School, Autonoma University of Madrid, Spain
[2] Instituto de Investigación Sanitaria Hospital de La Princesa, Hospital Universitario de La Princesa, Madrid
[3] Dept. Anatomy, Francisco de Vitoria University, Madrid, Spain

Sustained alterations of sensory experience induce lasting changes in representational maps, processing of sensory signals, and structure of synapses, dendrites and axons. It is known since the 1950s that an enriched environment could change the chemistry and the anatomy of the cerebral cortex. Haptic sensory deprivation also induces structural and molecular changes. This experience-dependent plasticity takes place not only in sensory cortical areas, where it is more prominent and has been more thoroughly investigated, but also at peripheral levels of the sensory pathways.

We investigated the effects of long-term exposure to sensory-enriched environment or unilateral trimming of the whole set of whiskers on the trigeminal ganglion (TG) in young adult rats. Our goal was to identify changes in the mRNA expression of glutamate receptors (AMPA, NMDA and mGLUR) and related molecules, using a commercial array focused on 84 key genes central to plasticity during learning and memory (RT² Profiler PCR Arrays, Quiagen). Our preliminary data show that the levels of most types of glutamate receptors, as well as the expression of Homer1, Pick1 and Grip1 (transcripts whose proteins are implicated in binding and clustering of glutamate receptors) in TG decrease after sustained haptic deprivation achieved by periodic whisker clipping over a two month period. In contrast, repeated daily exposure to an enriched environment causes an upregulation of metabotropic and NMDA receptor mRNA, with no changes in the AMPA receptor family or in the related proteins studied.

Our study points to a substantial gene expression response of TG neurons, and probably glia, to sustained changes in the intensity and/or spatiotemporal pattern of sensory input, without any direct manipulation of sensory receptors and/or nerves. This response could provide clues on the mechanisms by which an altered sensory input is capable of inducing structural and functional alterations upstream in the sensory paths.

Supported by Grant BFU 2012-39960 from the Plan Nacional I+D

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

BDNF AND TRKB ARE REGULATED BY BOTH PRE- AND POSTSYNAPTIC ACTIVITY AND ENHANCE PRESYNAPTIC CPKC I TO MODULATE NEUROMUSCULAR SYNAPTIC FUNCTION.

E. Hurtado, L. Nadal, T. Obis, A. Simón, V. Cilleros, N. Garcia, M. Santafé, M. Tomàs, M. A. Lanuza, J. Tomàs
Unidad de Histología y Neurobiología (UHN). Facultad de Medicina y Ciencias de la Salud. Universidad Rovira i Virgili. C/Sant Llorenç 21, 43201 Reus. Spain.

In the last few years, increasing evidence demonstrates that one of the benefits of physical activity/exercise on the health of the CNS is to improve the synaptic function (Van Praag-H et al., 1999). Additionally, it is well accepted the preponderance of BDNF in mediating these effects (reviewed by Gomez-Pinilla and Hillman, 2013). However, how an increase of synaptic activity-induced muscle contraction can modulate neuromuscular synaptic function through BDNF and its receptor, TrkB, remains unknown. We have recently identified that PKC family is involved in neurotransmitter release when continuous electrical stimulation imposes a moderate activity on the NMJ and that muscle contraction has an important impact on presynaptic PKC isoforms levels, specifically cPKCβI and nPKCƐ (Besalduch et al., 2010; Obis et al., 2015). Accordingly, the present study hypothesized that muscle contraction is a key regulator of BDNF/TrkB signaling pathway, activating presynaptic cPKC isoforms to modulate synaptic function. ELISA and Western blotting results show that pre- and postsynaptic neuromuscular activity are both responsible for the increase of BDNF levels in skeletal muscle and that nerve induced-muscle contraction regulates TrkB–T1 without affecting TrkB-FL and p75 levels. Moreover, an involvement of BDNF induced by pre- and postsynaptic activity on regulation of the presynaptic classical cPKC isoforms (Ɛ and βI) acting by a signaling pathway through TrkB. We also demonstrate by electrophysiological techniques that cPKCβI is decisively involved in ACh release induced by electrical stimulation. Together, these results provide a mechanistic insight into how synaptic activity-induced muscle contraction could regulate the BDNF/TrkB signalling at the NMJ by enhancing BDNF production and decreasing the TrkB-T1 levels. It further suggests that this signalling pathway could increase presynaptic levels of the cPKCβI isoform to affect neuromuscular neurotransmission.

This work was supported by 2014SGR0344 and 2014PFR-URV-B2-83.

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

DEPRESSED EXCITABILITY AND ION CURRENTS LINKED TO SLOW EXOCYTOTIC FUSION PORE IN CHROMAFFIN CELLS OF THE SOD1^{G93A} MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

J. A. Arranz-Tagarro[1,2], E. Calvo-Gallardo[1,2], R. de Pascual[1,2], J. C. Fernández-Morales[1,2], M. Maroto[1,2], C. Nanclares[1,2], L. Gandía[1,2], A. M. G. de Diego[1], J. F. Padín[1,2], A. G. García[1,2,3,4]
[1]. Instituto Fundación Teófilo Hernando de I + D del Medicamento, [2]. Departamento de Farmacología, Facultad de Medicina, Universidad Autónoma de Madrid, Madrid.
[3]. Servicio de Farmacología Clínica, [4]. Instituto de Investigación Sanitaria, Hospital Universitario de La Princesa, Madrid.

Altered synaptic transmission with excess glutamate release has been implicated in the loss of motoneurons occurring in amyotrophic lateral sclerosis (ALS). Hyperexcitability or hypoexcitability of motoneurons from mice carrying the ALS mutation SOD1^{G93A} (mSOD1) has also been reported. Here, we have investigated the excitability, the ion currents, and the kinetics of the exocytotic fusion pore in chromaffin cells from postnatal day 90 to postnatal day 130 mSOD1, when motor deficits are already established. Cell excitability and ion currents were recorded using the patch-clamp technique, $[Ca^{2+}]_c$ variations were measured using the probe Fura-2-AM, catecholamine secretion was studied using a carbon fibre electrode with an oxidation potential of +700 mV, and protein expression was researched using the western-blot technique. With respect to wild-type mice (WT), mSOD1 chromaffin cells had a decrease in the following parameters: 95% in spontaneous action potentials, 70% in nicotinic current for acetylcholine (ACh), 35% in Na^+ current, 40% in Ca^{2+}-dependent K^+ current, and 53% in voltage-dependent K^+ current. Ca^{2+} current was increased by 37%, but the ACh-evoked elevation of $[Ca^{2+}]_c$ was unchanged. Single exocytotic spike events triggered by ACh had the following differences (mSOD1 vs. WT): 36% lower rise rate, 60% higher decay time, 51% higher half-width, 13% lower amplitude, and 61% higher quantal size. The expression of the α_3-subtype of nicotinic receptors and proteins of the exocytotic machinery was unchanged in the brain and adrenal medulla of mSOD1, with respect to WT. A slower fusion pore opening, expansion, and closure are characteristics likely linked to the pronounced reduction in cell excitability and in the ion currents driving action potentials in mSOD1, compared with WT chromaffin cells. These drastic changes in cell excitability, ion currents, and exocytotic fusion pore suggest that ALS is a systemic disease that at later evolution stages affects excitable neurosecretory cells other than motoneurons.

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

HIGH CONSERVATION OF THE SYNAPTIC PROTEOME ALONG VERTEBRATE EVOLUTION.

À. Bayés[1,2]*, M.O. Collins[3], R. Reig[1,2], G. Gou[1,2], Golding D[4], JS Choudhary[3] and SGN Grant[5]
[1] Biomedical Research Institute Sant Pau, Barcelona, Barcelona
[2] Universitat Autònoma de Barcelona, Bellaterra, Barcelona
[3] The Wellcome Trust Sanger Institute, Hinxton, UK
[4] The Wellcome Trust Sanger Institute, Hinxton, UK.
[5] Centre for Clinical Brain Science, University of Edinburgh, Edinburgh, UK

Background: We have previously shown that the postsynaptic proteome has been highly conserved during mammalian evolution.
Objective: We wanted to investigate if this high conservation has been occurred since vertebrate evolution.
Methods: To achieve this goal we performed a comparative proteomics study on the synaptic proteomes of mouse and zebrafish.
Results: We have identified 3978 proteins in zebrafish and 3667 in mouse. These species display a highly similar synaptic proteome, as 80% of the proteins in one has an orthologue in the other. As observed in mammals, a high conservation on the synaptic proteome has occurred since vertebrates started to diverge. An analysis of protein sequence identity between orthologues indicates that synaptic proteins have been highly conserved, even more than other proteins expressed at the brain. We have seen that zebrafish synapses contain more paralogues per protein family than mouse. Surprisingly, this phenomenon, which is consequence of the third round of whole genome duplication (WGD) experienced by fish, is particularly noticeable for families of proteins with important synaptic roles. For instance, we have identified 8 subunits of the AMPA receptor or 8 NMDA type 2 subunits. This observation suggests that evolution has retained the extra copies of genes with a strong synaptic role, an observation that we are still validating.
Conclusions: We have performed the first proteomics study of the zebrafish synapse, which has allowed studding its vertebrate evolution. We have shown that the constraint on synaptic proteome evolution reported in mammals has actually occurred since vertebrates started to diverge 500 million years ago. Furthermore, we have data suggesting that zebrafish expresses more paralogues of key synaptic proteins than mouse. We hypothesize that the new copies of synaptic genes arising from the third WGD have been retained functional in the fish genome at a higher rate than other genes.

1. Neuronal excitability, synapses and glia: cellular mechanisms
2. Theoretical and Computational Neuroscience

THE DISCOVERY OF TWO ANCESTRAL GENE FAMILIES EXPANDS OUR CURRENT REPERTOIRE OF GLUTAMATE RECEPTORS.

D. Ramos[1,2], J. Ji[3], J. Luís[1,2], G. Gou[1,2], E. Navas[4], D. Burguera[4], J. García-Fernandez[4], H. Escrivà[5], N. Roher[3] and À. Bayés[1,2*]

[1]. Biomedical Research Institute Sant Pau (IIB Sant Pau), Barcelona, Barcelona.

[2]. Universitat Autònoma de Barcelona, Bellaterra, Barcelona.

[3]. Institut de Biotecnologia i de Biomedicina-Parc de Recerca UAB, Universitat Autònoma de Barcelona, Bellaterra, Barcelona.

[4]. Facultat de Biologia, Universitat de Barcelona, Barcelona, Barcelona.

[5]. Université Pierre et Marie Curie Paris 06; Observatoire Oceanologique; Banyuls-sur-Mer; Paris, France

Abstract

Background and Objectives: Glutamate is the major excitatory neurotransmitter in the central nervous system, acting on ionotropic (iGluRs) and metabotropic receptors (mGluRs). As these are key molecules to nervous system function we have studied their animal evolution. We have specifically looked into the genera *Branchiostoma*, commonly named amphioxus. Having experienced very few gene losses amphioxus has a very conserved genome, this characteristic makes amphioxus a very powerful tool in the study of protein family evolution.

Methods: We have searched for homologues of vertebrate glutamate receptors in 15 species from 9 phyla covering all major metazoan lineages. We have used these to perform phylogenetic analysis using a Bayesian method. Finally, the expression of genes identified in *Branchiostoma* have been validated by q-PCR in nerve cord tissue.

Results: Our initial results indicated that amphioxus has more gene families coding for iGluR subunits and mGluRs than vertebrates. Looking for these new genes in other metazoan phyla we have demonstrated that these are ancestral gene families that have been lost in vertebrates. This demonstrates that the repertoire of gene families coding for glutamate receptors is larger than currently recognized. The common ancestor of all bilaterians had one more iGluR gene family (termed A/K/D1) and one more mGluR gene family (termed Class IV) than modern vertebrates. The expression of these genes in the nerve cord of amphioxus further endorses their predicted molecular function as glutamate receptors.

Conclusions: We have established the metazoan phylogeny of glutamate receptors. In so doing we have discovered that the repertoire of genes coding for glutamate receptors is larger than what is currently known. Finally, we have shown that the ancestor to all vertebrates lost a group of genes coding for iGluR subunits and another one coding for mGluRs.

1. Neuronal excitability, synapses and glia: cellular mechanisms
2. Theoretical and Computational Neuroscience

THE $GABA_B$ RECEPTOR SUBUNITS B1 AND B2 OF THE SEA LAMPREY: CLONING AND EXPRESSION IN THE CENTRAL NERVOUS SYSTEM

D. Romaus-Sanjurjo[1], B. Fernández-López[1], A. Barreiro-Iglesias[1], MC. Rodicio[1]
[1]Facultad de Biología (CIBUS), Universidad de Santiago de Compostela, A Coruña.

γ-aminobutyric acid (GABA) is the main inhibitory transmitter in the central nervous system of vertebrates. It acts via ionotropic ($GABA_A$ and $GABA_C$) and metabotropic ($GABA_B$) receptors. The $GABA_B$ receptor addresses second messenger systems through the binding and activation of guanine nucleotide-binding proteins (G-protein-coupled receptors [GPCRs]), producing a slow inhibition and decreasing levels of AMPc. Lampreys are a key reference to understand molecular evolution in vertebrates. Many pharmacological and physiological studies in lampreys have shown the importance of GABA acting through $GABA_B$ receptors in the modulation of the circuits controlling locomotion and other behaviors. The aim of this work was to identify the sea lamprey $gaba_{b1}$ and $gaba_{b2}$ cDNAs and study the pattern of expression of these transcripts in the central nervous system (CNS). We cloned two partial sequences corresponding to the $gaba_{b1}$ and $gaba_{b2}$ cDNAs of the sea lamprey as confirmed by sequence analysis and comparison with known sequences of other vertebrates. We performed *in situ* hybridization to study the pattern of expression of these transcripts in the CNS. The *in situ* reaction appeared as a dotted labeling for both transcripts. We observed a broad and overlapping expression of both transcripts in the entire CNS. Expression of these transcripts was observed in all periventricular regions of the brain and in some of the ependymal and choroid plexus cells. Some expression was also observed in migrated cells. Expression of the $gaba_{b1}$ and $gaba_{b2}$ transcripts was also observed in identifiable cells of the brain like the Müller cells 1, 2 and 3. No expression was observed in identifiable fibers. Comparison of our results with those reported in other vertebrates indicates that a broad expression of the $GABA_B$ receptor in the CNS is a conserved character shared by agnathans and gnathostomes.

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

PRESYNAPTIC SPIKE TIMING-DEPENDENT LONG-TERM DEPRESSION IN THE CA1 REGION OF THE HIPPOCAMPUS.

Y. Andrade-Talavera, P. Duque-Feria, A. Rodríguez-Moreno.
Laboratory of Cellular Neuroscience and Plasticity, University Pablo de Olavide, Seville, Spain

Aims

Spike timing-dependent plasticity (STDP) is a model of synaptic plasticity that may underlie learning and memory. The aim of our research was to investigate the mechanisms of spike timing-dependent long-term depression (t-LTD) in the hippocampus.

Materials and Methods

Whole-cell recordings were made from individual CA1 cells from hippocampal slices prepared from P12-P18 mice. Two independent afferent pathways (Schaffer collaterals) were activated alternately by extracellular stimulation. To induce t-LTD, a post-pre pairing protocol (with the postsynaptic activity ocurring 10 ms before a presynaptic activity) was applied after a stable EPSP baseline period of 10 min and then EPSP slope was monitored for at least 30 min.

Results

We found that this protocol induced a robust input-specific t-LTD and that the induction of this form of LTD was completely blocked by D-AP5 (110 ± 7 %, n = 9, vs 71 ± 8 % in control experiments, n = 5). Interestingly t-LTP was not observed after pre-post pairing in cells loaded with MK-801 (103 ± 6 %, n = 6), but subsequent post-pre pairing in the same cell induced robust t-LTD (71± 7 %, n = 6). To further adress the locus of expression of this t-LTD we measured the decay time of NMDAR-EPSCs from both paired and unpaired pathways during MK801 aplication. The time constant (tau) was significantly reduced in the unpaired pathway (38 ± 7 stimuli in control vs 57 ± 5 in paired pathway). Fluctuation, failures and paired-pulse ratios analysis all indicated a presynaptic locus of expression for this t-LTD.

Conclusions

These results show that whereas t-LTP induction depends on postsynaptic NMDARs, the induction of t-LTD is independent of postsynaptic activation of NMDARs and likely requires presynaptic NMDA receptors. The results also show that the induction and expression of t-LTD at CA3-CA1 synapses is presynaptic.

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

IGF-1 DEFICIENCY INDUCES ASTROGLIOSIS IN THE MOUSE COCHLEAR NUCLEI

V. Fuentes-Santamaría[1], J.C. Alvarado[1], L. Rodríguez-de la Rosa[2,3,4], S. Murillo-Cuesta[2,3,4], J. Contreras[2,3,4,5], J.M. Juiz[1], I. Varela-Nieto[2,3,4]

1. Instituto de Investigación en Discapacidades Neurológicas (IDINE), Albacete, Spain. Facultad de Medicina, Universidad de Castilla-La Mancha, Albacete, Spain.
2. Grupo de Neurobiología de la Audición, Instituto de Investigaciones Biomédicas Alberto Sols, Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid, Madrid, Spain.
3. Centro Investigaciones Biomédicas en Red de Enfermedades Raras (CIBERER). Instituto de Salud Carlos III, Madrid, Spain.
4. IdiPAZ Instituto de Investigación en Salud, Madrid, Spain.
5. Facultad de Veterinaria, Universidad Complutense Madrid, Spain.

Insulin-like growth factor 1 (IGF-1) has an essential role in modulating the morphology and function of peripheral and central auditory neurons in the mature mouse brain. Chronic deficiency of IGF-1 leads to hearing loss, increases in auditory thresholds and latencies and to multiple anomalies in the inner ear and spiral ganglion. Recent findings have also demonstrated that mice lacking the *Igf1* gene have an abnormal neuronal cytoarchitecture and impaired presynaptic excitatory neurotransmission in the cochlear nucleus, which might be the result of multiple structural defects and functional adaptations in peripheral and central connectivity. An issue that remains unknown however, is whether glial cells are also involved in this process. In this study, we evaluated the expression of microglia and astrocytes in the cochlear nuclei of a 4-month-old mouse model of IGF-1 deficiency and sensorineural 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 GFAP-immunostaining in the cochlear nuclei of $Igf1^{-/-}$ when compared to $Igf1^{+/-}$ and $Igf1^{+/+}$ animals. However, there were no differences in the expression of microglia cells among genotypes in any cochlear nucleus subdivision. These findings provide evidence of astrogliosis in the cochlear nuclei of IGF-1 null mice that might be associated with synaptic dysfunction and / or remodeling due to an IGF-1 deficient cochlea.

Acknowledgements: This work has been supported by grants from from the Ministerio de Ciencia e Innovación (SAF2011-24391), the Seventh Framework Programme projects FP7-HEALTH-2012-INNOVATION-2 304900 AFHELO and FP7-PEOPLE-2013-IAPP 612261TARGEAR to IVN and and Ministerio de Ciencia e Innovación (BFU2009-13754-C02-01) and PROHEARING project of the 7th Framework Programme (FP7-HEALTH-2012-INNOVATION 304925) to JMJ.

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

CPT1C ROLE IN AMPAR TRAFFICKING

E. Gratacòs-Batlle[1,2], N. Yefimenko[1,2], C. Grau[3], X. Altafaj[3], D. Soto[1,2]
[1]. Laboratori de Neurobiologia, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL) L'Hospitalet de Llobregat, Barcelona
[2]. Department of Pathology and Experimental Therapeutics. Faculty of Medicine, University of Barcelona. L'Hospitalet de Llobregat. Barcelona
[3]. Institut de Neuropatologia, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de Llobregat, Barcelona

AMPA-type glutamate receptors (AMPARs) mediate fast excitatory synaptic transmission in the central nervous system. The biophysical and trafficking properties of AMPARs depend on their subunit composition and on several post-transcriptional and post-translational modifications. Besides, AMPARs associate with numerous auxiliary subunits in the brain, which finely control the properties of the channel. Recent studies have identified novel proteins interacting with AMPAR macromolecular complex, that could potentially regulate AMPAR activity. Amongst these, carnitine palmitoyltransferase 1C (CPT1C) has been demonstrated to form an integral part of native AMPAR complexes in adult brain. Given that AMPAR interacting proteins have been shown to modulate the trafficking and the biophysical behaviour of the receptor, here we have investigated whether CPT1C might be able to regulate AMPAR function. After confirming the physical interaction of CPT1C-AMPAR in heterologous expression systems by co-immunoprecipiation, we performed AMPA-evoked current recordings and showed that CPT1C enhances whole-cell currents of GluA1-containing AMPARs without altering their biophysical properties. Further co-localization experiments revealed that AMPARs and CPT1C strongly colocalize intracellularly, whereas they do not associate at the plasma membrane. By means of immunocytochemistry, we established that increased surface GluA1 receptor number was responsible for the enhanced AMPAR mediated currents in the presence of CPT1C, both in cell lines and in primary cortical neurons. Additionally, we revealed that the palmitoylable cysteine 585 of GluA1 is a key residue in the enhancement of AMPAR trafficking to the cell surface by CPT1C. This work supports a role of CPT1C as a novel regulator of AMPAR physiology and suggest a potential role in the regulation of physiological synaptic activity and in plasticity processes.

Acknowledgements:
Work supported by: the Spanish Ministry of Science and Technology (grant BFU2011-24725), the European Commission (FP7-PEOPLE-2011-CIG; grant 293498). DS is supported by the "Ramón y Cajal" Programme (RyC-2010-05970).

NEUROTRANSMISSION ALTERATIONS RELATED TO THE PROGRESSION OF ALZHEIMER´S DISEASE IN 3xTG-AD TRANSGENIC MICE

C. Nanclares[1], I. Colmena[1], A. Crespo-Castrillo[1], E. Calvo-Gallardo[1], J.A. Arranz-Tagarro[1], A. G. García[1] and L. Gandía[1]
[1]. Instituto Teófilo Hernando. Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, España

Alzheimer´s disease (AD) is the most common form of dementia. It has been reported that although initially the clinical features of AD correlates well with a deficit of cholinergic neurotransmission, behavioural changes that appear in the advanced stages of the disease comprises the involvement of other neurotransmitter systems. These alterations in neurotransmission processes could be correlated with changes in the synthesis, storage or release of neurotransmitter.
In this study we have used a triple transgenic murine model of AD (3xTg-AD). This animal model contains mutations in the gene encoding the amyloid precursor protein (βAPP_{Swe}), presenilin-1 ($PS1_{M146V}$) and tau_{P301L}, which determines a progressive development of the disease.
We propose to study here the last steps of exocytosis in chromaffin cells of 3xTg-AD mice using the amperometric technique, and possible changes that may occur with the development of AD, comparing the characteristics of the exocytotic events present in mice of different ages. The release of catecholamines was induced applying the physiological agonist acetylcholine (ACh) or by means of a depolarizing solution enriched in K^+.
We have found significant changes in the exocytosis of catecholamines that occur in mice of 6 and more than 12 months of age, where the pathology is already established and consolidated, respectively, when compared with prepathologic and cognitively unimpaired mice (2 months). These changes show an increase of the amperometric spikes during the development of the disease, both in response to ACh and K^+, although the quantal catecholamine content of each spike is lower. Kinetic analysis of secretory spikes shows that as the disease progresses amperometric spikes are faster and shorter in duration.
These preliminary data indicate the existence of alterations in the neurosecretory process in chromaffin cell of 3xTg-AD mice, which could form the basis of the various neurotransmitter deficits that occur with the progression of AD.

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

OVEREXPRESSION OF grik4 MODIFIES SYNAPTIC TRANSMISSION FLOW TROUGH MOSSY FIBER-CA3 SYNAPSES

V. Pecoraro[1], A. V Paternain[1], M. I.Aller[1] and J. Lerma[1],
[1]Instituto de Neurociencias, CSIC-UMH. San Juan de Alicante, 03550 Alicante

Glutamate is the most abundant excitatory neurotransmitter in the brain, and distinct classes of glutamate receptors coordinate synaptic transmission whose regulation is critical for normal brain function. KARs constitute one of these classes: they mediate post-synaptic depolarization carrying part of the synaptic current at some synapses; they can modulate the synaptic release of neurotransmitters such as GABA and glutamate at different sites; they play an influential role in the maturation of neural circuits during development. Recent studies have highlighted the role of GluK1, GluK2/3 and GluK5 KAR subunits, but less is known on GluK4 subunit. Abnormalities in the *GRIK4* gene (coding for GluK4 in humans) are present in individuals with schizophrenia and learning disability (mental retardation) and recent studies indicate that copy number variants, including a duplication of *GRIK4*, is implicated in cases of autism.
we have found that overexpression of *grik4* in mice lead to severe alterations in behaviour such as depression and anxiety. Therefore, we wondered how synaptic transmission could be affected by higher levels of GluK4 protein in the brain. For this reason, we explored the influence of GluK4-containg KARs at Mossy fiber (mf) to CA3 synapses, where this subunit is localized.
To study the efficacy of GluK4-containig KARs to modulate mossy fiber facilitation when activated both pharmacologically and synaptically, we used different patterns of stimulation. These experiments evidenced that GluK4 overexpression causes a significant increase in the amplitude of the evoked AMPAR-mediated EPSCs and a reduction of short-term synaptic plasticity (both frequency and pair pulse facilitation). Additionally long term-potentiation (LTP) and depression (LTD) were studied, given that mf-CA3 synapses are well known to express presynaptic long term changes in the probability of glutamate release. We also measured NMDAR-mediated currents and found a NMDAR hypofunction in GluK4-overexpressing mice versus wild type.
These results lead us to conclude that overexpression of the GluK4 subunit produces significant changes in the gain of synaptic transmission at mf-CA3 synapses, highlighting the essential role of GluK4 subunit for information transfer in the hippocampus. This action on circuit activity may underlay the role of *GRIK4* as a susceptibility gene for mood disorders.

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

NOVEL BENZOTHIAZEPINE ITH12662, A BLOCKER OF MITOCONDRIAL NCX, SECURES EXOCYTOSIS TRIGGERED BY REPEATED ACEYLCHOLINE PULSES IN MOUSE CHROMAFFIN CELLS PERIFUSED AT 37 °C

López-Gil, A., Calvo-Gallardo, E., Punzón, E., de los Ríos, C. and García, AG.
Instituto Teófilo Hernando de I+D del Medicamento, Universidad Autónoma de Madrid

We have recently synthesised the benzothiazepine derivative ITH12662 that happens to be more selective and potent than head compound CGP37157 in blocking the mitocondrial Na^+/Ca^{2+} exchanger (mNCX) in HeLa cells (de los Ríos et al., ACS Chem Neurosci. 2015 submited). We investigated here how ITH12662 affect exocytosis triggered by repeated acetylcholine (ACh) pulses applied to adrenal medullary mouse chromaffin cells (MCCs). Twelve (P1 to P12), sequential ACh pulses (100 µM for 2 s at 30 s intervals) were applied to single cells fast-perifused with Tyrode at 37 °C and catecholamine release was on-line monitored with a carbon fibre microelectrode. We found that the exocytotic responses decayed to about 20% during the first 4-5 pulses: when introduced from pulses P5-P8, CGP 37157 at 1 µM further decreased secretion. In contrast, 1 µM ITH12662 significantly doubled the P3-P8 responses to respect P4. When ITH12662 was applied from the very beginning (30 s before P1 and throughout P1-P12), P1 had smaller secretion but the decay was very slow in subsequent pulses. In contrast to secretion, ACh-elicited whole-cell currents (I_{ACh}) did not undergo a decay with repeated ACh pulses. However both, CGP37157 and ITH12662 at 1 µM blocked I_{ACh} by 20%. We conclude: (1^{st}) secretion decay is not due to I_{ACh} decay; (2^{nd}) decreased of steady-state secretion elicited by CGP37157 is surely due to its known non-specific blockade of various ion channels including voltage activated Ca^{2+} channels; (3^{rd}) ITH12662 augments steady-state secretion likely due to more selective blockade of the mNCX thereby prolonging the $[Ca^{2+}]_C$ transients, vesicle transport, and augmenting exocytosis; and (4^{th}) as previously suggested for HeLa cells. ITH12662 reveals as a more selective blocker of the mNCX, not only in HeLa cells but also in excitable neurosecretory MCCs. Hence, ITH12662 may become an invaluable selective pharmacological tool to inquire in the functional consequences of altering mitochondrial Ca^{2+} efflux into the cytosol through the mNCX.

Supported by SAF2013-44108-P to Antonio G. García.

SIGNALLING PATHWAYS VIA NON-CONVENTIONAL NMDA RECEPTOR CONTAINING THE GLUN3A SUBUNIT

Dey, P.N[1], Martinez-Turrillas, R[1], Conde- Dusman, M.J[1], Perez-Otaño, I[1]
[1]. Cellular Neurobiology Laboratory, Centro de Investigación Medica Aplicada (CIMA) and Universidad de Navarra, Pamplona, Spain.

A tightly orchestrated balance between synapse maturation and pruning processes is a hallmark of critical periods of postnatal brain development. This balance is determined by the levels of synaptic activity sensed by individual synapses, and depends on signaling via NMDA glutamate receptors (NMDARs). Mature NMDARs composed of NR1 and NR2 subunits drive synaptic stabilization by detecting coincident pre- and postsynaptic activity and coupling this activity to signaling pathways that promote synapse maturation and growth. A counterbalance is provided by GluN3A subunits, which are typically expressed at juvenile stages and have been proposed to act as a dominant negative regulator of synaptic maturation and stabilization by inhibiting classical NMDAR signaling. Whereas these inhibitory GluN3A roles seem to be critical for directing the targeted pruning of certain synapses and preventing premature synapse maturation/stabilization in developing brains, adult reactivation of GluN3A expression can be pathological and triggers synapse loss (1, 2).

We have begun to map the signaling pathways that underlie the synapse loss and memory deficits associated with enhanced GluN3A expression. To do so, we infected primary neuronal cultures with lentiviral vectors driving neuronal GluN3A overexpression and analyzed the expression of activity-regulated genes (Arc/Arg3.1, cFos, Zif268) and the response of signaling pathways involved in cognition. GluN3A overexpression impaired the increases in expression of Arc and cFos to brief treatment with the GABA antagonist bicuculline, without affecting the induction of other IEGs such as Zif268. Selective inhibition of a set of activity and NMDAR-regulated pathways was also observed in GluN3A overexpressing neurons: major defects in CamKII, MAP38K or the Akt/mTOR/pS6 pathways were detected without changes in ERK1/2 or CREB phosphorylation. This was in contrast to the general inhibitory effect observed in the presence of NMDAR antagonist AP5. Current studies are directed to delineate the mechanisms underlying the selectivity of GluN3A effects on NMDAR signaling. Of note, the mTOR-activating GTPase Rheb and the actin cytoskeleton scaffold GIT1 have been shown to interact directly with GluN3A subunits (3,4), suggesting a possible downstream mechanisms.

1. Roberts, A.C., et al. Neuron 63, 342-356 (2009).
2. Marco, S., et al. Nature Medicine 19, 1030-1038 (2013).
3. Sucher, N.J., et al. Neurosignals (2010)
4. Fiuza, M., et al. PNAS 110 (51):20807-12 (2013)

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

NEURAL STEM CELL-DERIVED REACTIVE ASTROCYTES INDUCED BY SEIZURES IN LPAR1-EGFP MICE

R. Valcárcel-Martín[1,2], S. Martín-Suárez[1,2], J. M. Encinas[1,2,3.]
[1] Achucarro Basque Center for Neuroscience, Bizkaia Science and Technology Park, Zamudio, Spain.
[2] University of the Basque Country (UPV/EHU), Leioa, Spain.
[3] Ikerbasque Foundation, Bilbao, Spain.

Radial neural stem cells (rNSCs) persist in the hippocampus of most mammals and are able to generate neurons through adulthood, a process known as adult neurogenesis. We have recently discovered that seizures originated in the hippocampus massively activate rNSCs inducing them to switch to symmetric cell division in order to generate reactive astrocytes (RAs), as at the same time they also differentiate into RAs. In addition, the neurogenic program of rNSCs becomes abolished.

Because RAs are key players in the brain's response to injury as they are proinflammatory and disrupt synaptic transmission, we aim to characterize rNSC-derived RAs and compare them with those RAs differentiated from parenchymal astrocytes in the hippocampus after seizures. For this purpose we resorted to a transgenic line of mice (LPAR1-EGFP) in which the lysophosphatidic acid receptor 1 (LPAR1) drives the expression the enhanced green fluorescent protein (EGFP).

In these transgenic mice LPAR1-EGFP labels specifically the rNSCs of the adult hippocampus as well as the rNSC-derived RAs generated after seizures. Interestingly, those RAs derived from parenchymal astrocytes do not express LPAR1-EGFP. Thus, this transgenic line is a most valuable tool to study the changes in the neurogenic niche after epileptic seizures.

The differential expression of LPAR1-EGFP readily suggests that indeed the rNSC-derived RAs are different from parenchymal astrocytes-derived RAs, and their contribution to tissue damage/repair might be different. This holds true at least for several weeks after the initial period of seizures but afterwards LPAR1-EGFP expression is lost in rNSC-derived RAs, and intriguingly starts on granule cells. Together our observations point out to LPAR1 playing a role in the regulation of the hippocampal neurogenic niche in basal and physiopathological conditions, and to the existence of a new type of RA in the damaged hippocampus.

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

CHANGES IN ELECTROPHYSIOLOGICAL MEMBRANE PROPERTIES IN CA1 PYRAMIDAL CELLS OF THE HIPPOCAMPUS IN THE *Fmr1* KO MOUSE

M.A. Luque, B. Torres and L. Herrero
Facultad de Biología, Universidad de Sevilla, Sevilla

Fragile X syndrome is the most common form of human inherited intellectual disability and is caused by the altered expression of a single gene located on the X chromosome, the Fragile X Mental Retardation 1 (*FMR1*) gene. The *FMR1* gene encodes the fragile X mental retardation protein (FMRP), which is an RNA-binding protein that controls RNA translation. Numerous neurophysiological studies of *Fmr1* knockout (KO) mice have concentrated on the analysis of synaptic function and plasticity in hippocampus; by contrast, available information about changes on electrophysiological membrane properties is lacking. Aimed at elucidated if FMRP is also involved in determining cell excitability, we compared passive and active electrophysiological membrane properties of CA1 pyramidal cells of the hippocampus. Brain slices were prepared from 8-10 weeks old wild type (WT) and *Fmr1* KO mice. A total number of 8 (from five WT animals) and 11 neurons (from four *Fmr1* KO) were recorded at 33ºC by the whole-cell patch-clamp technique. *Fmr1* KO mice exhibited a depolarized resting membrane potential (~8mV) and subtle increase in the input resistance (12%) in comparison to WT group. The voltage threshold to evoke a single spike remained almost unaltered about -50 mV in both groups of mice. Action potential duration was longer in KO mice (~15%). The firing rate, measured by injecting depolarizing current pulse of 1s at 200pA, in KO mice doubled that of WT animals. This increment was attributable to both a diminishing in current threshold to evoke a repetitive discharge and an increment on the gain of the F-I relationships. These results suggest that FMRP could contribute to control the translation of membrane proteins leading electrophysiological membrane properties. Alternatively, synaptic inputs modifications in *Fmr1* KO mice could modulate the kinetics of channels involved on passive and active membrane properties.

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

LIS1 MUTATION CAUSES ELECTROPHYSIOLOGICAL ALTERATIONS IN FAST-SPIKING GABAERGIC INTERNEURONS OF THE MOTOR CORTEX.

E. Domínguez-Sala[1], S. Martínez[2], E. Geijo-Barrientos[1]
[1]Instituto de Neurociencias, Universidad Miguel Hernández-Consejo Superior Investigaciones Científicas, Sant Joan d'Alacant, Alacant, España.
[2] Instituto Murciano de Investigación Biosanitaria, Murcia, España and CIBERSAM.

LIS1 protein is implicated in different cellular processes related to CNS development and function. We have studied whether alterations in LIS1 expression levels cause functional alterations in the cortical GABAergic interneurons of the fast-spiking (FS) subtype. We used the *Lis1/sLis1* mutant mouse (heterozygous deletion of the first exon in the LIS1) bred with GAD67-GFP mice to obtain a *Lis1/sLis1* line with GFP expressing interneurons. We used brain slices of wild type (WT) and heterozygous (HE) mice (P27-P32) for whole cell recordings from GFP positive FS interneurons in layer 2/3 of the primary motor cortex. Values given as mean ± s.e.m. and compared with the Student's t test. The action potential peak amplitude was larger in WT (72.8 ± 1.9mV; n=12) than in HE neurons (63.5 ± 2.0mV; n=13, p=0.003). The firing rate in response to depolarizing current pulses was higher in HE than in WT neurons: a threefold threshold current pulse produced steady firing at 90.8 ± 8.9Hz in WT neurons (n=12) and at 145.56 ± 19.7Hz in HE neurons (n=12; p=0.019). This different current-firing rate relationship was also quantified comparing the current needed to reach a firing frequency of 150 Hz, which was higher in WT neurons (480.9 ± 49.1pA, n=11 vs 347.0 ± 37.0pA, n=12; p=0.04). We found also differences in the spontaneous EPSCs: their amplitude was larger in HE neurons (26.3 ±1.3pA, n=6 vs 22.8 ± 0.8pA, n=9; p=0.025). These results show alterations in both the functional properties of these interneurons and in their excitatory input, which suggest a dysfunction of the cortical inhibitory system in this animal model of human diseases caused by alterations of the cortical development and function. Funding: Generalitat Valenciana, grant PROMETEOII/2014/014.

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

CREB REGULATES CALCIUM EXCITABILITY IN ASTROCYTES VIA MITOCHONDRIA

A. Eraso-Pichot[1], E. Vicario[2], R. Villalonga[1], L. Pardo[1], E. Galea[1], R. Masgrau[1]
[1]. Institut de Neurociències, Universitat Autònoma de Barcelona, Bellaterra, Barcelona.
[2]. University of California, San Diego, CA.

Astrocytes are calcium-based excitable cells that modulate neurotransmission, learning and memory; although how they do it is not completely understood. Our working hypothesis is that experience induces CREB-mediated long-lasting changes in astrocytes that, in turn, modulate the activity of astrocyte-neuronal circuitries. In this study we have asked the straight question of whether CREB modulates the gliotransmitter-elicited increases in cytosolic calcium in rat cortical astrocyte cultures. To do so, CREB-dependent transcription was triggered by 1 hour-pulses with noradrenaline (10µM) or ATP (100µM), which we have previously shown that activate CREB-dependent transcription in astrocytes within 6 hours, and cytosolic calcium responses were assessed by calcium imaging in Fluo-4 loaded astrocytes challenged by gliotransmitters (ATP, NA and ET-1). Agonist-induced calcium responses were lowered 15-38% and this reduction was reverted by the viral transduction of a dominant negative form of CREB (A-CREB). Likewise, viral transduction of a constitutively active form of CREB (VP16-CREB) caused a similar decrease in gliotransmitter-induced calcium response, as compared to astrocytes infected with an empty virus (Null). There were no alterations either in resting cytosolic calcium levels, extracellular calcium entry or calcium release from acidic stores. Interestingly, ATP induced higher endoplasmic reticulum calcium release and mitochondrial calcium uptake in VP16-CREB overexpressing astrocytes than in Null astrocytes, as measured with CEPIA-ER and CEPIA-MITO2 calcium dyes. The later results were also confirmed monitorizing mitochondrial calcium with Rhod-2 and indirectly with a mitochondrial proton gradient uncoupler FCCP. In conclusion, our results show that CREB changes calcium excitability in astrocytes by altering calcium compartmentalization between endoplasmic reticulum, cytosol and mitochondria.

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

TWO –PHOTON IMAGING OF MICROGLIAL LYSOSOMES IN THE MOUSE CORTEX IN VIVO

Estibaliz Capetillo-Zarate[1, *(2, 3)], S. Solé Domenech [1, 4], D. Cruz[1], C. Matute[2], F.R. Maxfield[1]
[1] Department of Biochemistry, Weill Cornell Medical College, New York, NY 10065, USA
[2]Achucarro Basque Center for Neuroscience, 48170, Zamudio, Spain; Neuroscience Department, University of the Basque Country UPV/EHU, Leioa, Spain
[3]IKERBASQUE, Basque Foundation for Science, 48011, Bilbao, Spain
[4]Experimental Dementia Research Unit, Dept. of Experimental Medical Science, University of Lund, Lund, Sweden
*current position and location

The study of microglial lysosomes in vivo in brain tissue is limited, among other factors, mainly due to lack of selective marker delivery to the brain. The blood brain barrier (BBB) selectively allows or excludes molecular transport in and out of the brain. Dextrans conjugated with dyes are suitable markers to study uptake and internal processing of exogenous materials by phagocytic and endocytic pathways, thus making them suitable as long-term tracers. In culture, microglia take up anionic dextran molecules selectively as compared to other cell types, thereby allowing for the study of lysosomal pH and trafficking as well as endocytic pathways. However, dextran delivery to the brain is limited due to their low BBB permeability. To study microglial lysosomes in vivo we applied intrathecal spinal cord injection for delivery of dextran molecules directly into the cerebrospinal fluid. Intrathecal injection of dextrans, conjugated with FITC and Rhodamine fluorophores, was performed in C57Bl/6 mice followed by two-photon imaging and immunohistochemistry at various time points after injection. Intravital two photon microscopy imaging of dextran delivery to mouse cortex was performed through a cranial window. Additionally, in order to identify microglia and determine whether cells had been loaded with dextran: 1) Brain tissues were sectioned and stained with an Iba1 antibody, and 2) Intrathecal injection of dextrans was also applied to a GFP-microglia mouse model. Immunofluorescence showed internalization of dextrans by lysosomes in Iba1-positive cells (by FITC-Iba1 spatial correlation) in cerebellum and cortical layer III 10 days after the last intrathecal injection. Moreover intravital imaging showed dextran dyes within lysosomes in GFP-microglia in the mouse cortex. These results provide a valuable methodology to be used for in vivo study of microglial lysosomes and provide new information regarding compound delivery to the brain relevant for drug discovery.

Supported by:
Appel Discovery grant, NIH DK27083 grant, Swedish Research Council (VR) Internationell Postdok Grant DRN 637-2013-503 and IKERBASQUE Foundation for Science.

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

SYNAPTIC ACTIVITY AND PKC/TRKB SIGNALING MODULATES THE PHOSPHORYLATION OF THE EXOCYTOTIC PROTEINS SNAP-25 AND MUNC18-1 AT ADULT NEUROMUSCULAR JUNCTION.

A. Simon, E. Hurtado, L. Nadal, V. Cilleros, N. Garcia, M. Santafé, M. Tomàs, M.A. Lanuza, J. Tomàs
Unitat d'Histologia i Neurobiologia (UHN). Facultad de Medicina y Ciencias de la Salud. Universidad *Rovira i Virgili*. Sant Llorenç 21, 43201 Reus. Spain.

In the synapses, several signaling pathways coordinate pre-, post-synaptic responses and associated glial cell. The relation between these signaling pathways modulate the voltage dependent calcium channels (VDCC) and the ready releasable pool of synaptic vesicles leading to neurotransmitter release (Südhof et al., 2013). In addition, the final functional outcome of a synaptic contact can be built by the confluence of the metabotropic receptor-mediated signaling on intracellular protein kinases as PKC and PKA (reviewed by Tomas et al., 2014). Although the mechanism underlying neurotransmitter release has been extensively studied there is still not fully understood the molecular machinery of synaptic vesicle exocytosis. Therefore, the present study is aimed to know (1) how Munc18-1 and SNAP-25 phosphorylation are affected by synaptic activity at the neuromuscular junction (NMJ), (2) how TrkB signaling pathway affects Munc18-1 and SNAP-25 phosphorylation in an activity-dependent way and (3) how presynaptic PKC□ and PKC□regulate Munc18-1 and SNAP-25 phosphorylation. We performed immunohistochemistry and confocal techniques to evidence the presynaptic location of Munc18-1, PKC□ and PKC□I in diaphragm muscle. To induce synaptic activity, we stimulated the phrenic nerve (1 Hz, 30min) with or without contraction (μ-conotoxin GIIIB was used to abolish muscle contraction). Specific inhibitory reagents were used to block tyrosine kinase receptor B (TrkB), PKC□ and PKC□I activity: anti-TrkB (clone 47/TrkB); εV1-2, nPKCε-specific translocation inhibitor peptide; □V1-3, cPKC□Ispecific translocation inhibitor peptide. Main results obtained from Western blot experiments showed a relationship of dependence between skeletal muscle contraction, TrkB signaling, presynaptic PKC□ and PKC□I and Munc18-1 and SNAP-25 phosphorylation at the adult rat NMJ. Together, these results provide a mechanistic insight into how phosphorylation of these exocytotic proteins are regulated to achieve the extraordinary speed, precision, and plasticity of neurotransmission.

This work was supported by 2014SGR0344 and 2014PFR-URV-B2-83.

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

LINOPIRDINE AND ITS ANALOG XE991 BLOCK THE TREK-2 CURRENT ACTIVATED BY RILUZOLE AND TEMPERATURE

P. Rivas-Ramírez[1], J.A. Lamas[1] and A. Reboreda[1]
[1]Laboratory of Neuroscience (CINBIO-IBIV). Faculty of Biology, University of Vigo. Vigo.

Introduction and Objectives: K2P channels have been proposed to contribute to the resting membrane potential (RMP) and excitability, much like M-channels do. Nevertheless the relative contribution of K2P and M channels to these important functions has been difficult to establish due to the lack of selective pharmacological tools. In this work we study the effect of selective M-current blockers linopirdine and XE991 on TREK-2 currents activated by riluzole and temperature.

Material and Methods: Voltage-clamp and current-clamp configurations of the perforated patch-clamp technique were used to record mouse superior cervical ganglion (SCG) neurons in primary culture. Temperature control was carried out using a Warner Instruments temperature controller.

Results: Rising the temperature causes a strong increment of the outward current recorded at -30 mV, a hyperpolarization of the RMP and a strengthening of the adaptation. Both the increase of the current and the hyperpolarization induced by the increase in temperature are reduced when we applied TEA (15 mM), that affects only M-current. The same parameters are further reduced by coaplication of TEA+XE991 (3 μM) that inhibits the remaining M-current and the temperature evoked TREK-2 current. Riluzole, another TREK-2 activator, induces an outward current through TREK-2 channels that was strongly blocked by XE991 and linopirdine, much like they do with the M-current.

Conclusions: Our results indicate that increasing temperature activates TREK-2 currents and that this activation hyperpolarizes SCG neurons and potentiates spike frequency adaptation. TREK-2 currents activated by temperature and riluzole, are inhibited by linopirdine and XE991 which also reduced the effects of the increase in temperature.

Aknowlegments: This research was supported by grants from MICINN/MINECO: CSD2008-00005 CONSOLIDER and BFU2011-25371; Xunta de Galicia: INBIOMED-CINBIO CN2012/273; EC FP7-316265-BIOCAPS. FEDER.

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

OPTOGENETIC BOOSTING OF ASTROCYTE-NEURON SIGNALING

S. Mederos, A. Hernández-Vivanco, G. Perea
Instituto Cajal (CSIC). Madrid, Spain

Astrocyte signaling has critical impact on brain physiology by releasing neuroactive substances, so-called gliotransmitters (Araque et al., 2014). However, its study is limited by standard experimental approaches to manipulate astrocyte activity. Recently, optogenetic has provided powerful method to non-invasively activate and control neuronal circuits. Here, we manipulate astrocytes with optogenetic tools to control their activity and evaluate their consequences on neuronal physiology. The ectopic expression of channelrhodopsin-2 (ChR2, a light-activated ion channel protein) was targeted specifically to astrocytes by viral transfection (Perea et al., 2014). Using electrophysiological techniques in brain slices, we found that optical activation of astrocytes enhanced local excitatory synaptic transmission in CA1 hippocampal pyramidal neurons. ChR2-stimulated astrocytes induced sustained potentiation of evoked synaptic responses according with the duration of light stimulation. Neuronal activity to light stimulation was recorded in control slices and no significant changes were observed. The pharmacological analysis indicated that astrocyte-induced modulation of synaptic transmission was mediated by activation of glutamatergic receptors at neuronal membranes. Then, optical activation of astrocytes stimulates glutamate release that controls the synaptic strength of pyramidal neurons influencing the operation of particular circuits.

Supported by MINECO: RYC-2012-12014; CSD2010-00045; BFU2013-47265R.

References:

Araque A. *et al.* Gliotransmitters travel in time and space. Neuron. 2014;81(4):728-39. doi: 10.1016/j.neuron.2014.02.007.

Perea G, Yang A, Boyden ES, Sur M. Optogenetic astrocyte activation modulates response selectivity of visual cortex neurons in vivo. Nat Commun. 2014;5:3262. doi: 10.1038/ncomms4262.

1st: Neuronal excitability, synapses and glia: cellular mechanisms
2nd: New methods and technologies

INVOLVEMENT OF MICROGLIA IN OVEREATING-INDUCED CHANGES IN NEUROPLASTICITY IN THE MESOCORTICOLIMBIC SYSTEM

M. Gutiérrez-Martos[1], R. Maldonado[1] and M. Martin[2]
[1]. Laboratori de Neurofarmacologia, Univeristat Pompeu Fabra, PRBB, Barcelona, Spain.
[2]. CNRS, UMR-5203, Institut de Génomique Fonctionnelle, Montpellier, F-34094, France; INSERM, U661, Montpellier, F-34094, France; Universités de Montpellier 1 & 2, UMR-5203, Montpellier, F-34094, France.

Overweight, obesity and related pathologies have a high prevalence and socio-economic impact worldwide. Modifications in neuronal plasticity and neuroinflamation in key areas of the brain are considered important mechanisms involved in the initiation and development of different eating disorders. However, little is known about the mechanisms involving alterations in neuroplasticity and neuroinflammation in the mesocorticolimbic system (MCL), a brain reward pathway very important modulating palatable food intake, which leads to the development of overeating and overweight. For these reasons, we have investigated the changes in dendritic spine density in neurons from the brain reward system in mice after the exposure to high fat/palatable food (HPF)-induced excessive eating and overweight. We have also studied the possible involvement of microglia/neuroinflammatory factors modulating this behavior and the associated alterations in neuronal plasticity.
Our results show that prolonged exposure to HPF led to a decrease in dendritic spine densities specifically in medium-sized spiny neurons of the nucleus accumbens shell and core, two main areas of the MCL system. Moreover, increased mRNA levels of microglial-released pro-inflammatory cytokines were also reported in these animals in the same brain areas. Chronic treatment with minocycline, a drug known to inactivate microglia reactivity, produced a decrease in food intake and body weight only in mice exposed to HPF. Moreover, chronic minocycline exposure also reversed the alterations in structural plasticity observed in this group of mice.
We hypothesize that HPF leads to alterations in neuronal plasticity in the MCL system that are responsible of the development of overeating. Microglia activation and neuroinflammation might be involved in these neuroplastic changes.

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

RETINAL MÜLLER GLIA HETEROGENEITY

X. Pereiro [1], N. Ruzafa[1], J. Pérez[1], S.C. Sharma [1,2] E.Vecino [1],
[1]. Dept. Cell Biology and Histology, University of the Basque Country UPV/EHU Spain
Experimental Ophthalmo-Biology Group (www.ehu.es/GOBE)
[2]. New York Medical College, Dept. Ophthalmology, Cell Biology and Anatomy.Valhalla, USA

The retinal Müller glia provide structural and trophic support to retinal ganglion cells (RGCs) in the healthy retina and may also have a function in promoting cell survival after injury. Defining glial heterogeneity is an important goal to open novel therapeutic avenues. Müller glia is implicated in inflammatory retinal pathologies and stimulating neuroprotection in retinal neuro-degenerative diseases like glaucoma (Vecino et al., Prog. Ret. Eye. Res 2015).

Objetives: The aim was to identify different phenotypes of Müller cells in primary cells cultures using different cell markers.

Material and Methods: Muller glia primary cell cultures from porcine retinas were studied. The heterogeneity of the Müller cells was characterized by culturing the cells in different substrates (clean glass, polylysine and polylysine-laminin) combined with different cultured media: DMEM or Neurobasal, plus F12 or B27 supplemented, with or without serum at different concentrations. The different morphological characteristics as well as molecular markers (glutamine synthetase GS, GFAP, Vimentin, p75 and CRALB) was analysed. The co-culture of retinal ganglion cells and Müller cells was also studied. The expression of the molecular markers was compared to Müller cells from control retinas.

Results: Not all Müller cells express the molecular markers with the same intensity. In primary Müller cell cultures, heterogeneity in marker expression of GS or GFAP, were observed. Müller cells expressing high amounts of GS or GFAP lie close to other Müller cells that do not express or express a low concentration of these markers. In addition to Müller cells heterogeneity, we have observed that RGCs prefer to grow on Müller cells that overexpress GS.

Conclusions: There is heterogeneity in Müller cells subpopulations *in vitro* that points to the specificity for GS and GFAP labelling which could be implicated in the different expression for neuroprotective factors.

Supported by Grupos Consolidados del Gobierno Vasco (IT437-10)

1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Nuevos métodos y tecnologías

ENDOCANNABINOID MEDIATED NMDAR INDEPENDENT LTD OF GLUTAMATERGIC SYNAPTIC TRANSMISSION AT LAYER V PYRAMIDAL NEURONS OF RAT INFRALIMBIC CORTEX.

JA. Noriega-Prieto[1], Y. Gallero-Salas[2], LE. Maglio[1], D. Fernandez de Sevilla[1].
[1]. Facultad de medicina, Universidad Autónoma de Madrid, Madrid
[2]. Faculty of Medicine, Imperial College London, London

Some forms of spike timing-dependent LTD at cortical glutamatergic synapses require the coactivation of presynaptic NMDA and CB1 receptors, being crucial the release of endocannabinoids by postsynaptic calcium spikes. Here we have analyzed the role of calcium spikes on the induction of LTD at glutamatergic synapses of layer V pyramidal neurons of rat infralimnic cortex. After recording the control postsynaptic currents (PSCs) evoked by electrical stimulation in the basal dendrites, we increased the stimulation intensity to generate a postsynaptic potential (PSP) followed by an action potential (AP) and a calcium spike (PSP-AP-Ca^{2+} spike responses). Repeating the stimulation 60 times at a frequency of 0.2 Hz induced a robust long-term depression (> 40 minutes) on the PSC peak amplitude (48.9 ± 4.15% compared to control). The LTD was blocked under Nifedipine (20µM) plus D-AP5 (50 µM) when APs were blocked by intracelullar QX-314 (5mM). However LTD was unaffected by intracelullar QX-314 (5mM) or Nifedipine (20µM) plus D-AP5 (50 µM) applied alone. A similar LTD was obtained when EPSCs were isolated under PiTX (50 microM). This LTD was mediated presynaptically because the decrease in the amplitude of the EPSCs was associated with a change in the coefficient of variation, being unaffected the postsynaptic currents evoked by local puff application of glutamate. The LTD of the PSCs was prevented by intracellular BAPTA or by AM251 bath superfussion. In addition, AM251 was not able to prevent the LTD of the EPSCs recorded under PiTX. Taken together our results suggest that the cytosloic calcium increase mediated by the PSP-AP-Ca^{2+} spike responses release endocannabinoids from layer V pyramidal neuron that reduce the $GABA_AR$ mediated inhibition allowing the induction of a NMDAR independent presynaptic LTD of the EPSCs.
Supported by MICINN to Dr. D. Fernández de Sevilla (BFU2011-23522; BFU2013-43668).

INSULIN-LIKE GROWTH FACTOR-1 MODULATES SYNAPTIC TRANSMISSION AND POSTSYNAPTIC EXCITABILITY IN LAYER II/III OF MOUSE SOMATOSENSORY CORTEX.

LE. Maglio[1], JA. Noriega-Prieto[1], P. Perez-Domper[2], I. Torres-Aleman[2], A. Nuñez[1], D. Fernandez de Sevilla[1].
[1] .Facultad de Medicina, Universidad Autónoma de Madrid, Madrid.
[2] .Instituto Cajal CSIC, Madrid.

Insulin-like growth factor I (IGF-I) is a neuromodulatory peptide released by specific populations of neurons. We had previously observed an enhanced electrocorticogram (ECG) activity in response to IGF-1 application. Because neurons of layer II/III contribute mainly to the electrical activity in the ECG, we have analyzed the effect of IGF-1 on synaptic efficacy and neuronal excitability at layer II/III pyramidal neurons of somatosensory cortex. Using coronal slices, we superfused IGF-1 (10 nM) and measured the changes in electrophysiological properties of pyramidal neurons using whole-cell patch clamp technique. IGF-1 increased the membrane resistance and reduced the action potential threshold. These results showed an excitatory effect of IGF-1. Moreover, we studied the modulation induced by IGF-I in the EPSCs and IPSCs evoked by electrical stimulation at layer IV. IGF-1 induced short-term potentiation or depression of EPSCs when it was applied during current clamp or voltage clamp recording of the layer II/III PN, respectively. These forms of synaptic plasticity were presynaptically mediated because a changed in the coefficient of variation paralleled the change of synaptic current amplitude. However, its induction depends on the postsynaptic calcium increase since they were absent when BAPTA was included in the patch pipette. This modulation was mediated by IGF1-R, because was absent in presence of selective antagonist NPV-EAW554. Pre- and postsynaptic actions of IGF-I are supported by the observation that IGF-I receptor immunoreactivity was found in close proximity to both presypnaptic (VGat) and postsynaptic (PSD95) markers. In conclusion, IGF-1 modulates neuronal excitability postsynaptically and synaptic transmission by presynaptic mechanism activated by retrograde messenger released by layer II/III PNs in calcium-dependent manner.

Supported by MICINN to Dr. D. Fernández de Sevilla (BFU2011-23522; BFU2013-43668).

GLIAL INFLAMMATORY RESPONSE IS IMPAIRED IN THE SENESCENCENT MOUSE SAMP8

R. Corpas[1], P. Molina-Martínez[1], M. Cosín-Tomás[1,2], P. Kaliman[1], C. Solà[1], M. Pallàs[2], C. Sanfeliu[1].
[1] Institute of Biomedical Research of Barcelona (IIBB), CSIC-IDIBAPS, Barcelona, Spain
[2] Faculty of Pharmacy, IBUB, University of Barcelona and CIBERNED, Barcelona, Spain.

Background: A chronic low grade neuroinflammatory process in the aging brain may contribute to the triggering and progression of neurodegenerative disorders. The senescence accelerated mouse SAMP8 shows neuroinflammation along with cognitive loss and other traits of pathological aging.
Objectives: We aimed to analyze the inflammatory response of microglia and astrocytes in cortex and hippocampus of SAMP8 mice compared with the control strain SAMR1.
Material & methods: The response to lipopolysaccharide (LPS) injection has been studied *in vivo* at the early age of 6 months and at the advanced stage of 12 months. *In vitro*, mixed glial cultures and pure microglia have been studied in response to LPS/interferon-γ (IFN). The levels of the proinflammatory cytokines IL6, IL1β and TNFα were determined by ELISA, their gene expression by qRT-PCR and the generation of nitric oxide (NO) by Griess method.
Results: Brain tissue of 6-months old SAMP8 showed higher levels of cytokines as compared to SAMR1, and LPS injection exacerbated the inflammatory response. In contrast, 12-months old SAMP8 showed lower inflammatory markers and lack of activation by LPS, whereas the response was preserved in SAMR1 mice. SAMP8 microglia and mixed glial cultures showed higher levels of cytokines and NO in response to LPS/IFN, and higher NO production in microglia under basal conditions, indicative of disturbed inflammatory mechanisms in the glial cells.
Conclusions: SAMP8 mice show an early chronic neuroinflammation that might depend on hyperactivated glia mechanisms. Furthermore, inflammatory response to proinflammatory external stimuli is nearly lacking at advanced senescence stage, indicating further glial derangement. Changes in glial inflammatory mechanisms associated with aging cause a reduction of neural defense mechanisms to external injuries, contributing to frailty and pathological brain aging.
Acknowledgements: CSD2010-00045 and SAF2012-39852, MINECO, Spain, and European Research Development Fund.

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

ALTERED CORNEAL NERVE ACTIVITY AFTER NERVE DAMAGE AND CHRONIC TEAR DEFICIENCY

S. Quirce[1], C. Luna[1], M.C. Acosta[1], I. Kovacs[1,2], C. Belmonte[1,3], J. Gallar[1].
[1] Instituto de Neurociencias, Universidad Miguel Hernández-CSIC, Sant Joan d'Alacant, Alicante.
[2] Department of Ophthalmology, Semmelweis University, Budapest, Hungría.
[3] Instituto Oftalmológico Universitario Fernández-Vega, Universidad de Oviedo and Fundación de Investigación Oftalmológica, Oviedo.

Objectives. To characterize the activity of the different functional types of corneal sensory nerve fibers after surgical injury or chronic dryness of the ocular surface (DE).
Material and methods. Corneal lesions were performed in anesthetized guinea-pigs with a custom-made microkeratome. A separate group of animals was subjected to surgical removal of the main lachrymal gland to reduce tearing. The spontaneous and stimulus-evoked electrical activity of the different functional types of corneal sensory nerve fibers was recorded "in vitro" from corneal nerve terminals and ciliary nerves in control and experimental eyes at different times after surgery, using conventional electrophysiological equipment. Thermal stimulation was made by changing the bath solution temperature from 34ºC (basal) down to 20ºC or up to 50ºC. Mechanical threshold was measured with calibrated Von Frey. Chemical stimulation was made applying 98% CO^2 pulses during 30s.
Results. Removal of the main lachrymal gland caused a significant reduction of basal and reflex tearing, as well as a reduction of the density of corneal epithelial and sub-basal nerves. Ongoing impulse activity and response to cooling pulses of corneal cold thermoreceptor endings increased with time after reduced tearing, being significant at 4 weeks after DE. Corneal polymodal nociceptors of tear-deficient eyes exhibited only mild sensitization to CO^2 stimulation, while mechano-nociceptors were not affected by chronic tear deficiency. Similar results were obtained at 2-4 weeks after corneal lesion with the microkeratome.
Conclusions. Chronic dry eye condition and surgical lesion of corneal nerves alter markedly the activity of corneal sensory nerve fibers as result of ion channel expression and/or activity, leading to the development of spontaneous activity and of abnormal responsiveness to natural stimuli. These changes are more prominent in cold thermoreceptor fibers, whose injury-evoked neuropathic firing appears to underlie the unpleasant sensations experienced by dry eye disease patients.

Supported by SAF2014-54518-C3-1-R and in part by SAF2014-54518-C3-2-R, Ministerio de Economía y Competitividad, Spain.

AXON GLYCOPROTEIN TRANSPORT-RELATED GALECTIN-4 REGULATES MYELINATION

J. Abad-Rodríguez[1], A. Higuero-Romero, N. Díez-Revuelta[1], H-J. Gabius[2], H. Kaltner[2], S. André[2]
[1]Hospital Nacional de Parapléjicos de Toledo, Toledo, Spain.
[2]Tierärztliche Fakultät, Ludwig-Maximilians-Universität, Munich, Germany

Galectin-4 (G4), expressed in discrete segments of the axonal membrane, displays two binding domains, N-terminal (NT) with high affinity for N-acetyllactosamine (LacNAc) epitopes in glycoproteins, and C-terminal (CT) for sulfated headgroup of sulfatides. These binding characteristics underlie its capacity to organize axon transport of glycoproteins. G4 has been recently involved in myelination.
Objective: We aimed to assess the role of G4 in myelination and to define its mechanism.
Methods: Cultures of rat hippocampal, cortical neurons, astrocytes, rat oligodendrocytes (OD), and neuron/oligo co-cultures. OD maturation on stripped substrata of recombinant G4 forms. Overexpression of wt and mutant forms of G4. Membrane fractionation, raft purification and analysis. Brain myelin purification.
Results: G4- or G4-NT domain-covered substrata enhance OD maturation, while G4-CT domain retards it. Surprisingly, ODs deposit myelin preferentially on G4 or G4-NT-free surfaces, as shown in striped carpets OD cultures. In fact, mature OD tend to myelinize G4-free axon segments, as shown by the quantification of G4 vs MBP-expressing tracts in neuron/OD co-cultures, and 3D reconstruction of myelinated tracts. We hypothesized that myelination starts at the limit of G4-expressing axon segments and that interaction of myelin with G4-CT could be inducing G4 elimination from axon membrane, permitting the spreading of the myelin sheath. HA-labelled G4 expressed in astrocyte membrane (that do not express endogenous G4) was reduced in the presence of purified myelin, while a similar form of G4 bearing a single mutation at the CT remained unaltered on astrocyte membrane upon myelin exposure. In all, G4 stimulates OD maturation and myelin production by OD interaction with its NT domain, likely through NAcLac epitopes, while myelin deposition on G4-free axon segments is regulated by myelin interaction with G4-CT, likely through sulfatides, which induces G4 removal from the membrane.

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

CALCIUM-INDUCED CALCIUM RELEASE SUPPORTS RECRUITMENT OF SYNAPTIC VESICLES IN AUDITORY HAIR CELLS

Manuel Castellano-Muñoz[1,3], Michael E. Schnee[1] and Anthony Ricci[1,2].
Department of Otolaryngology[1] and Molecular and Cellular Physiology[2]. Stanford University School of Medicine. 300 Pasteur Drive. Stanford, CA 94305 (USA).
Current address: Institute of Bioengineering. Miguel Hernández University. Avenida de la Universidad , s/n 03202 Elche, Alicante (Spain). [3]

Hair cells from auditory and vestibular systems transmit continuous sound and balance information to the central nervous system through the release of synaptic vesicles at ribbon synapses. The high activity experienced by hair cells requires a unique mechanism to sustain recruitment and replenishment of synaptic vesicles for continuous release. Using pre and postsynaptic electrophysiological recordings, we explored the potential contribution of calcium-induced calcium release (CICR) in modulating the recruitment of vesicles to auditory hair cell ribbon synapses. Pharmacological manipulation of CICR reduced spontaneous postsynaptic multiunit activity as well as the frequency of excitatory postsynaptic currents (EPSCs). Pharmacological manipulation of calcium stores had no effect on hair cell resting potential or activation curves for calcium or potassium channels; yet a reduction in vesicle release was observed using dual-sine capacitance measurements. This reduction contrasted with the release enhancement observed in control experiments during repeated stimulation. In addition, calcium substitution by barium reduced release efficacy by delaying release onset and diminishing vesicle recruitment. Together these results suggest a novel contribution of CICR in the recruitment of vesicles to the hair cell ribbon synapse. We hypothesize that calcium entry via calcium channels is tightly regulated to control timing of vesicle fusion at the synapse; thus CICR is used to maintain a tonic calcium signal to modulate vesicle trafficking.

1. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2. Sistemas homeostáticos y neuroendocrino

MODULATION OF SYNAPTIC PLASTICITY MARKERS BY CHRONIC EXPOSURE TO WNT LIGANDS IN THE HIPPOCAMPUS IN VIVO.

A. E. Ortiz Matamoros, C. Arias
Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, México, D.F.

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

This work was supported by PAPIIT, 202615, CONACYT 166482 and CONACYT 269645.

MECHANISMS UNDERLYING DECREASED SEIZURE SUSCEPTIBILITY IN SGK1.1 TRANSGENIC MICE.

N. Armas[1], L. Pérez[2], A. Reboreda[3], D. Alvarez de la Rosa[1], L.C. Barrio[2] and T. Giraldez[1].
[1]. Facultad de Medicina. Universidad de La Laguna, Tenerife.
[2]. Hospital Ramón y Cajal, Madrid.
[3]. Facultad de Biología. Universidad de Vigo, Pontevedra.

SGK1.1 a neuronal isoform of the ubiquitous expressed serum- and glucocorticoid-regulated kinase 1 (SGK1) has been proposed by our group as a physiological regulator of M-channels, members of the Kv7/KCNQ gene family that control membrane resting potential and excitability (Miranda et al., 2013). We have found that SGK1.1 upregulates the Kv7.2/3 current in heterologous expressions systems. Superior cervical ganglion (SCG) neurons isolated from transgenic mice expressing a constitutively active form of SGK1.1 (Tg.sgk) showed a significant increase in M-current levels, paralleled by reduced excitability and more negative resting potentials.
We have now assessed seizures susceptibility using the kainic acid (KA) model by either behavioral observation or EEG recordings. Seizure severity was evaluated according to previous scale (Racine 1972). Wild types mice showed higher rates of severe clonic seizures and 40% of mortality in contrast to reduced seizure activity and no mortality in Tg.sgk. Electroencephalographic studies were made to record changes in electrical activity in the hippocampus and cortex after systemic administration of KA. Analysis of the EEG data revealed that the Tg.sgk displayed a significant lower number of ictal episodes following KA application. The number of ictal discharges, and their total time spend in ictal seizure was significantly reduced when compared with wild type, with no difference in seizure onset. Ours finding reveals that SGK1.1 is involved in the maintenance and ending of seizures. Whole cell recordings in Tg.sgk hippocampal slices are underway to study the involvement of SGK1.1 in counteracting hyperexcitability.

Miranda P., Cadaveira-Mosquera A., González-Montelongo R., Villarroel A., González-Hernández T., Lamas J. A., et al. (2013). The neuronal serum- and glucocorticoid-regulated kinase 1.1 reduces neuronal excitability and protects against seizures through upregulation of the M-current. J.
Racine RJ (1972) Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol 32:281–294.

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

GSK3β INHIBITION PROMOTES SYNAPTOGENESIS IN DROSOPHILA AND MAMMALIAN NEURONS

G. Cuesto[1,2], S. Jordán-Álvarez[4,5], L. Enriquez-Barreto[2,3], A. Ferrús[4], M. Morales[2], A. Acebes[1,4].
1. Centre for Biomedical Research of the Canary Islands, Institute of Biomedical Technologies, University of La Laguna, La Laguna, Tenerife, Spain.
2. Structural Synaptic Plasticity Laboratory, Department of Neurodegenerative Diseases, Centro de Investigación Biomédica de La Rioja, Logroño, La Rioja, Spain.
3. Neuroscience Institute, Department of Biochemistry and Molecular Biology, Universitat Autònoma de Barcelona, Barcelona, Spain.
4. Department of Cellular, Molecular and Developmental Neurobiology, Cajal Institute, Consejo Superior de Investigaciones Científicas, Madrid, Spain.
5. Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Universidad Autónoma de Madrid, Cantoblanco, Madrid, Spain.

Glycogen synthase kinase 3 (GSK3) is a serine/threonine kinase that regulates several processes, including neuronal polarization, neuritogenesis, migration, axon guidance and formation of new synapses. In this work, we have addressed the role of GSK3β in synaptogenesis in primary hippocampal rat neuron cultures with tools that inhibit directly GSK3 activity, using AR-A014418 and SB-415286, two pharmacological specific inhibitors and PTD4-PI3KAc, a transduction phospho-peptide that activates PI3K-AKT signaling pathway and inhibiting indirectly GSK3. The pharmacological inhibition of GSK3 as well as PTD4-PI3KAc treatment leads in three weeks cultured rat hippocampal neurons to an increase in synaptic density measured by immunocytochemistry and Western blot. However, in experiments performed on younger cultures (12 days), both AR and SB yield an opposite effect, a reduction of synapse density, while PTD4-PI3KAc increases the number of synapses. Considering dendritic spine density, in Actin-GFP transfected primary hippocampal neuron cultures, only the lower concentration of SB results in an increase in spine density whereas higher concentrations were anti-spinogenic. On the other hand, PTD4-PI3KAc, again, yields to an increase in spine number. All together, these unexpected findings seem to unveil an age- and dosage-dependent differential response of mammalian neurons to the stimulation/inhibition of GSK3β, a feature that must be considered in the context of human adult neurogenesis and pharmacological treatments for Alzheimer's disease based on GSK3β antagonists.

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

SEX -DEPENDENT EFFECTS OF UNPREDICTABLE POSTNATAL MATERNAL STRESS PLUS MATERNAL DEPRIVATION ON GLIAL CELLS, AND CB1 CANNABINOID RECEPTOR IN THE HIPPOCAMPAL FORMATION OF ADULT RATS

L. Arnaldo[1], R. Nadal[2], S. Fuentes[2], A. Armario[3]; MP. Viveros[1]; M. López-Gallardo[4]
[1]. Department of Physiology (Animal Physiology II), Faculty of Biology, Universidad Complutense, Madrid, Spain.
[2]. Institut de Neurociències and Psychobiology Unit (School of Psychology), Universitat Autònoma de Barcelona, Spain.
[3]. Institut de Neurociències. Animal Physiology (School of Biosciences), Universitat Autònoma de Barcelona, Spain.
[4]. Department of Physiology, Faculty of Medicine, Universidad Complutense, Madrid, Spain.

Traumatic experiences during childhood can increase the risk of suffering neuropsychiatric diseases in the adulthood. In animals, neonatal stress models have been developed to mimic early life stress in humans and to investigate the neural basis of its long term effects. The hippocampal formation appears to be one important target of the deleterious effects caused by these stress protocols. In the present study, we have investigated the effects of a combination of unpredictable postnatal maternal stress (electric shock, restraint, forced swim) 20 minutes/day; and unpredictable maternal separation, 3 hours/day, from postnatal day (PND) 1 to 14, on the hippocampal formation of adult Long-Evans rats (PND 90) of both sexes. We analyzed by immunohistochemistry, neuronal and glial markers, CB1 receptor expression and several synaptic plasticity players. Notably, the present neonatal stress model increased active maternal behavior. In treated animals of both sexes, a decreased number of GFAP+ cells was found in the polymorphic layer of the Dentate Gyrus. In all areas analyzed, the number of Iba1+ cells tended to decrease in males and to increase in females. In relation with the stage of activation of microglia (I corresponding to ramified resting microglia and V to ameboid microglia, the most active one), an increase of morphotype II versus morphotype I cells was found in all areas and in both sexes. A decreased expression of CB1 cannabinoid receptor was found in treated males, mainly in CA3 area. No alterations were found in the expression levels of NeuN or synaptophysin. In conclusion, the present combined model of neonatal stress induced long term sexually dimorphic effects in glial cells and CB1 receptors in the hippocampal formation of adult rats.

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

LOSS OF MEF2c TRANSCRIPTION FACTOR CONTRIBUTES TO THE REDUCTION OF SYNAPTIC PLASTICITY AND BDNF LEVELS IN HIPPOCAMPAL HD NEURONS.

S. Fernández[*1,2,3], L.Vidal-Sancho[*1,2,3,4], A.Badia[1,2,3], J.Alberch[1,2,3], X.Xifró[1,2,3,4].
1 Facultat de Medicina, Universitat de Barcelona, Spain
2. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
3 Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain
4 New Therapeutic Targets Group, School of Medicine, Universitat de Girona, Spain
*The authors contributed equally to this work.

The family of transcription factors Myocyte Enhancer Factor 2 (MEF2) has been recently involved in memory formation process. Cognitive deficits have been highlighted as an important component of the Huntington's Disease (HD) and related to hippocampal dysfunction. Therefore, we studied the role of MEF2 in the hippocampus of two HD mice models, the R6/1, an exon-1 mouse model, and the knock-in mice (Hdh^{Q111}), a full-length mouse model. The analysis of MEF2 protein levels at different ages revealed a decrease of these transcription factors in both mice models from the onset of cognitive dysfunction. The transfection of mutant huntingtin (htt-94Q-GFP) in the neural cells ($STHdh^{Q7/Q7}$) resulted in an important decrease of MEF2 protein levels. We also observed by immunohistochemistry and immunoprecipitation assay that MEF2 neither colocalizes nor interacts with mutant huntingtin, suggesting that MEF2 decreased levels observed in HD mouse models could be directly related to its transcriptional dysregulation. Among the MEF2 genes expressed in the brain, we observed that loss of MEF2 is associated with a decrease in MEF2c, with no changes in MEF2a protein levels. To find out whether MEF2c is implicated in the reduction of hippocampal BDNF levels, an important hallmark of HD, we transfected $STHdh^{Q7/Q7}$ cells with a constitutively active form of MEF2c (mtMEF2C219). We observed that BDNF protein levels are enhanced in cells expressing mtMEF2C219. Moreover, hippocampal primary neurons treated with an inhibitor of MEF2c displayed a reduction in the number and length of dendritic spines. Our results suggest that the loss of MEF2c factor is involved in the reduction of BDNF and synaptic plasticity that occurs in the hippocampus of HD.

Funding:
Ministerio de Economía y competitividad (SAF2011-29507).
CIBERNED (Instituto Carlos III).
Generalitat de Catalunya (2014SGR-968).

1st: Disorders and nervous system repair.
2nd: Neuronal excitability, synapses and glia.

SYNCHRONIZED PRIMING AND RELEASE OF HETEROGENOUS VESICLES VIA A STATIC READILY RELEASABLE POOL AT MATURE MOUSE CALYX OF HELD

K. Mahfooz[1], J. Wesseling[1]
[1]. CIMA, Universidad de Navarra, Pamplona

The readily releasable pool (RRP) of vesicles is a core concept in studies of presynaptic function. Here we focus on two operating principles that seem to differ according to synapse type or developmental stage. First, the capacity for storing vesicles is thought to be a dynamic function of Ca^{2+} at the calyx of Held, but static at hippocampal synapses. And second, reluctantly-releasing vesicles are thought to be recruited to the RRP much more quickly than fast-releasing vesicles at immature synapses, but with similar timing at mature hippocampal synapses. We show here that RRP capacity does not depend on Ca^{2+} at mature calyces of Held when both reluctant and fast-releasing vesicles are included. We then use 100 Hz trains of action potentials to preferentially exhaust the fast-releasing supply. Subsequent jumps to 300 Hz revealed a standing steady state supply of reluctant vesicles that were recruited with timing that was similar to fast-releasing vesicles, and released in synchrony with action potentials. Quantitative analysis of the size and timing of release of the standing supply indicated that reluctant and fast-releasing vesicles are likely recruited to separate sets of release sites. If not, any intermediate reluctant state upstream of fast-releasing must be short-lived during ongoing 100 Hz stimulation, but highly variable between preparations. Together, the results support the idea that RRPs at mature synapses are made up of stable, autonomous release sites. Probability of release varies between release sites, but independently of the timing of vesicle recruitment and how tightly release is synchronized to action potentials.

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

DEVELOPMENTAL DISTRIBUTION OF RGS7/Gβ5 IN THE HIPPOCAMPUS AND THEIR RELATIONSHIP WITH $GABA_B$ RECEPTORS.

A. Fajardo Serrano [1], K. Martemyanov[2], R. Shigemoto[3], R. Luján[1], R.
[1]. Facultad de Medicina. Instituto de Investigación en Discapacidades Neurológicas (IDINE), Universidad de Castilla-La Mancha. Albacete.
[2]. The Scripps Research Institute. Jupiter, Florida. USA.
[3]. Institute of Science and Technology (IST). Klosterneuburg. Austria.

G protein-coupled signaling is a major cellular mechanism for controlling excitability in central nervous system. An integral part of G protein-coupled signaling pathway is the regulator of G-protein signaling (RGS) protein, which accelerates rates of G-protein deactivation. We have recently shown that RGS7 and Gβ5 share the same distribution relative to the glutamate release site, and it is virtually identical to that of GIRK2 and $GABA_{B1,}$ supporting the idea that RGS7/Gβ5 complexes modulate $GABA_B$ receptor signaling through the deactivation of GIRK channels in adult hippocampus (Fajardo-Serrano, 2013). Now, we analyze with the histoblot technique the expression pattern of these proteins during postnatal development. RGS7, Gβ5 and $GABA_B$ receptors have similar distribution pattern along development in different postnatal ages (P0, P5, P10, P15, P21, P60). The expression of RGS7 is low at P0, but it is increased at P10 and it is maintained in all analyzed regions (hippocampus, thalamus, caudate putamen and cerebellum) throughout all developmental ages. The Gβ5 expression shows an increase along development that it is stabilized at P21 and P60. Both $GABA_{B1}$ and $GABA_{B2}$ show an increase at P15 that it is maintained until P21 and P60 ages, where a second increase occurs in the different regions. The virtually identical distribution pattern of RGS7/Gβ5 and $GABA_B$ receptors suggests that RGS7/Gβ5 complexes are necessary to form macromolecular complexes with $GABA_B$ receptors during development. Altogether, these data support the idea that RGS7/Gβ5 complexes are involving in the deactivation rate of $GABA_B$ receptors since early stages of postnatal development.
Fajardo-Serrano et al. *Hippocampus* 23(12):1231-45. (2013)

This work is supported by MICINN BFU-2012-38348 and European Union (HBP - Project Ref. 604102)

DELTA-9-TETRAHYDROCANNABINOL CAUSES SYNAPTIC AND MOTOR COORDINATION IMPAIRMENTS THROUGH A MECHANISM UNDERLYING COX-2 ACTIVATION AND MICROGLIAL REACTIVITY

L. Cutando[1], V. Salgado-Mendialdúa[1], R. Maldonado[1], A. Ozaita[1].
[1]Laboratori de Neurofarmacologia. Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, 08003-Barcelona

Chronic use of cannabis has been associated to cerebellar dysfunction in humans. We previously demonstrated in mice that exposure to delta-9-tetrahydrocannabinol (THC), the main psychoactive component of cannabis, triggers microglial reactivity in the cerebellar molecular layer, and enhances the expression of neuroinflammatory molecules, such as interleukin 1β (IL-1β) cytokine or cyclooxygenase-2 (COX-2) enzyme. In the cerebellum, COX-2 is found in Purkinje cells and its expression is generally up-regulated following brain insults, via glutamatergic and inflammatory mechanisms. COX-2 synthesizes prostaglandins, which modulate synaptic plasticity and microglial activation through their action on EP prostaglandin receptors, such as EP2 receptors, that are mainly expressed on activated microglia. We evaluated the role of COX-2 in the cerebellar deficits associated with cannabis consumption and withdrawal. THC administration in mice (5mg/kg; 5.5 days; twice per day) produced COX-2 and EP2 enhanced expression in the cerebellum, which were still increased 5 days after THC-treatment. THC withdrawal was characterized by fine motor coordination deficits in the footprint, beam walking and hanger test performance. We evaluated the expression of ionotropic glutamate receptor 2 (GluR2) and glutamate receptor delta 2 (GluRδ2), both involved in cerebellar long-term depression (LTD) performance. Both receptors were modified in the cerebellum of THC-withdrawn mice. Interestingly, the sub-chronic administration of the COX-2 inhibitor NS-398 (10mg/kg; 5 days; once per day) after THC exposure, prevented the alterations in fine motor coordination observed in the THC-withdrawn mice and normalized EP2, GluR2 and GluRδ2 expression levels. In addition, NS-398 also prevented microglial reactivity in the cerebellar molecular layer. These results suggest that COX-2 plays an important role in the control of microglial reactivity in the cerebellum during THC-withdrawal conditions, revealing COX-2 as crucial in the cerebellar deficits associated to repeated cannabis exposure.
Supported by BFU2012-33500 (MINECO, Spain). V S-MAN-R was supported by a FPI fellowship (MINECO, Spain).

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

SYNAPTIC TAGGING OF SINGLE POTENTIATED SPINES BY A STABLE COFILIN-ACTIN MACROMOLECULE

M. Bosch[1,4], T. Saneyoshi[2], H. Matsuno[2], Y. Hayashi[1,2,3]
[1]. The Picower Institute for Learning and Memory, Massachusetts Institute of Technology (MIT), Cambridge, Massachusetts, USA
[2]. Brain Science Institute (BSI), RIKEN, Wako, Saitama, Japan
[3]. Saitama University Brain Science Institute, Saitama University, Saitama, Japan
[4]. Present address: Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain

Synapses can persistently modify their function independently of each other. These two properties –persistence and selectivity- can be explained together by the generation of a molecular tag that identifies the modified synapse and captures the components required for the consolidation of that modification. The nature of this synaptic tag remains totally unknown. We studied postsynaptic protein dynamics after the induction of long-term potentiation (LTP) in single dendritic spines by two-photon glutamate uncaging. We found that the actin depolimerization factor Cofilin is the only protein to be rapidly, massively and persistently enriched in the spine after induction of LTP. Cofilin slows down its turn-over, binds to actin filaments and, instead of depolymerizing them, forms a new macromolecule that gradually migrates to the base of the potentiated spine and remains stable for long-term. This synaptic capture of cofilin shares most of the intracellular signaling pathways that regulate LTP expression. Cofilin activity is not required for the induction but necessary for the consolidation of the structural plasticity of the dendritic spine. We propose that this cofilin-actin macromolecule could serve as a synaptic tag that selectively consolidates the potentiated state in spines.

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

ROLE OF RRas2 IN OPTIC NERVE MYELINIZATION

M. Sanz Rodríguez, J.R. Perea Úbeda Portugués and B. Cubelos Álvarez
Centro de Biología Molecular Severo Ochoa, CSIC-Universidad Autónoma de Madrid, Madrid

RRas2 is a member of the Ras-related GTPase subfamily (RRas), closely related to the classic Ras family. Some studies had shown that in culture, the expression of a constitutively-active RRas resulted in a dramatic increase in sheet formation by the oligodendrocytes while using a dominant-negative RRas reduces processes and myelin sheets.

Here, we will show that, in adult mice, RRas2 is expressed in oligodendrocytes and retinal ganglion cells (RGCs). *RRas2*$^{-/-}$ null mice present a decrease in the number of oligodendrocyte of the optic nerve and a tendency to a decreased content of myelin. These defects correlate with an abnormal delay of the response of Lateral Geniculate Nucleus (LGN) neurons upon stimulation of the RGCs. Together these results suggest that RRas2 contributes to myelin formation in the optic nerve and thus to a functional visual system.

CIRCUIT-SPECIFIC SIGNALING IN ASTROCYTE-NEURON NETWORKS IN BASAL GANGLIA PATHWAYS

R. Martín[1*], R. Bajo-Grañeras[1*], R. Moratalla[1], G. Perea[1], A. Araque[2]
[1] Instituto Cajal, CSIC, Madrid
[2] Department of Neuroscience,University of Minnesota, Minneapolis, USA.
* These authors contributed equally to this work.

Astrocytes are non-neuronal cells that are emerging as important regulatory elements in brain function by actively exchanging signals with neurons. They respond to neurotransmitters and release gliotransmitters that modulate synaptic transmission. However, the cell- and synapse-specificity of the functional relationship between astrocytes and neurons in particular brain circuits remains unknown. Here we show that in the dorsal striatum, which mainly comprises two subtypes of intermingled neurons (striatonigral and striatopallidal medium spiny neurons, MSNs) and synapses belonging two distinct neural circuits (the basal ganglia direct and indirect pathways), subpopulations of striatal astrocytes selectively respond to the activity of specific MSN subtypes. In turn, these subpopulations of astrocytes release glutamate that selectively activates NMDA receptors in homotypic, but not heterotypic MSNs. Likewise, subpopulations of astrocytes lead to the selective regulation of homotypic synapses through activation of group I metabotropic glutamate receptors. Therefore, bidirectional astrocyte-neuron signaling selectively occurs between specific subpopulations of astrocytes, neurons and synapses, which establish circuit-specific functional astrocyte-neuronal networks.

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

CYTOSKELETON ACTIN DYNAMICS REGULATES VESICULAR CYCLE IN CEREBELLAR GRANULE CELLS.

A. Rampérez[1,2], D. Triguero[1,2], A. García-Pascual[1,2], J. Sánchez-Prieto[1,2], M. Torres[1,2]
[1]Facultad de Veterinaria, Universidad Complutense, Madrid
[2]Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), Madrid.

Synaptic transmission depends on the regulated release of neurotransmitter from specialized domains of the axonal plasma membrane. This process involves synaptic vesicle (SV) exocytosis and endocytosis, as well as mobilization from the reserve to readily releasable pool during periods of sustained neuronal activity. Many proteins are required for these processes and actin seems to modulate several of these steps. The aim of this study was to analyze how actin dynamics regulates exo–endocytotic turnover of synaptic vesicles (SVs) at synapses between cerebellar granule neurons in culture. Vesicular cycle was examined by imaging techniques using FM1-43 dye and by electron microscopy (EM) coupled to the photoconversion (PC) of the fluorescent endocytotic marker FM1-43$_{FX}$ (fixable FM1-43 analog) by using 3,3´-diaminobenzidine, which allows to label with a luminal dark precipitate those vesicles that had undergo recycling upon stimulation. Our results show that vesicular cycle is clearly affected by disturbing actin dynamics with different pharmacological approaches, such as F-actin stabilization caused by Jasplakinolide or actin depolymerization caused by Latrunculin A. When cells were incubated with these drugs prior to dye loading, a significantly reduction in the fluorescence accumulated into the boutons was observed, indicating that less vesicles are labeled when actin dynamics is impaired. Moreover, the density of functional boutons also decreased, may well be as the result of a complete inhibition of vesicles loading in a subset of boutons. These results suggested a marked heterogeneity in exo-endocytotic activity and a wide range of susceptibility to these drugs in the whole population of boutons. EM coupled to the PC of the FM1-43$_{FX}$ showed that electron-dense products were located exclusively in SVs and endosomes-like structures, and the proportion of PC vesicles versus non-PC vesicles varied greatly from one bouton to another and is affected by actin dynamics disturbing, supporting the results obtained with imaging techniques.

Supported by MINECO (BFU2021-32105/BFI). A. Rampérez FPI hired from MINECO.

1ª: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2ª: Neurobiología del desarrollo

CSP-□MAINTAINS THE QUIESCENCE OF RADIAL-GLIA LIKE STEM CELLS IN POSTNATAL NEUROGENESIS

J.L. Nieto-González, L. Gómez-Sánchez, F. Mavillard, P. Linares-Clemente, J. A. Martínez-López, R. Pardal and R. Fernández-Chacón
Instituto de Biomedicina de Sevilla (IBiS) HUVR/CSIC/Universidad de Sevilla, Dpto. de Fisiología Médica y Biofísica and CIBERNED, Seville, Spain

Cysteine String Protein-□ (CSP-□) is a synaptic co-chaperone that prevents activity-dependent degeneration of nerve terminals. Mutations in the human CSP-□ gene cause neuronal ceroid lipofuscinosis characterized by progressive dementia and seizures. Synapses formed onto granule cells by parvalbumin (PV)-expressing basket cells progressively degenerate in CSP-□ KO mice. On the other hand, adult quiescent neural stem-cell fate decision is regulated by PV+ basket cells. It is, however, unknown if adult neurogenesis is deregulated in the absence of CSP-□. Here, we have used BrdU injections to find a significant increased in neuronal proliferation at the hippocampus of CSP-□ KO mice. We have observed a dramatic loss of quiescence in radial-glial like cells occurring within the two first post-natal weeks that turned into a dramatic depletion of the radial-glial like cell pool at postnatal age P30. In the absence of CSP-□, neural stem cells in culture undergo a very high proliferation rate that leads to stem cell depletion. Such an unanticipated finding unveils a novel and direct role for CSP-□ in the control of neural stem cell proliferation that is not secondary to GABAergic dysfunction.

Support: MINECO BFU2013-47493, Junta de Andalucía P12-CTS-2232, ISCIII and FEDER. We are grateful to A. Arroyo and M. C. Rivero for excellent technical assistance.

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

AMPA RECEPTOR EXPRESSION REGULATION BY CPT1C IN HIPPOCAMPUS

R Fadó[1,2], N Casals[1,2]
[1]NeuroLipid Group, Basic Sciences Department, Facultat de Medicina i Ciències de la Salut, Universitat Internacional de Catalunya, Sant Cugat del Vallés, Barcelona.
[2]CIBERobn, Instituto de Salud Carlos III, Barcelona.

AMPA-type receptor (AMPAR) abundance in the postsynaptic membrane is a key factor in synaptogenesis and post-synaptic plasticity. Recent data shows that carnitine palmitoyltransferase 1 C (CPT1C), a neuron specific isoform, is one of the constituents of the AMPAR macromolecular complexes, with a role in AMPAR trafficking from ER to the cell surface. Previously, our group had demonstrated that CPT1C deficiency disrupted spine maturation and impaired spatial learning. The main aim of the present study is to analyse the role of CPT1C in AMPAR synaptic expression in hippocampus and cultured neurons during development. We show that AMPAR GluA1 and GluA2 subunits have the same expression profile than CPT1C during development and demonstrate that their expression is dependent on CPT1C levels. Total GluA1 and GluA2 protein levels were diminished in CPT1C KO and increased in CPT1C overexpressing cultured neurons. Interestingly, mRNA levels of AMPARs were unchanged suggesting a post-transcriptional regulation of AMPAR expression by CPT1C. Synaptic AMPAR subunits GluA1 and GluA2 and synaptic transmission were clearly reduced in cultured hippocampal neurons of CPT1C knockout (KO) mice as a consequence of total reduced expression. These data identify CPT1C as a new regulator of AMPAR expression, in addition to its role in AMPAR trafficking. CPT1C could become a new target in the treatment of neurodegenerative diseases associated with decreased AMPAR levels.
This work has been funded by Ministerio de Ciencia y Innovación (NC: SAF2011-30520-C02-02, SAF2014-52223-C2-2-R), and by CIBERobn from Spain.

ELECTROPHYSIOLOGICAL RESPONSES OF LATE-SPIKING PYRAMIDAL NEURONS OF THE GRANULAR RETROSPLENIAL CORTEX

R. Robles, E. Domínguez-Sala, D. Echevarría, E. Geijo-Barrientos
Instituto de Neurociencias, Universidad Miguel Hernández-Consejo Superior Investigaciones Científicas, Sant Joan d'Alacant, Alicante, España.

The granular retrosplenial cortex (GRSC) is implicated in functions such as memory and spatial learning. Most pyramidal neurons of the superficial layers of the GRSC show a late-spiking (LS) firing pattern due to the presence of A-type potassium currents. We have studied the responses of these LS pyramidal neurons during the propagation epileptiform discharges.

We made intracellular recordings with patch electrodes in LS and non-LS pyramidal neurons of the layer 2-3 of the GRSC in brain slices (350 □m thick) prepared from mice of 14-16 days of postnatal age. Recordings were made at 32-33ºC using standard ACSF and a potassium gluconate based intracellular solution. Epileptiform discharges were induced, in the presence of 10□ M bicuculline, by electrical stimuli applied to layer 1 and propagated along the layer 2-3 of the GRSC at an average speed of ~80mms^{-1}.

LS and non-LS pyramidal neurons were identified by their firing pattern and by the higher input membrane resistance and shorter action potential duration of LS neurons (262.1±157.7 vs. 125.9±61.6 M□; 1.17±0.28 vs. 3.55±2.66 ms; n=12, $p<0.05$ for both parameters). During the propagation of epileptiform discharges almost all non-LS neurons fired large bursts of action potentials. In contrast, LS pyramidal neurons showed bursts of EPSPs whose latency was larger than in non-LS neurons and whose summated amplitude never reached the threshold for spike firing. The application of $GABA_B$ receptor blockers (CGP55845, 5 □M or CGP52432, 10 □M) increased the size of the summated synaptic responses in LS pyramidal neurons which in most cases resulted in the firing of bursts of action potentials.

These results show the presence of differences in the local microcircuits involving LS and non-LS pyramidal neurons in the GRSC and suggest the presence of an inhibitory component dependent on $GABA_B$ receptors in LS pyramidal neurons. Funding: Generalitat Valenciana, PROMETEOII/2014/014 and BFU2013-48230-P.

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

CLEC9A/DNGR-1+ DENDRITIC CELLS ARE LOCATED IN THE MENINGEAL MEMBRANES AND CHOROID PLEXUS OF THE NON-INJURED BRAIN.

Elena Quintana[1a], Andrés Fernández[1a];, Patricia Velasco[1], Belén de Andrés[2], Isabel Liste[3], David Sancho[4], María Luisa Gaspar[2] and Eva Cano[1*]

[1] Neuroinflammation Unit. Unidad Funcional de Investigación de Enfermedades Crónicas, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain..

[2] Department of Immunology. Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain.

[3] Neural Regeneration Unit. Unidad Funcional de Investigación de Enfermedades Crónicas, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain.

[4] Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain.

The role and different origin of brain myeloid cells in the brain is central to understanding how the central nervous system (CNS) responds to injury. C-type lectin receptor family 9, member A (DNGR-1/CLEC9A) is a marker of specific DC subsets that share functional similarities, such as CD8 DCs in lymphoid tissues and CD103+CD11b^{low}DCs in peripheral tissues. Here, we analyzed the presence of DNGR-1 in DCs present in the mouse brain (bDCs). *Dngr1* mRNA is expressed mainly in the meningeal and choroid plexus (m/Ch) membranes, and its expression is enhanced by fms-like tyrosine kinase 3 ligand (Flt3L), a cytokine involved in DC homeostasis. Using *Clec9a*$^{gfp/gfp}$ transgenic mice, we show that Flt3L induces accumulation of DNGR1-EGFP^{+} cells in the brain membranes. Most of these cells also express major histocompatibility.

Our results support a dendritic-like nature for Flt3L-dependent cells in the brain. First, they are responsive to Flt3L, a cytokine that has been shown to increase the dendritic repertoire. Second, they express DNGR-1, indicated by examination of *Cle9a* $^{gfp/gfp}$ mice and the expression of *Dngr1* mRNA. Finally, they express the transcription factors *Batf3* and *Irf8*, both of which are required for the maturation of CD8α+ classical DCs (Murphy et al., 2013).

The existence, regulation and role of APCs in the brain DCs, has implications for the development of strategies to target cargoes to these m/Ch brain DCs using receptor-specific antibodies. The expression of the highly specific receptor DNGR-1 in these cells makes such strategies especially attractive The similarity of these cells to lymphoid CD8 +DNGR-1+ cells suggests that they might be responsible for producing efficient CTL responses (Caminschi et al., 2008; Sancho et al., 2008), and therefore could be an important target in brain disorders such as neuroinflammation-based neurodegenerative diseases, viral-induced encephalitis and brain tumours such as gliomas.

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

IFN-GAMMA MEDIATED PRIMING LEADS TO MICROGLIAL ACTIVATION CONTRIBUTING TO DOPAMINERGIC DEGENERATION IN AN *IN VITRO* MODEL OF PARKINSON'S DISEASE.

P. Casanova, E. Saavedra, C. Barcia
Institut de Neurociències, Universitat Autònoma de Barcelona, Barcelona

Microglial cells constitute the first barrier of the innate immune response in the brain and act by continuously scanning the surrounding extracellular space and communicating with other cells, including neurons. Changes in microglial phenotype and function are observed during almost all neuropathological conditions like degenerative diseases such as Parkinson's disease (PD).

Our aim is to elucidate whether microglial cells phagocytose already damaged dopaminergic neurons or if, on the other hand, dopaminergic cells die while being phagocytosed by microglial cells. For this, we studied the microglial activation that leads to phenotypical changes using BV-2 microglial cell line. Firstly, we analyzed the response of these cells to pro-inflammatory agents such as IFN-gamma simulating the neuroinflammatory environment that occurs during PD. In order to measure microglia's activation, we evaluate their release of nitrites and simultaneously, we carried out immunocytofluorescence essays, which allowed us to see morphological changes due the activation. Furthermore, by means of confocal microscopy we analyzed in detail the response of these cells to the pro-inflammatory molecules. The experiments revealed that treatment with IFN-gamma alone resulted in a release of low concentrations of nitrites. However, the release of nitrites was highly elevated when priming cells with the antigenic molecule lipopolysaccharide (LPS) obtaining the full activation of the microglial cells. Surprisingly, we also saw that the density of BV-2 cells increases with higher concentrations of IFN-gamma. Additionally, previously activated microglial cells were set up in a co-culture with PC12 cells (dopaminergic neuron-like cells) as an *in vitro* model of the disease in order to observe their phagocytic interaction.

These results suggest that microglial priming and proliferation are important elements in the inflammatory-mediated dopaminergic neurodegeneration and will help us to understand the role that immune response plays in parkinsonism, highlighting the importance of the inflammatory component in PD therapy.

This work was supported through grants from the Spanish Ministry of Economy and Competitiveness (RYC2010-06729, SAF2010-21274 and SAF2013-45178-P).

1ª: Neuronal excitability, synapses and glia: cellular mechanisms.
2ª: Disorders and nervous system repair.

PROTEIN PHOSPHATASES ARE IMPORTANT TARGETS FOR NUCLEOTIDE RECEPTOR SIGNALLING IN NEURONS AND ASTROCYTES.

M. J. Queipo García, J. C. Gil Redondo, R. Pérez Sen, E. García Delicado, M. T. Miras Portugal.
Departamento de Bioquímica y Biología Molecular IV. Facultad de Veterinaria. Universidad Complutense de Madrid.

Objectives*:* Nucleotide receptors are widely distributed at the nervous system and regulate a great deal of functions. In granule neurons and astrocytic cell populations of rat cerebellar cortex, the metabotropic $P2Y_{13}$ and the ionotropic P2X7 receptors activated signaling kinases cascades promoting neuroprotection against different apoptotic stimuli. Concerning MAPK cascade, we investigated whether nucleotide receptors also regulated their inactivation mechanisms. We focused on dual specificity protein phosphatases (DUSPs), also termed MKPs (MAPK phosphatases), which specifically dephosphorylate Ser/Thr and Tyr residues of MAPKs.
Methods*:* We used primary cultures of cerebellar granule neurons and astrocytes obtained from P7 rat pups. We investigated the expression of DUSPs by QPCR and western blot experiments, and analyzed the phosphorylated forms of MAPKs by western blot and immunocytochemistry. Survival studies were performed by MTT assay.
Results*:* $P2Y_{13}$ receptor agonist, 2MeSADP, induced expression of the nuclear inducible DUSP2 protein phosphatase, showing similar kinetics in both granule neurons and astrocytes. DUSP2 expression was early detectable, consistent with its nature as an early immediate gene. In granule neurons, 2MeSADP-induced expression of DUSP2 contributed to the recovery of basal levels of phosphorylated p38 protein at the nucleus, after the increases produced by exposition to cytotoxic drug, cisplatin. As well, P2X7 receptors regulated the expression of the ERK1,2 specific phosphatase, DUSP6, which also followed a biphasic profile in granule neurons and astrocytes. Early stimulations with P2X7 agonist, BzATP, led to DUSP6 proteasomal degradation and decreased cytosolic protein levels. In a second phase beyond 2-4 h stimulation, DUSP6 protein levels increased due to transcriptional expression. In addition, P2X7 receptors were able to promote survival after cisplatin treatment.
Conclusions*:* $P2Y_{13}$ and P2X7 nucleotide receptors participate in negative feedback mechanisms to assure MAPK signaling, regulating the expression of DUSP2 and DUSP6 proteins, respectively, in both neuronal and glial models.

1. Neuronal excitability, synapses and glia: cellular mechanisms
2. Disorders and nervous system repair

INSULIN-LIKE GROWTH FACTOR-1 INCREASES POSTSYNAPTIC EXCITABILITY AND INDUCES LONG-TERM DEPRESSION OF EXCITATORY SYNAPTIC TRANSMISSION IN LAYER V OF RAT INFRALIMBIC CORTEX.

I.B. Maroto[1], LE. Maglio[1], JA. Noriega-Prieto[1] D. Fernandez de Sevilla[1].
[1]. Facultad de Medicina, Universidad Autónoma de Madrid, Madrid.

We have analyzed the effect of Insulin-like growth factor I (IGF-I) on synaptic efficacy and neuronal excitability at layer V pyramidal neurons of infralimbic cortex (IL). In whole-cell patch clamp recordings using coronal slices, we superfused IGF-1 (10 nM) and measured the changes in electrophysiological properties of pyramidal neurons. In current-clamp mode at -60mV and action potential discharge in response to the injection of different depolarizing current pulses were induced to record fast, medium and slow AHP and we noted that IGF1 reduced medium and slow AHP. Moreover, in voltage-clamp mode we record these Ca2+-dependent K+ currents and we observed that IGF1 reduced medium and slow kinetic K+ currents. Beside this IGF1 facilitated a generation of calcium spikes since the number of spikes were increased compared to control. Furthermore, we studied if IGF1 modulated EPSCs and IPSCs evoked by electrical stimulation at layer VI and we noted that IGF1 induced a long-term depression of both excitatory and inhibitory currents. These forms of synaptic plasticity were presynaptically mediated because a changed in the coefficient of variation paralleled the change of synaptic current amplitude. . In conclusion, IGF-1 increases neuronal excitability and modulates synaptic transmission in pyramidal neuron in the IL subdivision of medial prefrontal cortex (mPFC).

Supported by MICINN to Dr. D. Fernández de Sevilla (BFU2011-23522; BFU2013-43668).

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

CHARACTERIZATION OF NOVEL FAIM ISOFORMS

B. Sanuy-Escribano[1], L. Planells-Ferrer[1], B. Sanz[1], J. Urresti[1], B. Barneda-Zahonero[1], J.X. Comella[1]
[1]. Cell signaling and Apoptosis. Vall d'Hebron Research Institute, Barcelona

Objectives: The Fas apoptotic inhibitory molecule (FAIM) is highly evolutionarily conserved and broadly expressed, suggesting that its gene product plays a key role in cellular physiology. To date, two *FAIM* transcript variants have been characterized: FAIM short (FAIM-S) and FAIM long (FAIM-L). FAIM-S, is widely expressed and its main function in the nervous system is the promotion of neurite outgrowth via NFkB and ERK signaling. On the other hand, FAIM-L expression is restricted to neurons and its principal role is the protection against Death Receptor (DRs)- induced cell death. We have identified two more isoforms: FAIM extra-long (FAIM-XL) and FAIM extra-extra-long (FAIM-XXL). As novel identified proteins, little was known about their functional implications and their subcellular localization. The objectives of this work are the following:
1. To confirm the endogenous expression of FAIM-XL and FAIM-XXL and study their expression patterns in cell lines and tissues.
2. To determine the subcellular localization of the longer FAIM isoforms.
3. Functional characterization of FAIM-XL and FAIM-XXL.
Materials and methods: The confirmation of the endogenous expression of the extra long FAIMs was performed by PCR and Western blot. The subcellular localisation was done using subfractionation procedures and immunocytochemistry. Treatments with NGF and the posterior neurite length measurement were assessed in order to evaluate differentiation. Neurite outgrowth was assessed using the Adobe Photoshop 6.0 software and measuring neurite pixels.
Results: FAIM-XL and FAIM-XXL are expressed endogenously at lower levels than their shorter variants and follow the same expression pattern as FAIM-S and FAIM-L, respectively. The extra N-terminal fragment does not modify their subcellular localization. Regarding their functionality, FAIM-XL and FAIM-XXL increase neurite outgrowth in PC12 cells after NGF stimulation.
Conclusions: These data contribute to a deeper characterization and a better understanding of the different FAIM isoforms in the nervous system.

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

THE PRO-APOPTOTIC PROTEIN SIVA-1 IN THE NERVOUS SYSTEM

E.Cocccia [1], L.Planells-Ferrer [1], B. Barneda-Zahonero[1], J.X. Comella[1]
[1]. Cell Signaling and Apoptosis Group, Fundacio Institut de Recerca de l'Hospital Universitari de la Vall d'Hebron, Edifici Collserola, Passeig Vall d'Hebron 119-129, Barcelona, 08035, Spain

Objectives: Apoptosis is the main type of programmed cell death, a complex process in which hundreds of regulative proteins are involved. During development, apoptosis shapes structure and connections of the nervous system, whereas in the adult phases neuronal apoptosis is generally involved in degenerative or pathological diseases. Our lab described FAIM-L, a neural specific antagonist of cell death stimuli triggered by death receptors (DRs). To protect from cell death, FAIM-L acts on a potent inhibitor of caspases, XIAP, impairing its degradation, thus allowing it to inhibit caspase activation. Here we study the role of the pro-apoptotic protein Siva-1 in neurons.
The main objectives of the work are the following:

- Characterize Siva-1 subcellular localization in neurons.
- Assess cell death induction by Siva-1 in neurons.
- Describe possible mechanisms through which Siva-1 promotes cell death: interaction with FAIM-L and interference in FAIM-L stabilization of the anti-apoptotic protein XIAP.

Methonds: For the purpose of the study we used primary hippocampal neurons to explore Siva-1 subcellular localization by immunocytochemistry and induction of cell death by Siva-1 overexpression, by Hoechst staining, Western blot and DEVDase activity assay. We used HEK293T cells to report Siva-1 interaction by immunoprecipitation and its promotion of XIAP ubiquitination by Ubiquitin pull-down.
Results: We have described that Siva-1 locates in cell membrane and in cytoplasm of primary neurons. We report that Siva-1 promotes Caspase-3-dependent cell death. Moreover we have found that Siva-1 interacts with the DR antagonist FAIM-L and promotes XIAP ubiquitination.
Conclusions: In summary, our findings reveal that Siva-1 overexpression induces cell death in neurons by an activation of Caspase-3. We propose that Siva-1 action promoting cell death could be through XIAP modulation, enhancing its ubiquitination and following proteosomal degradation. Therefore Siva-1 and FAIM-L could have an opposite role in regulating XIAP, respectively inhibiting or promoting its ubiquitination and following degradation.

1ª: Neuronal excitability, synapses and glia: cellular mechanisms
2ª: Diseases and reparation of the nervous system

THE GAP JUNCTIONS OF THE THERMOSENSORY CIRCUIT IN THE NEMATODE *C. ELEGANS* ARE RESPONSIBLE FOR ISOTHERMAL TRACKING BEHAVIOR

A.C. Calvo[1], J. Hawk[1], A. Almoril[1] and D.A. Colon-Ramos[1]
[1]Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, USA

The nematode *Caenorhabditis elegans* moves towards the cultivation temperature when placed in a thermal gradient, tracking isotherms once this temperature is reached (isothermal tracking behavior). AFD is the principal thermosensory neuron described until now and is required to perform all modes of thermotactic behavior. But how one neuron can drive the distinct sensorimotor transformations that underlie movement down or up gradients towards the cultivation temperature vs. isothermal tracking near the cultivation temperature is not well understood. To study this question, we explored the downstream synaptic pathways from AFD. AFD has direct synaptic output to only two interneurons, chemical synapses to AIY and electrical synapses to AIB. We have identified a deletion mutant in an innexin gene, *inx-1*, that displays a constitutively isothermal tracking behavior, regardless of cultivation temperature. Innexins are the proteins responsible for gap junctions in invertebrates. We have cultivated *inx-1* mutant animals at different temperatures, and they track isotherms at any temperature within a thermal gradient, suggesting that the mechanism of isothermal tracking, which is usually inhibited when the animals are far from the cultivation temperature, is constitutively activated. *Inx-1* expression is mainly neuronal, and more importantly *inx-1* gene is expressed in several neurons of the thermotaxis circuit, such as AIB and AIY, suggesting a possible implication of these interneurons in the mechanism of isothermal tracking. We are currently characterizing the neuronal activity of AFD, AIY and AIB using the genetically expressed calcium indicator GCaMP6 in response to different temperature regimes. Whereas AFD shows a similar pattern of neural activity in both wild type and *inx-1* backgrounds, AIY has an increased probability of neuronal firing at any given temperature in *inx-1* defective worms. Taking together, our results suggest that electrical synapses within the thermotaxis circuit are implicated in the regulation of isothermal tracking behavior around cultivation temperature.

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

Topic 3

Systems Neuroscience

OLFACTORY BULB PROTEOME DYNAMICS DURING THE PROGRESSION OF SPORADIC ALZHEIMER'S DISEASE

J. Fernández-Irigoyen[1], M.V. Zelaya[1], E. Pérez-Valderrama[1], E. Santamaría[1]
[1]Proteored-ISCIII, Proteomics Unit, Clinical Neuroproteomics Group, Navarrabiomed, Fundación Miguel Servet, Instituto de Investigacion Sanitaria de Navarra (IDISNA), Pamplona, Spain

Olfactory dysfunction is present in up to 90% of AD patients. Although deposition of hyperphosphorylated tau and β-amyloid neuropathologic substrates are present in olfactory areas, the molecular mechanisms associated with decreased smell function are not completely understood. To gain new insights into the underlying pathogenic mechanisms in the olfactory system, we have applied mass spectrometry-based quantitative proteomics to probe additional molecular disturbances in postmortem olfactory bulbs (OB) dissected from pathologically confirmed AD cases respect to neurologically intact controls. More than 4,000 proteins have been identified in human OB. Global protein profiling, significant pathways, and functional categories were analyzed. Relative proteome abundance measurements have revealed specific protein interaction networks progressively disturbed across Braak stages, suggesting impaired mitochondrial function, an imbalance in the cycling of neurotransmitters, and a disturbance in neuron-neuron adhesion and neurite growth in the OB during AD pathogenesis. Moreover, our study uncovers 25 potential novel therapeutic targets that are modulated in specific Braak stages, unveiling targetable mechanisms underlying the progression of AD.

Presenting author:
Enrique Santamaria (esantamma@navarra.es)

ANATOMO-PROTEOMIC CHARACTERIZATION OF HUMAN BASAL GANGLIA: FOCUS ON STRIATUM AND GLOBUS PALLIDUS

J. Fernández-Irigoyen[1], M.V. Zelaya[1], E. Santamaría[1]
[1]Proteored-ISCIII, Proteomics Unit, Clinical Neuroproteomics Group, Navarrabiomed, Fundación Miguel Servet, Instituto de Investigacion Sanitaria de Navarra (IDISNA), Pamplona, Spain

The basal ganglia (BG) are a complex network of subcortical nuclei involved in the coordination and integration of the motor activity. Although these independent anatomical structures are functionally related, the proteome present in each isolated nucleus remains largely unexplored. In order to analyse the BG proteome in a large-scale format, we used a multi-dimensional fractionation approach which combines isolation of anatomically-defined nuclei, and protein/peptide chromatographic fractionation strategies coupled to mass spectrometry. Using this workflow, we have obtained a proteomic expression profile across striatum and globus pallidus structures among which 1681 proteins were located in caudate nucleus (CN), 1329 in putamen, 1419 in medial globus pallidus (GPi), and 1480 in lateral globus pallidus (GPe), establishing a BG reference proteome to a depth of 2979 unique proteins. Protein interactome mapping highlighted significant clustering of common proteins in striatal and pallidal structures, contributing to oxidative phosphorylation, protein degradation and neurotrophin signalling pathways. In silico analyses emphasized specific pathways represented in striatal and pallidal structures highlighting 5-hydroxytryptamine degradation, synaptic vesicle trafficking, and dopamine, metabotropic glutamate and muscarinic acetylcholine receptor pathways. Additional bioinformatic analyses also revealed that: i) nearly 4% of identified proteins have been previously associated to neurodegenerative syndromes, ii) 11% of protein set tends to localize to synaptic terminal, and iii) 20% of identified proteins were also localized in cerebrospinal fluid (CSF). Overall, the anatomo-proteomic profiling of BG complements the anatomical atlas of the human brain transcriptome, increasing our knowledge about the molecular basis of the BG and the etiology of the movement disorders.

Presenting author:
Enrique Santamaria (esantamma@navarra.es)

NEW INSIGHTS INTO THE HUMAN BRAIN PROTEOME: PROTEIN EXPRESSION PROFILING OF DEEP BRAIN STIMULATION TARGET AREAS

J. Fernández-Irigoyen[1], M.V. Zelaya[1], E. Pérez-Valderrama[1], E. Santamaría[1]
[1]Proteored-ISCIII, Proteomics Unit, Clinical Neuroproteomics Group, Navarrabiomed, Fundación Miguel Servet, Instituto de Investigacion Sanitaria de Navarra (IDISNA), Pamplona, Spain

Deep brain stimulation (DBS) is a neurosurgical procedure that provides therapeutic benefits for movement and affective disorders. The nucleus basalis of Meynert (NBM) and substantia nigra (SN) are considered target areas to apply DBS. Even though the degeneration of NBM and SN underlies the cognitive decline observed in neurological diseases, the protein knowledge derived from both areas is scarce. We have characterized the proteome present in both subcortical brain areas using the Triple TOF 5600 mass spectrometer, identifying 2775 and 3469 proteoforms in NBM and SN respectively. Data mining of MS-generated proteomic data have revealed that: i) 675 proteins tend to localize to synaptic ending, ii) 61% of the global dataset is also present in human CSF and/or plasma, and iii) 894 proteins have not been previously identified in healthy brain by mass-spectrometry. The correlation of NBM and SN proteomic expression profiles with human brain transcriptome data available at Allen Brain Atlas has revealed protein evidence for 250 genes considered with brain-wide expression and 112 genes with region-specific expression in human brain. In addition, protein datasets have been classified according to their chromosomal origin, increasing the current proteome coverage in healthy human brain.

Presenting author:
Enrique Santamaría (esantamma@navarra.es)

INDUCING BETA/LOW-GAMMA OSCILLATIONS IN THE MEDIAL PREFRONTAL CORTEX USING A BRAIN-MACHINE INTERFACE IN AN OPERANT CONDITIONING PARADIGM

S. Hernández-González[1], C. Andreu-Sánchez[2], M.A. Martín-Pascual[2], A. Gruart[1] and J.M. Delgado-García[1]*
[1]. Universidad Pablo de Olavide, Sevilla
[2]. Universitat Autònoma de Barcelona, Barcelona

The prefrontal cortex (PFC) constitutes the highest level of cortical hierarchy dedicated to the representation and execution of adaptive behaviors. None of its cognitive functions can be understood if taken out of a broad connectionist context (Fuster, 2001). Here, we studied what kind of neurophysiological processes occur during the volitional control of a specific oscillatory pattern of local field potentials (LFPs) recorded in the medial PFC (mPFC) and, in addition, how different cortical and subcortical regions are involved during its generation. We recorded LFPs from six brain regions widely connected with the mPFC [primary motor cortex, mediodorsal thalamic nucleus, ventral tegmental area (VTA), nucleus accumbens and hippocampal CA1 area] during the performance an operant conditioning task. Firstly, Lister Hooded rats were trained to touch a visual display in an iPad to obtain a piece of food. Then, we used a brain-machine interface (BMI) protocol to train rats to generate a previously selected oscillatory beta/gamma patter in the mPFC to activate the visual display at the iPad which they had to touch to obtain the food reward. Additionally, animals were stimulated with a high frequency train of pulses in these six brain regions to prove if they were or not involved in these ongoing cognitive processes. Rats learned to generate the selected beta/ gamma pattern, which increased its presentation rate across training sessions. Preliminary results indicate some signs of coherence between LFPs recorded in the mPFC and those recorded in the other regions. Only mPFC stimulation prevented rats from preforming the operant conditioning task, while VTA stimulation evoked a self-stimulation behavior. Our findings show that the mPFC has the capability of generating an oscillatory pattern able of being used to deal with environmental constrains. The selected beta/gamma pattern could be a useful tool to activate and control BMIs.

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

MODULATION OF SENSORY CORTICAL AREAS BY BASAL FOREBRAIN CHOLINERGIC NEURONS DEMONSTRATED BY NEURONAL TRACING AND OPTOGENETIC STIMULATION IN TRANSGENIC MICE

Irene Chaves-Coira, Natali Barros-Zulaica, Angel Núñez, Mararita Rodrigo-Angulo
Departamento de Anatomía, Histología y Neurociencia. Facultad de Medicina. Universidad Autónoma de Madrid

Acetylcholine transmission is mainly guaranteed by disperse cholinergic neuron groups within basal forebrain (BF): medial septum, horizontal/vertical (HDB/VDB) limbs of diagonal band of Broca and nucleus basalis magnocellularis (B) providing most cholinergic innervation to sensory, motor and prefrontal cortices. Early anatomical descriptions of BF cholinergic projections were consistent with the notion of diffuse pathways to the cortex; however, previous results suggest a refined anatomical and functional topographical organization of BF-cortical projection system that may control cortical sensory processing in a specific manner.
Our goal is to elucidate if there are common or separate pathways linking BF with sensory cortices in B6.Cg-Tg(Chat-COP$_4$*H$_{134}$R/EYFP,Slc$_1$8a$_3$)5$^{Gfng/\ J}$ mice using retrograde fluorescent tracers and optogenetic stimulation procedures. Animals were anesthetized with Isofluoranne; Fluoro-Gold (FlGo) and Fast-Blue (FB) were injected/deposited into primary somatosensory (S1) and primary auditory/visual (A1, V1) cortices. For optogenetic stimulation, mice were anesthetized with urethane (1.6g/kg); single-unit recordings were performed with tungsten microelectrodes on BF; cortical field potential was recorded through tungsten macroelectrodes.
Anatomical results revealed FlGo and FB labeled-neurons in BF: 62% in VDB/HDB were double-labeled while 38% were single-labeled (65% FlGo, 35% FB); double-labeled neurons in B averaged 32%, while single-labeled neurons by either tracer was roughly 50%. Optogenetic results indicate that light stimuli applied on BF neurons induced spike firing, cortical desynchronization and an increase of auditory and tactile cortical responses. Stimulation of VDB/HDB mainly induces an increase of tactile responses in S1 cortex while stimulation of B area increased both auditory and tactile responses in A1 and S1 cortices. Our studies suggest that cholinergic projections to the cortex are organized into segregated and overlapping pools of neurons that may modulate specific cortical areas.

Supported by BFU2012-36107

STRUCTURAL GUIDED MEG FUNCTIONAL CONNECTIVITY IN THE CHARACTERIZATION OF AMNESTIC MILD COGNITIVE IMPAIRMENT

J.A. Pineda-Pardo[1,2], R. Bruña [2], M. Woolrich [3,4], A. Marcos [5], A.C. Nobre [3], F. Maestú [1], D. Vidaurre [3,4]
[1]. CINAC, HM Puerta del Sur, Hospitales de Madrid, Móstoles, and CEU-San Pablo University, Madrid
[2]. Centre for Biomedical Technology, Universidad Politécnica de Madrid, Madrid
[3]. Oxford Center for Human Brain Activity (OHBA), University of Oxford, Oxford, United Kingdom
[4]. The Oxford Centre for Functional MRI of the Brain (FMRIB), University of Oxford, Oxford, United Kingdom
[5]. Department of Neurology, Hospital Clínico San Carlos, Madrid

Objectives. Amnestic mild cognitive impairment (aMCI) is a stage of cognitive decline preceding Alzheimer's disease (AD). Changes in brain functional (FC) and structural connectivity (SC) take place along the disease [1,2]. We propose a multimodal approach for the estimation of MEG functional connectivity, which takes into account the structural connectivity (SC) beneath the functional interactions, and we evaluate its potential in the discrimination of aMCI subjects.

Materials & Methods. The sample comprised 81 individuals (HE – 29; aMCI – 52). aMCI were divided in single-domain (sdMCI; 22) and multiple-domain aMCI (mdMCI; 30).

SC was estimated between 66 cortical regions, as the number of streamlines obtained from diffusion-tensor deterministic tractography. Resting-state MEG recordings were transformed to source-space using a beamformer spatial filter. Power envelopes of the source time-series were obtained using the Hilbert transform, and were averaged within each cortical region.

We employed a graphical lasso (GL) to estimate a sparse precision matrix, whose elements measure direct functional interactions, thus penalizing indirect interactions [3]. Our approach (GL-SC) consists on redefining the regularization term of the graphical lasso, to include SC as a weighting factor, thus penalizing also connections with low SC.

Results. We estimated the classification performance of the precision matrices. Best accuracies to discriminate HE-aMCI were for GL-SC (sdMCI – 86.27%; mdMCI – 81.36%), whereas between aMCI subtypes, the best accuracy was for GL (84.62%). Connections that contributed more to these accuracies included temporal, frontal and cingulate regions. Frontal cortex connections gained relevance in the classification between HE and mdMCI. In the discrimination between aMCI subtypes, the most relevant connections included the posterior cingulate and occipital regions.

Conclusions. This study highlights the benefits of multimodal brain connectivity characterization, with increments in classification accuracies up to the 10%. Identified connections agree with the cognitive deficits of these patients, providing robustness to the proposed methodology.

[1] Bajo et al. 2012. Differential Patterns of Connectivity in Progressive Mild Cognitive Impairment. Brain connectivity 2(1):21-4
[2] Shao et al. 2012. Prediction of Alzheimer's Disease Using Individual Structural Connectivity Networks. Neurobiology of aging 33(12): 2756–65
[3] Marrelec et al. 2006. Partial Correlation for Functional Brain Interactivity Investigation in Functional MRI. NeuroImage 32: 228–37

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

José Angel Pineda Pardo joseangel.pineda@ctb.upm.es

INCREASING GLUTAMATERGIC TRANSMISSION IN THE INFRALIMBIC CORTEX EVOKES ANTIDEPRESSANT-LIKE EFFECTS IN RATS

J. Gasull-Camós[1,3], F. Artigas[1,2,3], A. Castañé[1,2,3].
[1]. Institut d'Investigacions Biomèdiques de Barcelona (CSIC), Barcelona.
[2]. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona.
[3]. Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), ISCIII, Madrid.

Administration of sub-anesthetic doses of the NMDAR antagonist ketamine produces rapid antidepressant effects in humans and rodents. Recently, it has been suggested that a glutamate surge could have a crucial role on ketamine's antidepressant effect by increasing AMPAR neurotransmission. However, the mechanisms by which glutamate modulates mood remain unclear. The medial prefrontal cortex (mPFC) is involved in the processing of emotional and cognitive signals. In particular, neuroimaging studies reveal the presence of an altered energy metabolism in ventral cingulate areas of depressed patients. We aimed to investigate possible dissociable roles of glutamatergic neurotransmission in ventral (infralimbic cortex, IL) and dorsal (prelimbic cortex, PrL) areas of the rat mPFC on the regulation of depressive behaviors and serotoninergic function. We characterized the effects of the bilateral infusion of veratridine (100 µM), a depolarizing agent, or dihydrokainic acid (DHK, 3mM and 10 mM), a selective inhibitor of the glutamate transporter 1 (GLT1), into IL or PrL on depressive-like behaviors, using the forced swimming test (FST) and the novelty suppressed feeding tests (NSFT). In addition, the neurochemical effects of DHK on serotonin and glutamate were determined by *in vivo* microdialysis in freely-moving rats. Veratridine infusion into IL, but not PrL, evoked rapid antidepressant-like effects in the FST. Pharmacological inhibition of astrocytic glutamate uptake with DHK also produced antidepressant-like responses on the FST and the NSFT when infused into IL, but not PrL. Interestingly, blockade of AMPA receptors with NBQX (20 mM) prevented DHK-induced antidepressant-like effects on the FST. DHK perfusion increased extracellular glutamate levels equally in PrL and IL, but extracellular serotonin was only increased when DHK was applied in IL. Our results indicate that an acute increase of glutamatergic neurotransmission in the IL mPFC produces a rapid antidepressant-like effect in rats, which is likely mediated by a PFC-driven increase in serotonergic activity.

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

C-FOS IMMUNOREACTIVITY IN THE INFERIOR COLLICULUS AFTER PURE TONE STIMULATION

S. Estévez[1], R. Santamaría[2], V. Lamas[1], M. A. Merchán[1]
[1]. Instituto de Neurociencias de Castilla y León. Universidad de Salamanca. Spain
[2]. Departamento de Informática y Automática. Facultad de Ciencias. Universidad de Salamanca. Spain

The inferior colliculus (IC) is a relay station in which the ascending and descending connections of the auditory pathway interact for prethalamic processing of the spectral properties of sound. Its laminar organization has been considered an anatomical foundation for frequency analysis. To date, previous attempts to correlate pure tone processing with specific c-Fos immunoreactive bands in the central nucleus of the IC have not been conclusive.
Objective: To understand IC organization for pure tone analysis.
Materials and methods: Three groups of five Wistar rats were stimulated in close field with pure tones of 800, 8000 or 20000 Hz at 50 dB SPL for 45 minutes under anesthesia. After stimulation, rats were maintained in silence for 100 minutes and were perfused. Brains were serially sectioned in the coronal plane and immunostained for c-Fos. Mosaics of the IC were taken at X10 and analyzed to obtain the optic density value and the coordinates for each immunopositive nuclei. These data were used to build optical density maps or to analyze the density of positive neurons. Bands detection was estimated by a new mathematical method base on linear regression.
Results: In the control animals, positive neurons were organized randomly with a homogenous distribution of particles. In stimulated groups, we found a high concentration of positive neurons in the dorso-lateral part and band-shaped groups of positive neurons in the ventro-medial colliculus. A common immunoreactive principal band of neurons was always present regardless of the frequency used for stimulation. The main differences depending on the stimulus were observed in secondary bands and in the rostro-caudal distribution, both by density mapping and by applying our mathematical predictive bands detection methodology.
Conclusions: The analysis of clouds of points and density maps of c-Fos immunoreactive neurons demonstrate a complex non cocleotopic organization in the IC after pure tone stimulation.

1. System Neuroscience
2. Disorders and nervous system repair

This research was supported by the grants from the Ministry of Economy and Competitiveness of the Government of Spain, BFU2012-39982-C02andBFU2012-39982-C01.

Sheila Estévez Martín, e-mail: schanila@usal.es

SLOW FREQUENCY MODULATION OF HIPPOCAMPAL THETA OSCILLATIONS AS A CARRIER FOR ENCODING IN A SPATIAL-CUED RUNNING TASK IN RODENTS

Daniel Gómez-Domínguez [1,2], Kotaro Mizuta [1], François Laurent [1], Masaaki Sato [2], Takashi Takekawa [3], Masamichi Ohkura [4], Junichi Nakai [4] , Tomoki Fukai [2], Yasunori Hayashi [2], Liset Menendez de la Prida [1]
[1]. Instituto Cajal, CSIC, Madrid, Spain
[2]. Brain Science Institute, RIKEN, Wako, Saitama, Japan
[3]. Kogakuin University, Shinjuku, Tokyo, Japan
[4]. Saitama University Brain Science Institute, Saitama University, Saitama, Japan

Recent studies suggest the relevance of the rhythmic modulation of faster neuronal oscillations (nested oscillations) for different neural processes in rodents, monkeys and humans, but the mechanisms and functional significance remain elusive. While much of work is concentrated in theta-gamma coupling, less is known about the slow modulation (<1 Hz) of the hippocampal theta rhythm (4-12 Hz). Here, we focus on studying slow-theta modulation in the hippocampus of behaving rats and mice using a combination of multi-site silicon probes and multi-cellular calcium imaging. In freely moving rats, a robust slow-theta modulation was similarly detected across hippocampal layers both at proximal and distal locations of the dorsal hippocampus. Despite reduced theta power in epileptic rats, no difference was quantified in the slow modulatory process. In head-restrained mice running on a virtual linear track, we observed similar results suggesting that slow-theta modulation is constrained to the hippocampal formation in rodents and not dependent on the behavioral paradigm and/or the epileptic condition. We subsequently aimed to evaluate the role of slow-theta modulation in memory function using multi-cellular two-photon calcium imaging and genetically-encoded calcium indicators in the mouse line Thy1 G-CaMP7. For this purpose, we simultaneously visualized the activity of hundreds of CA1 pyramidal cells and recorded local field potentials while the animal learned to run in a virtual linear track with spatial cues for water reward. We found that activity of cells at the reward position (goal cells) was phase-locked to the slow modulation of theta oscillations. Slow phase-locking of goal cells reorganized along the task in parallel with the learning process. These findings support the relevance of hippocampal slow-theta modulation in cognitive processes during exploratory tasks.

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

THE ROLE OF THE NUCLEUS INCERTUS IN THE HIPPOCAMPAL THETA ACTIVATION

S. Martínez Bellver [1,2], A. Cervera Ferri [1], J. Martínez Ricós [1], A. Luque García [1], J. Guerrero Martínez[3], M. Bataller Mompeán[3], V. Teruel Martí [1]
[1]. Departamento de Anatomía y Embriología Humana. Facultad de Medicina y Odontología. Universitat de València, Valencia
[2]. Departamento de Biología Celular y Parasitología. Facultad de Biología. Universitat de València, Burjassot
3.Departamento de Ingeniería Electrónica, Escuela Técnica Superior de Ingeniería, Universitat de València, Burjassot

Theta rhythm is a relevant oscillatory process for cognitive tasks such as spatial navigation and memory processing. Theta oscillation in the hippocampus demands the cooperation of a large-scale, distributed subcortical network. In recent years, a body of evidence has shown that the nucleus incertus (NI), in the dorsal tegmental pons, is a key node of the brainstem circuitry involved in hippocampal theta rhythmicity. In the last years, our group have demonstrated that the NI shows theta activity coupled with the observed in the hippocampus. Moreover, in a recent paper, we have shown that some of the NI neurons display a theta firing activity in situations of hippocampal theta activation.
The objective of this work is to better understand the temporal relation between the theta neurons of the NI and the hippocampus theta activity and, at the end, evaluate the role of the NI activity on the hippocampal activation. For this purpose, causality analyses were performed in order to determinate whether the hippocampal theta activity depends on the regular activity of the NI neurons or *vice versa*. A sensory stimulation model was applied in order to generate theta activity in the anesthetized rat. In addition, electrical stimulation pulses in the NI were used in order to analyse the possible theta generation ability of this nucleus.
Under the sensory experimental conditions, there was a significant increase of the theta band information flow in the direction NI to hippocampus, highlighted by an increment of causality in the brainstem-hippocampal direction. The electrical pulses in NI evoked an increase of the hippocampal theta power as well as a phase theta reset.
These findings suggest that the NI has a key role in the direct generation and modulation of the theta activity in the hippocampus.

1ª: Systems Neuroscience
2ª: Theoretical and Computational Neuroscience

EFFECTS OF DEEP BRAIN STIMULATION OF THE INFRALIMBIC CORTEX OVER THE OSCILLATORY ACTIVITY IN THE HIPPOCAMPUS AND BASOLATERAL AMYGDALA

V. Teruel Martí[1], A. Cervera Ferri[1], M. Barceló Molina[1], J. Martínez Ricós[1], S. Martínez Bellver[1,2], A. Luque García[1], A. Adell Calduch[3]
[1]Departamento de Anatomía y Embriología Humana, Facultad de Medicina y Odontología, Universitat de València, Valencia.
[2]Departamento de Biología Celular y Parasitología, Facultad de Biología, Universitat de València, Burjassot.
[3]Institute of Biomedicine and Biotechnology of Cantabria, IBBTEC (CSIC, Universidad de Cantabria), Santander.

Deep Brain Stimulation (DBS) of the prefrontal cortex has shown to exert an antidepressant influence, both on behavioural experiments and lesion cases. The network including prefrontal cortex, hippocampus and amygdala is altered in depression.
In this study, we analyse the effects of the DBS in the infralimbic prefrontal cortex, over the oscillatory activity of this network. For that, local field recordings in hippocampus CA1 and the amygdala were performed in the anesthetized rat, under the electrical stimulation applied at the infralimbic prefontal cortex. The stimulation pattern employed was the same in intensity and frequency that of used in clinical treatments. The wavelet time-frequency analyses, and information theory methods, allow us to better understand the effect of the DBS treatment in the functional relation between the hippocampus and the amygdala.
The main findings of this study indicate that the DBS increases the presence of slow (0.1-1.5 Hz) and theta (3-10 Hz) waves both in the hippocampus and the amygdala. The DBS also increases the coupling between both structures in the range of frequencies previously cited, as well as the information flow between both areas. Moreover, we observed a modulation between slow waves and different higher frequencies in the hippocampus-amygdala network, under the DBS conditions.
Our findings suggest a better synchronization in the hippocampal-amygdalar network upon the influence of the DBS of the infralimbic cortex.

1ª: Systems Neuroscience
2ª: Disorders and nervous system repair

ANATOMICAL SEGMENTATION OF THE HUMAN MEDIAL PREFRONTAL CORTEX

M. Córcoles-Parada[1], N. Müller[2], M. Dresler[2], F. Mansilla[3], J. González-Fuentes[1], S. Cebada-Sánchez[1], E. Artacho[1], P. Marcos[1], R. Insausti[1], G. Fernández[2], M. Muñoz-López[1].
[1]Medical School, University of Castilla-La Mancha, Albacete, Spain.
[2]Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Centre, Nijmegen, The Netherlands.
[3]University Hospital, Albacete, Spain.

The medial prefrontal cortex (mPFC) is implicated in diverse studies of executive function, decision-making and memory. The anatomical segmentation of its subareas (i.e. anterior cingulate cortex or Brodmann´s area 24; prelimbic area 32, infralimbic area 25, and area 14 of the gyrus rectus) is not completely defined, but will be critical to better understand results obtained in functional magnetic resonance imaging (fMRI) studies involving the mPFC. The aims of this study were (1) to identify anatomical references, mainly sulci, visible in ex-vivo MRI scans that could guide the identification of boundaries of specific mPFC areas; (2) to supplement this segmentation with a larger sample of in-vivo MRI scans. For this issue, 11 ex-vivo hemispheres were first scanned in either an 1.5 or 3T MR system, frozen sectioned in the coronal plain perpendicular to the AC-PC axis at 50 μm thickness, mounted in glass slides, and processed for cyto (Nissl stain) and myelo-architectonic (Gallyas silver stain) analysis. Architectonic boundaries of mPFC areas were identified and two-dimensional unfolded maps were generated to determine the relationship of boundaries with sulci. Boundaries between mPFC areas were then transposed to MRI scans and volumes extracted. Next, mPFC sulci patterns and areas were analyzed in the larger sample of in-vivo MRI scans (N=20, males, mean age=25.7 years, SD=6.5) with area volumes compared to the ex-vivo cases. The results showed: a) area boundaries identified ex-vivo related consistently with the cingulate sulcus (cs) and superior rostral sulcus (srs); b) relative volumes of the mPFC areas were similar ex-vivo and in-vivo; c) probabilistic maps of mPFC areas were successfully generated. These results provide a valuable tool to investigate the function of mPFC areas in structural and functional MRI studies with greater accuracy. *Funded by Carlos III Institute, Erasmus, UCLM.*

1. Neurociencia de sistemas
2. Neurociencia cognitiva y conductual

Marta Córcoles Parada: corcoles.marta@gmail.com

DOPAMINE RECEPTORS MODULATE EXECUTIVE FUNCTION AND NEURAL ACTIVITY IN THE PREFRONTAL CORTEX: INSIGHTS FROM STUDIES IN NON-HUMAN PRIMATES

MV. Puig [1]
1. Institut Hospital del Mar d´Investigacions Mèdiques, Barcelona

Higher-order executive tasks such as associative learning, working memory, and behavioral flexibility depend on the prefrontal cortex, the brain region most elaborated in primates. The prefrontal cortex is an associational cortical area that receives remarkably dense inputs from dopamine neurons, suggesting that dopamine exerts powerful influences on its function. I will describe how dopamine, via D1 and D2 receptors, modulates executive function and neural activity in the prefrontal cortex of macaque monkeys. More specifically, I will show that both prefrontal D1 and D2 receptors play a role in modulating learning of new associations and cognitive flexibility, but not the performance of highly familiar associations. Moreover, D2 receptors also seem to influence motivation. Interestingly, D1 and D2 receptors modulate learning-related neural information both at single-neuron and network levels (i.e. alpha and beta oscillatory activity). Taken together, our results strongly suggest that prefrontal D1 and D2 receptors modulate executive function in a cooperative manner.

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

BRAIN STATE TRANSITIONS IN THE PEDUNCULOPONTINE NUCLEUS: COOPERATIVE PHASIC AND TONIC MECHANISMS

M. Valencia [1,2], A. Petzold [3], B. Pal [4], J. Mena-Segovia [3,5]
[1]. CIMA, University of Navarra, Pamplona, Spain
[2]. IdiSNA, Navarra Institute for Health Research, Pamplona Spain
[3]. MRC Anatomical Neuropharmacology Unit. University of Oxford, Oxford, UK
[4]. Department of Physiology, University of Debrecen, Debrecen, Hungary
[5]. Center for Molecular and Behavioral Neuroscience, Rutgers University, New Jersey, USA

Cholinergic neurons of the pedunculopontine nucleus (PPN) are most active during the waking state. Their activation is deemed to cause a switch in the global brain activity from sleep to wakefulness, while their sustained discharge may contribute to upholding the waking state and enhancing arousal. Similarly, non-cholinergic PPN neurons are responsive to brain state transitions and their activation may influence some of the same targets of cholinergic neurons, suggesting that they operate in coordination. Yet, it is not clear how the discharge of distinct classes of PPN neurons organize during brain states. Here we monitored the in vivo network activity of PPN neurons in the rat across two distinct levels of cortical dynamics and their transitions. We identified a highly structured configuration in PPN network activity during slow-wave activity that was replaced by a high level of disorganization during the activated state. During the transition, neurons were predominantly excited (phasically or tonically), but some were inhibited. Identified cholinergic neurons displayed phasic and short latency responses to sensory stimulation, whereas the majority of non-cholinergic showed tonic responses and remained at a high discharge rate beyond the state transition. In vitro recordings demonstrate that cholinergic neurons exhibit fast adaptation that prevents them from discharging at high rates over prolonged time periods. Our data shows that PPN neurons have distinct but complementary roles during brain state transitions, where cholinergic neurons provide a fast and transient response to sensory events that drive state transitions, whereas non-cholinergic neurons maintain an elevated firing rate during global activation.

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

EFFECTS OF TRANSCRANIAL DIRECT-CURRENT STIMULATION (TDCS) OF SOMATOSENSORY AND CEREBELLAR CORTICES ON WHISKER INPUT AND PLASTICITY IN ALERT MICE

C. A. Sánchez-León[1], J. M. Ausín-Azofra[2], C. Ammann[1], A. Gruart[1], J. M. Delgado-García[1], J. Márquez-Ruiz[1].
1. División de Neurociencias, Universidad Pablo de Olavide, Sevilla, España.
2. Laboratorio Europeo de Neurotecnologías Inmersivas, Instituto Labhuman, Valencia, España.

Recently, the classical role of cerebellum in motor learning and coordination has been reevaluated according to new evidences about the implications of cerebro-cerebellar pathways on cognitive functions and sensory processing. The aim of this study was to explore cerebro-cerebellar interactions affecting long-term plasticity in the somatosensory (SS) cortex by modulating cerebral and cerebellar cortical excitability in behaving mice. Mice were prepared for chronic recording of local field potentials (LFPs) in the SS cortex in response to whisker pad stimulation and for simultaneous transcranial direct-current stimulation (tDCS) over the cerebellum and SS cortex. tDCS stimulation was performed at different current intensities for 5 s to test the immediate effects, and during 20 min to show after-effects on SS-LFPs. To highlight tDCS effects on long-term plasticity in the SS cortex, two conditioning protocols consisting of 8 Hz and 50 Hz whisker stimulation during 10 min and 96 seconds respectively, were used during tDCS or sham condition. Regarding the immediate effects, anodal and cathodal tDCS increased and decreased, respectively, the amplitude of SS-LFPs when simultaneously applied to the ipsilateral SS cortex, whereas the opposite immediate effects were obtained when tDCS was applied to the contralateral cerebellar cortex. Concerning the long-term after-effects, cathodal tDCS over the SS cortex induced a long-term depression of LFP whereas no effects were observed after anodal currents. In addition, long-term potentiation of SS-LFPs was observed after both cathodal and anodal cerebellar tDCS. As expected, long-term depression and long-term potentiation of SS-LFPs induced by 8 Hz and 50 Hz whisker stimulation were modulated by simultaneous cerebellar tDCS. Present results demonstrate tDCS's capability for SS and cerebellar cortices modulation and strongly suggest an important role of the cerebellar cortex in the control of plastic changes occurring in the sensory cortex of behaving animals.

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: Neurociencia cognitiva y conductual

DOPAMINE D_4 RECEPTOR COUNTERACTS MORPHINE-INDUCED CHANGES IN M OPIOID RECEPTOR SIGNALING IN THE STRIOSOMES OF THE RAT CAUDATE PUTAMEN.

Alicia Rivera [1], Alejandra Valderrama-Carvajal [1], Ruth Roales-Buján [1], Diana Suárez-Boomgaard [1], José Medina-Luque [1], Kirill Shumilov [1], Kjell Fuxe [2], Adelaida de la Calle [1]

[1]. Facultad de Biología. Universidad de Málaga, Málaga

[2]. Department of Neuroscience. Karolinska Institute, Stockholm

Morphine is one of the most potent analgesic drugs used to relieve moderate to severe pain. After long-term use of morphine, neuroadaptive changes in the brain promotes tolerance, which result in a reduced sensitivity to most of its effects with attenuation of analgesic efficacy, and dependence, revealed by drug craving and physical or psychological manifestations of drug withdrawal. The mu opioid receptor (MOR) is critical, not only in mediating morphine analgesia, but also in addictive behaviors by the induction of a strong rewarding effect. We have previously shown that dopamine D_4 receptor (D_4R) stimulation counteracts morphine-induced activation of dopaminergic nigrostriatal pathway and accumulation of Fos family transcription factors in the caudate putamen (CPu).

In the present work, we have studied the effect of D_4R activation on MOR changes induced by morphine in the rat CPu on a continuous drug treatment paradigm, by analyzing MOR protein level, pharmacological profile, and functional coupling to G proteins. Furthermore, using conditioned place preference and withdrawal syndrome test, we have investigated the role of D_4R activation on morphine-related behavioural effects.

MOR immunoreactivity, agonist binding density and its coupling to G proteins are up-regulated in the striosomes by continuous morphine treatment. Interestingly, co-treatment of morphine with the dopamine D_4 receptor (D_4R) agonist PD168,077 fully counteracts these adaptive changes in MOR, in spite of the fact that continuous PD168,077 treatment increases the [^{3}H]DAMGO B_{max} values to the same degree as seen after continuous morphine treatment. In addition, the administration of the D_4R agonist counteracts the rewarding effects of morphine, as well as the development of physical dependence. The present results give support for the existence of antagonistic functional D_4R-MOR receptor-receptor interactions in the adaptive changes occurring in MOR of striosomes on continuous administration of morphine and preventing morphine-related behaviour.

Financiación: P09-CVI- 4702 (Proyecto de Excelencia de la Junta de Andalucía)

1ª: Neurociencia de sistemas

2ª: Trastornos y reparación del sistema nervioso

LOCAL SHORT AND LONG-TERM SYNAPTIC PLASTICITIES MODULATE GLOBAL NETWORK ACTIVATION.

A. Moreno[(1)], Richard G. M. Morris[(1)], Santiago Canals[(2)]
[1] Centre for Cognitive and Neural Systems, The University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ, UK.
[2] Instituto de Neurociencias, Consejo Superior de Investigaciones Científicas & Universidad Miguel Hernández, Sant Joan d'Alacant 03550, Spain

RATIONALE:
The possibility that experimentally induced synaptic potentiation, in addition to causing local changes, could trigger wider network alterations is relevant to hippocampal-neocortical interactions in memory processing. This project, involving combined in vivo electrophysiology and functional Magnetic Resonance Imaging (fMRI) experiments, focused on CA3-CA1 synapses and downstream extrahippocampal communications.
METHOD:
Sprague-Dawley rats (n=15) were used, with electric microstimulation of the Schaffer collaterals or CA3 pyramidal cell layer and subsequent monitoring of the activation pattern using both multichannel electrophysiology recordings and fMRI imaging (7 Tesla). We investigated the effects of a range of stimulation frequencies (5, 10, 20 and 40 Hz) and intensities (500 and 800 uA). Local activation patterns increased monotonically with applied intensity, but frequency modulation followed a biphasic pattern. Whilst activity within the hippocampal formation increased gradually with higher frequencies, the extrahippocampal spreading of activity was only seen at 10 and 20 Hz giving an inverted U-shaped function. Induced synaptic potentiation modulated the extrahippocampal spreading, allowing greater propagation at 5 and 40 Hz.
INTERPRETATION:
These results identify frequency-dependent information channels in brain-wide networks that appear to fulfill the dual needs of local independency and global integration through segregating activity propagation in the frequency domain.

Supported by grants to SC from the Spanish MINECO (BFU2012-39958). The Instituto de Neurociencias is a "Centre of Excellence Severo Ochoa". AM is supported by a studentship from the Univ. Edinburgh, and RM by a Royal Society International Exchange Grant and by the European Research Council

1. Systems Neuroscience / Neurociencia de Sistemas.
2. Neuronal excitablity, sinapsis and glía / Excitabilidad neuronal, sinapsis y glía.

MODULATION OF PREFRONTO-HIPPOCAMPAL SYNCHRONY BY SEROTONIN 5-HT1AR IN MICE DURING EXECUTIVE FUNCTION.

T. Gener [1], M. Alemany [1], M.V. Puig [1],
1. Institut Hospital del Mar d'Investigacions Mèdiques, Barcelona

The communication between the prefrontal cortex and the hippocampus is critical for higher order executive functions such as associative learning and memory. This is accomplished via neural oscillations generated by the synchronization of neural networks. Abnormal neural oscillations have been observed in schizophrenia patients in several frequency bands, both in prefrontal cortex and hippocampus. Moreover, a genetic murine model of schizophrenia shows impaired prefronto-hippocampal synchrony. However, little is known about this particular prefronto-hippocampal communication during executive function in schizophrenia and how pharmacological interventions normalize it. Currently used treatments for schizophrenia include typical (preferential action on dopamine D2 receptors) and atypical (preferential action on serotonin 5-HT1A and 5-HT2A receptors) antipsychotics. Here, we aim at better understanding the cellular mechanisms underlying the action of atypical antipsychotic drugs. More specifically, we examine the modulation of neural oscillations and synchrony of the prefronto-hippocampal circuit by the acute administration of the 5-HT1AR agonist 8-OH-DPAT and antagonist WAY100635 (0.1 mg/kg, intraperitoneally [IP]).This was assessed in mice chronically implanted with multiple stereodes during resting state and while they perform the novel object recognition test (NOR) that evaluates short and long-term memory. Neural data was recorded with the novel open source "Open Ephys" data acquisition system and analyzed with custom software written in Matlab and the video tracking system "Bonsai".

1. Neurociencia de sistemas
2. Neurociencia cognitiva y conductual

ROLE OF STRIATAL PROJECTION NEURONS IN THE GENERATION OF L-DOPA-INDUCED DYSKINESIA

C. Alcacer[1], I. Sebastianutto[1], T. Fieblinger[1], J. Jakobsson, and M.A. Cenci[1]
[1]Basal Ganglia Pathophysiology Unit, Department of Experimental Medical Science, Lund University, Sweden, [2]Laboratory of Molecular Neurogenetics, Wallenberg Neuroscience Center, Lund University, Sweden.

L-DOPA is the reference treatment for Parkinson's disease (PD). However, this treatment is complicated by L-DOPA-induced dyskinesia (LID). Current theories assume that LID results from an imbalance in the activity of the direct and indirect pathway favoring an overactivity of the direct pathway. However the above theory has never been verified experimentally.

To selectively activate the striatal projection neurons of the direct pathway (dSPN) or indirect pathway (iSPN) in a mouse model of PD and LID we developed a chemogenetic approach based on targeting the expression of designer receptors exclusively activated by designer drugs (DREADD) selectively to dSPN or iSPN. The G-coupled DREADD are only activated by systemic administration of the inert compound, clozapine-N-oxide (CNO). Adeno-associated viral vectors (AAV) coding for Cre-inducible mCherry-tagged DREADD constructs were injected in the striatum in D1-Cre and A2a-Cre transgenic mice (to target dSPN or iSPN, respectively). Here we show experiments performed with the modified Gq-coupled human M3 muscarinic receptor (hM3Dq) whose stimulation by CNO triggers the activation of Gq-mediated signaling.

AAV-hM3Dq exerts functional effects *in vivo.* As expected, CNO treatment induced opposite effects on general motor activity in D1-Cre and A2a-Cre hM3Dq-transfected mice, i.e. increased and decreased activity respectively. In PD mice, hM3Dq-mediated activation of dSPN by CNO induced very mild dyskinesia but potentiated established dyskinesia in L-DOPA treated D1-Cre mice. Conversely, hM3Dq-induced activation of iSPN reduced established LID in A2a-Cre mice.

This is the first study using DREADD-based chemogenetic approach to explore the pathophysiology of PD and LID. Our results show that AAV-hM3Dq integrated in both SPNs and mediated robust changes in motor activity. In PD mice, activation of Gq-mediated signaling in dSPN induced very mild dyskinesia but potentiated already established LID. Conversely, activation of iSPN reduced established dyskinesia, thus considering activation of the indirect pathway a new route for treating LID.

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

OLFACTORY AND VOMERONASAL STIMULI PROCESSING IN THE MOUSE OLFACTORY BULBS AND CORTICOMEDIAL AMYGDALA

C. Pardo-Bellver[1], S. Martínez-Bellver[2], F. Martínez-García[3], E. Lanuza[1], V. Teruel-Martí[2]
[1]. Facultat de Ciències Biològiques. Universitat de València, Valencia.
[2]. Facultat de Medicina, Universitat de València, Valencia.
[3]. Predepartamental Medicine Unit, Universitat Jaume I, Castelló.

Olfactory and vomeronasal information is relayed to the main and accessory olfactory bulbs, respectively, which in turn project to the amygdala, a group of nuclei involved in processing the emotional value of chemical signals (such as sexual pheromones). To investigate how this system processes chemosensory information, we recorded the electrophysiological activity in the main (MOB) and accessory (AOB) olfactory bulbs, medial and posteromedial cortical amygdala of freely behaving female mice. In order to assess the correlation between the recorded signal and chemoinvestigatory behavior, different stimuli were supplied: neutral stimuli (clean bedding or geraniol-scented bedding) and conspecific-derived stimuli (such as female- and male-soiled bedding). Local field potentials were processed off-line by continuous wavelet transform in the time-frequency domain.

During the exploratory behavior the MOB and AOB showed dominant, coupled theta rhythmicity. The theta oscillation of the AOB showed phase preference and was highly phase-coupled with that of the MOB. In the AOB, theta waves modulate higher frequency bands, the alpha (14-20Hz) and high gamma (80-100Hz). Moreover, in the posteromedial cortical amygdala the theta oscillation in the AOB modulates alpha (12-20Hz), low gamma (40-50Hz) and high gamma (70-90Hz) frequency bands.

In the MOB, the theta rythmicity associated to very-fast gamma oscillation, has been described as a “sniffing” oscillation, as it is coupled with the respiratory frequency. This pattern is also observed in the AOB under exploratory behavior. The similar activation of the bulbs suggests a coupling between the internalization of the stimuli in the nasal cavity and the vomeronasal pumping. The theta modulation within the AOB suggests an intra-nucleus processing of the stimuli. In addition, this wave modulates the posteromedial cortical amygdala activity suggesting that primary theta oscillation in the AOB directs the local processing of the vomeronasal stimuli in the amygdala.

Funded by MINECO-FEDER (BFU2013-47688-P) and JCCLM-FEDER (PEIC11-0045-4490).

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

EEG ALPHA RHYTHM IN RETINITIS PIGMENTOSA

R. Mancebo Azor [1], J. M. Rodríguez Ferrer [1]
[1] Laboratorio de Neurociencia Visual y Cognitiva, Instituto de Neurociencias. Universidad de Granada, Granada

Background: In previous electrophysiological studies we showed that visual evoked potentials recorded in patients with retinitis pigmentosa mainly differs from normal subjects in that the P100 component of the recording has lower amplitude and longer latency. This reveals the attenuation of inputs to the visual cortex from a degenerated retina. Contrary to visual evoked potentials, the alpha rhythm obtained in electroencehalographic (EEG) recordings of the occipital lobe, due to the synchronic activity of the thalamus-cortical cells, represents the activity of the visual cortex at rest.
Purpose: To further study the effect of the altered retina in the electrophysiological activity of the visual cortex we investigated the EEG alpha rhythm in retinitis pigmentosa patients.
Methods: EEG alpha activity, characterized by a pattern of slow brain waves (8-12 Hz) in persons at rest with closed eyes, was analyzed, with consent, in a group of 24 patients with retinitis pigmentosa (7 men, mean age of 38.67 ± 10.88 years) and in 24 normal subjects (6 men, mean age of 39.71 ± 13.91 years). The electrophysiological signal was recorded by surface electrodes, according to international 10-20 system, O_Z-F_Z dipole.
Results: Alpha rhythm was absent in the 50% of the patients, while in the control group the percentage was 12.5%. In most of the patients (83.3%) with alpha activity, the recording had altered morphology and low amplitude waves, in comparison with that obtained in normal subjects.
Conclusions: The results show that in patients with retinitis pigmentosa the EEG activity of the visual cortex at rest is altered, as it is under the performance of visual tests, and the alpha rhythm is absent or abnormal in the majority of the patients studied. Secondly, they reveal that the degenerated retina also impedes the activity of thalamic cells in generating the alpha activity in the visual cortex.

1ª: Systems Neuroscience
2ª: Developmental Neurobiology

NORADRENERGIC INNERVATION OF THE PRIMATE THALAMUS: ANTERIOR, MIDLINE, INTRALAMINAR, AND VENTRAL MOTOR NUCLEI

I. Pérez-Santos, C. Cavada
Facultad de Medicina. Universidad Autónoma de Madrid, Madrid

In Parkinson's disease (PD) there is significant loss of noradrenalin (NA) neurons in *locus coeruleus* in addition to the loss of dopamine neurons in *substantia nigra*. In the thalamus of PD patients there is NA loss in almost all nuclei; it is particularly outstanding in ventral motor nuclei, in limbic (anterior group) and association nuclei, and in the centromedian nucleus. In the monkey MPTP model of PD, there is NA loss in the ventral motor nuclei. These abnormalities warrant the need to understand the normal NA innervation of the primate thalamus. At present this is incomplete because only limited thalamic regions have been analyzed and reported.

We have explored the NA innervation in the macaque monkey thalamus using immunohistochemistry against dopamine-β-hydroxylase (DβH), the NA synthesizing enzyme. Maps of DβH immunoreactive (-ir) axons were generated using Neurolucida® Kodalith filter, which transforms the real images in monochromatic representations of the immunostained axons. The borders of thalamic nuclei were traced using sections processed for acetylcholinesterase adjacent to the immunoreacted ones.

The thalamic midline nuclei were the most densely innervated by DBH-ir axons. Patches of dense labeling were also present in some intralaminar nuclei, including the paracentral nucleus and the centromedian-parafascicular complex, where the densest innervation was in the parafascicular region. Moderate labeling density was present in the anterior and the ventral motor nuclei. These nuclear complexes showed some heterogeneity in the NA innervation: it was densest in the densocellular subdivision of the anteromedial nucleus, in the ventromedial subdivision of the ventral anterior nucleus, and in area X.

The distribution of NA innervation in the monkey thalamus suggests a prominent role for NA in thalamostriatal circuits, since the intralaminar and midline nuclei are the main origin of thalamostriatal projections.

Supported by the Chair UAM - Fundación Tatiana Pérez de Guzmán el Bueno.

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

NEURAL SUBSTRATES OF COGNITIVE IMPAIRMENT IN THE DOWN SYNDROME MOUSE MODEL Ts65Dn: STUDY OF THE PREFRONTO-HIPPOCAMPAL CIRCUIT

M. Alemany [1], T. Gener [1], M.V. Puig [1,]
[1]. Institut Hospital del Mar d'Investigacions Mèdiques, Barcelona

Learning and memory are two of the major cognitive processes impaired in Down syndrome. These higher-order executive functions require a rapid and precise communication between the prefrontal cortex and the hippocampus that is accomplished via neural oscillations. However, so far research in Down syndrome has focused mainly on the effects produced by the genetic imbalance caused by the trisomy 21 both at the cellular and behavioural levels, but not on the alterations at the overall brain neural network level. The most used animal model of Down syndrome, the partial trisomic Ts65Dn mouse, exhibits multiple dendritic and synaptic abnormalities in the hippocampus and prefrontal cortex and also shows severe cognitive impairments in hippocampal- and prefrontal cortex- dependent tasks such as learning and memory. Yet, whether these cellular alterations impact neural network activity and communication between these two structures and results in impaired cognitive performance is still unknown. To address this, we recorded neuronal network activity simultaneously in the prefrontal cortex and the hippocampus in Ts65Dn mice while they performed a novel object recognition (NOR) task that evaluates short and long term memory, two disrupted cognitive functions in Down syndrome. During the performance of the NOR task, that quantifies the time mice spend exploring a new object versus a familiar one, local field potentials were recorded using a 32-channel headstage connected to the Open-Ephys system, a novel and open-source electrophysiology acquisition system. Behavioural data was acquired simultaneously with the new open source video tracking system Bonsai. Finally, in order to assess the synchrony between the neural networks of the prefronto-hippocampal circuit, we examined the degree of coherence between the local field potentials recorded in both structures.

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

LEARNING ABOUT NEOCORTICAL MICROCIRCUITS BY LOOKING FROM THE CONTRALATERAL SIDE

A. Sempere Ferrandez, E. Geijo Barrientos
Instituto de Neurociencias, Universidad Miguel Hernández-CSIC, Sant Joan

Unravelling the neuronal connectivity diagram is a key step in our understanding of brain function. In the neocortex, the quantitative dominance of the local circuit does not preclude the importance of long-range projections, which are necessary to integrate different sensory and motor commands with internal states to properly adapt to the environment. The callosal pathway, halfway in between short and long-range connectivity, mediates the transfer of information between homotopic regions of both cortical hemispheres. With a combination of axonal tracing methods and whole-cell patch-clamp recordings in mice cortical slices, we address the contribution of callosal axons from superficial pyramidal neurons to the neocortical circuitry by studying how different pyramidal and parvalbumin-fast spiking (PV-FS) interneurons from the entire cortical depth integrate these inputs. Our results show that callosal input is layer and cell-type specific. Both, direct callosal excitation (cEPSPs/cEPSCs) and disynaptic inhibition (dIPSCs) were larger in L2/3 and thick-tufted L5B pyramids than in thin-tufted L5 and L6 ones. Surprisingly, the pattern changed for PV-FS interneurons, in which cEPSPs amplitude decreased with columnar depth. These results challenge the current view of an unspecific inhibitory matrix covering all nearby pyramidal cells, suggesting the existence of different inhibitory subnetworks for different pyramidal types (L2/3 and L5B thick-tufted vs. L5 thin-tufted pyramids). In addition, we noticed that while superficial pyramids fired scarcely in response to callosal input, action potentials were elicited in a large proportion of L5B thick-tufted neurons; probably do to the reduced recruitment of L5B PV-FS interneurons. According to this, the excitation to inhibition balance was larger and the reversal potential of the compound callosal response closer to the firing threshold in L5B pyramids. This difference was further increased with 40Hz train stimuli, indicating that callosal projecting neurons are well suited to recruit this important output pyramidal population in the contralateral cortex.

Funding: Generalitat Valenciana, grant PROMETEOII/2014/014; Programa JAE Pre-DOC 2010

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

CAN A SUSTAINED INCREASE IN INHIBITION MODIFY THE STRUCTURE OF PYRAMIDAL NEURONS IN THE MEDIAL PREFRONTAL CORTEX?

Y. Curto Sastre, I. García Mendoza, J. Nácher Roselló
Facultad de Biología. Universitat de València, Burjassot (Valencia)

The adult brain retains a substantial capacity for synaptic reorganization, which includes a wide range of modifications from molecular to structural plasticity. Previous reports have demonstrated that structural changes seem to occur in parallel to changes in GABAergic neurotransmission. The physiology of neuronal inhibitory networks can be modified through the GABAa receptor, which has a binding site for benzodiazepines (BZ). Although BZs are among the most prescribed drugs, it is not yet known whether they have an effect on the structure and connectivity of pyramidal neurons. In the present study we wish to elucidate the impact of a chronic treatment of 21 days with the BZ diazepam (2 mg/kg, ip) on the structural plasticity of pyramidal neurons of the medial prefrontal cortex (mPFC) of adult mice, specifically in its cingulate region, using a strain of transgenic mice with fluorescent excitatory neurons (B6.Cg-Tg(Thy1-YFP)HJrs/J). To do this, we examined the density of dendritic spines in the principal apical dendrite of layer III pyramidal neurons. We have also evaluated the density of en passant axonal boutons in layer I. Confocal analysis after immunohistochemistry has revealed that most of these axonal projections have an extracortical regions. Two behavioral tests were conducted at the beginning (light/dark) and the end (open field) of the BZ treatment to determine its influence on anxiety levels. Animals treated with diazepam showed a decrease in the total number of spines in the principal apical dendrites of pyramidal neurons. No changes were found in the density of axonal boutons. Our study has demonstrated that the chronic intake of benzodiazepines can cause important structural alterations in pyramidal neurons. Whether these changes are permanent, whether they can occur after a shorter exposure to BZP or whether they can be reversed after the end of the treatment will be the subject of future studies.

Spanish Ministry of Economy and Competitiveness BFU2012-32512, Generalitat Valenciana Prometeo Excellence Program PROMETEO2013/069 and the Fundación Alicia Koplowitz.

1ª: Developmental Neurobiology
2ª: Disorders and nervous system repair

LOCAL INHIBITION AND ACTIVITY PROPAGATION IN THE DENTATE GYRUS IS CONTROLED BY VTA INPUTS.

C. Cobos, L. Pérez-Cervera, S. Canals
Instituto de Neurociencias de Alicante (CSIC-UMH), San Juan de Alicante, Alicante.

Dopaminergic neurotransmission in the hippocampus is essential for learning and memory. An influential model of memory consolidation suggests a critical role of the dopaminergic input from the ventral tegmental area (VTA) (Lisman et al., TINS, 2011). Previous studies demonstrated that the enhanced learning occurring when a novelty stimulus is presented around the time of memory encoding is blocked by dopamine receptor antagonists in the rodent hippocampus (Wang et al., PNAS, 2010). However, how and where the VTA input modulates information transfer in hippocampal circuits is not known. Here we combine electrophysiological recordings and fMRI to investigate the role of the VTA on hippocampal effective connectivity.

In a first set of experiments we implanted urethane anesthetized rats with both, electric microstimulation electrodes in VTA and the perforant pathway, and linear multi-recording probes in the dorsal hippocampus spanning the CA1 and dentate gyrus regions. Activation of the VTA facilitates the spiking of granule cells when the stimulus precedes an entorhinal input. The effect is maximal when pairs of pulses are applied to the VTA at 2.5-5 ms ISI and followed by perforant pathway stimulation by 10-15 ms delay.

Analysis of sub-threshold potentials in the hilus evoked by perforant pathway stimulation, and spontaneous activity using independent component analysis (ICA), demonstrate that feed-forward inhibition is depressed by VTA stimulation. This des-inhibitory effect enhances activity propagation in the trisynaptic circuit. Previous results from the laboratory have shown that both, LTP induction in anesthetized and novelty exposure in freely moving animals induce a similar decrease of feed-forward inhibition in the dentate gyrus (Álvarez-Salvado et al. SENC 2013).

Overall these results suggest that VTA inputs to the dentate gyrus facilitate information transfer in the hippocampus by decreasing feed-forward inhibition. Ongoing fMRI experiments in this project investigate the consequences of such modulatory effect on large scale network dynamics.

1.- Systems neuroscience.
2.- Cognitive and behavioral neuroscience.

ANATOMICAL WEIGHT OF CORTICAL VS. SENSORY DRIVER INPUTS TO "HIGHER-ORDER" RELAY NUCLEI OF THE THALAMUS: A QUANTITATIVE ANALYSIS IN MICE.

D. Casas-Torremocha, C. Porrero and F. Clascá
Department of Anatomy & Neuroscience. School of Medicine, Autónoma University of Madrid

Inputs from thalamic nuclei to the cerebral cortex determine the content and dynamics of information flow in cortical circuits. Two main functional categories of thalamic nuclei have been proposed: First Order (FO) relay nuclei, whose cells are powerfully driven by subcortical structures, and Higher Order (HO) relay nuclei whose driving input is come from axon collaterals of cortical layer 5 (L5) cells. HO nuclei such as the Posterior nucleus (POm), however, are known to receive, in addition to cortical L5 driver input, subcortical somatosensory input supposed to be "driver". Recent studies suggest that L5 and subcortical "driver" inputs can actually converge on the same POm neurons. The precise origin, convergence and relative anatomical weight of both types of input have not been assessed yet with quantitative methods.
To reveal the number and location of all cells innervating POm in adult mice, we made small stereotaxic retrograde tracer deposits in POm or in the ventroposteromedial nucleus (VPM, a FO nucleus, for comparison), and subsequently plotted the localization of retrograde labelled somata throughout entire neuraxis, from cortex to sacral spinal cord. We then counted and compared labeled somata numbers in somatosensory-related subcortical nuclei with those arising from cortical L5. To detect possible differences in the dispersion within POm and/or in the number of varicosities per axon of the neurons innervating this nucleus, we analyzed individual terminal arborizations into POm labeled by BDA microiontophoresis in L5 of the somatosensory cortex or in the trigeminal nuclei of the brainstem. Our results reveal that the HO POm receives ~ 70% of its driver input from cortical L5 but still a 30% from somatosensory nuclei. In contrast, the FO VPM receives more of 90% of its driver inputs from subcortical sensory nuclei.

Funding: MINECO (BFU2010-19695) and the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 604102 (Human Brain Project).

3. Systems Neuroscience

THETA COUPLING IN THE HIPPOCAMPUS AND THE NUCLEUS INCERTUS DURING REM SLEEP AND ACTIVE WAKEFULNESS.

I. San Martín-Molina [1], S. Martínez-Bellver[1,2], A. Cervera-Ferri[1],J. Martínez-Ricós[1], A. Luque-García[1], A. Blasco-Serra[1], E. González-Soler[1], A. Núñez Molina[3], V. Teruel-Martí[1]
[1]. Facultad de Medicina. Universitat de València, València
[2]. Facultad de Biología, Universitat de València, València
[3]. Facultad de Medicina, Universidad Autónoma de Madrid, Madrid

The hippocampal theta rhythm is an oscillatory process, between 3-12 Hz, which is recorded during behavioral states like exploration or navigation and during the REM sleep. Theta rhythm is involved in numerous functions of the hippocampus as long-term potentiation (LTP). The presence of this rhythm during REM sleep is associated with the retrieval and memory consolidation, through the replay of neuronal sequences in the same network activated under the learning process.
This rhythm is modulated by septal and brainstem areas, like the nucleus incertus (NI), which presents a role in stress and modulating arousal. Previous studies under urethane anesthesia demonstrate that the NI exhibits theta activity in the same arousing conditions that generate this oscillation in the hippocampus. Moreover, this theta activity is highly coherent between both structures, in the anesthetized rat.
The aim of this study is to determine whether theta synchronization is also present between the NI and the hippocampus in freely behaving rats during REM sleep and active wakefulness.
In order to perform this analysis, chronic field recordings in unanesthetized rat were made through of the implantation of monopolar electrodes in both structures, as well as electromyogram electrodes in the neck muscles. The experiments were recorded in video for helping in the identification of the sleep-wakefulness states.
Data were analyzed using spectral and time-frequency analyses (wavelet analysis). The results confirmed that theta rhythm is present in the NI frequency both during REM sleep and active wakefulness. Also, our study conclude that both the NI and the hippocampus are highly coupled during these states. In conclusion, this study reinforce the role of the NI in the theta network.

1. Systems Neuroscience
2. Cognitive and conductual Neuroscience

RELEVANCE OF GABAERGIC NEURONS IN THE MODULATION OF WHISKER RESPONSES IN THALAMOCORTICAL NEURONS

N. Barros-Zulaica[1,2], A. Nuñez[1], A. Villa[2]
1. Depto. Anatomía, Histología y Neurociencia. Universidad Autónoma de Madrid, Madrid
2. Comunication Systems Department of Lausanne University, Lausanne, Switzerland

The somatosensory pathway is characterized by a high degree of order from periphery to the cortex. Sensory information from the whiskers passes via the brain stem and thalamus to the barrel cortex, which also project to the thalamus. The aim of the present work is to study the relevance of the inhibitory system in the modulation of thalamocortical responses to whisker stimulation. At all levels of this thalamocortical network there are GABAergic neurons expressing the calcium-buffer parvalbumin (PV). Thus, we performed unit recordings in urethane anesthetized wild-type and parvalbumin-deficient (PVKO) mice. Recordings were performed in supra- or infragranular layers of the barrel cortex simultaneously with a unit recording of thalamic activity in the ventro-posteriormedial thalamic nucleus or in the posterior medial nucleus of the thalamus. We found that whisker responses were increased in PVKO mice, mainly in barrel cortical neurons. Thalamic neurons also increased their responses in PVKO mice. Differences were also evident when different stimulation patterns were applied. Synchronous activity of thalamocortical neurons with the same receptive field was enhanced, as well. In conclusion, our data show that modulation of inhibition in the thalamocortical network may control the flow sensory information to the cortex. Since inhibitory neurons in the thalamocortical network are controlled by cholinergic afferents from the brain stem and the basal forebrain, the flow of sensory information may change in the awake state or during attentional processes, in which cholinergic system is enhanced, by modulation of inhibitory activity in the thalamocortical network.

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

CONTROL OF SOMATOSENSORY CORTICAL PROCESSING BY THALAMIC POSTERIOR MEDIAL NUCLEUS: A NEW ROLE OF THALAMUS IN CORTICAL FUNCTION

C. Castejón, N. Barros-Zulaica, A. Núñez
Depto. Anatomía, Histología y Neurociencia. Universidad Autónoma de Madrid, Madrid

Current knowledge of thalamocortical interaction comes mainly from studying lemniscal thalamic systems. Less is known about paralemniscal thalamic nuclei function. In the vibrissae system, the posterior medial nucleus (POm) is the corresponding paralemniscal nucleus. POm neurons project to L1 and L5A of the primary somatosensory cortex (S1) in the rat brain. It is known that L1 modifies sensory-evoked responses through control of intracortical excitability and that blocking activity in L1 increases whisker responses, suggesting that L1 exerts an inhibitory influence on sensory responses. Therefore, thalamocortical pathways targeting L1 could modulate cortical firing. Here, we have determinate POm modulation of cortical processing, using a combination electrophysiology and pharmacology techniques *in vivo*. In our experiments, unit recordings performed in urethane-anesthetized rats showed that POm electrical stimulation reduced magnitude and duration of the cortical whisker responses meanwhile POm inactivation enhanced them. These effects were more prevalent in the second, NMDA-mediated component of the responses and changed with different POm electrical stimulation intervals before sensory stimulus. Our findings demonstrate that L1 inputs from POm impose an intensity dependent regulation on cortical sensory processing. Moreover, we found that L1 GABAergic inhibition mediates this process because L1-evoked inhibition was blocked by picrotoxin. L1-evoked inhibition was reduced when P/Q-type Ca2+ channels were blocked, suggesting that parvalbumin neurons may be involved. POm neurons also control sensory processing in the secondary somatosensory cortex by corticofugal activity from L5 in S1 to POm. Taken together, our data demonstrate the relevant role exerted by the POm in the adjustment of somatosensory cortical processing and in the regulation of cortical relationship between S1 and S2 somatosensorial areas. We propose that this adjustment could be a thalamocortical gain regulation mechanism also present in the processing of information between cortical areas.

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

SENSORY INTERFERENCE IN THE MEDIAL PREFRONTAL CORTEX. IMPLICATION IN ATTENTIONAL PROCESSES

J. Martín-Cortecero, A. Núñez
Depto. Anatomía, Histología y Neurociencia. Universidad Autónoma de Madrid, Madrid

The medial prefrontal cortex (mPFC) plays an important role in attentional control of incoming sensory inputs. The mPFC comprises several structures such as agranular medial cortex, anterior cingulate cortex, prelimbic (PL) and infralimbic (IL) cortex. We have studied event related potentials (ERPs) evoked by tactile or auditory stimuli in mPFC as well as interference processes when both stimuli were applied simultaneously in anesthetized adult rats. The mPFC displayed ERP when whisker or click stimuli were applied. To test if mPFC tactile responses were due to S1 activity we applied lidocaine (0.1 □l) on the S1 barrel cortex. In presence of lidocaine, mPFC responses were reduced up to 39% in PL and 31% in IL respect to control values, suggesting that mPFC may receive sensory inputs from the thalamus. Moreover, the results show and important ERP attenuation in infralimbic cortex respect to control conditions when white noise auditory stimuli (14%) or contralateral whisker stimulation (21%) were applied simultaneously (14% or 21% of attenuation, respectively). Nevertheless, PL or S1 cortices did not show attenuation. Since S1 did not reveal attenuation the decreased amplitude observed IL cannot be due to a response reduction of S1. We also studied the response of mPFC neurons to a novel stimulus in the whiskers (5 Hz train). Novel stimuli had about 30% of amplitude increment in IL respect to control values while PL (<17%) or S1 (<6%) displayed lower amplitude increments. In conclusion, mPFC may be exerting an attentional control between sensory modalities since sensory responses are modulated according to the stimulus relevance.

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

POPULATION ACTIVITY MODULATES NEURONAL TUNING AND AFFECTS ENCODED INFORMATION

Iñigo Arandia-Romero [1,2], Seiji Tanabe[4], Jan Drugowitsch[3], Adam Kohn[4,5] and Rubén Moreno-Bote[1,2]
[1] Research Unit, Parc Sanitari Sant Joan de Deu and Universitat de Barcelona, Esplugues de Llobregat, Barcelona, Spain, 08950
[2] Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Esplugues de Llobregat, Barcelona, Spain, 08950
[3] Département des Neurosciences Fondamentales, Université de Genève, CH-1211 Geneva 4, Switzerland
[4] Dominick Purpura Department of Neuroscience and [5]Ophthalmology and Visual Science, Albert Einstein College of Medicine, Bronx, New York, 10461

Many studies have shown that stimuli modulate neuronal responses, but responses are also affected by the state of the local network. Attention or arousal changes are able to modify the tuning of sensory neurons, and fluctuations of the ongoing population activity have been shown to play many roles. However, little is known about how intrinsic fluctuations of stimulus evoked population activity modulate the sensory tuning of cells and whether they affect their encoded information. We found that fluctuations of population activity in monkey V1 modulate the tuning of individual neurons in a multiplicative and additive manner, with minor effects on the broadening or displacement of the tuning curves. Neurons with strong multiplicative effects tended to have little additive modulation, and the reversed was observed for neurons with strong additive effects. We also found that the last 100 milliseconds of ongoing activity before stimulus onset is able to modulate the tuning of individual neurons both multiplicatively and additively, but with a weaker impact on the tuning curves than the stimulus evoked activity. Then, we analyzed whether these fluctuations of the state of the network had any impact on the amount of encoded information using state-of-art decoding techniques from simultaneously recorded neuronal populations. As predicted by a model based on a multi-gain model of population activity we found that the information encoded by multiplicatively-modulated neurons increased with greater population activity, while that of additively-modulated neurons decreased. These effects cancel each other in such a way that fluctuations of population activity had little effect on total information. Therefore, our results suggest that the global state of the network acts as a 'traffic light' that controls which subset of neurons are most informative without affecting total information.

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

POSSIBLE BILATERAL MODULATION OF HIPPOCAMPAL SHARP WAVES.

G. Martín Vázquez [1], Ó. Herreras[1]
[1]. Instituto Cajal. CSIC, Madrid

Hippocampal sharp-wave ripples (SWRs) participate in memory formation, but the mechanism of initiation of these irregularly occurring events still remains obscure. SWRs recorded in the CA1 subfield reflect synchronous population bursts of the CA3 region, while it is widely assumed they occur unilaterally. However, there is strong connection between CA3 subfields in both hippocampi through the commissure and it has been reported strong interhemispheric transfer necessary for the buildup of epileptiform afterdischarges. We here investigated the role played by bilateral connections in the generation of these physiological hypersynchronous SWR events.
We recorded the local field potential (LFP) of the CA1 region of both hippocampi using linear multielectrode in anesthetized rats. The pathway-specific contributions were extracted using a spatial discrimination technique (Independent Component Analysis, ICA). The ipsilateral CA3-CA1 (Schaffer) glutamatergic input consists of SWRs and bouts of gamma activity. The contribution of the contralateral CA3 region was blocked through local injection of lidocaine using a cannula, and the extension of the drug was monitored by evoked potentials.
Lidocaine injections drastically reduced the power of the ipsilateral Schaffer input to the CA1, while in the contralateral side only affected the low frequencies, hence mantaining the Schaffer gamma activity unaltered. In average, the individual gamma waves or elementary excitatory events (µfEPSPs) in the side contralateral to the injection did not change, neither in duration or amplitude. However, SWR events showed significant reductions in amplitude, duration and frequency of occurrence. We also noticed a significant reduction of the amplitude of the associated ripples.
These results indicate a bilateral crosstalk for the generation of SWRs in the CA3 region. Even if there may be ignited unilaterally their progression may require a cooperative process between hippocampi.

Financed by MEC: BFU2013-41533-R

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

CANNABINOID AND LYSOPHOSPHATIDIC ACID SYSTEMS IN THE TRIPLE TRANSGENIC MICE MODEL OF ALZHEIMER'S DISEASE

[1]Gónzalez de San Román E, [1]Garrofe E, [1]Manuel I, [1]Martínez-Gardeazabal J, [1]Moreno M, [1]Llorente A, [1]Lombardero L, [1]Giralt MT, [2]Giménez-Llort L, [1]Rodriguez-Puertas R
[1]Department of Pharmacology, Faculty of Medicine and Odontology. University of the Basque Country.
[2]Department of Psychiatry and Forensic Medicine, Institute of Neuroscience, Universitat Autónoma de Barcelona.

Alzheimer's disease (AD) is the most common cause of dementia in aging populations. The triple transgenic mice (3xTg-AD) initiate the accumulation of senile plaques and neurofibrillary tangles at 6 months of postnatal development. The present study analyzes the involvement of neurolipids, such as endocannabinoids (eCB) and lysophosphatidic acid (LPA) in this mice model of familial AD using functional and receptor autoradiography combined with the identification of phospholipid (PL) precursors of the endogenous ligands by Imaging Mass Spectrometry (MALDI-IMS).
The autoradiographic distribution of functional CB_1 receptors in the mice model showed a decreased activity in cortex layer VI (WT 435.4 ± 58.2% over basal levels; 3xTg-AD 238.4 ± 22.9%) and posterior amygdala (WT 295.5 ± 41.7%; 3xTg-AD 112.8 ± 28.9%). On the contrary, LPA_1 activity was increased in transgenic mice at striatum (WT 3.3 ± 7.2%; 3xTg-AD 23.1 ± 3.8%), motor cortex (WT 6.2 ± 12.4%; 3xTg-AD 26.1 ± 7.6%), corpus callosum (WT 90.8 ± 12.3%; 3xTg-AD 189.6 ± 17.4%) and hippocampal CA1 area (WT -18.7 ± 7.8%; 3xTg-AD 22.7 ± 4.4%).
Quantitative densitometry showed a higher density of CB_1 receptors in 3xTg-AD mice than in WT at brain areas where intracellular Aβ has been build up, such as hippocampal CA1 area (WT 193.8 ± 33.8 fmol/mg t.e.; 3xTg-AD 284.6 ± 19.8 fmol/mg t.e.; $p<0.05$) and cingulate cortex (WT 166.8 ± 27.7 fmol/mg t.e.; 3xTg-AD 236.8 ± 17.1 fmol/mg t.e.; $p<0.05$).
The application of IMS to the transgenic mice model identified modification in different PL species and one of the possible LPA precursors, the PA (34:1), was up-regulated in the same brain areas where the LPA_1 activity was increased.
The eCB and LPA neurolipid systems would activate in familial AD at initial stages by increasing the synthesis of the endogenous ligands and the efficiency of their target receptors.

Acknowledgements: This work was Supported by ISCIII (PI10/01202), co-funded by European Research Development Fund and Basque Government grant (IT584-13) to the “Neurochemistry and Neurodegeneration” Research group. E. G. de S. R. has a postdoctoral contract from UPV/EHU.

1. Neurociencia de sistemas
2. Neurociencia cognitiva y conductual

WHITE MATTER OF THE CEREBELLO-THALAMO-CORTICAL NETWORK IS DAMAGED IN ESSENTIAL TREMOR

A. López-del Rio [1], J.P. Romero [2], E. Rocón [3], J. Benito-León [4], J.A. Pineda-Pardo [1,5]
[1]. Centre for Biomedical Technology, Universidad Politécnica de Madrid, Madrid
[2]. Facultad de Ciencias Biosanitarias, Universidad Francisco de Vitoria, Madrid.
[3]. Neural and Cognitive Engineering group, CAR, UPM-CSIC, Madrid
[4]. Departamento de Neurología, Hospital 12 de Octubre, Madrid
[5]. CINAC, HM Puerta del Sur, Hospitales de Madrid, Móstoles, and CEU-San Pablo University, Madrid

OBJECTIVES: Essential tremor (ET) is a neurological disorder whose underlying mechanisms are not completely known to date. The main aim of this study is the characterization of essential tremor (ET) through the study of white matter integrity using Diffusion Tensor Imaging (DTI).

MATERIALS & METHODS: MRI data from 21 controls (age=60±13 years, 53.4% females) and 23 subjects with ET (age=63±13 years, 52% females, average duration of tremor=8414±6019 ms) was acquired. Probabilistic tractography, using a Ball&Stick model (Behrens et al. 2007), was performed between different regions of the cerebello-thalamo-cortical network, including primary motor cortex, premotor cortex, primary somatosensory cortex, cerebellum and thalamus, in order to identify the extent of the anatomical connections. Then, two different types of analysis were performed: 1. tract-based, in which DTI scalar measures [fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD)] were averaged along the anatomical connections and compared between groups and 2. voxel-wise statistical analysis, in which anatomical connections were compared group-wise by a nonparametric permutation statistical test (5000 permutations) performed for FA, MD, RD, AD and probabilistic tractography maps. The significance threshold for between group differences was set at $p<0.05$ applying a FDR correction for multiple comparisons ($q < 0.05$).

RESULTS: Tract-based analysis revealed that patients with ET in comparison to controls showed significant ($p_{corrected} < 0.05$) increase of MD, RD and AD in studied white matter tracts of cerebello-thalamo-cortical network. AD increment was seen in cerebello-thalamic, thalamus-primary motor cortex and thalamus-primary somatosensory cortex on both hemispheres. Voxel-wise analysis also revealed significant increase of AD in these tracts. No significant change in FA was observed in none of the analysis.

CONCLUSIONS: There is in vivo evidence for axonal disintegration of white matter fibers in patients with ET, specifically in the tracts connecting the thalamus with primary motor cortex, cerebellum and primary somatosensory cortex. FA change by its own it's not enough to characterize white matter degeneration on ET.

[1] Behrens et al. 2013. Probabilistic Diffusion Tractography with Multiple Fibre Orientations: What Can We Gain?. *NeuroImage* 34(1): 144–55.

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

MECHANISMS OF CORTICAL NETWORK COMPLEXITY AND ITS EXOGENOUS MODULATION IN VITRO

M. D'Andola[1], B. Rebollo[1], A.G. Casali[2], J.F. Weinert[1],A. Pigorini[3], M. Massimini[3] & M.V. Sachez-Vives[1,4]

[1] IDIBAPS (*Institut D'Investigacions Biomèdiques August Pi i Sunier*), Barcelona, Spain.

[2] Federal University of São Paulo, Institute of Science and Technology, São José dos Campos, SP, Brazil.

[3] Department of Biomedical and Clinical Sciences "L. Sacco", Università degli studi di Milano, Milano, Italy

[4] ICREA (*Instituciò Catalana de Recerca i Estudis Avançats*), Barcelona, Spain

Recently a perturbational complexity index (PCI) was introduced to quantify the information content of deterministic patterns evoked in the brain by cortical stimulation with TMS (Casali et al., 2013). This scalar index shows a significant correlation with the level of consciousness in individual human subjects across different conditions (sleep, anesthesia, coma) thus having a great diagnostic value. Intracranial stimulation and recordings have shown that deterministic responses are impaired by stability during non-REM sleep (Pigorini et al., 2015). In these conditions, upon receiving inputs, cortical neurons entered a Down state after which deterministic activations were lost.
Here we aimed to investigate the mechanisms underlying these cortical responses under different brain states. To this end we used an in vitro preparation of the cerebral cortex that generates temporally structured spontaneous activity. We then perturbed the activity by electrical pulses while recording with arrays of 16 electrodes the spatiotemporal profile of the responses. By means of pharmacology we simulated different brain states. We first adapted the PCI algorithm and performing phase-lock analysis we were able to reproduce the base-specific changes observed with cortical stimulation in humans. In particular, we investigated 3 network states: 1) spontaneous slow oscillations (SO), 2) activity in the presence of kainate, and 3) activity in the presence of norepinephrine and carbachol. During SO we observed a response similar to what observed in nREM sleep: an initial activation, followed by a silent state and break-off of deterministic activations, while in kainate we observed no restoration of phase-locking notwithstanding the increase in the activity level, resulting in an unvaried level of PCI. Under the effect of norepinephrine and carbachol we observed a significant increase in PCI correlated with prolonged deterministic effects similar to those corresponding to the awake state. This *in vitro* results support the hypothesis that bistability might be responsible for loss in complexity and demonstrate that complexity can be modulated pharmacologically.

Supported by EU FP7 FET CORTICONIC contract 600806.

1°: System Neuroscience
2°: Theoretical and Computational Neuroscience

DETECTION OF NEURON POPULATIONS AND PATHWAYS INVOLVED IN DOWNSTREAM PROPAGATION OF EPILEPTIC ACTIVITY THROUGH CORTICO-HIPPOCAMPAL CIRCUITS

T. Ortuño Silva[1], S. Hernández Recio[2], D. Torres Romero[1], J. Makarova[1], O. Herreras Espinosa[1]
[1]Cajal Institute, CSIC, Madrid, Madrid
[2]Facultad de Biología, Universidad Autónoma, Madrid, Madrid

Epileptogenic activity spreads downstream neuron populations through synaptic chains and may affect several local and long range networks. Abnormal reverberations make difficult to identify the faulty population/network. This is particularly relevant in cases where macroscopic structural defects are not obvious and cannot inform the clinician to guide treatment to the appropriate targets. Electrophysiological studies can detect EEG spikes from the scalp or intracerebrally, but the spread of electrical fields far from their sources hampers the precise identification of the specific neuron populations involved in the different structures. We use an optimization of spatial discrimination techniques to disentangle pathway-specific threads from multisite local field potentials (LFP) that allows precise delimitation of the activated synaptic territories and the back-tracking of the populations of origin. We generated small epileptic foci through local disinhibition using microinjections of Bicuculline (1mM) in the CA3 region of the Hippocampus or the entorhinal cortex (EC). Recordings were performed simultaneously in Motor Cortex (M2) and the CA1/CA3-DG regions in anesthetized rats. In control conditions we identified 5-6 main hippocampal LFP generators, including the CA3-CA1 (Schaffer) and EC-Dentate inputs, plus three main LFP generators in the Motor Cortex (M2), all of them with distinguishable physiology and independent activity. After CA3 disinhibition we found large epileptic spikes as well as changes in baseline activity in the CA1, and some cortical LFP generators. Notably, some of these showed interictal spikes with own frequency apparently detached from the CA3 focus. Some LFP generators reduced drastically the activity while new ones appeared, indicating the silencing and activation of formerly silent populations, respectively. In conclusion, we were able to establish direct spread of abnormal spike and baseline activity from CA3 to the Motor cortex downstream the synaptic chains that involve multiple cell populations identifiable through pathway-specific LFPs. Financed by MEC: BFU2013-41533-R

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

OLFACTORY TYPE G-PROTEIN α SUBUNIT EXPRESSION IN THE STRIATUM IS REGULATED BY DOPAMINERGIC SYSTEM

I. Ruiz-DeDiego[1,2], J.R. Naranjo[2,3], D. Hervé[4,5,6] and R. Moratalla[1,2]
[1]Cajal Institute, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain.
[2]CIBERNED, Instituto de Salud Carlos III (ISCIII), Madrid, Spain.
[3]Centro Nacional de Biotecnología, CSIC, Madrid, Spain.
[4]Inserm UMR S-839, Paris, France.
[5]Institut du Fer à Moulin, Paris, France.
[6]Université Pierre et Marie Curie, F-75005 Paris, France

Background: In the normosensitive striatum, the olfactory type G-protein α subunit (Gαolf) couples the dopamine D1 receptor (D1R) to adenylyl cyclase, triggering intracellular signaling and neuronal activation. Previous findings have reported that Gαolf is enriched in the striosomes and that dopamine deregulation induces alterations in Gαolf protein and cAMP-signaling pathway. However, the regulation of Gαolf expression by striatal dopamine and dopamine receptors is not fully understood. The work presented here is focused on elucidation of the regulation of Gαolf expression by manipulating the dopaminergic system upstream and downstream Gαolf.
Methods: Striatal Gαolf was studied in wild-type and genetically engineered mice lacking D1R, D2R (D2 receptor) and DREAM (downstream regulatory element antagonist modulator) protein whose dopamine levels were manipulated. Dopamine depletion was accomplished by 6-OHDA striatal lesion or by Pitx3 ablation (aphakia mice), and dopamine replacement by chronic L-DOPA treatment. Gαolf levels were analyzed by immunohistochemistry, Western-Blot and RT-qPCR.
Results: Dopamine depletion or genetic inactivation of D1 receptors increased Gαolf protein levels, abolishing the striosomal-enriched pattern of Gαolf expression. Dopamine replacement by L-DOPA in denervated wild-type mice reestablished both, the expression pattern and protein levels, but paradoxically increased Gαolf mRNA. In $D1R^{-/-}$ mice, dopamine depletion decreased striatal Gαolf expression, while L-DOPA did not restore either Gαolf levels or its expression pattern. By contrast, inactivation of D2R or changes in the cAMP/PKA signaling pathway downstream of Gαolf did not modify its expression.
Conclusion: Our results show a homeostatic, negative regulation of Gαolf expression by dopamine and by D1R stimulation, which are also required for the striosomal Gαolf expression pattern. These results shed light on the regulation of Gαolf by dopamine-signaling that could be involved in the pathophysiology of the maladaptive response to chronic L-DOPA treatment in Parkinson's disease.

This work was supported by grants from the Spanish Ministerios de Economía y Competitividad and of Sanidad, Política Social e Igualdad, ISCIII: SAF2013-48532-R, PNSD #2012/071, RedRTA (RD06/0001/1011), CIBERNED ref. CB06/05/0055, and Comunidad de Madrid ref. S2011/BMD-2336 to **RM** and ANR09-MNPS-014 to **DH** and ISCIII/CIBERNED, Madrid Community/Neurodegmodels and grant SAF2010-21784 from Spanish Ministerio de Economía y Competitividad to **JRN**.

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

EFFECTS OF CLOSED-LOOP DEEP BRAIN STIMULATION IN THE 6-OHDA RAT MODEL OF PARKINSON DISEASE

I. Cordon, M.J. Nicolás, Sandra Arrieta, Eneko Lopetegui, M.Alegre, J. Artieda, M.Valencia
Neurophysiology Laboratory, CIMA. University of Navarra. Pamplona, Spain

Implantable devices for electrical stimulation of the brain have been in routine clinical use since 1997, when the first commercial deep brain stimulation (DBS) system was approved for the treatment of tremor. These DBS devices provide an invariant train of stimulatory pulses at a fixed frequency (~130 Hz). The shortcomings of this therapy are multiple and vary from the impossibility of adapting the bran stimulation to the stage of the illness to a continuous consumption of the pacemaker battery life. In this context, the design of closed-loop stimulation systems represents the next frontier to achieve in neuromodulation. In this study we present the first DBS closed-loop system successfully tested on rats and compare it against classical DBS stimulation.
We recorded the cortical brain activity of 15 control and 12 parkisonian rats. We designed a closed-loop stimulation system that takes into account the phase of the ongoing cortical brain activity of the rat to perform DBS in the subthalamic nucleus (STN). The effect of the DBS was assessed by means of spectral analysis of the electrophysiological signals and behavioral tracking.
While classical DBS increased the locomotor activity of animals, closed-loop stimulation based on the phase of the activity worsened the behavioral activity of control rats reducing the degree of locomotion. During classical DBS, a reduction of beta activity was observed while the opposite occurred when closed-loop stimulation was applied.
Closed-loop stimulation produced a decrease in the locomotion of the animals together with an increase on the beta activity. These results were contrary to the obtained when delivering classical DBS (high frequency stimulation). Our results suggest that closed-loop paradigms, far from ameliorating the rats' behavior, might deteriorate it.

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

A REM SLEEP-SPECIFIC HIGH FREQUENCY BAND: CORTICAL AND SUBCORTICAL ORGANIZATION

A. Sánchez-López and M. Escudero
Facultad de Biología. Universidad de Sevilla, Sevilla

Electroencephalographic activity (EEG) remains one of the main physiological activities used in the identification of neurofunctional states. Recently, we found a frequency band peaking between 120 and 140 Hz which was characteristic of REM sleep in rats and showed the highest amplitude in the centro-parietal cortex just rostral to lambda. Here we study the internal organization of this high frequency band and its relationship with distant cortical and subcortical structures. Thirteen animals were implanted with electrodes for the recording of muscular and EEG activities on 18 cortical locations and nineteen additional animals were implanted with bipolar electrodes to study field activities in the hippocampus, parafascicular thalamic nucleus (PFTN), Meynert's basal nucleus (MN), central amygdala (CA) and bed nucleus of stria terminalis (BNST). The cortical high frequency oscillations between 120-140 Hz displayed a high coherence among unilateral locations but not between hemispheres and occurred in synchrony with oscillations in the theta band. A similar frequency band was found in the hippocampus were the oscillation also nested in theta. Although a similar frequency band was found in CA and BNST, this oscillation neither showed REM sleep specificity nor theta modulation. The PFNT displayed an activity of lower frequency than the cortex but also nested in theta. Finally, the MN did not show high frequency oscillations. Electrolytic lesions in any of these subcortical structures induced no changes in the amplitude or the internal organization of the cortical high frequency activity, indicating that there must exist a generator independent from these structures.

Support: MICINN (SAF2009-10560), JJAA (P09-CVI-4712) and FEDER funds.

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

MODELS OF RAPID EYE MOVEMENTS (REM) SLEEP DEPRIVATION: DESIPRAMINE

M. Silva-Pérez, A. Sánchez-López, M. Escudero
Facultad de Biología. Universidad de Sevilla, Sevilla

Although sleep deprivation constitutes one of the most classical approaches to study sleep function, there exist no good models of REM sleep deprivation. Some antidepressants are known to selectively inhibit REM sleep, thus becoming potentially good tools to study REM sleep function. In the present study, the effects induced by desipramine –a first-generation tricyclic antidepressant– on the sleep-wake cycle of the rat were analyzed. For that, 7 adult Wistar rats were prepared for neck muscles and electroencephalographic recordings. After a 24 hours control recording period, rats were intraperitoneally injected with 15 mg/kg of desipramine and its effects on the sleep-wake cycle were studied throughout 4 days. The injection of desipramine did not modify the total amounts of sleep and wake but induced changes in sleep architecture. Rats showed a complete REM sleep deprivation and a proportional increase in the time spent in non-REM sleep during the first 19.88 ± 4.59 hours. During the following 24 hours, animals slowly began to produce REM sleep periods of longer duration than control episodes. Finally, during the next 24 hours a 36.27% increase in the amount of REM sleep was observed, exclusively due to an increase in the number but not in the duration of episodes. Transition periods from non-REM to REM sleep were of similar length after deprivation. The increased length of the REM sleep episodes can be considered a good indicator of the REM sleep pressure. Altogether, these results support that the single dose of desipramine could constitute a good tool to study physiological changes induced by REM sleep deprivation.

Support: SAF-2009-10560, P09-CVI-4712 and FEDER Funds.

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

AFFERENT PROJECTIONS TO THE VOMERONASAL BED NUCLEUS OF THE STRIA TERMINALIS

J. Vega-Pozo, C. Pardo-Bellver, B. Cádiz-Moretti, M. Otero-García, F. Martínez-García, E. Lanuza
Facultad de Biología. Universidad de Valencia, Valencia

Pheromones are the main signals responsible of sociosexual behaviours. They interact with receptors localized in sensory neurons present in the main olfactory and vomeronasal epithelia. Some (unknown) chemical cues released from ill or infected males are detected by the vomeronasal organ and are aversive for females. Since the bed nucleus of the stria terminalis (BST) has been shown to be involved in the aversive response to predator odours, we hypothesize that connections from the vomeronasal system through vomeronasal BST (BSTmpm) may participate in this aversive response. Apart from the direct input from the accessory olfactory bulb (AOB), the rest of afferent projections to the BSTmpm has not been characterized. In this work, we have studied the afferent projections to the BSTmpm using iontophoretic injections of the retrograde tracer fluorogold. Retrograde labelling showed that the structures giving rise to major projections to the BSTmpm are the mitral layer of AOB and the vomeronasal amygdala, including the medial amygdala (in all its subnuclei), the posteromedial cortical amygdala and the bed nucleus of the accessory olfactory tract. Other amygdaloid areas, such as the basomedial and basolateral amygdala, the amygdalohippocampal transition area and the anterior amygdala, also showed retrograde labelling. In addition, retrograde tracer also revealed neurons labelled in: 1) Olfactory structures: nucleus of the lateral olfactory tract, cortex-amygdala transition area, olfactory tubercle, endopiriform nucleus and a few neurons in piriform cortex 2) Cortical areas: infralimbic and cingulate cortex, ventral subiculum, 3) Midline and intralaminar thalamic nuclei 4) Hypothalamic areas 5) ventral tegmental area and periaqueductal grey. Thus, these results indicate that the BSTmpm not only receives vomeronasal inputs, but is also the target of thalamic and hypothalamic inputs. These projections may modulate neural processing in the BST as a function of the behavioural response.

Funding: MINECO/FEDER (BFU2013-47688-P) and Government of Castilla-La Mancha/FEDER (PEIC11-0045-4490)

HILAR INTERNEURONS IN CONTEXTUAL LEARNING AND MEMORY

J.M. Caramés[1], E. Álvarez-Salgado[1], E. Pérez-Montoyo[1], S. Canals[1].
[1]. Instituto de Neurociencias de Alicante (CSIC-UMH), San Juan de Alicante, Alicante.

Granule cells in the dentate gyrus (DG) are critically important for the encoding of contextual memories. Interestingly, electrophysiological recordings *in vivo* (Bragin et al. J. Neurosci 1995; Permía-Andrade et al. Neuron 2014) demonstrated that these cells presents largely hyperpolarized resting membrane potentials and very rare firing. However, when the voltage threshold is reach, they frequently fire in burst, having a strong influence on its postsynaptic CA3 targets. Previous results from our lab have demonstrated that either LTP induction in anesthetized animals or the exposure to a novel environment in behaving animals, decrease the inhibitory tone in granule cells by reducing feed-forward inhibition (Álvarez-Salvado et al. SENC, 2013). This increase in the excitation/inhibition balance correlates with enhanced activity propagation in the tri-synaptic circuit. Here we investigate the role of different subpopulations of DG interneurons in the control of granule cells firing, its influence on functional connectivity and the encoding of contextual memories. We use cell type-specific tools to selectively control activity in genetically defined populations. In a first set of experiments, we have expressed DREADD (Designer-Receptor-Exclusively-Activated-by-Designer-Drugs) to increase (GqH3) or decrease (GiH4) neuronal activity (Ambruster et al. 2007; Urban and Roth, 2015) in parvalbumin $(PV)^+$ interneurons, using adenoviruses and a PV-Cre mouse line. We have performed a novel place recognition task in which only during the memory encoding phase and selectively on the PV^+ population the activity was modulated by activating DREADDs with clozapine-N-oxide (CNO, i.p.). In comparison to genetic controls and saline injected animals, the CNO injected GiH4 mice (inhibiting PV^+ cells) demonstrate a significant improvement of contextual discrimination when tested 24h after encoding. Ongoing experiments using fMRI investigate the consequences of this manipulation on the long-range functional connectivity of the hippocampus. Overall our results suggest that PV-cells in the DG contribute to a synaptically operated gating mechanism that regulates memory formation.

1ª: Neurociencia de Sistemas.
2ª: Neurociencia Cognitiva y Conductual.

EFFECT OF LESIONS OF THE CINGULATE CORTEX ON THE REGULATION OF ADULT NEUROGENESIS

A. Lozano Ureña[1], A. C. Delgado[2], M. Otero-García[1], I. Fariñas[1], M. Kirstein [1] and E. Lanuza[1].
[1]. Faculty of Biological Sciences. University of Valencia, Valencia
[2]. Biozentrum, University of Basel, Klingelbergstrasse 50/70, Basel, Switzerland

In the adult mammalian brain, neurogenesis takes place only in two specific regions: the subventricular zone (SVZ) and the subgranular zone of the dentate gyrus in the hippocampus. Neural stem cells, or type-B cells, are responsible for the generation of these new neurons.
The neurogenesis process is affected by many external and internal factors including neurotransmitters such as GABA and glutamate. The residing astrocytes are the main source of glutamate in the SVZ, and secrete the neurotransmitter in a vesicular fashion. It is known that the cortex is a major source of glutamate for the striatum, which is adjacent to the SVZ. In this work, we have mapped the projections from the cingulate cortex to the striatum by injecting the anterograde tracer biotinylated dextranamine. The results revealed a light projection from the cingulate cortex to the SVZ, mainly targeting the intermediate rostro-caudal levels of the neurogenic zone.
To study the possible involvement of the cingulate cortex in the modulation of SVZ neurogenesis, we performed lesions of glutamatergic neurons residing in the cingulate cortex using ibotenic acid. To determine the percentage of proliferating B cells in the SVZ we performed immunohistochemical studies of the SVZ using a proliferation marker (Ki67) and a neural stem cell marker (glial fibrillary acidic protein). The results showed that the proliferation of these cells in lesioned mice is significantly increased compared to the controls (sham animals injected with saline in the cingulate cortex).
Therefore, corticostriatal (probably glutamatergic) projections reach the SVZ and apparently play a role in the regulation of the proliferation of neural stem cells. The upregulation of this proliferation after cortical injury may have important consequences in the cerebral plasticity during brain damage.

Funding: MINECO/FEDER (BFU2013-47688-P) and Generalitat Valenciana (PROMETEOII/2013/020).

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

SLEEP AND PAIN DISORDERS IN A RESERPINE- INDUCED MODEL OF FIBROMYALGIA IN RAT

A. Blasco Serra, A. Cervera Ferri, EM. González Soler, F. Escrihuela Vidal, F. Martínez Expósito, S. Martínez Bellver, A. Luque García, M.C. Blasco Ausina, J. Martínez Ricós, V. Teruel Martí, A.A. Valverde Navarro.
Dept. Of Human Anatomy and Embryology. Faculty of Medicine and Dentistry. Universitat de València, Valencia.

Objective: The aim of this work is to test whether rats undergoing the reserpine-induced model of fibromyalgia show two of the most relevant symptoms of this pathology: sleep and pain disorders. C-Fos studies were performed in order to correlate electrophysiology and behavioural data obtained in sleep and pain analysis and tests with the neural activity pattern underlying reserpine condition.
Methods: Male Sprague-Dawley rats were used. Reserpine was administrated at doses of 1 mg/kg for three consecutives days. Sleep parameters were obtained through chronic field electrodes implanted in hippocampus (CA1), primary somatosensory cortex and dorsal raphe nucleus, as well as an EMG electrode in neck musculature. Motor impairment and neuromuscular spasms during sleep periods were analysed by direct observation. Electrovonfrey and Randall and Selitto tests were performed to measure pain thresholds. C-Fos immunohistochemistry was revealed with DAB protocol and monoaminergic nuclei were selected for image analysis.
Results: Reserpine-treated rats show an increase in total sleep time regarding control registers. High number of neuromuscular spams and microarousals are observed during the sleep periods after reserpine injections until day 21. Electrophysiology evidences an increment of theta activity in hippocampus, in both sleep and wakefulness conditions. Furthermore, animals administered with reserpine exhibit allodynia ($t=0.000$; $p<0.001$) and hyperalgesia ($t=0.000$; $p<0.001$) symptoms. Neural activity of monoaminergic nuclei observed in C-Fos is coherent with these results.
Conclusion: These results manifest the presence of sleep and pain disturbances in reserpine-induced model of fibromyalgia (Nagakura et al., 2009). Sleep impairments and decreased pain thresholds are two symptoms presents in new diagnostic criteria of American College of Reumathology (2010). Our data are a step in the establishment of a valid model of fibromyalgia, which is highly necessary to study its pathogenesis and to move forward in the development of more specific, effective treatments.

1. Systems neuroscience
2. Disorders and repairment of nervous system

SPATIO-TEMPORAL MODULATION OF CORTICAL EMERGENT ACTIVITY BY WEAK DC ELECTRIC FIELDS

J. F. Weinert [1], M. D'Andola [1], P. Boada-Collado [1], R. Villa[3,4], M. V. Sanchez-Vives[1,2]
[1]. Insitut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona
[2]. Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona
[3]. Instituto de Microelectrónica de Barcelona, IMB-CNM (CSIC), Bellaterra, SPAIN
[4]. CIBER-BBN, Networking Center on Bioengineering, Biomaterials and Nanomedicine, SPAIN

Objective:
Transcranial direct current stimulation (tDCS) is currently being widely tested as non-invasive treatment for a large number of conditions: depression, stroke rehabilitation and pain management, between others. Furthermore, there is a large interest in its use even in the healthy for cognitive enhancement. Even when products are already available on the market, the detailed cellular mechanisms of its action are not yet fully understood.
In this study we used a well established *in vitro* model of cortical slices that display spontaneous slow oscillations (<1 Hz) and applied uniform electric fields (EFs) at varying intensities to study the ongoing network dynamics during DC stimulation.
Methods:
Cortical slices were obtained as previously described (Sanchez-Vives et al. 2010 JNeurophys 104:1314) and EFs were generated by passing current between two parallel aligned Ag/AgCl electrodes that were placed perpendicular to the axis of pyramidal neurons. Extracellular local field potentials were recorded with 16 channel multielectrode arrays designed to this end.
Results:
The frequency of slow oscillations was linearly related to the EF intensity in a log scale. While Up state durations were not influenced by DC electric fields, their initiation was facilitated or prevented depending on EF orientation by a direct modulation of firing during Down states. Furthermore, a correlation between EF intensity and the characteristic high frequency activity (15-90 Hz) during Up states was apparent in a subset of slices. A 2D analysis of wave propagation properties revealed a significant spatio-temporal modulation with a greater impact on the horizontal (parallel to layers) rather than the vertical (columnar) components of the propagation speed.
Conclusions:
Proper manipulation of the electric fields can result into a highly specific spatio-temporal control of the activity. This provides a valuable testbed for the mechanistic study of cortical stimulation for the intervention in different models of neurological alterations.

This work was supported by EU FP7 FET CORTICONIC contract 600806

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

LONG LASTING PAIN RELIEF INDUCED BY THETA BURST TRANSCRANIAL MAGNETIC STIMULATION OF MOTOR CORTEX

Y. Perez-Borrego[1], L. Mordillo-Mateos[1], I. Rodriguez-Carrión[1], M. Campolo[1], MC Carrasco-Lopez[1], A. Brocalero[1], A. Oliviero[1]
[1]FENNSI Group and Clinical Neurology and Neurophysiology Sections, Hospital Nacional de Parapléjicos, SESCAM, Finca La Peraleda s/n, 45071, Toledo, Spain

Objectives: Motor cortex stimulation has emerged as a potential therapy for chronic pain and has been reported to be highly successful in a proportion of patients that have been treated. **Materials and methods:** To explore the effects of five consecutive daily sessions of repetitive Transcranial Magnetic Stimulation, given as an intermittent or continuous Theta Burst Stimulation (iTBS or cTBS), on pain in patients with drug-resistant chronic pain of different origin. Outcomes included pain measures with visual analogic scale, the amount of daily dose of "rescue treatments" and a patient's global impression of changes.
Results: We observed that five daily sessions of monolateral or bilateral iTBS can produce long lasting relief of pain for about 10 days after the end of treatment (30% reduction in VAS scores). Many patients also reduced the amount of their pharmacological "rescue treatments" and there was a significant overall improvement in patients global impression of their overall clinical status . Interestingly, only the bilateral application of cTBS significantly reduced the pain in the treated patients (VAS score reduction), while the monolateral cTBS had no effects. "Sham" stimulation produced a significant placebo effect but the effect of "real" iTBS (and of bilateral cTBS) was much greater and lasted longer. No major side effects were encountered.
Conclusions: iTBS and bilateral cTBS over the motor cortex decreased pain intensities. TBS protocols may represent a useful and "quick" rTMS protocol to apply to the motor cortex for pain management.

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

ACTIVATION OF CENTRAL AMYGDALA SHIFTS MEDIAL PREFRONTAL CORTEX DYNAMICS TO A CHARACTERISTIC SLOW OSCILLATORY PATTERN VIA DORSAL RAPHE NUCLEUS ACTIVITY

A Luque García[1], V Teruel Marti[1], A Cervera[1], L Parra Santos[1], S Martinez Bellver[1,2], Arantxa Blasco Serra[1], Eva González Soler[1], J Martinez Ricós[1].
[1] Faculty of Medicine. University of Valencia, Valencia.
[2] Faculty of Biology. University of Valencia, Valencia.

Many limbic structures are regulative of physiological systems and behavioural functions so their dysfunctions are involved in numerous neuropsychiatric diseases that imply stress or anxiety. The central nucleus of the amygdala (CeA) activates the hypothalamic-pituitary, what triggers stress responses, while medial prefrontal cortex (mPFC) function is important for limiting fear responses. The dorsal raphe nucleus (DRN) presents ascending serotonergic projections to multiple brain regions, including CeA and mPFC so it may coordinate distinct responses to a stressor.

The purpose of this study is to investigate in depth the relationship between these structures in stress management. In particular, we test the hypothesis that stimulation of the CeA affects mPFC electrical dynamics and this fact is dependent on DNR activity.

To do so, we recorded the field activity in mPFC in urethane-anesthetized rats before and after an electrical activation of CeA. Next, pharmacological blockade (muscimol; 0,5µl/ 0,01min 75ng/0,5µl) of the DRN was performed to test the effect of its inactivation on mPFC changes induced by CeA activation. CeA stimulation parameters (0.2ms pulses and 0.08 mA to 5 - 8Hz, for 200s) were based on those recorded from the CeA of rats during restraint stress (Henke, 1983) so emulate firing rates induced by stress (Forster et al, 2008).

Within the following 45’ after CeA activation, a significant increase in slow waves (<1Hz) power and time proportion is observed in mPFC. Interestingly, DRN muscimol injection returns mPFC activity back to basal levels, proving its relevance for the changes observed in the mPFC.

Therefore, we conclude that mimicking an acute stress by means of CeA electrical activation elicits the mPFC to acquire a specific oscillatory pattern, probably related to its role in managing this stressful situation. Interestingly, DRN activity, presumably its serotonergic projections, is required to induce this effect.

SUPPRESSION OF V1 FEEDBACK PROVOKES A SHIFT IN THE RECPETIVE FIELD POSITION OF LGN CELLS IN THE AWAKE MACAQUE

Aguilá J[1], Cudeiro J[1], Rivadulla C[1]
[1]. Neuroscience and Motor Control Group (NEUROcom), Faculty of Health Sciences and INIBIC, University of A Coruña

Objective: We investigated the influence of feedback from the primary visual cortex (V1) on the spatial organization of the receptive fields of cells in the lateral geniculate nucleus (LGN) in awake monkeys, suppressing the cortical feedback via repetitive transcranial magnetic stimulation (rTMS).
Methodology: We recorded neuronal activity in the LGN of two awake behaving monkeys. Animals were trained to maintain visual fixation within a 0.5° window. Control visual stimuli consisted of circular patches of different diameters (0.6° and 6°) centered over the receptive field of the cell under test. Patches were filled with a grating of optimal spatial frequency (drifting or static). The stimulus presentation was then repeated (i) during blockade of V1 induced by rTMS at low frequency (0.8Hz for 4 minutes) and (ii) after a recovery period that lasted up to 20 minutes.
Results: We recorded 75 cells before and after induction of the cortical blockade. 33% of the sample appeared to "displace" the RF centre position 4.5 degrees on average while simultaneously increased the inhibitory component of the RF and the latency of the response. We found a clear correlation between the position of the RF in the visual space and the direction of the displacement during cortical blockade, which indicates that the effect is influenced by the precise retinotopic organization of the connections between cortex and thalamus. Furthermore, those cells that change the position have a lower eccentricity ($p<0.01$).
Conclusions: The rTMS create a short-term cortical blockade that induces a reorganization in the RF of the LGN cells displacing the RF centre and, presumably, changing the RF size. This shows that cortical feedback to the thalamus dynamically regulates the responses to stimuli over the RF by modulating center surround interactions, including the spatial distribution of separate RF elements.

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

Supported by: Programa de Consolidación y estructuración de Unidades Competitivas. XUGA and MINECO BFU2013-45343-P

EFFECTS OF STATIC MAGNETIC STIMULATION (SMS) AND TRANSCRANIAL MAGNETIC STIMULATION (TMS) ON SPONTANEOUS AND VISUALLY EVOKED ACTIVITY IN THE ANAESTHETIZED MONKEY

Sandra Prieto-Soler[1], Jordi Aguila[1], Juan Aguilar[2], Javier Cudeiro-Blanco[2,3], Oscar Herreras[4], Julia Makarova[4], Casto Rivadulla[1], Javier Cudeiro[1]
[1]Neuroscience and Motor Control Group (NEUROcom), Faculty of Health Sciences and INIBIC, University of A Coruña
[2]Experimental Neurophysiology Group, Hospital de Parapléjicos, Toledo
[3]FENSI Group, Hospital de Parapléjicos, Toledo
[4]Cajal Institute, CSIC, Madrid

Non invasive neuromodulatory techniques have became really relevant for clinical purposes in the last decade. While the therapeutic effects of those techniques seems to be clear, the underlying mechanisms are still a matter of debate.
We applied TMS (five trains; 4 seconds @ 10 Hz each , intertrain interval 26 seconds) and SMS (0.6T, 30 minutes) on primary visual cortex (V1) and the effects on spontaneous and visually evoked responses were studied at the level of the thalamus and V1 in the anaesthetized monkey. Neural activity was recorded by mean of tungsten electrodes. Putative interactions between both techniques were studied by comparing the effect of TMS alone with those obtained by TMS when applied immediately after SMS. Two different analyses were performed: Frequency distribution of the recorded signal and evoked potentials (amplitude, duration and latency) by full field flashing stimulation.
FFT analysis has shown two peaks at 2 and 7 Hz in control conditions. Both, TMS and SMS, have reduced frequencies around 7 Hz and have increased the power of lower frequencies; during spontaneous and visual evoked activity. When applied together the effect of both techniques suggests summation, being stronger than when individually applied.
Despite this uniform effect on the frequency domain, the results achieved on visual evoked potentials showed a much more complex profile. At V1, TMS reduced the amplitude of the EP without modifying other attributes, while at the LGN TMS has produced an increase in the amplitude of the second EP component and a decrease in the latency. SMS produced a less clear effect and when applied together, TMS dominated the effect.
Our results seem to indicate that TMS and SMS operate using different mechanisms, even when the effects can be similar and in some cases can be added. Metaplastic effects can be also obtained.

1ª: Neurociencia de sistemas

Supported by: Programa de Consolidación y estructuración de Unidades Competitivas. XUGA and MINECO BFU2013-45343-P

THE ROLE OF FEEDFORWARD INHIBITION IN THE FACILITATION OF INFORMATION FLOW FROM THE PERFORANT PATHWAY ONTO DENTATE GRANULE CELLS. EXPLORING THE MECHANISM BEHIND THE DISYNAPTIC INHIBITORY "SWITCH".

M.R. Popiel Jakobsen [1,2], E. Álvarez-Salvado [3], C. Mirasso[2], L.M. Martínez Otero[1], S. Canals[1]
[1]. Instituto de Neurociencias de Alicante (UMH-CSIC), Alicante
[2]. Instituto de Física Interdisciplinar y de Sistemas Complejos (UIB-CSIC), Palma de Mallorca
[3]. New York Neuroscience Institute, New York University School of Medicine, New York.

Induction of LTP (long-term potentiation) by electrical stimulation has been linked to mechanisms of memory formation. We and others have shown that activity propagation in the trisynaptic hippocampal circuit is enhanced by potentiation of the entorhinal input to the dentate gyrus (e.g. Canals et al., Curr Biol 2009). Our study focuses on the local circuit composed of glutamatergic stellate processes from the superficial medial entorhinal cortex synapsing onto granule cells as well as inhibitory interneurons which in turn, connect in a feedforward manner to the granule cells. This layer of interneurons also displays feedback inhibition. The interneurons in this microcircuit act as a "switch" by regulating the degree of inhibition onto the granule cells and hence the information flow through the hippocampal trisynaptic pathway. Electrophysiological recordings combined with Independent Component Analysis (ICA) showed that hilar interneuron-granule cell connectivity is significantly depressed after induction of LTP (Álvarez-Salvado et al., SENC 2013) facilitating the propagation of activity. Pharmacological-fMRI experiments with local aplication of gabazine or bicuculine in the hilus support the theory that the increased flow of information through the granule cells is indeed due to a decrease in feedforward inhibition. A computational model of the circuit was implemented using Izhikevich's Simple Model to simulate the network's dynamics and elucidate the functioning of the interneuron "switch", namely the roles of the excitatory synapses onto the interneurons proceeding from the perforant pathway and the resulting feedback inhibition versus that of the interneurons' onto the granule cells. Preliminary findings show that the recurrent feedback inhibition seems to be an important factor and may alter the oscillation dynamics of the interneuron population, potentially leading to a phase shift relative to that of the granule cells (as described by Akam and Kullmann, Neuron 2010), allowing these in turn to take advantage of an increased time window for integration and firing therefore causing a facilitation in information transfer.

1: Systems Neuroscience
2: Cognitive and behavioral neuroscience

DISTRIBUTION OF OXYTOCIN AND CO-LOCALIZATION WITH ARGININE-VASOPRESSIN IN THE BRAIN OF MICE

Marcos Otero-Garcia[2], Carmen Agustin-Pavón[1], Enrique Lanuza[2] and Fernando Martinez-Garcia[1]
[1]. Facultat de Ciències de la Salut, Universitat Jaume I, Castelló de la Plana, Spain
[2]. Facultat de Ciències Biològiques, Universitat de València, València, Spain

Oxytocin (OT) and vasopressin (AVP) play a major role in social behaviours. Mice have become the species-of-choice for neurobiology of social behaviour due to identification of an increasing number of pheromones and the advantage of genetically modified mice. However, neuroanatomical data on nonapeptidergic systems in mice are fragmentary, especially concerning the central distribution of OT. Therefore, we analyse the immunoreactivity for OT and its neurophysin in the brain of male and female mice (strain CD1). Further we combine immunofluorescent detection of OT and AVP to locate cells co-expressing both peptides and their putative axonal processes.
The results indicate that OT is present in the neurosecretory paraventricular and supraoptic hypothalamic cells. From the anterior supraoptic nucleus OTergic cells extend into the medial amygdala, where a sparse cell population occupies its ventral anterior and posterior divisions. Co-expression of OT and AVP in these nuclei is rare.
Moreover, a remarkable OTergic cell group is found near the ventral bed nucleus of the stria terminalis, distributed between the anterodorsal preoptic nucleus and the nucleus of anterior commissure (ADP/AC). This cell group and the rostral edge of the paraventricular nucleus display frequent OT+AVP double labelling, with a general dominance of OT over AVP immunoreactivity. Fibres with similar immunoreactivity profile innervate the accumbens shell and core, central amygdala, and portions of the intervening bed nucleus of the stria terminalis. These data, together with data in the literature on rats, suggest that the projections of ADP/AC nonapeptidergic cells onto these brain centres would promote pup-motivated behaviours and inhibit pup avoidance during motherhood.
Funding: MINECO/FEDER (BFU2013-47688-P) and Government of Castilla-La Mancha/FEDER (PEIC11-0045-4490).

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

SPECIFICITY OF NEURONAL POPULATIONS ACTIVATED BY TWO DIFFERENT, PREDOMINANTLY EMOTIONAL, STRESSORS

Ignacio Marín-Blasco, A. Armario
Institut de Neurociències and Unitat de Fisiologia Animal, Facultat de Biociències, Universitat Autònoma de Barcelona

The expression of immediate early genes, particularly c-fos, has been extensively used to characterize brain areas activated by stressors. However, emotional or predominantly emotional stressors resulted in common activation of a wide range of brain areas. The extent to which different emotional stressors activate the same or distinct neuronal populations is at present unknown. In the present work we used a novel approach to this problem using double labeling for c-Fos protein (immunofluorescence, IF) and c-fos mRNA (fluorescent in situ hybridization, FISH) in the same sections. The rationale for this approach was that short exposure (20 min) of rats to a particular stressor (immobilization, IMO) would result in neurons having low or undetectable levels of protein, but high levels of mRNA, whereas prolonged exposure of rats for 4 h to the same stressor will result in the opposite (due to the typical decline of c-fos mRNA after prolonged exposure). If rats exposed to prolonged IMO are then release from the situation and exposed to a novel stressor (forced swim, SWIM), those neurons previously activated by prolonged IMO, but not activated by SWIM would be IF+/FISH-, those activated initially by IMO and again by SWIM would be IF+/ FISH+, and those only activated by SWIM would be IF-/FISH+. In the prelimbic mPFC some neurons were activated by the mere release of the animals from IMO, but an additional number was specifically activated by SWIM; in the lateral septum and the medial amygdala some neurons were specifically activated by SWIM; and (iii) in the paraventricular nucleus of the hypothalamus (PVN) there were no neurons specifically activated by SWIM. In conclusion, different emotional stressors activate common neuronal population, but a minority of neurons is specific for each particular stressor in high-level processing brain areas, but not in low-level processing ones (PVN).

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

CORTICAL EVENT RELATED POTENTIALS ELICITED BY DEEP BRAIN STIMULATION OF THE SUBTHALAMIC NUCLEUS IN THE 6-OHDA RAT MODEL OF PARKINSON DISEASE.

Sandra Arrieta, I. Cordon, M.J. Nicolás, Eneko Lopetegui, M.Alegre, J. Artieda, M.Valencia
Neurophysiology Laboratory, CIMA. University of Navarra. Pamplona, Spain

High-frequency stimulation of around 130 Hz delivered to the subthalamic nucleus (STN-DBS [deep brain stimulation]) is an effective treatment of Parkinson's disease (PD). Prior to delivering the stimulation the motor threshold (defined as the minimum intensity at which a motor response is produced) must be estimated. The motor cortex has been proposed as a possible contributor (via antidromic activation) in the mechanisms of DBS suggesting an important role of this area on the DBS effect. In this experiment we aimed to study the electrophysiological and behavioral responses produced by STN-DBS in the cortex during the process of motor threshold estimation in control and parkinsonian rats.
We recorded the evoked potentials from four different areas of the motor cortex (primary and secondary cortex from both brain hemispheres) in 15 control and 12 parkinsonian during the estimation of the motor threshold. Biphasic trains of 130 Hz and 60 µs pulse width were delivered increasing the intensity of stimulation in 20 µA steps until achieving the motor threshold. Evoked potentials were characterized by averaging cortical responses over pulse sweeps. Correlations were measured by means of the Pearson correlation coefficient.
As the intensity of stimulation increased, the cortical evoked response rose arriving at its maximum amplitude at the motor threshold. Cortical responses were observed on the four recording cortex sites but with different amplitudes depending on the distance to the stimulation site. Parkinsonian rats showed a significant lower intensity threshold compared with control animals.
Antidromic responses were observed in all the areas recorded in both groups of animals, control and Parkinson. The fact that parkinsonian rats show a lower intensity threshold suggests that their cortex may be in a state of hiperexcitability. No differences were observed on the morphology of the evoked potentials and their components between groups.

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

COMPARATIVE STUDY OF THE CYTO- AND CHEMOARCHITECTONIC ORGANIZATION OF THE AMYGDALA IN RODENTS AND CHIROPTERS

M. Abellán-Álvaro[1], C. Portfors[2], F. Martínez-García[3] and E. Lanuza[1]
[1]. Facultat de CC. Biològiques. Universitat de València, Burjassot, Spain.
[2]. Neuroscience Dept., Washington State University, Vancouver, USA.
[3]. Facultat de CC. de la Salut. Universitat Jaume I, Castelló, Spain.

We present the cyto and chemoarchitectonic description of the chemosensory system and the telencephalic amygdala of a chiropter species, the Seba's short-tailed bat (*Carollia perspicillata*). The comparative study has been carried out using Nissl's staining, histochemistry for acetylcholinesterase (AChase) and immunostaining for tyrosine hydroxylase (TH) and calretinin. The results reveal that while the broad subdivisions of the amygdala of *Mus musculus* are present in *Carollia perspicillata*, there are some differences in the chemoarchitecture between them. The most important differences are observed in structures related with the processing of vomeronasal stimuli, such as the posteromedial cortical amygdaloidal nucleus (PMCo) and the medial amygdaloidal nucleus (Me), which present a much smaller acetyl cholinesterase-positive layer 1 (a marker of the vomeronasal projection from the accessory olfactory bulb) in *Carollia perspicillata* than in *Mus musculus.* In the Me, the vomeronasal input appears restricted to the anterior and posterodorsal subdivisions, whereas, according to the calretinin immunostaining (a marker of the projections of both the main and accessory olfactory bulbs), the posteroventral subdivision of the Me apparently receives an olfactory input, something that does not occur in mice. Regarding the deep nuclei of the amygdaloid complex, all of them (pallial derivatives: lateral, basolateral, basomedial and amygdalo-hippocampal area and subpallial derivatives: central amygdala) are well developed in the bat brain. Calretinin immunostaining shows that the neuropil of basolateral nucleus is not stained in *Carollia perspicillata*, in contrast to what happens in mice. This study has revealed some differences between the amygdala of rodents and chiropters, but these results are consistent with the idea that the amygdala is a telencephalic structure very conserved over the evolution of the mammalian brain.
Funding: MINECO/FEDER (BFU2013-47688-P) and Government of Castilla-La Mancha/FEDER (PEIC11-0045-4490).

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

EFFERENT PROJECTIONS OF THE CORTEX-AMYGDALA TRANSITION ZONE AND THE ANTERIOR CORTICAL NUCLEUS OF THE OLFACTORY AMYGDALA IN MICE

M. Abellán-Álvaro[1], * B. Cádiz-Moretti[1], F. Martínez-García[2] and E. Lanuza[1]
[1]. Facultat de Ciències Biològiques, Universitat de València, Valencia.
[2]. Facultat de Ciències de la Salut. Universitat Jaume I, Castelló.

The anterior cortical nucleus (ACo) of the amygdala and the cortex-amygdala transition zone (CxA) are structures of the chemosensory amygdala in mice which receive a major input from the main olfactory bulb and a minor input from the accessory olfactory bulb. The aim of this study is to provide a complete description of their efferent projections, which is necessary to understand their behavioural role. This study has been carried out using anterograde neuronal tracing with biotinylated and tetrametylrhodamine-conjugated dextranamines. The results show that the CxA and ACo are strongly interconnected with the rest of the chemosensory amygdala. Moreover, they show a moderate output to other olfactory-related structures like the piriform cortex (Pir) and a dense projection to striatal structures. In addition, each structure has a number of differential outputs: the CxA projects to some cortical areas and to the lateral entorhinal cortex (LEnt), which is part of the olfactory cortex and also of the hippocampal formation. On the other hand, the ACo presents widespread projections, including a dense projection to some structures of the tuberal hypothalamus like the magnocellular nucleus of the lateral hypothalamus (MCLH), a moderate projection to the mammillary hypothalamus and also a moderate output to the midbrain and brainstem. These results suggest that CxA is part of the intratelencephalic circuitry processing olfactory information, while ACo is involved in the processing of multimodal information, including olfactory and vomeronasal stimuli, and projects to hypothalamic and midbrain and brainstem structures, and therefore it probably plays a role in activating appropriate behavioural responses to the incoming stimuli.
Funding: MINECO/FEDER (BFU2013-47688-P) and Government of Castilla-La Mancha/FEDER (PEIC11-0045-4490).

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

MITOCHONDRIAL DIVISION INHIBITOR 1 (MDIVI-1) PROTECTS NEURONS AGAINST EXCITOTOXICITY THROUGH THE MODULATION OF INTRACELLULAR Ca^{2+} SIGNALING AND MITOCHONDRIAL FUNCTION.

A. Ruiz, E. Alberdi, C. Matute
Departamento de Neurociencias, Achucarro Basque Center for Neuroscience, Universidad del País Vasco (UPV/EHU) y Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED), Leioa, Bizkaia.

Overactivation of the dynamin related protein 1 (Drp1) triggers an imbalance in mitochondrial dynamics towards fission and has been implicated in brain ischemia and neurodegeneration. However, how mitochondrial fission contributes to intracellular Ca^{2+} homeostasis disruption and neuronal death during excitotoxicity is not fully understood. In this work, we have analyzed the effects of Drp1 inhibition by mdivi-1 on NMDA-induced excitotoxicity in primary cortical neurons. NMDA triggered Drp1 dephosphorylation at Ser637 and mitochondrial fission that was inhibited by mdivi-1. Drp1 inhibitor strongly attenuated NMDA-induced calpain activation and neuronal death, suggesting a modulation of cytosolic Ca^{2+} ($[Ca^{2+}]_i$) signals by mdivi-1. Indeed, live cell imaging experiments showed that mdivi-1 depleted ER Ca^{2+} stores and reduced both NMDA-induced $[Ca^{2+}]_i$ and mitochondrial ($[Ca^{2+}]_m$) overloads. In addition, after mdivi-1 incubation neuronal mitochondria were depolarized, basal mitochondrial respiration reduced, and eventually NMDA-produced spare respiratory capacity drop attenuated. However, mdivi-1 turned out to protect neurons against kainate and thapsigargin, which did not trigger mitochondrial fission, whereas knock-down of Drp1 did not prevent NMDA-induced either mitochondrial fragmentation nor cell death. In summary, our results provide evidence that mdivi-1 protects neurons from excitotoxic Ca^{2+} homeostasis disruption by regulating intracellular Ca^{2+} signaling and mitochondrial function through a Drp1-independent mechanism.

Supported by MINECO (SAF2013-45084-R), Gobierno Vasco and CIBERNED

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

SAMP8: AN AGING MODEL FOR THE STUDY OF HIPPOCAMPAL INTERNEURONS

M.M Arroyo Jimenez [1], M.J. Lagartos [1], J. González Fuentes [1], M.E. Legidos [1], M.P. Marcos Rabal [2], R. Insausti Serrano [2]
[1]. Facultad de Farmacia. CRIB. Universidad de Castilla la Mancha, Albacete
[2]. Facultad de Medicina de Albacete. CRIB. Universidad de Castilla la Mancha

Alterations of memory and other cognitive processes occur in aging and are one of the most prominent effects in neurodegenerative diseases. However, not all types of memory are affected in the same way. Spatial and declarative memory subtypes are especially susceptible to these changes. The hippocampus is the anatomical structure that facilitates this type of memory that is affected in normal as well as pathological aging. Some of the changes in aging are associated with the decrease of inhibitory GABAergic interneurons what leads to a higher excitability in the hippocampal circuitry.

Here we used the mouse model SAMP8, which has been described as an accelerated aging model. SAMP8 mice present learning and memory deficits, as well as other markers of premature aging such as increased oxidative stress and shorter life expectancy. We have analyzed different groups of GABAergic interneurons: parvalbumin, (PV), somatostatin (1-24), (SOM) and calretinin (CR) in the hippocampus of mice SAMP8 and SAMR1 (control group) from 6 months to 16months.

It was observed a significantly decrease of CR+ cell density between 6 months and 16 months-old SAMP8 mice (p-value= 0.01). SST+ interneurons decreased by 48% from 6 months to 16 months-old SAMP8 mice (p-value < 0.001). However, no difference in the pattern of distribution of PV cells were found between SAMR1 and SAMP8 mice at any of the studied ages. CR+ interneuron revealed no changes in density in CA1 region relative to SAMR1.

The quantitative analysis of interneurons showed no significant differences in SAMR1 in aging.

We showed a decline in hippocampal populations of SOM + and CR + in the older SAMP8 mice while PV + interneurons are kept constant. Our results point to the existence of different susceptibility and function of hippocampal interneurons in aging in this mouse model of aging.

Supported by the Consejería de Educación y Ciencia de la Junta de Comunidades de Castilla la Mancha (PEII 14-0348-8331)

CALRETININ NEURONS IN THE SEPTAL AREA SEND DESCENDING PROJECTIONS OVER THE NUCLEUS INCERTUS IN THE RAT

FE. Olucha-Bordonau[1], A. García Avilés[1], F. Ros-Bernal[1], H. Albert-Gascó[1], S.Moustafa[1], I. Arnal-Vicente[1], AM Sánchez-Pérez[1]
Departamento de Medicina, Universidad de Jaume I, Castellón de la Plana, (Castellón).

The nucleus incertus send ascending connections over the septal area that has been found as a keystone in modulating hippocampal theta rhythm. However, little is known on the descending connections over that area. Brainstem-induced hippocampal theta rhythm depends on the integrity of this nucleus, which is in turn influenced by various neural inputs. In this study, we identified a novel projection, from the medial septum to the nucleus incertus, which may provide feedback modulation of the circuitry. Fluorogold injections into the nucleus incertus resulted in retrograde-labeling of cells in the medial and posterior septum that was concentrated in the horizontal medial septum, a few in the vertical medial septum and some more in the triangularis septalis and septofimbrialis. 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-labeling was observed in some parvalbumin, calbindin and glutamic acid decarboxylase (GAD)67-positive neurons. Also a high grade of double loabeling occurred between calretinin and FG in medial septum, triangularis septalis and septofimbrialis.. Anterograde miniruby injection into the medial and posterior 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 NI. 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.

1. Sistemas homeostáticos y neuroendocrino
2. Neurociencia cognitiva y conductual

DISTRIBUTION OF RELAXIN3 FIBERS IN THE AMYGDALA REVEALS A ROLE IN EMOTIONAL PROCESSING

F. Ros-Bernal[1], A. García Avilés[1], H. Albert-Gascó[1], S.Moustafa[1], AM Sánchez-Pérez[1], FE. Olucha-Bordonau[1],
Departamento de Medicina, Universidad de Jaume I, Castellón de la Plana, (Castellón).

Modulation of amygdala function depends on ascending subcortical projections. Amongst these, an important regulatory projection arises from the nucleus incertus (NI) located in the brainstem, innervate the amygdala using GABA and peptide relaxin 3 (RLN3) neurotransmitter. We have already shown that the lesion of the NI impairs extinction of amygdala-dependent conditioned fear. Thus, the detailed distribution of RLN3 in the amygdala may provide an anatomic background for NI modulation of specific amygdala functions. In this work, we have studied in detail the specific distribution of NI projections and RLN3 positive fibers over the amygdala complex using the pattern of neuronal calcium binding protein labeling as an anatomic reference. We show that the distribution pattern of fibers from anterograde tracer injections in the NI and RLN3 ICC positive fibers were almost identical. The highest density of anterograde and RLN3 positive fibers concentrated in the endopiriform nucleus, the medial amygdala and medial divisions of the bed nucleus of the stria terminalis. There was a particular accumulation in the caudal lateral amygdala. On the contrary, only disperse labeling occurred in other amygdala nuclei. Double labeling for RLN3 and calcium binding proteins showed consistent putative contacts between RLN3 fibers and soma and dendrites of parvalbumin, calbindin and calretinin positive neurons. Double immunofluoresecence for RLN3 and synaptophysin indicates that in all nuclei studied, RLN3 fibers made synaptic contacts with amygdala neurons. All these results indicate an important projection of the NI over specific nuclei of the amygdala, suggesting a putative modulator role of this system over amygdala dependent social and emotional behaviors.

1. Sistemas homeostáticos y neuroendocrino
2. Neurociencia cognitiva y conductual

DISYNAPTIC INHIBITION ALTERS THE SPATIAL PROFILES OF EXCITATORY PATHWAY-SPECIFIC GAMMA OSCILLATIONS IN THE HIPPOCAMPUS.

J. Makarova, G. Martín-Vázquez, O. Herreras
Cajal Institute, CSIC, Madrid

Local field potentials (LFPs) reflect synchronous activity from neuron assemblies. Due to volume conduction, LFPs are mixed signals contributed by multiple local and remote currents at any site and their discrimination has been a major technical problem not easily addressable until the arrival of spatial discrimination techniques. The so obtained spatial profiles generated by individual afferent pathways should match during spontaneous and evoked activity, but there are certain discrepancies in some pathways that point to differential interference with unknown factors. We investigated the analogies and differences of spontaneous and evoked field potentials specifically elicited by activation of CA3 pyramidal cells in the hippocampus of the anesthetized rats. The spontaneous activity consists on bouts of excitatory gamma oscillations mixed with occasional sharp waves. We found that Schaffer-elicited and CA3 recurrent gamma waves separated by independent components analysis (ACI) are coherent, but not fully, displaying amplitude differences in individual waves. The CA3 gamma oscillations are composed of the CA3 excitatory and inhibitory recurrent waves, outphased by about half a cycle. Inhibitory gamma waves are not present in the cell body layer of CA1. Ipsi and contralateral CA3-CA1 evoked potentials differed in spatial profile and relative amplitude, and the somatic fields uncorrelated with dendritic ones at minimal intensities eliciting responses of similar size as natural gamma waves. Blockade of perisomatic inhibition with GABA-A blockers produces consistent and different effects on spontaneous and evoked potentials and CSD profiles that unequivocally demonstrates co-activation of excitation and inhibition from a common origin, albeit with a marked pathway-specific impact. Financed by MEC: BFU2013-41533-R

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

Topic 4

Cognitive and Behavioral Neuroscience

APPETITIVE RESPONSES IN HUMAN NEWBORNS: ROLE OF FATTY ACIDS

A.G. Gutiérrez-García [1,2], C.M. Contreras [2,3]
[1]. Facultad de Psicología, Universidad Veracruzana, México.
[2]. Laboratorio de Neurofarmacología, Instituto de Neuroetología, Universidad Veracruzana, México.
[3]. Unidad Periférica Xalapa, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, México

The nature of some cue guiding newborns towards their feeding source is a controverted theme. Certainly, all newborn mammals, soon after delivery, look for and find out the maternal breast. In consonance with other authors we have hypothesized the possibility of an early intrauterine learning. During embryo development there is a direct contact with amniotic fluid which may contain some substances acting as cues and seemingly there is a developing but functional olfactory system. The question is if amniotic fluid share some compounds also present in colostrum and maternal milk which may act as cues after delivery. We have demonstrated that: *i*) these three biological fluids have in common the presence of 8 fatty acids (from C12: 0 to C18: 2); *ii*) the exposure of cotton swabs impregnated with its own amniotic fluid or an artificial mixture of these fatty acids, produces in very young human newborns (18 hours), orientating and feeding movements towards these swaps; *iii*) subcutaneous injections of human amniotic fluid or a similar artificial mixture in the Wistar rat reduce all indicators of anxiety; and, *iv*) the fatty acid producing the highest amount of orientating-feeding responses is the myristic acid (C14: 0). We conclude that this finding represents an adaptive evolutionary character through the process of natural selection giving a highly efficient process that enables immediate newborn nutrition, and may be used for the design of biologically based cosmetics.

Topic:
1st: Cognitive and Behavioral Neuroscience
2nd: Developmental Neurobiology

IBOTENIC LESIONS IN THE ANTERIOR PIRIFORM CORTEX DISRUPT THE ACQUISITION OF CONDITIONED FLAVOR PREFERENCE

C. Mediavilla[1], M.Martín Signes[2], S. Risco [3]
[1] Facultad de Psicología, Universidad de Granada.
[2] Facultad de Medicina, Universidad de Cádiz
[3] Facultad de Farmacia, Universidad de Granada.

Objectives. Previous immunohistological study showed a significant higher number of activated cells in the anterior piriform cortex (aPC) after acquisition of *flavor-taste* learning. This type of learning develops a preference for a neutral flavor that is associated with a previously preferred taste. This appear to indicate a role of aPC in taste learning. Thus, present study analyzed the effect of bilateral lesions in the aPC on Conditioned Flavor Preference (CFP) induced by saccharin and on Flavor Aversion Learning (FAL) induced by LiCl.
Methods. Rats were trained over 8 alternating one-bottle sessions to acquire a CFP flavor-taste induced by pairing a flavor with saccharin. Two choice-test (Test 1 and Test 2 -reversal test-) were performed, in which the flavors used as CS + and CS – were simultaneously presented. 48 h after Test 2, a FAL procedure induced by a single administration of LiCl took place and the aversion was tested by a choice test.
Results. Outcomes indicate that neurotoxic lesions block flavor-taste preference given that the two-bottle choice test showed no significant difference between CS + and CS – intake in lesioned rats. The differences between CS + and CS – intake were only significant in the sham-lesioned group. In contrast, the aPC lesions did not interrupt FAL in a two-bottle test.
Conclusions. aPC may function not as a primary olfactory region but as an association cortex necessary to some learning such as flavor-taste learning in which the positive hedonic value of the flavor is increased.

Supported by MEYC grant PSI2012-37987

Áreas Temáticas:

1. Neurociencia cognitiva y conductual
2. Neurociencia de sistemas

GENDER DIFFERENCES IN SPATIAL LEARNING AND LONG-TERM POTENTIATION IN HIPPOCAMPUS IN RATS. MOLECULAR MECHANISMS

B. Gómez-Giménez, P. Monfort, M. Llansola, V. Felipo
Centro de Investigación Príncipe Felipe. Valencia

The hippocampus is the main area involved in modulation of spatial learning and memory. It is considered that the main mechanism by which hippocampus modulates spatial learning and memory is long-term potentiation (LTP).
The aim of this work was analyze differences between male and female rats in spatial learning and memory and assess whether these differences are associated with alteration in LTP in hippocampus as well as the mechanisms involved.

We used female and male rats (2-3 months old) and analyzed learning and memory using two tests:
a) Radial Maze
b) Morris water maze (MWM)
We analyzed LTP and some of the molecular mechanisms modulating it in males and females.

Male rats performed significantly better than females in the Morris water maze and radial maze:
a) In radial maze, female rats performed more reference and working errors on day 4 than males. The learning index was also significantly lower.
Escape latency was longer in female than in male rats in the MWM.
The magnitude of tetanus-induced LTP in CA1 of hippocampus was lower in females than in males. Differences in AMPA receptor expression in post-synaptic densities could be involved in these gender-differences in LTP.

In conclusion, the results reported show that there are important gender differences in spatial learning and long-term potentiation in hippocampus.
The mechanisms leading to reduced LTP in female rats would be also involved in the reduced ability to learn the spatial tasks in the radial and Morris water mazes.

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

COMPARISON OF THE PRO-COGNITIVE EFFECTS OF AN ENRICHED ENVIRONMENT AND THOSE PRODUCED BY A LOW DOSE OF 17-BETA ESTRADIOL IN OVARIECTOMIZED RATS

O. Picazo, J. Espinosa-Raya, A. Ortiz-Pérez
Escuela Superior de Medicina, Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón s/n, Col. Sto. Tomás, 11340 México, D.F., México. Phone: (52) 5557296000 Ext. 62761. E-mail: rifo99mx@gmail.com

Estrogen depletion due to aging, surgery or pathological events can cause a multitude of problems, including neurodegenerative alterations. In a rodent model of menopause, 17-beta estradiol has been shown to produce beneficial effects on cognition, stimulating brain regions (e.g., the neocortex, hippocampus and amygdala) related with cognition and learning. Another treatment that stimulates these brain regions is an enriched environment, which is a complex set of external factors in the immediate surrounding that facilitates a greater stimulation of sensorial, cognitive and motor circuits of the brain. The aim of the present study was to test the relative effect of 17-beta estradiol (with a time-released pellet; 1 ug/rat/day), an enriched environment, and a combination treatment on the impairment of memory induced by an ovariectomy. Experimental and control groups were submitted to two memory tests 18 weeks after surgery; the autoshaping learning task (ALT), used to measure working memory, and the novel object recognition test (NORT), used to study short- and long-term memory. The evaluation of rats for possible impairment in motor abilities after the ALT showed that no treatment produced an alteration in this sense. The clear impairment of cognitive abilities produced by the ovariectomy could be overcome by chronic estrogenic treatment or exposure to an enriched environment. Estrogen treatment produced better results on the ALT. The combination of treatments did not improve the results of either of the two individual treatments. Possible action mechanisms are proposed.

THE 5-HT$_6$ RECEPTOR ANTAGONIST LU AE58054 ALONE AND IN COMBINATION WITH DONEPEZIL POTENTIATES GAMMA OSCILLATIONS IN RAT MEDIAL PREFRONTAL CORTEX

M. Amat Foraster, K.F. Herrik, N. Richard, J.F. Bastlund, I.E.M. de Jong, A. Mørk
Lundbeck A/S, Neuroscience Drug Discovery, Copenhagen, Denmark

Background: The 5-HT$_6$ receptor is primarily localized in the brain, in areas relevant for cognition. Combining a 5-HT$_6$ receptor antagonist and an acetylcholinesterase inhibitor (AChEI) represents a promising new approach to the symptomatic treatment of Alzheimer's disease. In a recent phase II trial, the selective 5-HT$_6$ receptor antagonist Lu AE58054 improved cognition in patients with moderate Alzheimer's disease on stable donepezil (AchEI) treatment. In rats, the increased power of oscillatory activity in deep brain regions has been linked to increased cognitive performance. In the present study, we investigated the ability of Lu AE58054 alone and in combination with donepezil to modulate neuronal rhythms in the medial prefrontal cortex (mPFC).
Methods: Cortical activity was recorded using intracranial mPFC electrodes in urethane anesthetized male Sprague Dawley rats. Electrical stimulation was applied to the brainstem reticular formation (0.3 ms square pulses delivered over 6 s at 250 Hz, repeated every 100 s). The power of oscillatory activity was compared between treatments to quantify the strength of the oscillation.
Results: Intravenous (i.v.) injection of 2 mg/kg Lu AE58054 alone produced a transient increase in electrically-induced gamma power. Furthermore, Lu AE58054 (2 mg/kg, i.v.) significantly potentiated the effect of donepezil (0.3 mg/kg i.v.) on brain-stem stimulation induced gamma oscillations. At 1 mg/kg i.v., Lu AE58054 alone did not increase gamma power in the mPFC, but did significantly potentiate the effect of donepezil (0.3 mg/kg i.v.).
Conclusions: Lu AE58054 (2 mg/kg, i.v.) increases gamma oscillations in the mPFC by electrical stimulation, such network activity believed to play a role in higher cognitive function. In addition, we demonstrated an add-on effect of Lu AE58054 (1 and 2 mg/kg i.v.) to donepezil (0.3 mg/kg i.v.). Such potentiation may contribute to the procognitive effects of Lu AE58054 in Alzheimer's disease.

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

NEURAL MECHANISMS UNDERLYING INTERACTIVE BEHAVIORS BETWEEN ANIMALS AND THEIR ENVIRONMENTS

A.R. Conde-Moro, F. Rocha-Almeida, J.M. Delgado-García, A. Gruart
Division of Neurosciences, Pablo de Olavide University, 41013-Seville Spain

Brains are continuously exchanging information and generating behaviors together with other brains to avoid survival threads and to satisfy basic needs. Brain activities not only allow animals to interact with other subjects, but also to manipulate environmental clues to obtain the needed resources (food, water, heat, and mate). Although there are attempts to reproduce these interactive behaviors in laboratory contexts, most of these studies have been carried out at a behavioral level, but the ones looking directly at what happens in the brain during these behavioral sequences are still scarce. This study was aimed to identify the neural mechanisms that allow the interaction between two animals (rats) to find adaptive solutions in a changing environment. Animals were bilaterally implanted with recording electrodes in cingulated, prelimbic, and infralimbic cortices, because we have shown that these areas are related to cooperative and synchronized behaviors (Jurado-Parras et al., Learn. Mem., 19: 99, 2012). Local field potentials (LFPs) were recorded during all experimental procedures. We have developed a behavioral procedure to reproduce interactive behaviors using two modified and adjacent Skinner boxes.It took 3-5 days for a rat to learn pressing a lever in order to obtain a food pellet in an 1:1 fixed ratio schedule. When placed together, rats took also 3-5 days to learn pressing the lever by pairs in adjacent Skinner boxes, and an average of 3.6 days to learn to climb on a platform that will release the lever so they can press it and obtain food. After this period, both rats were progressively trained to stay in the platform simultaneously for 4 s to release the lever so they can press it and eat. Preliminary analysis of collected LFPs indicated the selective involvement of these three prefrontal areas in the proper solution of environmental and social constrains.

Área Temática:
1ª: Neurociencia cognitiva y conductual

FOOD OR COMPANION? - AN EXPERIMENTAL STUDY OF CORTICAL AND SUBCORTICAL ACTIVITIES FOR TWO TYPES OF REINFORCEMENT WITH RATS

F. Rocha-Almeida, A.R. Conde-Moro, J.M. Delgado-García, A. Gruart
Division of Neurosciences, Pablo de Olavide University, 41013-Seville, Spain

Social interaction has a really important role in rodents and it could be used as a positive reinforcer, proving that rodents can press a lever to gain access to social interaction with littermates. However, it will be interesting to know which a rat's choice will be when presented at the same time, with a consummatory reinforcement (for example to receive a pellet of food) and a social interaction reinforcement (for example, to get contact with a familiar or unfamiliar rat). In order to study rats' preferences between these two types of reinforcements, we used two modified and adjacent Skinner boxes divided in two equal-size compartments by a guillotine door. One of these Skinner boxes has two available levers: i) one lever that, when pressed, provides access to food pellets, with a 1:1 fixed ratio schedule; and ii) another lever that, when pressed, allows 10 s of visual and partial physical contact with a rat located in the adjacent box. This access is achieved by the mechanical opening of a guillotine door that give access to a stainless steel grid separating the 2 boxes. Every test session has a duration of 20 min for 10 consecutive days. Rats are implanted with recording electrodes in cingulated, prelimbic, and infralimbic cortices and in the shell of the subcortical accumbens septi nucleus. Some of us have already shown that these brain areas are related to appetitive, cooperative, and vicarious behaviors in rodents (Jurado-Parras et al., Learn. Mem., 19: 99, 2012). Selected rats are previously placed in social isolation or under food deprivation (80% of body weight) to increase the motivation for social interactions or food intake. Preliminary results indicate that previous motivation determines the pressing rate of each lever and the characteristics of local field potentials recorded at the selected brain sites.

Área Temática:
1ª: Neurociencia cognitiva y conductual

EFFECTS OF tDCS ON CLASSICAL EYEBLINK CONDITIONING AND OPERANT CONDITIONING IN BEHAVING RABBITS

R. Leal-Campanario, J.C. López-Ramos, A., Gruart, J.M. Delgado-García
Division of Neurosciences, Pablo de Olavide University, 41013-Seville, Spain

Transcranial direct-current stimulation (tDCS) is a non-invasive brain stimulation technique that has been successfully applied in both basic and clinical research. tDCS induces changes in neuronal membrane potentials in a polarity-dependent way. Nevertheless, little is known about tDCS 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, but no effects were observed on day 8. 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 conditioned lever responses was reduced by the application of cathodal or anodal tDCS, during both days 2 and 8. To determine if anodal or cathodal stimulation was modulating different pathways that could explain the results in operant conditioning, we did immunohistochemistry for c-fos and recorded heart rate when applying one or the other polarity. In conclusion, results reported here confirm earlier studies in humans regarding the effects of tDCS on prefrontal cortex functions, highlighting the potential of this technique for modulating associative learning using either classical or operant conditioning paradigms.

Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Nuevos métodos y tecnologías

PARTICIPATION OF CEREBELLAR NUCLEI IN CLASSICAL EYEBLINK CONDITIONING IN WILD-TYPE AND LURCHER MICE

J.C. López-Ramos[1], Z. Houdek[2], J. Cendelín[2], F. Vožeh[2], J.M. Delgado-García[1]
[1]Division of Neurosciences, Pablo de Olavide University, 41013-Seville, Spain
[2]Department of Pathophysiology Faculty of Medicine, Charles University, Pilsen, Czech Republic.

Classical eyeblink conditioning is one of the experimental models more widely used for the study of the neuronal mechanisms underlying the acquisition of new motor and cognitive skills in behaving mammals. Currently, there are two different interpretations of the role of the cerebellum in the acquisition and storage of eyelid responses learned through classical conditioning. One of them proposes that the cerebellum is the place where this type of associative learning takes place and it is stored, while the opposite suggest that the cerebellum is involved in the proper performance of both palpebral reflexes and learned movements. Our aim was to check these two opposite theories, using for that the Lurcher mice, a well-known model of cerebellar degeneration (see Porras-García et al., Eur. J. Neurosci., 21, 979, 2006). Mice were prepared for classical eyeblink conditioning and for recording of the unitary activity of cerebellar interpositus neurons. Chronic electrodes for stimulation and recording were implanted in the eyelid, and a craniotomy was carried out in the cerebellar skull. Eyelid movements were recorded with the help of a magnet fixed to it, and with the help of a high velocity camera. Neurons were identified by their antidromic activation from the contralateral red nucleus. Animals were conditioned with a tone as conditioned stimulus, and an electric shock as unconditioned stimulus in simultaneity of the recording of interpositus neurons. Trace and delay conditioning were made. Preliminary results indicate a lower learning index in Lurcher mice, probably due to its higher muscle excitability. Antidromically identified interpositus neurons presented firing rates related with both reflex and conditioned eyelid responses. The cerebellum seems to be related with the realization of the movement more than with its acquisition. The loss of Purkinje cells characterizing Lurcher mice represents an important deficit for the proper execution of conditioned eyelid responses.

Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual

BEHAVIORAL AND METABOLIC EFFECTS OF CAFETERIA DIET AND/OR HIGH OR LOW INTENSITY EXERCISE IN YOUNG FEMALE RATS

I. Cigarroa[1,2], A. Caimari[3], JM. del Bas[3], JF. Lalanza[1,4], Ll. Capdevila[4],Ll. Arola[3,5] RM. Escorihuela[1*]
[1]Institut de Neurociències, Departament de Psiquiatria i Medicina Legal, Universitat Autònoma de Barcelona, Catalonia.
[2]Carrera de Kinesiología, Facultad de Salud, Universidad Santo Tomás, Los Ángeles, región del Bio-Bio
[3]Centre Tecnològic de Nutrició i Salut (CTNS), TECNIO, CEICS, Reus.
[4]Laboratori de Psicologia de l'Esport, Departament de Psicologia Bàsica, Universitat Autònoma de Barcelona, Catalonia.
[5]Departament de Bioquímica i Biotecnologia, Nutrigenomics Research Group, Universitat Rovira i Virgili, Tarragona.

Introduction: Nowadays, obesity and syndrome metabolic are increasing as a consequence of unhealthy lifestyles and the lack of exercise. There is little knowledge about the behavioral effects produced by an unhealthy diet and the ability of exercise to counteract its deficits. **Objectives:** The aim of this study was to examine the behavioral, eating patterns and metabolic effects of a cafeteria diet (CAF) combined with different intensities of treadmill running. **Material and Method:** Female Sprague Dawley rats were randomly assigned to eight groups: sedentary-standard diet (SED-ST), sedentary-cafeteria diet (SED-CAF), control-standard diet (CON-ST), control-cafeteria diet (CON-CAF), treadmill-low intensity-standard diet (TML-ST), treadmill-low intensity-cafeteria diet (TML-CAF), treadmill-high intensity-standard diet (TMH-ST), treadmill-high intensity-cafeteria diet (TMH-CAF). After 7 weeks of intervention we tested adolescent rats in the Open Field (OF) and in Shuttle-box (SB). Besides, we measured body and liver weight, retroperitoneal white adipose tissue (RWAT) and metabolic parameters. **Results:** In the OF, Rats fed with the CAF diet reduced locomotor activity, mainly SED group, whereas the TMH-CAF group showed reduced anxiety-like behavior. In SB, the CAF groups, mainly SED-CAF, was slower in avoidance learning than the ST groups. Moreover, rats fed with the CAF diet developed metabolic syndrome, showed overweight, higher RWAT and liver weight, increased plasma levels of triglycerides (TG), insulin, leptin, as well as glucose and R-QUICKI in comparison with ST groups. Exercise showed a significant decrease in circulating TG and NEFAs as well as in RWAT weight and increased solid food consumption compared with ST groups. **Conclusions:** CAF diet consumption produced avoidance learning disturbances, changed eating patterns, induced metabolic syndrome, overweight and insulin resistance in female young rats. The treadmill running and/or handling partly counteracted the negative behavioral and metabolic effects of CAF diet.

Keywords: cafeteria diet, treadmill, high and low exercise, behavior, rats.

1. Neurociencia cognitiva y conductual
2. Sistemas homeostáticos y neuroendocrino

Acknowledges: This works was supported by Dirección General de Investigación (PSI2011-29807-C02_01). IC was supported by a pre-doctoral fellowship from Universidad Santo Tomás, Chile and a fellowship from CONYCYT/BECA CHILE/PAI 72150035. JFL received a pre-doctoral fellowship from the Generalitat de Catalunya (FI-DGR 2011).

HOW TIME INTERVALS ARE DETERMINED IN THE MEDIAL PREFRONTAL CORTEX OF BEHAVING RABBITS?

C.R. Caro-Martín, R. Leal-Campanario, R. Sánchez-Campusano, J.M. Delgado-García, A. Gruart
Division of Neurosciences, Pablo de Olavide University, Seville, Spain

A still unanswered question is how medial prefrontal cortex (mPFC) neurons determine time intervals, a property related to working memories. For this, rabbits were classically conditioned with a delay paradigm consisting of a tone (600 Hz, 90 dB) as CS. The CS started 50, 250, 500, 1000, or 2000 ms before and co-terminated with a corneal air puff (100 ms, 3 kg/cm^2) used as US. Eyelid movements were recorded with the magnetic search-coil technique and by the EMG activity of the orbicularis oculi muscle. Unitary recordings of pyramidal neurons were carried out following procedures described elsewhere (Leal-Campanario et al., *J Neurosci*, 33: 4378, 2013). For unitary analysis we developed a customized spike sorting algorithm that determines the number of neuronal spikes distributed across time according to up to twenty-two physiological parameters characterizing each action potential. Reflex, spontaneous, and conditioned eyelid responses presented a dominant oscillatory frequency of ≈ 10 Hz. At its time, neuronal firing presented a peak of activity with a frequency dependent on the CS-US interval (i.e., ≈ 12 Hz for 250 ms, ≈ 6 Hz for 500 ms, and ≈ 3 Hz for 1000 ms). Interestingly, rmPFC neurons presented their peak firing rates at 3 different moments with respect to CS start for middle range intervals (250, 500, and 1000 ms). No significant neural responses were recorded at short (50 ms) or large (2000 ms) CS-US intervals. We can conclude that mPFC neurons do not encode the oscillatory properties characterizing eyelid responses in behaving rabbits (Gruart et al., *J Neurophysiol*, 83: 836, 2000), but are probably involved in the determination of CS-US intervals of an intermediate range (250-1000 ms). We proposed that this variable oscillator underlies the generation of working memories in rabbits, in a way probably different to neural mechanisms underlying working memories in primates.

Área temática:
1ª: Neurociencia cognitive y conductual

FUNCTIONAL STATES OF HIPPOCAMPAL AND PREFRONTAL CIRCUITS DURING UNPREDICTABLE SITUATIONS

R. Sánchez-Campusano, I. Fernández-Lamo, J.M. Delgado-García, A. Gruart
Division of Neurosciences, Pablo de Olavide University, Seville, Spain

Real life situations involve the presence of unpredictable changes in cues or contexts, introducing additional difficulties for the appropriate acquisition of the selected task. Thus, it would be important to understand the involvement of hippocampal and prefrontal circuits in unpredictable situations. Wistar rats were implanted with stimulating and recordings electrodes in selected brain sites. Rats were trained with an operant conditioning paradigm using a fixed ratio 1:1 schedule. When criterion was reached, the lever could be removed randomly during a session. Electrodes were used to record local field potentials (LFPs) and the field potentials (fPSPs) evoked during trials type A (lever IN) and type B (lever OUT). LFPs were recorded simultaneously from five selected sites (hippocampal CA1 area; subiculum, SUB; medial prefrontal cortex, mPFC; nucleus accumbens, NAc; and basolateral amygdala, BLA) during trials type A and B. Spectral analysis of LFPs recorded in the lever OUT situation presented significant changes in power as compared with those computed during lever IN occasions for all recording sites, apart from CA1. Coherograms of LFPs indicate significant changes in phase synchrony between SUB (increase) and CA1 (decrease) and mPFC, NAc, and BLA from lever IN to lever OUT situations. The resulting time-frequency patterns allowed us the optimal timing control to record activity-dependent changes in synaptic strength. Thus, fPSPs were chronically evoked in perforant pathway-CA1 (PP-CA1), CA1-SUB, CA1-mPFC, mPFC-NAc, and mPFC-BLA synapses during lever IN and lever OUT situations. While lever presses evoked a significant increase in synaptic strength at the five synapses, the unpredictable absence of the lever decreased synaptic strength in all, except PP-CA1. Results strongly support the theory that phase synchrony is directly involved in the acquisition of new motor and cognitive skills. We expect that a functional map of rat cortical circuits can be shorty offered for complex and unpredictable situations.

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

METHYLPHENIDATE INDUCES C-FOS IN CALRETIN NEURONS IN RAT MEDIAL SEPTUM

AM Sánchez-Pérez, A. García Avilés H. Albert-Gasco, FE. Olucha-Bordonau
Facultad de Medicina, Universitat Jaume I, Castellón

AIMS

The aim of this study was to map neuronal activity of brain areas involved in control and regulation of awareness and attention as a consequence of acute administration of Methylphenidate (MPD) at low but clinically relevant doses. MPD is a commonly administered drug to treat children suffering from attention deficit with hyperactivity disorder (AD/HD).

MATERIAL AND METHODS

Rats were orally administered with 1.3 and 2.7 mg/Kg of MPD. c-fos expression in different areas was detected by immunohistochemistry. Double immunofluorescence was used to characterize positive c-fos neurons with calcium-binding (parvalbumin, calretinin and calbindin 28kD) proteins. In addition, tyrosine hydroxylase (TH) immunostaining was performed to delimit dopaminergic areas.

RESULTS

We did not observed significant differences in any of the studied areas when administering 1.3mg/Kg MDP dose. However, at the higher dose, MPD induced an increase in c-fos activation specifically in two septal nuclei: the medial septum (MS) and the dorsal part lateral septum (LSd). We found little co-localization with parvalbumin or choline acethyl transferase. In contrast, a high percentage (over 30%) of c-fos activated neurons in the medial septum was calretinin positive neurons that are typically GABAergic, either interneurons or projecting to hypothalamus.

CONCLUSION

These results indicate that administration of methylphenidate at low but clinical relevant doses specifically targets medial and dorso lateral septal neurons, specifically calretinin.

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

CORRELATION OF POTENTIALS EVOKED BY IAPS IN PEDIATRIC POPULATION WITH CEREBRAL PALSY AND HIS GROUP CONTROL

S. Belmonte Darraz [1] , F.J.Hernández Arredondo[2], I.Riquelme Agulló[3].
[1]. Universidad de las Islas Baleares, Palma de Mallorca.
[2]. Hospital Universitario de Son Espases, Palma de Mallorca.
[3]. Universidad de las Islas Baleares, Palma de Mallorca.

Summary: To determine if the emotional regulation occurs similarly in subjects with Cerebral Palsy (PC) and subjects controls (SC), have carried out the work records of evoked potentials, while subjects exposed to images of the International Affective Picture System (IAPS). We examined the recognition of facial expressions in pediatric population with Cerebral Palsy (PC) and using control subjects evoked potentials (EP).
Objective: To evaluate the hypothesis that patients with PC would take place in poorer shape identification and expression of the basic emotions front (SC)
Results: The parameters determined using the Pearson correlation test were analyzed statistically. We studied the correlations between the records of EEG, its amplitude and latency for N100 (50-150 ms), P2 (150-250 ms) and N2 (200-300 ms), as well as its topography. There were significant differences in the amplitude of the P100 component between the two groups [F (2.70) = 2.93, p < 0,05, ε = 0, 075, set p = 0, 05]. In terms of topography differences associated with regions of interest in left hemisphere mainly (F7, F3, C3, P3) against components of the right hemisphere (F8, F4, C4, P4) [F (1.9) = 10, 24, p = 0, 01].
Conclusions: On the basis of the above, we understand that there are significant differences in the processing of emotions among subjects with PC and the control subjects primarily in N100 (PE early between 50-150 ms) and especially in left temporal-lateral regions. In the literature reviewed such reports are not. These changes allow us to infer that the emotional processing may be affected in patients with PC.

Thematic areas:
1. Cognitive neuroscience and behavioral.
2. Disorder and nervous system repair.

DIFFERENTIAL RATE AND BLINKING INHIBITION IN SCREENS AND REAL-WORLD BETWEEN MEDIA PROFESSIONALS AND NON-MEDIA PROFESSIONALS

C. Andreu-Sánchez[1], M.A. Martín-Pascual[1,2], J.M. Delgado-García[3], A. Gruart[3]
[1]. Audiovisual Communication and Advertising Department, Universitat Autònoma de Barcelona, Barcelona, Spain
[2]. Instituto RTVE, Radiotelevisión Española, Barcelona, Spain
[3]. Division of Neurosciences, Pablo de Olavide University, Seville, Spain

Since recently, eyeblink has been used as a marker of attention, in clinical diagnosis and in communication contexts. Inhibition of spontaneous eyeblink seems to be a system to minimize the loss of important and useful information for the viewer. It is also known that the blinking rate (BR) decreases and synchronizes between subjects, during a task that requires visual attention. The aim of our study was to learn about the BR differences in apparent movement and in real-world. It was also of our interest to find out if different types of cut in film editing reflected brain differences between media professionals and non-media professionals. Our study measured the BR in relation to four different stimuli in 40 healthy volunteers. Half of the subjects were media professionals, the rest were non-media professionals. Three of the displays were videos showing the same events but with a different editing style: one was a single run (one shot); the second was edited through stereotypical continuity-editing rules (33 shots), according to the standard institutional mode of representation (IMR); the third was a clip plenty of perceptual discontinuity according to a video clip editing style (79 shots); and the fourth display was a theatrical play, performed with the same events. The four stimuli were presented randomly. Brain activity was recorded with EEG synchronized with the shots and the events in the displays. High definition video cameras captured the viewers and the performed play. Preliminary results indicate a different BR between media professionals and non-media professionals. These showed a higher BR. Displays in screen caused a lower BR than the play in real-world, in both professionals and non-professionals. The higher eyeblink inhibition in media professionals suggests a possible distinct pattern in perceiving apparent motion. This difference may be a consequence of extensive training with screens.

Topics:
1. Cognitive and Behavioral Neuroscience
2. New methods and technologies

LOOKING REALITY AND WATCHING SCREENS: DIFFERENCES IN THE MU RHYTHM SUPPRESSION BETWEEN A PLAY AND VIDEO STIMULI WITH DIFFERENT EDITING STYLE

M.A. Martín-Pascual[1,2], C. Andreu-Sánchez[1], A. Gruart[3], J.M. Delgado-García[3]
[1]. Audiovisual Communication and Advertising Department, Universitat Autònoma de Barcelona, Barcelona, Spain
[2]. Instituto RTVE, Radiotelevisión Española, Barcelona, Spain
[3]. Division of Neurosciences, Pablo de Olavide University, Seville, Spain

The mu rhythm is suppressed when tactile stimulation, contralateral movement, and movement imagery are performed. Its desynchronization has been related to the activation of the Mirror Neuron System (MNS) in humans, suggesting the existence of a mechanism for simulation and learning, and it is being used as a noninvasive technique in Brain-Computer Interface (BCI) protocols. The mu rhythm suppression also occurs when we see movement generated by the actions of others. We studied differences in mu rhythm suppression when seeing a play in real world and watching videos on screens. We also studied differences according with editing styles in videos. The study was performed on ten media professionals and on ten non-media experts. Each subject saw a short play and three short movies with different editing styles: one was a one shot movie through a establishing shot; another was edited according to the standard institutional mode of representation (IMR) or Hollywood style, making a total of 33 shots; the last movie was conformed by 79 shots according to an avant-garde or experimental film style. The action was identical in the four stimuli, with several movements of grasping objects. Brain activity was recorded wireless, with EEG synchronized with the shots and the events in the displays. High definition video cameras captured the viewers and the performed play. Although there was a mu rhythm suppression in both the performed play and the videos on the screen, the suppression was higher with close-up shots and with the repetition of the action (grasps) from different points of view. The editing style of the videos seemed to be relevant to mu rhythm suppression. Results are important to understand brain processes related to apparent movement. Differences in mu rhythm suppression between a play and video stimuli suggests that brain distinguishes between real world and on-screen simulation.

Topics:
1. Cognitive and Behavioral Neuroscience
2. New methods and technologies

SYNAPTIC AND MOLECULAR PLASTICITY TAKING PLACE IN STRIATUM DURING BEHAVIORAL FLEXIBILITY

M.A. Gómez-Climent, M.T. Jurado-Parras, A. Gruart, J.M. Delgado-García
Pablo de Olavide University, Seville, Spain

The shell of nucleus accumbens (NAc-s) receives glutamatergic inputs from the medial prefrontal cortex (mPFC) and the ventral subiculum (vSub), whilst the caudate putamen (CPu) receives inputs from the lateral orbitofrontal cortex (lOFC). Both, NAc-s and CPu regulate cognitive-related functions in the reward circuit. We analyzed functional changes in these neural connections during an operant conditioning test of behavioral flexibility —by switching from one task to a related one. We recorded field potentials (fPs) evoked at these connections and quantified molecules related with synaptic transmission, using immunohistochemical techniques and image analysis. The day of task switching there was a significant decrease in the amplitude of fPs at both (mPFC-NAc-s, vSub-NAc-s) synaptic connections. However, vSub-NAc-s connections showed an increase in the amplitude of evoked fPs four days later, at the time of reaching the selected task criterion. Only lOFC-CPu connections showed an increase in the amplitude of evoked fPs the day after task switching. Tetanic stimulation delivered to mPFC and vSub evoked antagonistic interactions with respect to long-term potentiation (LTP) induction in the NAc-s. In all three pathways, LTP/LTDs evoked by electrical stimulation were in the same direction as those collected during task switching. Molecules related with synaptic transmission were also studied in the striatum. Only after learning the first task, there was an increase in the density of dopamine transporter expressing puncta in NAc-s and a decrease of tyrosine hydroxylase in the NAc-s and CPu. Moreover, there was a decrease in the number of Calcium/Calmodulin Kinase II phosphorylated expressing somas in the CPu and NAc-s after learning the first task and, finally, a significant decrease in NAc-s after learning the second task. These results indicate that switching from one task to another induces functional synaptic and molecular changes and suggest a specific role of the lOFC-CPu synapse in behavioral flexibility.

DRR1 EXPRESSION IN MOUSE CA3 IS ESSENTIAL FOR OPTIMAL COGNITIVE FUNCTION AND SYNAPTIC PHYSIOLOGY

M Masana[1,2], J. Dine[1], S. Filsen[3,4,5], L. Blazquez-Llorca[3,4,5], A. Kretzschmar[1], M.V. Schmidt[1], J. Bock[6], J. Herms[3,4,5], M. Eder[1], T. Rein[1], M.B. Müller[1,2]

[1]Max Planck Institute of Psychiatry, Munich, Germany

[2]Translational Psychiatry, Department of Psychiatry and Psychotherapy & Focus Program Translational Neuroscience (FTN), Johannes Gutenberg University Medical Center, Mainz, Germany

[3]German Center for Neurodegenerative Diseases (DZNE), Ludwig Maximilian University Munich, Munich, Germany

[4]Center for Neuropathology, Ludwig Maximilian University Munich, Munich, Germany

[5]Munich Cluster of Systems Neurology (SyNergy), Ludwig Maximilian Munich, Munich, Germany

[6]Institute of Biology, Otto von Guericke University Magdeburg, Magdeburg, Germany

Background

The Down-Regulated in Renal cell carcinoma 1 (DRR1) gene is a glucocorticoid-regulated gene in the central nervous system which modulates complex behavior, most probably through fine-tuning of actin dynamics. In the adult mouse brain, DRR1 is constitutively highly expressed in the hippocampal CA3 region, septum, cortex, and cerebellum. The unique increase in DRR1 in the CA3 and septum –mimicking stress induced increase-induces pro-active behaviors known to be negatively affected by stress, such as cognition and sociability, respectively. This places DRR1 as a potential molecular candidate promoting stress resilience.

Methods

In order to dissect the role and relevance of the CA3 high DRR1 expression in synapse physiology and behavior under basal conditions, we combined a CA3 region-specific AAV-induced DRR1 knock-down in the mouse with a comprehensive characterization of behavior, electrophysiological and morphological aspects.

Results

Knocking-down DRR1 in the mouse CA3 region induced deficits in the acquisition of novelty-place associated information (one-trial place-object test), but not in strict spatial memory tasks (Y-maze and water cross-maze), while anxiety, and other physiological and endocrine parameters were unaltered compared to control. These deficits were accompanied by increased LTP magnitude and reduced paired-pulse facilitation at CA3-CA1 synapses. These changes were associated to unaltered axon dynamics, but reduced AMPA receptors expression and increased spine density in CA1 dendrites.

Conclusions

The strong DRR1 expression in the CA3 region is necessary for optimal cognitive function. DRR1, probably by modulation of actin dynamics, modulate synapse physiology which impacts on the acquisition of associative learning but not spatial learning.

Áreas Temáticas:

1ª: Neurociencia cognitiva y conductual

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

TRADE-OFF BETWEEN FREQUENCY AND PRECISION DURING STEPPING MOVEMENTS: KINEMATIC AND BOLD BRAIN ACTIVATION PATTERNS

Martínez M.[1,*], Valencia M. [2,*], Vidorreta.M.[1], Luis E.O.[1], Castellanos G.[1], Villagra F.[1], Fernández-Seara M.A.[1], Pastor M.A.[1, 3]

[1]. Neuroimaging Laboratory, Division of Neuroscience, Centre for Applied Medical Research (CIMA), University of Navarra, 31008, Pamplona, Spain.

[2]. Neurophysiology Laboratory, Division of Neuroscience, Centre for Applied Medical Research (CIMA), University of Navarra, 31008, Pamplona, Spain.

[3]. Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, 28030, Madrid, Spain.

[*]. Equal contribution.

Human locomotion constitutes a coordinated process involving alternating movements of different segments of the body. Depending on the needs, the central nervous system has the ability to adapt this pattern to produce a wide range of modalities of locomotion and velocities. In reacting to external pacing stimuli, deviations from an individual preferred cadence provoke a concurrent decrease in accuracy that suggests the existence of a trade-off between frequency and precision; a compromise that could result from the specialization within the control centers of locomotion to ensure a stable transition and optimal adaptation to changing environment. Here we explore the neural correlates of such adaptive mechanisms by visually guiding a group of healthy subjects to follow three comfortable stepping frequencies while recording their kinematic and BOLD responses. In following the visual stimuli, subjects adopt a common pattern of symmetric and anti-phase stepping movements. When increasing the stimulus frequency, there is an increase in pace stability and task performance that suggest a change in the mode of the motor control from predictive (less precise/stable) to reactive (more precise/stable) schemes. For the first time, we are able to examine the neural correlates of such transition in humans and uncover a distributed network that includes midbrain structures previously proposed in models coming from animal studies. In addition we detect the modulation of occipito-parietal and paravermal cerebellar regions that had been proposed to be essential for visuomotor adaptation during locomotion. All these results suggest that the effect of increasing frequency with visual stimulation evokes a transition in the brain from a predictive to a reactive control mode and that brain activity is modulated depending on the current strategy.

Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual
2ª: Nuevos métodos y tecnologías

Presenting author: Martín Martínez (tininmartinez@gmail.com)

IS THE ADULT HIPPOCAMPAL NEUROGENESIS INVOLVED IN RECONSOLIDATION OF OBJECT RECOGNITION MEMORIES?

I. Suárez-Pereira[1], S. Bachiller[1], S. Canals[2], AM. Carrión[1].
[1]. Departamento de Fisiología, Anatomía y Biología celular. Universidad Pablo de Olavide, Sevilla.
[2]. Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-CSIC, Alicante.

The adult hippocampal neurogenesis is the process of continuous generation of new neurons in the adult hippocampus, a brain area related to cognitive and mood processes. These newly generated neurons migrate and mature in the subgranular layer of the dentate gyrus (DG), to be integrated into functional circuits. There is evidence that adult hippocampal neurogenesis influences hippocampal function, although the specific role of the neuronal precursors cells in learning and memory processes begin to be understood.

Our previous studies using X-ray fast and selective neurogenic depletion protocol demonstrated that adult hippocampal neurogenesis is required for acquisition and storage of object recognition (OR) information (*Suárez-Pereira, I. et al. Hippocampus 2015*). Here we focus in the possible role of neurogenesis on stored memories modifications. Irradiating mice at different times with respect to the reactivation session for OR, we found that depletion of adult neurogenic cells produced deficiencies in reconsolidation only when reactivation session included novelty. On the other hand, we study the adult immature neurons activation during OR memory formation, by immunocolocalization of neuron activation (c-Fos or Egr1) and immature neurons (doublecortin) markers. We discovered that novelty, in new information acquisition or during an old memory modification, increased the number of activated adult immature neurons in the DG.

These results highlight new and unexpected aspects of neurogenesis in cognitive processes: a, adult neurogenic cells participate in storage of new information or integrating new information into stored memories; and b, the adult hippocampal immature neurons specifically detect novelty in order to store new information as well as updating stored memories. Then immature neurons are an essential component in the stabilization of hippocampal new memory traces.

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

Contact: Irene Suárez Pereira
isuaper@upo.es

SUCCESSFUL WORKING MEMORY PROCESSES AND CEREBELLUM IN AN ELDERLY SAMPLE: A NEUROPSYCHOLOGICAL AND fMRI STUDY

Elkin O. Luis[1] , Gonzalo. Arrondo[1,4], Marta. Vidorreta[1] , Martin. Martínez[1], Francis. Loayza[1], Federico Villagra[1], María A. Fernández-Seara[1,3], María A. Pastor[1,2,3].

[1] Neuroimaging Laboratory, Division of Neurosciences, Center for Applied Medical Research (CIMA). University of Navarra, Pamplona. Navarra.

[2] Department of Neurology, Clinica Universidad de Navarra, University of Navarra School of Medicine, Pamplona. Navarra.

[3] CIBERNED, Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas, Instituto de Salud Carlos III, Spain.

[4],Department of Psychiatry, University of Cambridge, Cambridge, UK.

Introduction: Imaging studies help to understand the evolution of key cognitive processes related to ageing, such as working memory (WM). This study aimed to test three hypotheses in older adults. First, that the brain activation pattern associated to working memory processes in elderly during successful low load tasks is located in posterior sensory and associative areas; second, that the prefrontal and parietal cortex and basal ganglia should be more active during high-demand tasks; third, that cerebellar activations are related to high-demand cognitive tasks and have a specific lateralization depending on the condition.

Methods: We used a neuropsychological assessment with functional magnetic resonance imaging and a core N-back paradigm design that was maintained across the combination of four conditions of stimuli and two memory loads in a sample of twenty elderly subjects.

Results: During low-loads, activations were located in the visual ventral network. In high loads, there was an involvement of the basal ganglia and cerebellum in addition to the frontal and parietal cortices. Moreover, we detected an executive control role of the cerebellum in a relatively symmetric fronto-parieto network. Nevertheless, this network showed a predominantly left lateralization in parietal regions associated presumably with an overuse of verbal storage strategies. The differential activations between conditions were stimuli-dependent and were located in sensory areas.

Conclusions: Successful WM processes in the elderly population are accompanied by an activation pattern joint between cerebellar regions and the fronto-parietal network.

Thematic areas:
1st: Cognitive and Behavioral Neuroscience
2nd: Systems Neuroscience

DNA DAMAGE AND REPAIR MECHANISMS ARE REQUIRED IN MEMORY FORMATION

S. Bachiller[1], I. Suárez-Pereira[1], Y. del-Pozo-Martín[2], AM Carrión[1].
[1]. Faculty of Experimental Sciences. Pablo de Olavide University, Seville.
[2]. Cancer Research UK, London.

In recent years, some studies suggest that permanent memory may be codified by new proteins expression generated by genomic reorganization mechanisms, a hypothesis consistent with the transiently increase of neuronal DNA breaks observed after physiologic neuronal activation in cerebral areas related with memory formation and storage. This data suggest that DNA metabolism and repair mechanisms may be necessary for the formation and/or storage of memories.
DNA repair processes and genomic reorganization involved the activity of some DNA associated proteins. Expression analysis in hippocampus for several genes that encoding for proteins related with DNA reorganization and repair revealed that exploration of enriched environment increased their expression quickly and transiently. Also, in hippocampus the same stimulation protocol provoked a transient increase in the number of cells with 53BP1 foci, a marker of DNA breaks, and in recombinase activity. To verify the functional role of DNA metabolism in memory formation, we systemically administrated araC, an inhibitor of DNA metabolism process, at different times with respect the training session for object recognition or for passive avoidance, two hippocampus-dependent paradigms. Our results indicated that DNA metabolism was necessary in memory formation in two temporal waves: during the first 2 hours and 24 hours after a training session of both cognitive paradigms. Also, we performed local administration of araC in hippocampus and prefrontal cortex at different times, and we obtained that the early and late inhibition wave of DNA metabolism occurred differentially in hippocampus and prefrontal cortex, respectively. Consequently, changes in the expression of gene encoding for proteins related with metabolism and repair of DNA in hippocampus and prefrontal cortex were displaced in time.
All these data suggest that DNA metabolism and repair mechanisms are critical for memory formation and stabilization of system consolidation.

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

Sara Bachiller.
Email de contacto: sbacsan@upo.es

ALCOHOL AVERSIVE EFFECTS IN ALCOHOL-INDUCED CONDITIONED FLAVOR AVOIDANCE: DOSE/CONCENTRATION OF ETHANOL AND THE ROLE OF THE GUSTATORY THALAMUS

Á. Agüero Zapata[1], M. D. Escarabajal Arrieta [1] , M. L. de la Torre Vacas [1]
[1]. Facultad de Humanidades y Ciencias de la Educación. Departamento de Psicología (Área de psicobiología). Universidad de Jaén, Jaén

Flavor Avoidance Learning (FAL) occurs when rats suppress intake of a taste cue following pairings with known aversive agents. This phenomenon has been interpreted as a reaction established by the development of an association between a flavor and activation of gastrointestinal discomfort. The present study aimed to obtain FAL in female and male Wistar rats paired with different doses and concentrations of ethanol and a palatable taste cue as conditioned stimuli (CS). The rats appear to be more responsive to the rewarding properties of the lowest dose of ethanol; they avoid intake of the CS when paired with the highest dose of ethanol in a manner comparable to the suppression of the CS induced by a known aversive agent such as lithium chloride (LiCl). Evidence suggests that, at least for high doses of ethanol, those suppressive effects are mediated by aversive consequences of the unconditioned stimuli. Some authors, such as Liu and colleagues (2009), consider that bilateral lesions of the gustatory thalamus, a structure traditionally involved in reward-learning paradigm, can be used to identify the rewarding or aversive properties of drugs as ethanol. Thus, they suggest that the effects of lesions of the taste thalamus in rats subjected to ethanol-induced FAL may serve to discern whether ethanol-induced suppression of CS intake is based on the rewarding or aversive properties of ethanol. In this regard, bilateral electrolytic lesions of the gustatory thalamus have been done in this study to obtain additional support and to compare their effects on ethanol-induced FAL and LiCl-induced FAL. Altogether, the results seem to support the notion that ethanol-induced FAL is based on the aversive properties of the drug. Nonetheless, future studies need to be designed to clarify which of these properties, the aversive or the rewarding ones, are responsible for ethanol-induced FAL.

Ángeles Agüero Zapata, aaguero@ujaen.es

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

CONCOMITANT HDAC and PDE5 INHIBITION SYNERGISTICALLY PREVENTS DISRUPTION IN SYNAPTIC PLASTICITY AND REVERSES COGNITIVE IMPAIRMENT IN ALZHEIMER´S DISEASE MICE

M. Cuadrado-Tejedor [1,5], C. Garcia-Barroso [1], J. Sanzhez-Arias [2] , O. Rabal [2], S. Mederos [3], A. Ugarte [2], R. Franco [1] , V. Segura [4], G. Perea [3], J. Oyarzabal [2], A. Garcia-Osta [1]
[1]. Neurosciences Division, CIMA, University of Navarra, Pamplona.
[2]. Molecular Therapeutics Program, CIMA, University of Navarra, Pamplona.
[3]. Cajal Institute, CSIC, Madrid.
[4]. Bioinformatics, CIMA, University of Navarra, Pamplona.
[5]. School of Medicine, University of Navarra, Pamplona.

Objective: The goal of this study was the validation of a novel mode of action to treat Alzheimer´s disease (AD). We postulated that the simultaneous inhibition of histone deacetylase (HDAC) and phosphodiesterase 5 (PDE5), both involved in independent pathways related to AD pathology, could have a synergistic effect and restore memory deficits in AD mouse models.
Methods: Firstly, two FDA-approved drugs (vorinostat and tadalafil) were utilized as reference compounds for in-vitro and in-vivo proof-of-concept studies. First, a possible synergistic effect of the aforementioned combination was tested using neuronal cultures and hippocampal slices of APP/PS1 mice. Next, the effects of chronic treatments on AD-related cognitive decline were evaluated using the fear conditioning and Morris water maze tests and aged Tg2576 mice. After behavioural testing, animal brains were used to analyze amyloid and tau pathology. Spine density in apical dendrites in pyramidal neurons was determined using Golgi-Cox method. Affymetrix microarrays technology was used to analyze gene expression analysis. The same methodology was used with the lead compound CM-414.
Results: In vitro assays demonstrated that the combination of vorinostat and tadalafil had a synergistic effect increasing the levels of the epigenetic mark AcH3K9 and also, at functional level, restoring LTP in APP/PS1 mice. We demonstrated that beneficial effects on reversing AD-phenotype, including memory deficits, synaptic loss and amyloid and tau pathology, were only achieved when both pathways were target simultaneously. A lead compound (CM-414) hitting both targets simultaneously was synthesized and tested in vivo, confirming the beneficial effects observed by using reference compounds. Microarray expression analysis identified differential genes involved in the beneficial effect observed in treated animals.

Conclusions: A novel dual approach, hitting two different pathways, involved in memory formation and other AD-related features, leads to a synergistic therapeutic effect in AD mice.

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

CHANGES IN BOLD SIGNAL AFTER UNILATERAL SUBTHALAMOTOMY IN PARKINSON'S DISEASE DURING REACTIVE INHIBITION

I. Obeso[1,2], F. Loayza[3], F. Villagra[4], R. Gonzalez[4], M.C. Rodríguez-Oroz[5], M. Jahanshahi[6], J. A. Obeso[1,2], A. Pastor[4]
[1] Centro Integral en Neurociencias A. C. (CINAC), Fundación Hospitales Madrid-Puerta del Sur, Madrid, Spain
[2] Center for Networked Biomedical Research on Neurodegenerative Diseases (CIBERNED), Madrid, Spain
[3] Escuela Superior Politécnica del Litoral, Guayaquil, Ecuador
[4] Clinica Universidad de Navarra, University of Navarra, Pamplona, Spain
[5] BioDonostia Research Institute, San Sebastian, Spain
[6] UCL Institute of Neurology, UK

Introduction: Reactive inhibition is achieved via fronto-striatal networks and the subthalamic nucleus (STN) exerts its inhibitory role in joint activation with frontal regions. The stop signal task measures participant's ability to inhibit an action when a stop signal is presented. We aim to investigate the role of the STN in response inhibition as investigated by unilateral subthalamotomy in Parkinson's disease (PD) patients using a stop signal task and functional magnetic resonance imaging (fMRI). **Methods**: The stop signal task was completed by 5 PD patients treated with right unilateral subthalamotomy in two identical fMRI experiments (pre and post surgery). Patients were on similar pharmacology state in both sessions. Imaging data were evaluated using 2-way anova ($p<0.05$) cluster corrected with factors surgery and hand. **Results:** A marked clinical improvement was seen in every patient after subthalamotomy. Behaviorally, subthalamotomies were associated with speeding of initiation times (Go reaction times). In contrast, reactive inhibition turned worse in the hand contralateral to the lesion as compared to the non-operated hemibody. The imaging results show dysfunctional inhibition was achieved with significant activation in right medial frontal gyrus and bilateral head of caudate extended to capsula interna, anterior pallidum and thalamus, acting as compensatory mechanisms after subthalamotomy. **Conclusions**: When STN activity is no longer available, a critical network for inhibition consisting of medial PFC, the caudate nucleus and thalamus has been highlighted here. Thus, this provides strong evidence that the STN is a key node responsible in stopping of actions.

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

LACK OF E2F1 PROTEIN ENHANCES LEARNING AND MEMORY

A. Fontán-Lozano; A. Pallé; JM; Frade; JL. Trejo
Instituto Cajal, CSIC, Madrid

Aberrant expression and activation of the cell cycle protein E2F1 in neurons has been implicated in many neurodegenerative diseases. E2F1 is a transcription factor that regulates G1 to S phase progression in proliferative cells. E2F1 is often up-regulated and activated in models of neuronal death. However, little is known about the role of E2F1 in physiological conditions in the mature brain. Hence, the objective was to study behavioural effects of the lack of E2F1 in mice [E2F1-KO]. E2F1 expression is significantly elevated with aging; therefore, we studied general health and cognitive behaviour in E2F1-KO and wild type [WT] animals at different ages. Moreover, we evaluated molecular basis of behavioral changes induced in E2F1-KO. In this study we used a combined approach to study the effect of E2F1 gene disruption in mice's behaviour and brain molecular changes. Cognitive impairment associated with age coincides with increased E2F1 protein. Our results indicate that E2F1-KO mice delay cognitive impairment, in respect to WT mice; however, the lack of E2F1 protein enhances learning and memory. These results point out E2F1 inhibitors as candidate agents for the palliative treatment of learning and memory impairments in aging and in neurodegenerative disorders.

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

INTENSE ODOURS INDUCE AVOIDANCE AND LEARNING IRRESPECTIVE OF THEIR BIOLOGICAL SIGNIFICANCE

C. Agustín-Pavón[1], Ll. Fortes-Marco[1,2§], E. Lanuza[2], F. Martinez-Garcia[1]
[1]. Fac. CC de la Salut, Universitat Jaume I, Castelló de la Plana.
[2]. Fac. CC Biològiques, Universitat de València, València.
[§]. Current adress: Instituto Cajal, Madrid.

Chemosignals mediate both intra- and inter-specific communication in most mammals. Pheromones elicit stereotyped reactions in conspecifics, whereas kairomones released by an individual produce a reaction in an allospecific animal that harms the emitter's interests. For instance, predator kairomones elicit anticipated defensive responses in its preys. Conversely, simple odorants can have indirect effects on conspecific or allospecific individuals through learning. In this work we tested the response of female mice to two biologically significant odorants: 2-heptanone (2-HP), a putative pheromone for rats and mice; and trimethyl thiazoline (TMT), a fox-derived putative kairomone, widely used to investigate fear and anxiety in rodents. The banana-like odour isoamyl acetate (IA), an odorant unlikely to act as an intra or interspecific communicative chemosignal, served as control. We first presented these chemicals at increasing concentrations in consecutive days, up to pure substance, in a test box in which the animal could explore or avoid the chemicals. Mice did not show attraction for any of the dilutions, and avoided all three compounds at the highest concentrations, with minimal significant effects in global locomotion and immobility. However, TMT and IA elicited avoidance at lower concentrations (10^{-5} to 10^{-2}) than 2-HP did. Second, we analysed whether repeated exposure to the pure chemicals in the same location of a two-chamber test cage resulted in conditioned avoidance for that place. Our results revealed that both TMT and IA (but not 2-HP) induced conditioned place aversion in the animals, and increased immobility in the safe compartment during the contextual memory test. Our results suggest that intense odours can induce contextual learning irrespective of their putative biological significance. Although our results challenge the kairomonal nature of TMT, they support the usefulness of synthetic predator-related compounds (like TMT) or other concentrated odorants to investigate the neurobiological basis of fear and anxiety in rodents.

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

TASTE AND OBJECT RECOGNITION MEMORY IMPAIRMENT BY EXCITOTOXIC LESIONS OF THE PERIRHINAL CORTEX

A, Grau-Perales, E. Morillas, B. Gómez-Chacón, and M. Gallo.
Institute of Neurosciences (CIBM). University of Granada. Granada. Spain.

Previous studies have shown the relevance of perirhinal cortex (PRh) integrity either in safe taste recognition memory and object recognition memory. Objective: This experiment was aimed to assess the relevance of PRh integrity for the acquisition of taste and visual memories. Thus, we investigated the effect of complete excitotoxic lesions of PRh on the performance of both a taste neophobia attenuation task and a spontaneous object recognition task (SOR). Method: Twenty-nine male Wistar rats were assigned to one of two surgical groups receiving i.c. bilateral infusions of either NMDA (lesioned) or vehicle (Sham). They were then subjected to a SOR task using a 24-hour retention interval followed by a taste neophobia attenuation task, receiving four consecutive exposures to a 3% cider vinegar solution. Results: PRh lesioned rats explored the same amount of time the novel and the familiar object in a 24-hour retention test, thus showing impaired visual memory. This group also exhibited a slower attenuation of neophobia than the sham-lesioned group. Conclusions: The results indicate that both object and taste recognition memory were impaired by PRh lesions. This is consistent with a role of PRh in the acquisition of long-term memories independent of the sensory modality involved.

Supported by PSIC2011-23702 and PSI2014-57643-P (MINECO. Spain)

Areas temáticas:
1. Neurociencia cognitiva y conductual
2. Neurociencia de sistemas

TASTE MEMORY-RELATED N-ETHYLMALEIMIDE-SENSITIVE FACTOR EXPRESSION IN AMYGDALA AND PERIRHINAL CORTEX

B. Gómez-Chacón[1], F. Gámiz [1], T.C. Foster[2] and M. Gallo[1]
[1] Institute of Neurosciences (CIBM).University of Granada.Granada. Spain.
[2] McKnight Brain Institute, University of Florida, Gainesville, Florida, USA.

Consolidation of safe taste memories has been linked to protein synthesis in temporal lobe areas, including the perirhinal cortex (PRh) and the basolateral amygdala (BLA).We have previously found that inhibition of PKMζ in BLA attenuates conditioned the formation of a safe taste memory. It has been demonstrated that PKMζ maintains LTP and memory by regulating GluR2-dependent AMPARs trafficking. N-ethylmaleimide factor (NSF) has a relevant role in this mechanism since it interacts with GluR2 to regulate AMPA receptors synaptic trafficking. Therefore, it could be proposed that NSF/GluR2 interactions in temporal brain areas might be involved in safe taste recognition memory. Objective: In the present experiments we investigate both the behavioral performance and the expression profile of NSF and GluR2 genes in several brain areas, including PRh, BLA and hippocampus. Method: Twenty eight naïve male Wistar rats were exposed to a saccharin solution (0,4%) during the first (novel), the second (familiar I) and the sixth presentation (familiar II).Total RNA was extracted and gene expression was measured by quantitative PCR (qPCR) using Taqman gene expression assays. In addition the expression of the synaptic plasticity related immediate early genes, Homer1 and Narp, was also assessed. Results: We have found increased expression of NSF gene in BLA and PRh but not in dorsal hippocampus 30 min after drinking a saccharin solution which was becoming familiar during the second presentation in comparison with the sixth exposure. No changes in the expression profile of GluR2, Homer 1 and Narp genes were found. Conclusions: The results support a role of NSF in the consolidation of the safe memory trace. Moreover, the results suggest the relevance of a potential network in the temporal lobe for taste recognition memory and open new possibilities for understanding the molecular mechanisms.

Supported by PSIC2011-23702 and PSI2014-57643-P (MINECO.Spain)

Areas temáticas:
1. Neurociencia cognitiva y conductual
2. Neurociencia de sistemas

GLUCOCORTICOIDS SIGNALING IS ESSENTIAL FOR OPIATE WITHDRAWAL-ASSOCIATED MEMORIES

D. García-Pérez[1,], C. Núñez[1,2], M.L. Laorden[1,2], M.V. Milanés[1,2]
[1]Group of Cellular and Molecular Pharmacology, University of Murcia, Campus de Espinardo, 30100 Murcia, Spain.
[2]IMIB (Instituto Murciano de Investigación Biosanitaria, Murcia, Spain).

Drug addiction is a relapsing disorder in part due to the strong associations formed between drugs and the stimuli associated with drug use. Reactivation of withdrawal memories has been suggested to trigger the relapse of drug seeking. However, the neural circuitry that underlies conditioned-cued relapse is not clarified. Interestingly, it is well established that the dentate gyrus (DG) is essential for memory encoding/consolidation but also for episodic memory retrieval. On the other hand, stress hormones such as glucocorticoids (GC) mediate and modulate memory consolidation.
We performed the conditioned-place aversion (CPA) paradigm, which is an animal model for measurement of the negative affective component of drug withdrawal. Therefore, we have used CPA in sham and adrenalectomized (ADX) morphine-dependent rats to study the plasticity-related processes that occur within the DG during the conditioning of the aversive properties of opiate withdrawal (memory formation), and after allowing their reactivation by re-exposure to the conditioned environment (memory retrieval). For that, we have measured the plasticity marker Arc in the DG.
During the test, morphine-dependent rats spent less time in the naloxone-paired compartment than controls. However, ADX diminished the naloxone-induced CPA in opiate-dependent animals. We detected that the levels of Arc mRNA and protein were enhanced in sham and ADX morphine-dependent animals during memory formation. In addition, there was an enhancement of Arc mRNA and protein levels in sham morphine-dependent animals that was not observed in animals with lack of GC during memory retrieval. Importantly, we observed a negative correlation between Arc levels and the score of animals dependent on morphine.
Our data seem to indicate that GCs are not involved in memory formation in the DG of morphine-withdrawn rats. However, Arc seems to be essential for the retrieval of memories related with morphine withdrawal and depends on GC homeostasis.

M.V. Milanés Maquilón (milanes@um.es)

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

ACUTE AND SUBCHRONIC TREATMENT WITH KETAMINE INCREASED CLIMBING BEHAVIOR, WHICH WAS REVERTED WITH THE 5-HT6 AGONIST E-6837

J.E. Suárez Santiago[1], M. Castellanos Pérez[2], J. Espinosa Raya[1], A. Briones Aranda[2]
[1]Escuela Superior de Medicina, Instituto Politécnico Nacional, México Distrito Federal, México
[2]Facultad de Medicina Humana, Universidad Autónoma de Chiapas, Chiapas, México

Schizophrenia is a chronic illness identified by positive and negative symptoms as well as cognitive alterations. In his catatonic form, stereotyped behaviors are observed, characterized by the execution of repeated movements without specific function. The administration of both dopamine agonists (D_2) and antagonists such as N-methyl-d-aspartate receptor (NMDAR) in rodents have been associated with the induction of stereotyped movements. Moreover, recently a relation has been demonstrated between the function of the 5-HT_6 receptor (R5-HT_6) and modifications in dopaminergic activity. The aim of the present study was to evaluate the effect of the administration of ketamine alone and in combination with the agonist of R5-HT_6 (E-6837) on climbing behavior. Male NIH mice (25-30 gr) were divided into groups (n=10) to test different doses. The climbing evaluation model consists of an acrylic rectangular box with a wall made of a metallic screen where the animals climb. Assays were recorded on video and later analyzed manually. Initially, ketamine was administered during 1 and 5 days (5 and 10 mg/kg, i.p.). Later, the acute and subchronic scheme of the NMDA antagonist (10 mg/kg) was combined with one of the two acute doses of the agonist E-6837 (5 and 10 mg/kg, i.p.). The acute and subchronic administration of ketamine (10 mg/kg) was able to increasing the climbing behavior, and this effect was reversed by the agonist E-6837 (5 and 10 mg/kg). The present results confirm that the NMDA antagonist induces climbing behavior and indirectly suggests a possible interaction between the NMDA receptors, 5-HT_6 and dopaminergic activity. Further research is necessary to provide more evidence of the latter interaction.

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

Mail: jeduardo.santiago@hotmail.com

PRO-COGNITIVE EFFECT OF TWO 5-HT6 DRUGS IN FEMALE OVARIECTOMIZED MICE WITH PREVIOUS ACUTE STRESS

M. Castellanos Pérez[1], J.E. Suarez Santiago[2], G. López Aguirre[1], A. Briones Aranda[1]
[1]Facultad de Medicina Humana, Universidad Autónoma de Chiapas, Chiapas, México
[2]Escuela Superior de Medicina, Instituto Politécnico Nacional, México Distrito Federal, México

Hypoestrogenism during menopause has been associated with changes in memory and the stress response, and the 5-HT_6 receptor (R5-HT_6) with pro-cognitive effects. The aim of the present study was to explore the effect of 5-HT_6 drugs on memory in ovariectomized mice (OVX) with previous acute stress. Female NIH mice (25-30 g) were ovariectomized 3 weeks before experiments. Acute stress was induced with a 15-min forced swimming session. Memory was evaluated with the novel object recognition task (NORT), which includes three phases: habituation (without objects), acquisition (with identical objects), and retention (with different objects). The period of time between the second and third phase varies according to experimental conditions. The experiment was divided into three stages. In the first, ovariectomized mice were evaluated for memory loss in different groups (n=10), each with a distinct interassay time between the second and third phase (1, 3, 6, 12, 24 and 48 h). In the second stage, mice were stressed 24 h before beginning the test, with two interassay times (1 and 24 h). In the last stage, ovariectomized mice with and without previous acute stress were tested after the administration of one of three drugs: 5-HT_6, the R5-HT_6 agonist E-6837 and the R5-HT_6 antagonist SB-271046 (each at 10 mg/kg administered i.p. 20 min. before the test). Memory was lost at 48 h post-ovariectomy in the animals. Stress by forced swimming deteriorated long term memory, but this could be reverted by the agonist and antagonist of R5-HT_6. This confirms the importance of R5-HT_6 in cognitive processes under vulnerable conditions such as post-stress and hypoestrogenism.

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

Mail: castellanos_manuela@yahoo.com.mx

PRE- AND POST-NATAL MELATONIN ADMINISTRATION REDUCED OXIDATIVE STRESS BUT DID NOT IMPROVE COGNITION, HIPPOCAMPAL NEUROGENESIS OR CELLULARITY IN A MOUSE MODEL OF DOWN SYNDROME

V. Vidal, E. B. Parisotto, A. Corrales, S. García-Cerro, S. Lantigua, C. Martínez-Cué, N. Rueda.
Facultad de Medicina, Universidad de Cantabria, Santander, Cantabria

Ts65Dn mice (TS), the most commonly used model of Down syndrome (DS), exhibit numerous phenotypic characteristics of DS, including cognitive deficits due to impairments in hippocampal morphology and function and to increased oxidative stress. A previous study from our laboratory demonstrated that melatonin treatment to adult TS mice improves spatial learning presumably by normalizing the function and morphology of the hippocampus and/or by reducing oxidative stress. **Objetive**: The aim of the present study was to assess whether early administration of melatonin induced the same cognitive enhancement in young TS mice. **Material and Methods:** Melatonin or vehicle were orally administered to TS females during pregnancy and lactation and to their TS and CO male pups from weaning until the age of 20--22 weeks. We analysed the effects of melatonin on cognition, hippocampal cellularity and neurogenesis and on the brain oxidative status of these animals. **Results**: Contrary to our previous results in adult TS mice, pre- and post-natal melatonin treatment did not improve hippocampal-dependent learning and did not increase the density of proliferating cells or of mature granule neurons. However, melatonin administration during early stages decreased lipid peroxidation in the hippocampus and the activity of the enzymes SOD1 and CAT, but not of PC, in the cortex. **Conclusion**: Pre- and post-natal melatonin administration did not promote the same cognitive and morphological (on hippocampal cellularity and neurogenesis) benefits found after this treatment to TS mice during adulthood However, during early stages melatonin administration partially regulated TS brain oxidative metabolism. These results suggest that the reduction of oxidative stress induced by chronic melatonin administration is not the main factor inducing the cognitive benefits found in adult mice.

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

OVEREXPRESSION OF *DYRK1A* IS IMPLICATED IN SEVERAL CEREBELLAR ALTERATIONS FOUND IN A MOUSE MODEL OF DOWN SYNDROME

S. García-Cerro, V. Vidal, S. Lantigua, M. Lafarga, M. Berciano, N. Rueda, C. Martínez-Cué
[1]Facultad de Medicina, Universidad de Cantabria, Santander, Cantabria

Down syndrome (DS) phenotypes result from the overexpression of a number of dosage-sensitive genes. One of the genes that have been proposed to play a role in different DS phenotypes is *DYRK1A* (dualspecificitytyrosine-(Y)-phosphorylation regulated kinase 1A), which has been implicated in the behavioral and neuronal alterations characteristic of DS, such as the impairments in neuronal progenitor proliferation and differentiation and long-term potentiation that contribute to the cognitive deficits found in DS. **Objectives:** The aim of this study was to evaluate the role of the *Dyrk1A* in different neuromorphological and behavioral cerebellar phenotypes in the Ts65Dn (TS) mouse model of DS and in control littermates. **Material and Methods:** TS females were crossed with heterozygous *Dyrk1A+/-* mice to obtain TS mice with three and two copies of this gene and control mice with two or one copy of *Dyrk1A.* The four groups of mice were assessed in several motor tasks. In addition, a neuromophological analysis of the size and cellularity of different cerebellar structures were analyzed by immunohistochemistry. **Results:** TS mice showed alterations in their walking pattern, reduced size of the cerebellar vermis, the internal granular layer and the molecular layer of this structure, the cell density in the internal granular layer and the linear density of Purkinje cells. Normalization of the *Dyrk1A* copy number in TS mice reduced these altered phenotypes. Control mice with reduced *Dyrk1A* gene dosage showed impairments of their walking pattern, a decreased size of cerebellar vermis and of the internal granular and the molecular layer, an increased cell density in the internal granular layer and a normal Purkinje cell linear density. **Conclusions:** These data provide evidence for the role of *Dyrk1A* in motor and cerebellar phenotypesand suggest that the overexpression of *Dyrk1A* is implicated in DS-associated motor and cerebellar alterations.

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

GAL(1-15) INDUCES A DEPRESSION AND ANXIOGENIC EFFECT TROUGH GALR1/GALR2 HETERORECEPTOR: siRNA GALR1/GALR2 KNOCKDOWN RATS

C. Millón [1], A. Flores-Burgess [1], M. Narváez [1], DO. Borroto-Escuela [3], L. Santín [2], C. Parrado [1], J. A. Narváez [1], K. Fuxe [3], Z. Díaz-Cabiale [1]
[1]. Faculty of Medicine, University of Málaga, Málaga
[2]. Faculty of Psychology, University of Málaga, Málaga
[3]. Department of Neuroscience, Karolinska Institute, Stockholm

The Galanin N-terminal fragment (1-15) [GAL(1-15)] induces depressant- and anxiogenic-like actions. In this work, we have studied the role of GALR2 and GALR1 on the effects of GAL(1-15) in the Forced Swimming Test (FST) and Open Field Test (OFT) using siRNA GALR2 and GALR1 knockdown rats.
Rats (n=6-14) were injected with GAL(1-15) 3nmol, GALR2 antagonist M871 3nmol in combination or alone 15 before the FST or OFT. The time of immobility, climbing and swimming were recorded during 5 min FST and Time and entries in the central square during 5min were scored in the OFT.
In other experiment, rats (n=6-14) were injected Intracerebroventricular (icv) with siRNA-GALR2 or siRNA-GALR1 to generate the GALR knockdown rats. These knockdown rats were used in the OFT and in the FST after receiving icv GAL(1-15) 3nmol 15 min before the test. Vehicle was used as control.
In the FST, M871 significantly blocked the increased immobility ($p<0.001$) and decreased climbing ($p<0.01$) induced by GAL(1-15). In the OFT M871 also significantly decreased the number of entries ($p<0.001$) and time spent in the center ($p<0.05$) mediated by GAL(1-15).
Down-regulation of GALR2 or GALR1 by siRNA was sufficient to block the effect of GAL(1-15) in behavioural tests. Thus, GAL(1-15) 3nmol lacked effect on the immobility, climbing and swimming time in the FST. The same effect was observed in the number of entries and time spent in the central square in the OFT.
These results indicated that GALR1 and GALR2 are involved in the GAL(1-15) depression- and anxiogenic-like effects suggesting that GAL(1-15) could act through GALR1/GALR2 heteroreceptor complex. These findings may give the basis for the development of novel therapeutic drugs targeting GAL(1-15) system for treatment of depression and anxiety disorders.
This study was supported by Junta de Andalucía CVI6476.

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

SOCIAL STRESS IS AS EFFECTIVE AS THE DRUG ITSELF IN REINSTATING COCAINE-INDUCED PLACE PREFERENCE IN MICE

R. Guerrero[1,2], J. Teruel[1], M.L. Laorden[1,4], M.V. Milanés[1,4], B.R. Ribeiro[3]
[1]Facultad de Medicina. Universidad de Murcia, Murcia
[2]Hospital General Universitario Santa Lucía. Servicio de Farmacia. Cartagena, Murcia
[3]Facultad de Psicología. Universidad de Murcia, Murcia
[4]Instituto Murciano de Investigación Biosanitaria (IMIB)

OBJECTIVES: Drug addiction can be considered as a chronic, recurrent disease characterized by relapse. Relapse can be produced by drug craving that is a subjective feeling experienced by human addicts that motivates drug-seeking behaviors.
It is very difficult to directly evaluate craving in laboratory animals, but it is possible to measure relapse directly if a laboratory animal reinitiates a previously acquired and subsequently extinguished response, which is often referred as reinstatement.
In experimental animals and with many drugs, this can be observed when exposed to drug-associated stimuli or cues, the drug itself, and stressful events.
In the present work we evaluated the role of social stressors (social defeat) and the effect of a priming dose of cocaine on the reinstatement of cocaine-induced conditioned place preference (CPP).
MATERIAL AND METHODS: Adult male C57 mice were conditioned with 25 mg/kg of cocaine. All mice underwent two weekly extinction sessions until the CPP was extinguished. Then, the effects of social stress or a priming dose of cocaine were evaluated on the reinstatement of CPP. Animals performed an agonistic encounter with an isolated mouse or receive a priming dose of cocaine 15 min before the reinstatement test.
RESULTS: Social defeat, in an agonistic encounter with an isolated mouse or a non-contingent priming dose of cocaine, produces the reinstatement of CPP in cocaine-conditioned animals.
CONCLUSIONS: These data demonstrate that social stress is as effective as the drug itself in reinstating cocaine-seeking.

Áreas Temáticas:
1ª: Neurociencia cognitiva y conductual.

Rocío Guerrero Bautista. Correo electrónico: rocio.guerrero3@um.es

EFFECTS OF TIBOLONE ON SOME METABOLIC DISORDERS AND MEMORY IN OVARIECTOMIZED RATS WITH METABOLIC SYNDROME

E. Flores Flores[1], J.G. Huerta Aguilar [1], O. Picazo Picazo [1], J. Espinosa Raya [1]
[1]. Escuela Superior de Medicina. Instituto Politécnico Nacional, México

Objective
To study the effects of tibolone on cognitive impairment and metabolic parameters associated with metabolic syndrome in ovariectomized rats fed with a high-fat diet.
Material & methods
Sprague-Dawley ovariectomized (OVX) rats were randomly assigned to one of the followings groups: a) standard diet (SD) + vehicle ; b) high fat diet (HFD) + vehicle; c) HFD + 0.1 mg/kg of tibolone (TIB); d) HFD + 1 mg/kg of TIB. These data were compared to those derived from sham OVX treated with SD + vehicle. Experimental groups were fed with HFD which supplies a 34% of energy derived from fat (palm oil) for 9 weeks. Two doses of TIB and vehicle were daily administered through oral gavage for 9 weeks. Food intake, body weight, mean arterial pressure, area under the curve (AUC) of glycemia after an i.p. glucose tolerance test, serum lipid profiles and serum insulin were analyzed. Additionally, we determined the effect of TIB on memory with the novel object recognition test, a hippocampus-dependent task.
Results
OVX rats subjected to HFD without TIB treatment exhibited characteristic features of metabolic disorder; hyperglycemia, hypertriglyceridemia, hypercholesterolemia, and lower high-density lipoprotein (HDL) cholesterol levels. In the novel object recognition test, these animals showed a deficit in learning and memory. In contrast, the OVX+HFD rats treated with both doses of TIB (0.1 and 1 mg/kg), showed a significant reduction in serum glucose and triglycerides together with an increase of HDL-cholesterol levels, learning and memory performance.
Conclusions
Our results indicate a possible novel therapeutic strategy to prevent the cognitive impairment associated with menopause and metabolic syndrome.

This study was supported by SIP-IPN. FFE received a grant from CONACYT-Mexico (182576)

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

GALANIN N-TERMINAL FRAGMENT (1-15) INDUCES AN ANXIETY- AND DEPRESSIVE-LIKE BEHAVIOURS IN THE LIGHT/DARK AND TAIL SUSPENSION TESTS

A. Flores-Burgess[1,2], C. Millón Peñuela[1], M. A. Narváez Peláez[1], L. Santín[2], J.A. Narváez Bueno[1], Z. Díaz-Cabiale[1]
[1].Faculty of Medicine, University of Málaga, Spain.
[2].Faculty of Psicology, University of Málaga, Spain.

Galanin N-terminal fragment (1-15) [Gal(1-15)] is involved in mood regulation. We have shown that intracerebroventricular (icv) administration of Gal(1-15) produces a depressive-like behaviour in the forced swim test (FST) and an anxiety-like behaviour in the open field test (OF) in rats. In this work we analyze the effect of Gal(1-15) in two more behavioural tests, the tail suspension test (TLT) and the light/dark test.
In light/dark test we studied during 5 min the latency time for entering the dark box, time spent in the light compartiment, and the latency time for re-entering the light box as parameters indicators of anxiety-like behaviour. In TLT total immobility time was analyzed during 6 min test as parameter indicator of depressive-like behaviour.
Groups of rats (n=5-8) were injected icv with Gal(1-15) 3nmol, a dose effective in FST and OF, or artificial cerebrospinal fluid 15 minutes before the test. Behavioural assessment were conducted with at least one week between tests. Student's *t*-test was used for comparation between experimental groups.
In the light/dark test Gal(1-15) 3nmol significantly reduced the time spent in the light compartiment by 52% ($p<0.05$) and the latency time for entering the dark box by 65% ($p<0.05$). An increased in the latency time for re-entering the light box was also observed ($p<0.05$). This pattern of results reflects an anxiogenic-like effects of Gal(1-15) in this test.
In the TST, the administration of Gal(1-15) 3nmol significantly increased rat immobility by 16% ($p<0.05$) indicating a depressive-like effect.
These results confirm the depressive- and anxiety-like effects of Gal(1-15) in rats. Future therapeutic strategies based on these results could be developed for depression and anxiety disorders. These results are published in PMID: 25522404.

This work has been supported by the Junta de Andalucia CVI6476 and TV3-Marató 090130/31/3.

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

POTENTIATION OF MORPHINE-INDUCED ANALGESIA BY σ_1 RECEPTOR BLOCKADE IN A MODEL OF VISCERAL PAIN IN MICE

A. Artacho-Cordón[1], L. Romero[1], I. Bravo-Caparrós[1], E. Portillo-Salido[2], J.M. Baeyens[1], C.M. Cendán[1].
[1]. Faculty of Medicine and Biomedical Research Centre, University of Granada, Granada.
[2]. Esteve Laboratories, Barcelona

Visceral pain is a major clinical problem whose treatment is challenging. Sigma-1 receptors (σ_1R) play a major role in several models of somatic pain where modulates opioid analgesia. We have confirmed that σ_1R are involved in visceral pain but the opioid modulation in visceral pain is unexplored. Therefore, the purpose of this study was to determine the potential use of σ_1R antagonists associated to opioids as a potential strategy for treating visceral pain. To test this possibility we performed studies in the intracolonic capsaicin-induced visceral pain model. Female wild-type (WT) and σ_1R knockout (σ_1R-KO) mice were used. The tested drugs were: the selective σ_1R antagonists S1RA, NE-100, BD-1063 and BD-1047; the μ-opioid agonist morphine (2 mg/kg); and the opioid antagonists naloxone and naloxone methiodide (a peripherally restricted opioid antagonist). Capsaicin (0.1%) was administered by inserting a fine cannula into the colon via the anus and the number of pain-related behaviors (licking abdomen, stretching and retractions of abdomen) were counted for 20 min and afterwards, the referred mechanical hyperalgesia was recorded using a range of von Frey filaments. In WT mice, the effect of 2 mg/kg of morphine, a dose that produces a moderate reduction of pain behaviors but has no analgesic effects in mechanical hyperalgesia, was enhanced by all the σ_1R antagonists evaluated. Naloxone (1 mg/kg) fully reversed the morphine potentiation induced by the σ_1R antagonists in both types of pain, whereas naloxone methiodide (2 mg/kg) totally abolished the referred pain but only partially the acute pain. None of the σ_1R antagonists potentiated the morphine-induced effect in σ_1R-KO mice. These results suggest that σ_1R antagonists might play a potentiation role in the morphine analgesia providing a therapeutic approach to visceral pain treatment. Supported by Junta de Andalucía (CTS-109 group), Esteve laboratories, FEDER funds, FPU program (IBC) and project SAF2010-15343.

LONG-TERM ANXIETY EFFECTS ARE ASSOCIATED WITH EPIGENETIC DYSFUNCTIONS INDUCED BY INTERMITTENT ETHANOL TREATMENT DURING THE ADOLESCENCE: ROLE OF TLR4 RECEPTORS

M. Pascual [1], J. Montesinos [1], M. Rodríguez-Arias [2], C. Guerri [1]
[1]. Department of Cellular Pathology, Centro de Investigación Príncipe Felipe, Valencia
[2]. Department of Psychobiology, Facultad de Psicología, Universitat de Valencia, Valencia

The adolescence is a vulnerable brain maturation stage to the neurotoxic and behavioral effects of alcohol. Although the mechanisms of these effects are largely unknown, we have shown that ethanol by activating innate immune receptors toll-like receptor 4 (TLR4), induces neuroinflammatory damage and causes cognitive behavioral dysfunction, events which are maintained in adult mice exposed to ethanol during adolescence. Therefore, the aim of this study is to evaluate whether the long-term effects induced by ethanol abuse in adolescent animals are associated with epigenetic chromatin changes and behavioral dysfunctions and whether these events correlate with ethanol-induced inflammatory environment. To answer these questions, wild-type (WT) and TLR4-deficient (TLR4-KO) adolescent mice (PND 30) were treated intermittently with ethanol (3 g/kg) for 2 weeks, followed by 21 days of abstinence during maturation to young adulthood (65 day-old). We show that ethanol treatment activates TLR4 signaling and triggers inflammatory mediators in the adolescent prefrontal cortex. These events were associated with changes in the histone acetylation (upregulation K9 H3, K5 H4 and K12 H4) and methylation (downregulation of 3me-K4 H3). Young adult mice, treated with ethanol during adolescence, showed long-term behavioral effects, as increased both the anxiety-like behavior (open field behavior and elevated plus maze), the rewarding effects (as demonstrated with conditioned place preference) and they also increased the amount of voluntary alcohol intake, using two bottle choice paradigm. These long-term effects in young adult mice were associated with changes in the histone H4 acetylation within the *bdnf* and *fosb* gene promoters, using chromatin immunoprecipitation studies. Interestingly, the elimination of the TLR4 receptors restores both the long-term behavior impairments and histone changes induced by ethanol treatment during the adolescence. These results support the role of the neuroimmune response and TLR4 signaling in the epigenetic and behavioral effects of ethanol in the adolescence. (Supported by SAF2012-33747, RTA-Network, RD12-0028-007).

EPIGENETIC MACHINERY MECHANISMS ARE ALTERED IN 5XFAD MICE

Griñan-Ferré, C[1]; Sarroca, S[2]; Ivanova, A.[1]; Camins, A[1]; Sanfeliu[2], C2; Pallas, M[1]
[1]Department of Pharmacology and Therapeutic Chemistry, Faculty of Pharmacy, IBUB, University of Barcelona and CIBERNED, Barcelona, Spain..
[2]Institut d'Investigacions Biomèdiques de Barcelona, CSIC-IDIBAPS, Barcelona, Spain.

The 5XFAD is an early-onset mouse transgenic model of Alzheimer`s disease (AD) in which amyloid plaques appear shortly after 2 months of age in the cortical layer five and in the subiculum of the hippocampal formation. Although cognitive and molecular alterations have been described in this mouse, there are no studies that focused on epigenetic changes produced as a result of Aβ-42 accumulation and the possible involvement in the different expression of related genes of AD.
For this reason, to identify the influence of amyloid plaques on epigenetic machinery we studied female and male 5XFAD at 2 and 8 months. Under several behavioural and cognition test, we found an impairment in memory and condition in 5XFAD mice in reference to wild type (Morris Water maze, Novel object recognition) and also psicoemotional changes that were worsen with age, evaluated by open field, four hole test and elevated plus maze.
Cognition changes correlated in female mice with alterations on Western blot analysis and gene expression of those related with oxidative stress, inflammation and amyloidogenic pathway as APP and BACE1, but not changes are found in non-amyloidogenic pathway as ADAM10.
In reference to epigenetics, increased levels in DNA methyltransferases family, especially in (DNMT3a and b) were found in the oldest mice, and a similar pattern was found with other methylstransferases as G9a and Setd1a. In addition, similar changes were developed in histone deacetylase 2 (HDAC2) but nor HDAC1. Epigenetically effectors as Hes1 in young mice and NeuroD1 in aged 5XFAD were differentially expressed in reference to age matched wild type mice. All together, these hallmarks presented by the 5XFAD model prompted its use to assess different potential therapeutic interventions based on epigenetic targets.

Acknowledgements: This study was supported by grant SAF2012-39852 from the Spanish MINECO, and the European Regional Development Fund.

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

ENHANCING ASSOCIATIVE MOTOR LEARNING ACQUISITION BY NON-INVASIVE TRANSCRANIAL DIRECT-CURRENT STIMULATION (TDCS) IN ALERT RABBITS

J. Márquez-Ruiz, C. Ammann, A. Gruart, J. M. Delgado-García
División de Neurociencias, Universidad Pablo de Olavide, Sevilla, España.

Transcranial direct-current stimulation (tDCS) is a non-invasive brain stimulation technique that has been successfully applied for cortical-excitability modulation. The aim of this study was to investigate whether tDCS over primary motor cortex (M1) could modulate the acquisition of an associative learning. Six male rabbits were prepared for classical eyeblink conditioning and simultaneous tDCS on M1. tDCS was applied through a disk electrode (0.5 cm^2) over the skull, with a saline-soaked sponge attached to the contralateral ear serving as a counterelectrode. We applied a delay paradigm using a 350 ms tone followed 250 ms from its onset by a 100 ms air puff to the left cornea. The presence of conditioned responses (CRs) was determined by recording the EMG activity of the ipsilateral orbicularis oculi muscle. Anodal and cathodal tDCS (1 mA, ± 2 mA/cm^2, ~30 min) were applied during conditioning session 3 (C3) and session 8 (C8), respectively. The results showed that anodal tDCS during C3 potentiated the acquisition of the classical eyeblink conditioning, whilst cathodal tDCS during C8 did not present a significant effect on the percentage of CRs, nevertheless this tDCS polarity reduced magnitude and increased latency of the CRs. To discard a potential thermal effect associated to tDCS, three male rabbits received anodal and cathodal tDCS on M1 during 60 or 120 min through the disk electrode over the skull. An epidural NTC thermistor was implanted on M1 for brain temperature measurement before, during and after tDCS (±0.029, ±0.29, and ±2.9 mA/cm^2). No significant changes of the epidural temperature were detected. The present work highlights the potential use of tDCS on M1 for modulating the motor learning acquisition and suggests that no thermal effect was induced over the brain cortex when density currents two orders of magnitude higher than those applied in humans were used.

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

1ª: Neurociencia cognitiva y conductual
2ª: Nuevos métodos y tecnologías

BILATERAL tDCS ON PRIMARY MOTOR CORTEX: EFFECTS ON FAST ARM REACHING TASKS

P. Arias[1], Y. Corral-Bergantiños[1], V. Robles-García[1], A. Madrid[1], A. Oliviero[2] and J. Cudeiro[1]
[1]. NEUROcom. Neuroscience and Motor Control Group. Department of Medicine, INEF Galicia and INIBIC. University of A Coruña. Spain
[2]. FENNSI Group, Hospital Nacional de Parapléjicos, SESCAM, Toledo, Spain.

Background: The effects produced by transcranial direct current stimulation (tDCS) applied to the motor system have been widely studied in the last decade, chiefly focusing on primary motor cortex (M1) excitability. However, the effects on functional tasks are less well documented. ***Objective***: This study aims to evaluate the effect of tDCS-M1 on arm-reaching movements (ARM), in a reaction-time protocol. ***Material and Methods***: 9 healthy subjects executed dominant ARM as fast as possible to one of two targets in front of them. Subjects executed ARM (Pre), received tDCS, and then executed a second set, Post, similar to Pre. Each subject received three different types of tDCS, one per session, one week apart: in AR-CL the anode was placed on right-M1, and the cathode on the left-M1; AL-CR reversed the montage; and Sham was applied likewise. Real stimulation was 1mA-10' while subjects were at rest. Reaction times (RT's) and movement times (MT's) were analyzed. ***Results***: RT's were significantly larger at Post only after Sham. Results obtained after real tDCS were not different depending on the montage used, in both cases RT's were significantly reduced compared to Pre. MT's were unchanged. The Sham after-effects were compatible with expressions of fatigability, which was prevented when using real stimulation. ***Conclusion***: We conclude that tDCS might have a role in optimizing cognitive processing and reducing fatigability during the execution of fast motor tasks.

1st: Cognitive and Behavioral Neuroscience
2nd: Systems Neuroscience

COGNITIVE FLEXIBILITY AND GRAMMATICAL COMPREHENSION IN AMNESTIC MILD COGNITIVE IMPAIRMENT PATIENTS

R. López-Higes [1], J. Mª Prados [1], D. Del Río [1], M. Galindo [1], I. Nieto [1], Mª. T. Martín-Aragoneses [2], B. Álvarez-Crespo [3], y A. García-Martín [3]
[1]. Departamento de Psicología Básica II. Universidad Complutense de Madrid.
[2]. Departamento MIDE II. Facultad de Educación. UNED. Madrid.
[3]. Centro de Prevención del Deterioro Cognitivo. Madrid Salud.

Mild cognitive impairment (MCI) is a syndrome susceptible of evolution. So although it may initially affect only memory; attention, executive functions or language could also be impaired over time. The main objective of this study was to analyze the role of cognitive flexibility and processing speed on sentence comprehension of patients with the amnestic type of MCI. For this purpose, we used the Trail Making Test (TMT), as well as the ECCO-Senior test (*Exploración Cognitiva de la Comprensión de Oraciones*), which assesses comprehension of sentences varying in complexity by using a picture-sentence verification task. To explore the cognitive status of all participants we used Mini-Mental State Exam. The Weschler Memory Scale-III Word List was also used as a measure of episodic memory. By means of cluster analyses, an initial sample of 51 patients with amnestic MCI profile was reduced to two subgroups differentiated by their performance in the TMT part B (time and correct responses). The two clusters had equal size (n = 19) and their members shared the same educational characteristics. In addition, there were no differences between them in mean age, nor in general cognitive status or in episodic memory level. The results revealed significant differences between the two clusters of patients in sentences fitted to the syntactic canonical word order and in sentences with only one proposition, that is, in the simplest sentences from the syntactic point of view and with the lowest propositional density. Differences between groups were observed in syntactic foils (items in which the thematic role assignment in the sentence are reversed in the picture) in the sentence types previously mentioned. It seems that the differences between clusters reside in the verification stage in which the meaning of the sentence is compared with the picture, and not in the thematic role assignment process.

1. Cognitive and Behavioral Neuroscience
2. Development

CHANGES IN THE PLASTICITY OF DOPAMINERGIC REWARD PATHWAYS AFTER MATERNAL SEPARATION WITH EARLY WEANNING

E. Martínez-Laorden[1,2], I. Gracia-Rubio[3], M.L. Laorden[1,2], O. Valverde[3,4], M.V. Milanés[1,2]
[1]Facultad de Medicina. Universidad de Murcia, Murcia, Spain
[2]Instituto Murciano de Investigación Biosanitaria (IMIB) , Murcia, Spain
[3]Neurobiology of Behavior Research Group (GReNeC), Department of Experimental and Health Sciences, Univertitat Pompeu Fabra, Barcelona, Spain
[4]Neuroscience Research Program. IMIM (Hospital del Mar Research Institute), Barcelona, Spain

Early life experiences play a role in shaping brain and behavior. Adverse events during childhood are a main vulnerability factor to develop mood disorders in the adulthood. Brain is particularly sensitive to stress during early childhood, due to lack of maturity and to the occurrence of plasticity phenomena. One of the key regulators for the maintenance of dopaminergic system are Nurr1 and Pitx3, which are crucial for the expression of the set of genes involved in DA metabolism (Th, Dat, Vmat2 and Drd2) in the mesolimbic pathway and are also associated with addiction pathology. In the present study we have investigated the changes in the dopaminergic markers DAT, DA2R and DA turnover in the NAc as well as Pitx3 and Nurr1 in the VTA.
The day of birth each litter of CD1 mice were assigned to three experimental groups: a) Maternal separation with early weaning (MSEW), b) standard nest (SN) and c) Communal nest (CN). During adolescence, mice were treated with cocaine (3 or 15 mg/kg ip) or saline. Seven days later mice were sacrificed and tissues of interest were dissected using a punching device. Nurr1, Pitx3, DAT and DA2R were determined using Western-bot. DA turnover were measured by HPLC.
MSEW mice showed a decrease in Nurr1 and Pitx3 in the VTA after cocaine (15 mg/kg) treatment VS saline. In parallel, an increase in DAT, DA2R and the turnover of DA was seen in the NAc. In addition, MSEW treated with saline (control) showed higher levels of Nurr1 and Pitx3 VS SN and CN saline-treated groups.
These data would indicate that early maternal separation and cocaine treatment induce changes in the dopaminergic reward pathways plasticity, which might be part of the neurobiological mechanisms involved in the development of long-lasting emotional alteration during adolescence and the adulthood.

1ª: Neurociencia cognitiva y conductual.

ROLE OF OMEGA-3 FATTY ACIDS AS VIABLE BIOMARKERS FOR A DEPRESSIVE-LIKE STATE IN A MURINE MODEL

K.R. McGreevy[1]; A. Fontán-Lozano[1]; S. Gradari[1]; J.L. Trejo[1]
1. Instituto Cajal, CSIC, Madrid

Major Depressive Disorder (MDD) causes premature deaths resulting from suicide, and is among the 10 leading causes of worldwide loss of disability-adjusted life years (DALY's). Many studies in humans have shown that depressive disorders are associated with alterations in serum polyunsaturated fatty acids (PUFAs) composition, with reduced omega-3 fatty acids and increased omega-6/omega-3 ratio. Furthermore, there is a large amount of evidence which show that omega-3 fatty acids play a key role in proper brain function and are involved in the regulation of emotional states. However, omega-3 fatty acids' possible role as biomarkers has yet to be determined. Identifying peripheral biomarkers of MDD would be of great help for diagnosis and in the prediction of treatment response. Additionally, the mechanisms by which omega-3 fatty acids could exert their influence remain unknown. Adult Hippocampal Neurogenesis (AHN) has been associated with mood disorders. Many studies have revealed a pro-neurogenic effect on chronic administration of antidepressants. The aim of the study was to evaluate the possible role of omega-3 fatty acids as viable biomarkers for a depressive-like state, as well as assessing the rate of neurogenesis in a murine model.

In order to induce a depressive-like state, all experimental animals underwent the Forced Swim Test (FST) following a two day protocol. To assess depressive-like behaviour and anxiety-like behaviour, the Tail Suspension Test (TST) and the Elevated Plus Maze (EpM) test were performed. To analyze the relation between levels of omega-3 fatty acids and the behavioural tests, blood was collected before and after the FST was carried out.

We analyzed the correlation between omega-3 fatty acids' serum levels with the depressive like state of the animals, together with the rate of AHN. The results of the behavioural tests, quantitative data tables and representative 5-Bromo-2´-Deoxyuridine's (BrdU) photographs are shown.

1ª: Neurociencia cognitiva y conductual
2ª: Neurobiología del Desarrollo

REGULAR ADMINISTRATION OF NON-CONTINGENT INTRACRANIAL SELF-STIMULATION AND LEARNING AND MEMORY IN RATS.

N. Biosca Simon, C. Tapias Espinosa, I. Morgado Bernal, P. Segura Torres
Facultat de Psicologia. Institut de Neurociències. Universitat Autònoma de Barcelona.

Objectives: Deep brain stimulation can modulate memory in some pathological conditions, such as Alzheimer's disease, in humans. Research with intracranial self-stimulation (ICSS) in rats can give clues about the best stimulation patterns for such therapeutic use in humans. The present study explores for the first time the effects of regular administration of non-contingent intracranial self-stimulation upon two paradigms of explicit and implicit memory in rats. We hypothesize that this kind of non-contingent stimulation generates a sustained state of the nervous system activation able to facilitate different kind of learning and memory tasks.
Materials and methods: To test our hypothesis, three daily ICSS sessions were conducted (8:00am/13:00pm/18:00pm), inserted between training sessions. Twenty-six male rats were distributed in a group undergoing ICSS for five days and a Control group. Two tasks were used to test the effects of ICSS non-contingent on training: two way active-avoidance (TWAA) and Morris water maze (MWM).
Results: Regular and non-contingent administration of ICSS was not effective to facilitate neither learning acquisition nor retention in TWAA and MWM. These results disagree with our previous ones with single post-training ICSS administration. Moreover, ICSS showed a tendency to difficult MWM. It is noteworthy the significant statistical differences between groups in variables related to anxiety in MWM, such as velocity and thigmotaxis behaviors, being the ICSS group the most anxious. The fact that MWM task requires a greater level of attention to surrounding keys than TWAA could explain the interference of ICSS in present conditions in this task.
Conclusions: We believe that the lack of effectiveness of the present ICSS treatment for improving learning and memory is due to the high level of anxiety that the pattern of ICSS that we have used generates in the stimulated subjects. News experiments must be designed to confirm this effect.

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

BRAIN TRAINING COGNITIVE IMPROVEMENTS REFLECT CHANGES IN FUNCTIONAL CONNECTIVITY

J.A. Pineda-Pardo[1], J. Contreras [2], J. Goñi [2], K. Martínez [3], F.J. Román [4], R. Colom [4]
[1] CINAC, HM Puerta del Sur, Hospitales de Madrid, Móstoles, and CEU-San Pablo University, Madrid.
[2] Center for Neuroimaging and Indiana Alzheimer Disease Center, Indiana University, Indianapolis, United States of America
[3] Department of Biological and Health Psychology. Universidad Autónoma de Madrid, Madrid
[4] Hospital Gregorio Marañón, Madrid

Objectives. There is strong interest in unraveling the biological mechanisms that are beneath cognitive training. We know that brain training induces functional connectivity (FC) changes in the brain (Martínez et al. 2012). Here we study changes in FC within and between cognitive systems that occur after cognitive training, and how these changes relate to psychometrics.
Materials & Methods. The sample comprised 46 young female subjects. Half of the sample received brain training consisting of an adaptive N-back task during three months. MRI acquisitions were performed before and after the training periods. Resting-state fMRI was pre-processed using classical pipelines. Linear correlations were estimated between average time-series using Shen's parcellation (Shen et al. 2013). Regions were later grouped following Yeo's cortical parcellation of 7 resting-state networks (Yeo et al. 2011), including also a subcortical and a cerebellar network.
Average FC was estimated intra- and inter-network, and z-scores compared to the basal sample were obtained. Changes in intra- and inter-network FC between the training and non-training groups that differed significantly were correlated with changes in psychometric evaluation [fluid intelligence (Gf), crystallized intelligence (Gc), working memory (WM), and attention (ATT).
Results. We observe that intra-FC in the ventral attention network decreased in the training group compared to the basal ($p<0.05$). These changes correlated with WM ($R_{intra\_Vatt}=-0.58$; $p<0.05$) indicating that the lower the connectivity within this network, the better performance of the task.
Inter network FC of ventral attention with visual and somatomotor network increased and decreased respectively in the training group ($p<0.05$), changes that correlated with Gc ($R_{inter\_Vatt\&Vis} = 0.59$; $R_{inter\_Vatt\&Som} = -0.52$; $p<0.05$). In addition we found significant changes in inter-network FC that did not correlated with intelligence measures: decrease (increase) of inter-network FC between limbic-subcortical (visual-subcortical).
Conclusions. This study provides biological evidence to cognitive changes caused by brain training. FC changes that are here reported could aid to quantify objectively the benefits of cognitive training.

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

THE BASAL FOREBRAIN CHOLINERGIC PATHWAY IS MODULATED BY CB_1 RECEPTORS

Moreno M., Madariaga M., Llorente A., González de San Román E., Lombardero L., Manuel I., Rodríguez-Puertas R.
Department of Pharmacology, Faculty of Medicine and Odontology. University of the Basque Country (UPV-EHU), Spain.

The selective vulnerability of the basal forebrain cholinergic pathway (BFCHOL) is responsible for most of the clinical alterations in learning and memory processes that are characteristic of the Alzheimer's disease (AD). The muscarinic receptor (MR) antagonism, e.g. using scopolamine, (Scop), is used as a memory impairment model in rodents. Biphasic effects induced by low or high doses of cannabinoids have been reported in relation to both cognitive functions and hippocampal acetylcholine (ACh) release.
The aim of the present study was to evaluate the effects on spatial and working memories induced by a subchronic treatment with WIN55,212-2 at a low dose in the Scop model of learning and memory deficit. The brains were dissected 48h after the last administration for [^{35}S]GTPγS autoradiographical evaluation of the functional activity of both CB_1 rand MR in the BFCHOL pathway.
The administration of WIN55,212-2 or the vehicle did not modify the learning and memory trials in the Barnes maze test, but a significant effect of WIN55,212-2 as memory-saver of impairment induced by Scop was recorded when the time spent in the target quadrant was measured (time in target quadrant: WIN + Scop: 78,7± 13 sec vs VEH + Scop: 45,6 ± 3 sec).
The *in vitro* experiments showed that the CB_1 activity was higher in the basal forebrain of WIN55,212-2-treated rats compared to vehicle (WIN: 553 ± 93 % stimulation over basal vs VEH: 273 ± 44 %) and at the layer VI of the motor cortex (WIN: 1201 ± 65 % vs VEH: 730 ± 86 % $p<0.05$, n=11). Moreover, the MR activity was also higher with the WIN treatment in the BFCHOL pathway. (*nbM*: VEH: 204 ± 26 % vs WIN: 366 ± 42 % of stimulation; *Hippocampus*: VEH: 112 ± 27. vs WIN: 219 ± 35 nCi/g t.e., $p<0.05$; n=11) A moderate stimulation of the eCB system at the BFCHOL pathway could have protective effects on the MR-mediated impairment in spatial memory.

This work was supported by ISCIII (PI10/01202)-co-funded by European Research development Foundation and Basque Government grant (IT584-13) to the "Neurochemistry and Neurodegeneration" Research group. To the SGIKER core research facilities of the UPV/EHU. The support by the J.B. Gangoiti foundation to M. Moreno is gratefully acknowledged

1. Neurociencia cognitiva y conductual
2. Trastornos y reparación del sistema nervioso

OX_1R SELECTIVE ANTAGONIST SB-334867 MODIFIES ERK ACTIVATION IN HIPPOCAMPUS AND AMYGDALA IN SELF-STIMULATED RATS.

Soleil García-Brito[1], Daniel Rico[2], Laura Aldavert-Vera[1], Gemma Huguet[2], Pilar Segura-Torres[1], Elisabet Kádár[2].
[1] Facultat de Psicologia. Institut de Neurociències. Universitat Autònoma de Barcelona.
[2] Facultat de Ciències. Departament de Biologia. Universitat de Girona.

Objectives: Intracranial self-stimulation (ICSS) in the lateral hypothalamus (LH) has been confirmed as a treatment capable of facilitating learning and memory in rats. The Orexin-producing neurons in the LH project throughout the brain, including the hippocampus (HPC), amygdala (AMG) and prefrontal cortex (PFC), where Orexin A and B (OX1/OX2) receptors are present. Ox1 is a neuropeptide implicated in reward behaviours, such as ICSS, and it affects neuroplasticity in the HC through the activation of long-term plasticity signal pathways. This study aims to determine the effect of ICSS on the activation of the memory and plasticity-related protein ERK42-44 and the implication of Ox1 in this effect, by administering the OX1 receptor selective antagonist SB-334867.
Materials and methods: Four experimental groups (ICSS+SB, ICSS, SB and Sham) were analyzed. Extraction of HPC, AMG and PFC was performed 30 min after the ICSS (or Sham) session. Levels of phosphorylation of ERK42-44 (pERK42-44) were determined by Western Blot and normalizing for ERK42-44 levels.
Results: In the HPC, ICSS significantly increased pERK42 level (P= .02) especially in SB condition (ICSS+SB>SB; P= .009) while SB does not have any effect. In the AMG, we found a significant interaction between SB and ICSS factors (P= .014): SB increases significantly pERK42 level only in the non-ICSS condition (SB>Sham; P= .002) and ICSS eliminates SB effects (ICSS+SB=ICSS; ICSS+SB<SB; P= .013). However, no effects were found between ICSS and sham groups. In the PFC, activation of ERK-42 was not observed after ICSS and/or SB. Similar results were obtained in pERK44 analysis for all regions.
Conclusions: Results suggest that while the ICSS on its own does not affect pERK42-44 level, ICSS blocks the up-regulating effect of SB-334867 on pERK42-44 in the AMG. On the contrary in the HPC the selective blocking of OX1 receptor seems to potentiate the ICSS activation of ERK42-44.

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

PHYCOPHYSIOLOGICAL CORRELATES OF MULTI-DIGIT ADDITIONS PROCESSING IN HIGH MATH-ANXIOUS INDIVIDUALS

M.I. Núñez-Peña [1], M. Suárez-Pellicioni [1]
[1]. Facultad de Psicología. Universidad de Barcelona, Barcelona

In the present study we investigated the time course of neural processing of multi-digit additions in high- (HMA) and low-math anxious (LMA) individuals. Seventeen HMA and 17 LMA individuals were presented with two-digit additions and were asked to perform a verification task. Behavioral data showed that HMA were slower and more error prone than their LMA peers, and that incorrect solutions were solved slower and less accurately that the correct ones. Moreover, HMA individuals tended to need more time and commit more errors when having to verify incorrect solutions than correct ones, as compared to their LMA peers. ERPs time-locked to the presentation of the addends (calculation phase) and to the presentation of the proposed solution (verification phase) were also analyzed. In the two phases, a P2 component of larger amplitude was found for HMA individuals as compared to their LMA peers. Moreover, in the verification phase, LMA individuals showed a larger late positive component (LPC) for incorrect solutions at parietal electrodes as compared to their HMA counterparts. Because the P2 component is considered to be a biomarker of the mobilization of attentional resources towards emotionally negative stimuli, these results suggest that HMA individuals might invest more attentional resources during the arithmetical task than their LMA peers. Moreover, the smaller LPC shown by HMA individuals when verifying incorrect solutions suggests that these solutions might have been more plausible for them than for LMA ones.

1. Neurociencia cognitiva y conductual
2. Historia, Docencia, Divulgación y Ética

GENERATIVE MODEL ESTIMATION THROUGH EXPECTATION-MAXIMIZATION ALLOWS INSIGHT INTO PERCEPTUAL ACCUMULATION MECHANISMS

Alexandre HYAFIL [1,2], Gustavo Deco[1], Jordi Navarra[2], Rubén Moreno-Bote[1,2]
[1]. DTIC, Universidad Pompeu Fabra, Barcelona
[2] Parc Sanitari Sant Joan de Déu, Barcelona

The mechanisms at play when the brain integrates sensory information over time to reduce uncertainty over percepts have been the focus of intensive study in the last two decades. Despite this effort, it has remained virtually impossible to experimentally tease apart distinct models for perceptual accumulation. This is largely due to the fact that in these models stochastic processes (either sensory or internal noise) play a large role and thus models only loosely constrain experimental data. Here we introduce a novel method based on generative models that reduces that part of stochasticity to its minimum and hence allows digging much more precisely into accumulation mechanisms. The method is applied in a context where perceptual information provided by each sensory sample can be quantified. Moreover, the method is novel in providing a common generative model for both response choices and reaction time. The method is based on parameter estimation through an Expectation-Maximization algorithm in generative models that combine three cognitive components: an accumulation stage, a decision stage and a post-decision stage. We first tested the method on synthetically generated stimuli, showing model parameters could be accurately estimated. Then the method was applied to a speeded-reaction time task where subjects must judge the overall orientation of successive visual stimuli. Results very clearly arbitrate between different variants of the distinct model components both in terms of log-likelihood and fitting to experimental data characteristics. Notably we show that the decision threshold remains constant throughout stimulus presentation while decision noise increases sub-linearly, in line with diffusion-to-boundary hypothesis. Overall, the results open a promising path towards understanding of the refined mechanisms of perceptual accumulation. More generally, they illustrate the power of fitting generative models of behavior to human psychophysics data for unveiling cognitive mechanisms in action.

1ª: Neurociencia cognitiva y conductual
2ª: Nuevos métodos y tecnologías

DEPRESSION-RELATED BRAIN NEUROPLASTICITY IN 5-HT$_4$ KNOCKOUT MICE

Amigó J[1,2,3], Díaz A[1,2,3], Compan V[4,5,6,7], Pilar-Cuellar F[1,2,3], Pazos A[1,2,3], Castro E[1,2,3].
[1]Instituto de Biomedicina y Biotecnología (IBBTEC) (UC-CSIC-SODERCAN), Santander, Cantabria, Spain
[2]Departamento de Fisiología y Farmacología. Universidad de Cantabria, Santander, Spain
[3]CIBERSAM, CIBER de Salud Mental, Instituto de Salud Carlos III, Madrid, Spain
[4]CNRS, UMR-5203, Institut de Génomique Fonctionnelle, Montpellier, F-34094, France
[5]INSERM, U661, Montpellier, F-34094, France
[6] Universités de Montpellier 1 & 2, UMR-5203, Montpellier, F-34094, France
[7]Université de Nîmes, Nîmes, F-30000, France.

5-HT$_4$ receptors appear to be involved in the neurobiology and treatment of affective disorders. Indeed, it has been reported that the partial agonist of 5-HT$_4$ receptor RS67,333 exhibits antidepressant properties with a faster onset of action than classical antidepressants. However, nothing is known about the role of this receptor in the modulation of those neuroplasticity pathways linked to depression pathophysiology and antidepressants effects.

This work was aimed to evaluate, in 5-HT$_4$ receptor knockout (KO) mice, the expression of neuroplasticity biomarkers (BDNF, TRkB and β-catenine) in brain areas implicated in the modulation of mood by using in situ hybridization.

5-HT$_4$ receptor KO mice showed a significant decrease of BDNF mRNA expression in the CA3 of the hippocampus (% red= 25.7± 8.7, $p<0.05$) as well as in the dentate gyrus (% red= 22.7± 11.6,$p=0.051$) with no changes in the others brain areas analyzed. TrkB mRNA levels were also lower in the hippocampus, with a reduction range between 12.3-18.4% among different hippocampal areas. In addition, a significant reduction of TrkB mRNA expression was also observed in other limbic structures such as the amygdala and the pyriform cortex (% red= 30.8±9.2 and 19.9±4.2 respectively; $p<0.01$). By contrast, 5-HT$_4$ receptor KO mice showed an increase in β-catenine expression levels in the CA3 hippocampal field (+26.2 ± 5.5% compared to WT mice; $p<0.01$).

In conclusion, we demonstrate the presence of changes on depression-related brain neuroplasticity biomarkers in 5-HT$_4$ receptor KO mice. Therefore, it could be speculated that the regulation of neurotrophic factors is one of the molecular mechanisms underlying the antidepressant effects induced by activation of 5-HT$_4$ receptors, though further pharmacological studies are needed.

Supported by Ministerio de Ciencia (SAF2007-61862), Ministerio de Economía y Competitividad (SAF2011-25020), ANR-SERFEED grant.

MICROGLIA IMPAIR NEW OBJECT INFORMATION STORAGE BY INHIBITION OF NEURONAL ACTIVITY-DEPENDENT GENE EXPRESSION

F.J. Aguilar-Montilla, Á.M. Carrión.
Facultad de Ciencias Experimentales. Universidad Pablo de Olavide, Sevilla.

Objectives

Synaptic plasticity changes and synthesis of new molecules are commonly used to explain long-term memory (LTM) formation in processes called consolidation and reconsolidation. Consolidation refers to the new information storage while reconsolidation is the modification of a lasting memory to be enhanced or updated. The most studies are designed to elicit the role of neurons in these processes. However the role of microglial cells in the different phases of the memory formation is poorly studied so we focus on their involvement.

Material & methods

To study the microglial role in learning, consolidation and reconsolidation we have used novel object recognition [NOR] protocols in mice under effect of minocycline, a tetracycline-analog antibiotic that can inhibit microglial activation and proliferation. Neuronal immediately-early gene, *c-Fos* and *Egr1* are associated to cognitive processes in hippocampus so we have tested the effect of minocycline administration after training and reactivation sessions on their expression.

Results

Systemic administration of minocycline around training task only disrupted long-term memory formation. Moreover, minocycline impaired reconsolidation of NOR when reactivation included novelty. Furthermore, minocycline administration just after training or reactivation with novelty sessions decreased immediately early gene expression, especially in dentate gyrus and CA3 field.

Conclusions

All these results suggest that microglia play a key regulating role in neuronal activity-dependent gene expression induced by novelty, and as consequence, microglial inhibition affects to new memory formation and updating of stored memory.

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

CBP DEPLETION IN THE MATURE BRAIN CAUSES ROBUST HISTONE DEACETYLATION AND TRANSCRIPTIONAL DOWNREGULATION, BUT ONLY RESULTS IN MILD COGNITIVE DEFICITS

M. Lipinski [1], J.P. Lopez-Atalaya [1], L.M. Valor [1], A. Medrano-Fernandez [1], D. Guiretti [1], M. Alcaraz-Iborra [2], A. Barco [1]

[1]. Instituto de Neurociencias de Alicante (Universidad Miguel Hernández-Consejo Superior de Investigaciones Científicas), Sant Joan d'Alacant, 03550, Alicante, Spain

[2]. Universidad de Almería, Almería 04120, Almería, Spain

CREB-binding protein (CBP) is a nuclear protein that plays an important role in transcriptional regulation. CBP together with its paralog P300 are one of the most essential lysine acetyltransferases (KAT) in the mammalian brain, targeting both histones and non-histone proteins. Loss-of-function hemizygous mutations in CBP are the cause of a severe intellectual disability known as Rubinstein-Taybi syndrome (RSTS). CBP heterozygous depletion in mice mimics the symptoms of RSTS, establishing mice as a good model for animal study of this disease. Although undoubtedly essential for the proper function of the central nervous system, the importance of the CBP for the adult versus developing brain is still unclear. Here, we report that the induced and neuronal-restricted knockout of this protein in the mature forebrain (icKO-CBP) results in a strong deacetylation (>40%) of the histone H2B tail, and a significant decrease in the acetylation of the canonical target residue for CBP's KAT activity H3K27 as well as of a number of non-histone substrates. Genome-wide analysis of H2B acetylation shows that the deacetylation is broadly distributed through the genome. In contrast, microarray analysis revealed a highly specific transcriptional downregulation. Interestingly, the comparison of genomic profiles for acetyl-H2B between genotypes revealed a large overlap between genes displaying a significant reduction in the acetylation of H2B in the hippocampus of the icKO mice, and those downregulated at the transcriptional level. In this subset of genes, we identified a strong neuronal footprint with significant enrichment for synapse, dendritic spine and behavior related genes. These transcriptional changes may underlay the mild cognitive deficits observed in icKO-CBP mice. Overall, the comparison of different CBP deficient strains allowed us to dissect the developmental and adult components of RSTS, suggesting that CBP and histone acetylation is more important during development than in terminally differentiated neurons of the adult brain.

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

AN ANIMAL MODEL OF DEPRESSION WITH IMPAIRED HIPPOCAMPAL PROLIFERATION: WHAT IS THE SEROTONERGIC SYSTEM TRYING TO SAY?

E. Garro-Martínez[1,2,3], E. Castro[1,2,3], F. Pilar-Cuéllar[1,2,3], A. Pazos[1,2,3]
[1]Instituto de Biomedicina y Biotecnología de Cantabria (IBBTEC) (UC-CSIC-SODERCAN), Santander, Spain
[2] Departamento de Fisiología y Farmacología, Universidad de Cantabria, Santander, Spain
[3] CIBERSAM, CIBER de Salud Mental, Instituto Carlos III, Spain

Objectives. Several hypotheses have been postulated in order to elucidate the pathophysiology of depression. Among them, the implication of the serotonergic system and the impairment of neurogenesis in the adult brain are widely accepted. We have evaluated in an animal model (β-catenin induced knock-out in hippocampal progenitor cells) with impaired hippocampal proliferation the depressive/anxious-like behavior and the changes associated to the serotonergic system.
Material and methods. Conditional inducible mice (KO) were crossed with a mouse expressing CreERT under the control of the astrocyte-specific glutamate transporter (GLAST) promoter inducible by tamoxifen administration for 5 days (1 mg/day; i.p.), producing β-catenin ablation in progenitor cells of the subgranular zone (SGZ) of the hippocampus. After 4 weeks of tamoxifen administration, we evaluated the effect on behavior (novelty suppressed feeding, NSF), and serotonergic system functionality (5-HT_{1A} and 5-HT_4 receptor subtypes).
Results. β-catenin KO animals presented an increased latency to feeding (403.7±58.5 s) compared to WT animals (221.5±32.3 s; $p<0.01$) in the NSF. Regarding changes on the serotonergic system, the functionality of cortical 5-HT_{1A} receptors (8-OH-DPAT induced stimulation of [^{35}S]GTPγS binding assay) was decreased in KO mice vs WT counterparts (Emax= 122.4±3.6% vs 155.2±9.8%, respectively; $p<0.05$). Moreover, a desensitization of striatal 5-HT_4 receptors (zacopride-induced cAMP accumulation assay) was also measured in KO (73.7±4.2%) compared to WT (154.0±12.1%, $p<0.001$) mice . The stress-induced hyperthermia (SIH) elicited a higher increase in the temperature of the WT vs KO, (1.06±0.18ºC vs 0.46±0.15ºC; $p<0.05$), suggesting changes associated to 5-HT_{1A}/5-HT_{1B} receptor functionality.
Conclusions. Our results suggest that β-catenin knock-out mice represent an animal model of depression which exhibits a depressive-like behavior and alterations parallel to changes reported in post-mortem human samples.
Supported by CYCIT SAF2007-61862 y SAF2011-25020.

CORTICAL CONTROL OF THE ANTISACCADIC RESPONSE

M. Escudero, I. Cordones
Facultad de Biología, Universidad de Sevilla, Sevilla

The antisaccade task is a simple but cognitively demanding oculomotor paradigm that requires the subject to inhibit a visually-directed saccade and to perform a saccade to the mirror location. Although it is known that the frontal lobe lesion impairs the ability to produce antisaccades and that the parietal lobe is involved in their control, very little is known about their cortical dynamics. In the present study, EEG activity and eye movements were recorded in 21 healthy subjects during prosaccadic, antisaccadic and no-go tasks in a mixed experiment. Subjects seated in front of a CRT monitor and fixed their gaze on a central color square (S1) for a random period of 1900-2500 ms. The color of S1 was the cue for the task in each trial. Afterwards, S1 disappeared for 370 ms (gap) and a black dot (S2) appeared at 8 deg on the right or the left side at random. Lateralized readiness potential showed a negativity on the contralateral parietal cortex during the three tasks in the 120-200 ms interval after S2. This activity was coincident with a synchronization in the 5-10 Hz band. The negativity became ipsilateral in antisaccadic and bilateral in the no-go task from 200 ms. In the prosaccadic task, the period between 150 and 220 ms was further characterized by a synchronization in the gamma-band in the parietal cortex contralateral to the stimulus. By contrast, in the antisaccadic task, gamma-band activity was located in the fronto-central cortex ipsilateral to the stimulus. These results suggest that antisaccades are top-down controlled by the frontal cortex that becomes synchronized at 5-10 Hz, inducing the mechanism underlying the vector of inversion in the parietal cortex. By contrast, prosaccades are exclusively controlled by the parietal cortex.

Support: SAF-2009-10560, P09-CVI-4712 and FEDER Funds.

COGNITIVE RESERVE INFLUENCES THE ALLOCATION OF READING TIME IN ELDERS.

D. del Río [1], R. López-Higes [1], J. Mª Prados [1], M. Galindo [1], I. Nieto [1], Mª. T. Martín-Aragoneses [2], B. Álvarez-Castro [3], de Andrés Mª. E. [3]
[1]. Departamento de Psicología Básica II. Universidad Complutense de Madrid.
[2]. Departamento MIDE II. Facultad de Educación. UNED. Madrid.
[3]. Centro de Prevención del Deterioro Cognitivo. Madrid Salud.

Cognitive Reserve is a key concept in modern cognitive neuroscience. It is based on the fact that previous life outcomes shape the effect of neuropathology and aging-related changes on cognitive performance. However, little studies have focused on the influence of Cognitive Reserve over language comprehension. Here we investigate the relationship between cognitive reserve and sentence reading in a group of healthy seniors. Elders seem to strategically allocate resources to the closure of syntactic clauses, which might help to compensate for difficulties associated to syntactic complexity. Forty healthy elders attending the Service for the Prevention of Cognitive Impairment at the Madrid City Council (mean age 71.65 ±4.65) volunteered to participate in the study. Exclusion criteria included a MMSE score lower than 26 and any previous or current psychiatric or neurological disease, including Dementia or Mild Cognitive Impairment. Cognitive Reserve was assessed using the Cognitive Reserve Questionnaire (Valls et al., 2011). Participants performed a self-paced reading task including sentences containing syntactically simple embedded subject-relative clauses and two kinds of syntactically more complex embedded object-relative clauses. Residual reading times per word at critical sentence regions, once lexical length was regressed out, were fitted to linear mixed effect models considering sentence complexity and Cognitive Reserve as fixed effects. Crucially, the model including a sentence complexity X Cognitive Reserve interaction showed a significant better performance at the closure region of the relative clause than a simpler additive model ($p < 0.01$). Interestingly, model parameters showed that higher Cognitive Reserve scores were associated with higher reading times for syntactically complex clauses. Results thus suggest that Cognitive Reserve allow for a better allocation of processing resources at the closure of complex syntactic clauses in order to compensate for age-related limitations in on-line sentence processing.

1. Cognitive and Behavioral Neuroscience
2. New methods and technologies

ROLE OF THE HISTONE H3 DEMETHYLASE KDM5C IN NEURONAL FUNCTION

M. Scandaglia, A. Medrano-Fernandez, R. Olivares, A. Barco.
Instituto de Neurociencias de Alicante (Universidad Miguel Hernández-Consejo Superior de Investigaciones Cientificas), Sant Joan d'Alacant, 03550, Alicante, Spain

Mutations in the gene encoding KDM5C are the cause of X-linked intellectual disability (XLID), Claes-Jensen type (Gonçalves et al., 2014). This gene encodes a lysine demethylase that acts on the di- and trimethylation of lysine 4 of histone H3 (H3K4me2/3). KDM5C is also known to interact with other proteins that play an important role regulating neuronal gene expression, such as the RE1-Silencing Transcription factor (REST). In this study, we aim to characterize the first mouse model for XLID, Claes-Jensen type. The analysis of $Kdm5c^{-/y}$ (KO) mice, in which the protein is missing in the whole organism, revealed a number of phenotypes that support the suitability of this strain to model the human syndrome. Thus, these mice show small body and brain size, and structures such as the corpus callosum and cerebellum seem underdeveloped. In addition, the mice show emotional and cognitive abnormalities in the fear conditioning and the Morris water maze tests, as well as important differences in the response to the painful stimulus, in terms of jumping and vocalization. In parallel, we are also in the process of characterizing pCaMKIIα-ER^{T2}Cre:: $Kdm5c^{flox/y}$, in which gene ablation takes place specifically in forebrain neurons of the adult mouse after tamoxifen treatment. This strain, referred to as icKO-KDM5C, show a milder impairment in learning and memory suggesting that the protein loss in the principal neurons of the adult forebrain is likely contributing to deficits in working memory. Importantly, the comparison of these two KDM5C deficient strains will allow us to dissect the developmental or adult components of the syndrome. Finally, we also aim to develop possible tools to recover wild-type protein function and rescue the disease-associated phenotype using lentiviral transduction. Towards this goal, experiments in cell cultures have shown that lentivirus expressing the wild-type protein efficiently increases di- and trimethylated H3K4.

1. Neurociencia cognitiva y conductual
2. Trastornos y reparación del sistema nervioso

PHOSPHATASE DUSP6 REGULATES HIPPOCAMPAL ERK1/2 ACTIVATION AND CONTEXTUAL MEMORY

C. Alcon Franch and C. Sindreu
Pharmacology Unit, University of Barcelona School of Medicine, Barcelona

Transient activation of the Erk/MAP kinase pathway in the hippocampus is essential for memory consolidation and long-term neuroplasticity. Although negative regulation of Erk1/2 may be just as important for memory as stimulatory mechanisms, little is known about the hippocampal phosphatases that directly inactivate Erk1/2 during memory formation. Here we show that Dusp6 (a dual-specificity Y/T-phosphatase) is a neuron-specific cytosolic protein enriched in pyramidal dendrites of the hippocampus. Notably, binding between Dusp6 and Erk1/2 increased during contextual memory formation, coinciding with the trough of Erk1/2 phosphorylation. Accordingly, Dusp6 knockout (KO) mice showed increased levels of Erk1/2 activation in CA1 pyramidal neurons compared with controls. Dusp6 KO mice showed normal body growth, brain anatomy, and basal behaviours compared with WT siblings. Remarkably, Dusp6 KO mice showed a severe deficit in long-term contextual fear memory, suggesting that Dusp6 maintains basal Erk1/2 activation low to allow for memory consolidation. Collectively, the results indicate that Dusp6 dynamically interacts with Erk1/2 to regulate the duration of learning-induced MAPK signaling in the hippocampus, and identify Dusp6 as a novel candidate regulator of memory formation.

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

HEALTH CONDITIONS, LEVEL OF INDEPENDENCE, COGNITIVE RESERVE AND AGE AS FACTORS ASSOCIATED WITH COGNITIVE PERFORMANCE IN SUBJECTIVE MEMORY COMPLAINTS

Martín-Aragoneses, M. T.[1], López-Higes, R.[2], Prados, J. M.[2], Nieto, I.[2], Del Río, D.[2], Galindo, M.[2], Montenegro, M.[3], y Álvarez, B.[3]
[1]. Departamento MIDE II. Facultad de Educación. UNED.
[2]. Departamento de Psicología Básica II. Universidad Complutense de Madrid.
[3]. Centro de Prevención del Deterioro Cognitivo. Madrid Salud. Ayuntamiento de Madrid

Few studies have explored what factors predict cognitive performance in elders with subjective memory complaints (SMC). We carried a study with 68 elders, between 65 and 80 years of age, which presented SMC. Inclusion criteria were: (1) SMC, which had been the reason for medical consultation; (2) normal performance on the Logical Memory subtest of the WMS III; (3) Global Deterioration Scale < 3; (4) Geriatric Depression Scale < 9; (5) MMSE > 27; (6) MFE > 13. A series of regression analysis were computed to predict subjects' performance in memory, executive function and language by using as predictor variables: age, cognitive reserve (CR) estimation, independence level as measured by the FAQ, emotion state as measured by Yesavage scale, and personal antecedents of diseases (PAD) as reflected by a factorial score combining diabetes, hypertension, cardio-vascular disease and dyslipidemia. R^2 between .33 and .40 were obtained for (the most powerful predictors in brackets): correct responses following semantic cue in the Boston Naming Test (PAD), time in the Simple Rey Figure Test-Memory (PAD), and delayed memory for topics in WMS-III Logical Memory (CR). Values between .28 and .18 were obtained for fluency for proper names (Age; negative slope), time in TMT part-A (CR; negative slope), and delayed memory for 4 histories in the Rivermead Test (FAQ; negative slope). In conclusion: (a) a significant percentage of the total variance for tasks that rely on visual recognition or memory like confrontation naming and figure reproduction from memory were associated with PAD; while (b) performance in an auditory-verbal memory task was strongly related to CR. In addition, (c) evocation of proper names was negatively predicted by age; (d) time in sustained attention was negatively predicted by CR; and (e) everyday memory was negatively predicted by FAQ score. The implications of these results are discussed.

1ª: Cognitive and Behavioral Neuroscience
2ª: Developmental Neurobiology

NEURAL MECHANISMS UNDERLYING RISK PROCESSING IN DRIVING SITUATIONS.

A. Megías, A. Cándido, D. Petrova, J.F. Navas, A. Maldonado, A. Catena.
Learning, Emotion and Decision Group. Mind, Brain and Behavior Research Center. University of Granada, Granada

The main aim of this research was to study the neural processing underlying risky decision making and risk perception in driving. To address this issue, we differentiated between two types of behavior in risky driving situations: urgent behaviors (decisions performed under high time-pressure, which can lead to severe negative consequences) and evaluative behaviors (a judgment of a situation, where the response is not imperative and does not involve negative consequences). A total of thirty-eight participants were asked to perform urgent behaviors -braking or not to avoid a hazard-, and evaluative behaviors -judging whether the situation was risky or not- in a set of driving scenarios. Brain activity was measured via an EEG recording. The results showed a dissociation of urgent and evaluative behavior both at the behavioral and at the neural level. Compared to the evaluative behavior, the urgent behavior showed a more cautious response bias, shorter reaction times, and worse sensitivity to risk. At the neural level, in the risk situations urgent behaviors showed higher activation in frontal electrodes compared to evaluative ones. Divergences between both types of behavior may be explained by differences in risk processing, and can be related to a more automatic processing and greater influence of heuristic and affective processes in the urgent behavior. On the other hand, risky situations in both tasks were characterized by a higher activation of centro-parietal electrodes. This component could be associated with a subjective evaluation of the emotional significance associated with the risk level. In summary, our findings provide valuable new information about the neural basis of risky behavior, and reflect the influence of emotion in processes considered mostly cognitive. Moreover, this research may have practical implications in the development of programs to assess risk perception skills and training programs for novice or offending/risky drivers.

This work was funded by the Spanish Directorate General of Traffic (DGT), SPIP2014-01341, to A. Cándido; and Spanish MICINN, PSI2012-39292, grant to A. Catena.

1ª: Neurociencia cognitiva y conductual

MEMORY COMPLAINTS, COGNITIVE RESERVE, AND SEMANTIC INTERFERENCE

Prados, J. M.[1], López-Higes, R.[1], Galindo, M.[1], Martín-Aragoneses, M. T.[2], Del Río, D.[1], Montejo, P.[3] y Nieto, I. [1]
[1] Universidad Complutense de Madrid.
[2] Universidad Nacional de Educación a Distancia.
[3] Centro de Prevención del Deterioro Cognitivo. Madrid Salud, Ayuntamiento de Madrid.

Objective. Subjective memory complaints (SMC) associated with age are been studied as a predictor of poor cognitive function. This study examined whether there were differences in cognitive reserve and semantic interference between two groups of older people, with and without SMC. Two hypotheses were suggested: (1) SMC are negatively correlated with cognitive reserve, and (2) SMC are positively correlated with semantic interference. *Method.* One hundred participants (70 women and 30 men), aged between 65 and 80 years (M = 71.16; SD = 3.8), voluntarily took part in the study. They were selected from a larger sample, depending on whether they have SMC or not (50%). The two groups were equivalent in age ($t(98) = -1.55$, $p = .123$) and proportion of men and women ($\chi^2 = 3.04$, $p = .081$). The Stroop Test (Golden, 1978) and the Cognitive Reserve Questionnaire (CRC; Rami et al., 2011) were used. *Results.* A significant lower score on cognitive reserve was obtained by SMC group in comparison with controls. Also a significant lower score on Stroop test was obtained by SMC group with respect to participants without SMC, specifically on both color and word-color subtests. **Conclusions.** SMC in aging are related to a lower cognitive reserve. This could be coherent with the cognitive reserve conception as a functional improvement on memory strategies. In addition, SMC in aging are related to a poorer cognitive function, especially a reduction on executive performance. As this pattern is also found in patients with right-hemispheric damage, more research is needed exploring brain-damage correlates when cognitive complaints are present.

1ª: Neurociencia cognitiva y conductual
2ª: Nuevos métodos y tecnologías

DISTRIBUTION OF ESTROGEN RECEPTORS ALPHA (ERα) IN THE AMYGDALO-HYPOTHALAMIC CIRCUITS IN MICE: CHANGES ALONG THE ESTROUS CYCLE.

M. Andrés Antón[1], M. Mulet Fernandez[1], G. Valera Marín[1], P. Marco-Manclús[1], H. Saláis-López[2], F. Martínez-García[2], E. Lanuza[1]
[1]. Facultad de Ciencias Biológicas, Universidad de Valencia, Valencia.
[2]. Facultat de CC. de la Salut. Universitat Jaume I, Castelló, Spain.

Estrogen plays an important role regulating the structure and function of neuronal systems in both male and female mice. Estrogen and progesterone levels vary during the estrous cycle leading to changes in sexual receptivity. This study analyses the distribution of estrogen receptor alpha (ERα) in the brain areas involved in sociosexual behaviour along the different phases of the estrous cycle, using 40 ovariectomised female mice treated with hormonal substitution. The behaviour of the experimental females was previously analysed in a test of attraction for the male sexual pheromone darcin, and the brains processed for the expression of ERα and Fos as an activity marker. In addition, the results obtained in females were compared with data of ERα distribution in castrated and non-castrated males. In particular, we focus in the anterior (MeA), posterodorsal (MePD) and posteroventral (MePV) subdivisions of the medial amygdala, beside other amygdaloid areas, bed nucleus of the stria terminalis (BST), septum and hypothalamus, some of which are sexually dimorphic structures. The results reveal that the diestrous phase (oil+oil) has the greatest density of ERα while the proestrous (estradiol+oil) has the lowest. The MePD nucleus has a great density of ERα in all phases of the cycle, consistent with its implication in the control of sexual behaviour; whereas the density of ERα in the MePV varies, being higher in the estrous phase. Additionally, the posteromedial division of the medial BST, medial preoptic nucleus, ventromedial hypothalamic nucleus and arcuate hypothalamic nucleus show intense labelling, with variable density along the cycle. In contrast, results in males reveal lesser labelling for ERα, which is increased in castrated males (probably because they cannot produce estradiol). As a conclusion, estradiol down-regulate the ERα expression, regulating the sociosexual behaviour by these amygdalo-hypothalamic connections.
Funding: MINECO/FEDER (BFU2013-47688-P) and Government of Castilla-La Mancha/FEDER (PEIC11-0045-4490).

1ª: Neurociencia cognitiva y conductual
2ª: Sistemas homeostáticos y neuroendocrinos.

DIFFERENTIAL MODULATION OF AMINOACID CONTENT DURING THE INCUBATION OF COCAINE, HEROIN AND SUCROSE CRAVING IN BASOLATERAL AMYGDALA AND VENTROMEDIAL PREFRONTAL CORTEX

D Roura-Martínez, A Marcos, M Ucha, R Santos-Toscano, A Contreras, R Capellán, J Orihuel, A Higuera-Matas, E Ambrosio
Department of Psychobiology. School of Psychology. UNED. Madrid, Spain.

Objectives. The incubation (progressive increase) of craving is a phenomenon commonly suffered by addicts during abstinence periods. Although reproduced in animal models with different drugs and natural reinforcers, most studies have focused on the incubation of cocaine craving. The aim of this study was to describe the changes in the content of several neuroactive aminoacids (L-glutamate, GABA, L-glutamine, L-aspartate, glycine, taurine, D-serine) in the ventromedial prefrontal cortex (vmPFC) and basolateral amygdala (BLA) during the incubation of cocaine, heroin and sucrose craving.
Materials and methods. Male Lewis rats underwent self-administration protocols that are known to induce incubation of craving: 6 h per day sessions (cocaine, heroin, saline), or 2 h per day (sucrose, water), for 10 consecutive days. Then, rats from each group were assigned to two withdrawal conditions: half of the animals were sacrificed after one day of withdrawal and the other half after one month of forced abstinence. The brains were collected and vmPFC and BLA were dissected, homogenized, derivatized with NBD-F and analyzed by capillary electrophoresis.
Results. Only the treatment with cocaine was able to produce changes in the measured analites: a decrease in L-glutamate in vmPFC in early withdrawal was followed by an increase one month after.
Conclusions. As seen by principal component analysis, the increased levels of L-glutamate could be interpreted as general activity changes in cocaine-exposed animals during abstinence. Such effect could be one of the mediators of the incubation phenomenon.

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

REACTIVATION OF ERK/MAP KINASE SIGNALING IN CA1 NEURONS IS REQUIRED FOR RETRIEVAL OF CONTEXTUAL MEMORY

C. Zamorano Ng Teajan, J. Fernandez Albert, C. Sindreu
University of Barcelona School of Medicine, Barcelona

The usefulness of stored memories relies on our ability to retrieve them. However, understanding of the molecular mechanisms supporting the recall of episodic memories is limited. We observed that when fear-conditioned mice are re-exposed to the context a major increase in Erk1/2 activation ensues in CA1 pyramidal neurons. In contrast, context re-exposure in mice with impaired memory did not activate Erk1/2, directly linking Erk1/2 activation with retrieval. Acute inhibition of Erk1/2 signaling in conscious mice shortly before re-exposure suppressed memory retrieval. Moreover, Erk1/2 inhibition confined to the CA1 subregion of the dorsal hippocampus was sufficient to block memory retrieval. Interestingly, local Erk1/2 inhibition impaired the retrieval of long-term, but not short-term, contextual memories. Analysis of the overlap between neuronal ensembles activated during learning and those showing Erk1/2 activation during retrieval indicated that Erk1/2 is preferentially reactivated in the same subset of CA1 neurons. Intriguingly, though, NMDAR antagonists blocked Erk1/2 activation in CA1 neurons during conditioning, but not during retrieval, suggesting distinct upstream mechanisms are involved in each memory phase. We conclude that reactivation of Erk1/2 signaling in the CA1 hippocampus is required for the retrieval of long-term memory.

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

EFFECTS OF AEROBIC TRAINING IN VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF), COGNITIVE FUNCTIONS AND AEROBIC CAPACITY IN ALZHEIMER'S DISEASE

T. Martins Vital[1,2], A. Miki Stein[1], F. Gomes de Melo Coelho[3], A. Veloso Rueda[4], R. Camarini[4], R. Ferreira Santos-Galduroz[1,5]
1. Institute of Biosciences, UNESP, University Estadual Paulista, Physical Activity and Aging Lab (LAFE), Rio Claro,SP, Brazil
2. Institute Federal Goiano, Morrinhos/GO, Brazil
3. Department of Sports Sciences, UFTM, Univ.Federal do Triângulo Mineiro, Uberaba/ MG, Brazil.
4. Institute of Biomedical Sciences, USP, University of São Paulo, Department of Pharmacology, Laboratory of Neurochemistry and Behavioral Pharmacology, São Paulo, SP, Brazil
5. Center of Mathematics, Computing and Cognition, UFABC, University Federal of ABC, Santo André SP, Brazil

The aim of this study was to investigate the effects of aerobic training in the concentrations of VEGF, cognitive functions and aerobic capacity in elderly in the mild stage of Alzheimer's disease. This study was composed of 34 elderly in the mild stage of DA which were divided into two groups. The Training Group consisted of 18 elderly who performed an aerobic training protocol of moderate intensity, three times a week, lasting 25-40 minutes for 12 weeks. The Control Group was composed for 16 elderly in the mild stage of AD who maintained your routine. Elderly of both groups performed an incremental test on a treadmill before and after 12 weeks, in which a blood sample was collected. The dosage of VEGF levels was performed using the ELISA method. The aerobic capacity was measured in incremental test by means of the following variables: inclination, test time, perceived effort and heart rate. To evaluate the cognitive functions the following instruments were used: Clock Drawing Test, Verbal Fluency Semantics, and Digits Span. After verifying the data distribution through the Shapiro-Wilk test, for those data that rejected the hypothesis of normality, we used z score. After the student t test and Anova repeated measures was used. The applied statistical analysis showed that twelve weeks of aerobic training does not alter the VEGF concentrations. It was also found that the training group improved immediate memory, increased the time and the inclination of the incremental test and reduced heart rate after twelve weeks. The applied aerobic training was able to improve aerobic capacity and maintenance of cognitive function in the elderly in the mild stage of Alzheimer's disease.

Financial Support: FAPESP (number process: 2012/21286-7)

1ª: Neurociencia cognitiva y conductual
2ª: Nuevos métodos y tecnologías

A CHRONIC Δ9-TETRAHYDROCANNABINOL TREATMENT DURING ADOLESCENCE INCREASES COCAINE SELF-ADMINISTRATION, COMPULSIVE DRUG SEEKING AND ESCALATION AT ADULTHOOD.

J. Orihuel [1], R. Capellán [1], M. Ucha[1], D. Roura-Martínez[1], A. Contreras[1], M. Fernández-Cabrera[1], E. Ambrosio[1] y A. Higuera-Matas [1]
[1]. Facultad de Psicología. UNED, Madrid

Objectives: Cannabis continues to be the illegal drug most widely consumed by adolescents. There is epidemiological evidence suggesting that early marihuana consumption might act as a gateway to addiction to other drugs during adulthood. Previous data showed that cannabinoid exposure during adolescence facilitates the acquisition of cocaine self-administration in adult female rats. The aims of this study was to validate these results using the main psychoactive component of marihuana THC, and to analyze not just the acquisition of cocaine self-administration but other behavioral patterns reminiscent of drug addiction.
Material and Methods: Male and female adolescent Wistar rats (PND28-42) were daily injected with THC (3mg/kg i.p.) or vehicle (ethanol:chremophor:saline) and left undisturbed until they reached adulthood (PND90). Subsequently, they underwent cocaine self-administration (0,5 mg/kg) under different conditions: fixed ratio 1 (12 days), progressive ratio (6 days), stabilization in FR1 (3 days) compulsive taking (drug seeking punished with electric shocks; 1 day) and extended access (6 hour sessions, 10 days).
Results: THC-treated rats (male and female) show a facilitation of cocaine self-administration acquisition during the first seven sessions, but there were no clear differences during the progressive ratio phase. THC-treated males also displayed more compulsive cocaine seeking than the other groups. Conversely, THC-exposed females exhibited higher cocaine intake during extended access.
Conclusions: These results provide the first experimental evidence supporting the existence of an addictive phenotype in rats with a chronic THC treatment during adolescence, providing experimental support to the Gateway Hypothesis of Drug Addiction.

Supported by: SAF2013-47520-P); RTA-RD12/028/0020 PNSD Grant nº: 2012I057; Proyecto nº: S-2011/BMD-2308; Programa de Actividades I+D+I CANNAB-CM); UNED (Plan de Promoción de la Investigación); and European Union (Grant nº: JUST/2013/DPIP/AG/4823-EU MADNESS).

1. Neurociencia cognitiva y conductual
2. Trastornos y reparación del sistema nervioso

DISRUPTION OF THE MIRROR NEURON SYSTEM AFTER SUBARACHNOID HEMORRHAGE.

J. Plata Bello[1,2], C. Modroño[1], E. Pérez-Martín[1], S. Acosta-López[2], Y. Pérez-Martín[3], F. Marcano[1], V. García-Marín[2], JL González-Mora[1].
[1] Grupo de Neuroquímica y Neuroimagen. Departamento de Fisiología Humana. Universidad de La Laguna. España.
[2] Servicio de Neurocirugía. Hospital Universitario de Canarias, Santa Cruz de Tenerife. España.
[3] Servicio de Neurología. Hospital Universitario de Canarias, Santa Cruz de Tenerife. España.

Background
Nearly 20% of patients who suffer a subarachnoid hemorrhage (SAH) present cognitive impairment even after a year of follow-up. In this sense, visuospatial and visuoperceptive domains have not been fully studied in these patients. Furthermore, they have been associated with the activity in the so-called mirror neuron system (MNS).
Objective
To analyze the pattern of brain activity with a MNS task-based functional magnetic resonance imaging (fMRI) study in SAH patients.
Methods
A complete neuropsychological assessment and fMRI study (with observation and execution conditions) were both performed in patients with a history of SAH registered in the database of the Hospital Universitario de Canarias. They must fulfill all the inclusion criteria for the study (less than 40 years old; SAH with Fisher score 1-3; no vasospasm or ischemia; minimum follow-up of one year).
Results
Twelve patients were completely neuropsychologically studied. Four of them presented visuospatial/visuoperceptive impairment. fMRI study demonstrated the presence of higher activity in MNS regions in these patients than in patients with normal visuospatial/visuoperceptive functions. Furthermore, there was a negative correlation between the test scores and the extent of activation in premotor regions of the studied patients.
Conclusion
SAH patients with visuospatial/visuoperceptive impairment present an increase of activity in the MNS regions. This may be associated with a subcortical dysfunction, leading to a disruption of neural activity and a less efficient behavior of this brain network.

1. Systems Neuroscience and Cognitive and Behavioral Neuroscience.

MORPHINE SELF-ADMINISTRATION AND EXTINCTION MODULATE THE EXPRESSION OF DIFFERENT ELEMENTS OF THE PI3K/AKT/MTOR SIGNALING PATHWAY IN THE AMYGDALA OF MALE LEWIS RATS

Ucha M, Coria SM, Roura-Martínez D, Contreras A, Capellán R, Higuera-Matas A & Ambrosio E
Department of Psychobiology, School of Psychology. UNED. 28040, Madrid, Spain.

Objectives: While the use of opioids, either as analgesic or as drug of abuse, is widely extended, the neurochemical adaptations that occur during the exposure and abstinence stages remain poorly understood. The aim of this study is to characterize the effects of morphine self-administration and extinction on the expression of several genes related to the PI3K/Akt/mTOR signaling pathway, which plays a role in learning and memory and has been recently linked to drug abuse.
Materials and methods: Male Lewis rats underwent either morphine or saline self-administration sessions (1mg/kg/injection, 18days, 12h/day). Half of the rats were sacrificed at this point and the rest of them endured 15 extinction sessions before being sacrificed. The brains were dissected and the prefrontal cortex and amygdala were stored for subsequent qPCR analysis.
Results: *Rptor* and *Akt2* gene expression showed a trend to increase after morphine self-administration. This effect was reverted after the extinction sessions. In the PFC, we found a decrease in the expression of *igf2r* after extinction independent of the treatment.
Conclusions: These results suggest a potential implication of the PI3K/Akt/mTOR signaling pathway in the neurochemical processes occurring with morphine administration.

Supported by: SAF2013-47520-P); RTA-RD12/028/0020 PNSD Grant nº: 2012I057; Grant nº: S-2011/BMD-2308; Programa de Actividades I+D+I CANNAB-CM); UNED (Plan de Promoción de la Investigación); and European Union (Grant nº: JUST/2013/DPIP/AG/4823-EU MADNESS)

1. Cognitive and Behavioral Neuroscience
2. Disorders and nervous system repair

CHRONIC SOCIAL ISOLATION ON AGED MALE WISTAR RATS ALTERS SPINE DENSITY AND LTP IN THE HIPPOCAMPUS AND INDUCES COGNITIVE IMPAIRMENT

Pereda-Pérez I [1]., Núñez A[2]., Valencia A.[1] ,Venero C[1].
1. Faculty of Psychology, National Distance Education University, Madrid
2. Faculty of Medicine, Universidad Autónoma de Madrid

Social isolation is known to alter cognitive function in young and adult rodents. However, no studies have evaluated the impact of social isolation on cognitive function in aged rats. In the present study, we investigated the electrophysiological, morphological and cognitive consequences of exposing aged male Wistar rats to chronic social isolation. 18 and 20 month-old male Wistar rats were individually housed for 1 month, short-term isolation or 3 months, long-term isolation, avoiding without visual contact with other rats. In three independent experiments, we evaluated; i) spatial and memory abilities of animals in the Morris water maze test; ii) dendritic spine density of granular neurons of DG and pyramidal neurons of CA1 and; iii) in-vivo Long-Term Potentiation (LTP) in the DG and CA1 areas of the hippocampus. Our data showed that both, short and long-term isolated groups of animals exhibited poorer spatial learning abilities compared to group-housed rats. However, spatial memory impairment was only observed in rats submitted to long-term isolation. Quantitative confocal microscopy of Alexa 595 labelled neurons showed a reduction in spine density of granular cells from animals submitted to short- and long-term isolation procedures. In the *stratum oriens* of CA1 pyramidal cells, long-term isolated rats showed a lower spine density compared to group-housed controls, while short-term isolated animals only displayed a tendency to a reduction of spine density. Electrophysiological studies indicated that, in the dentate gyrus of the hippocampus, LTP was weaker in both isolated groups of rats compared to controls. In CA1, LTP was reduced in short-term isolated animals compared to group-housed rats, but LTP was absent in animals exposed to long-term isolation. Taken together, our results indicate that social isolation at aging progressively induces morphological and electrophysiological changes in the hippocampus that may lead to the impairment in spatial learning and memory observed in isolated animals.

ARE BODY OWNERSHIP ILLUSIONS ANALGESIC?

B. Nierula[1,2], M. Martini[1,2], M. Matamala Gómez[1,2], M. V. Sanchez Vives[1,2,3]
[1]. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
[2]. EVENT-Lab, Facultat de Psicologia, Universitat de Barcelona, Spain
[3]. Institució Catalana Recerca i Estudis Avançats (ICREA), Barcelona, Spain

Objectives: Seeing one's own body has been reported to have analgesic properties (Longo et al. J Neurosci 29 (2009): 12125). However there is a controversy regarding whether seen an illusory owned body is also analgesic. Mohan et al. (PLoS One 7 (2012): e52400) showed no changes in pain perception during the rubber hand illusion. However, Martini et al. (2014) European JPain 18 (2014): 1040) found increased pain thresholds when participants looked at an embodied virtual arm compared to seeing a real or virtual object. A major difference between the two studies lies in the distance between the real and the illusory arm: while VR allows for the real hand to be co-located with the virtual surrogate, this is not possible for the rubber hand illusion due to physical constraints. We conducted this study in order to test whether the distance between the real and the virtual arm can explain this controversy. In it, we compared the pain threshold in between a co-located real versus virtual arm with a 30 cm distance between both.
Material & Methods: 20 male and right handed participants (mean age = 24.7, SD = 5.6) were investigated with a two-factorial within-subject design. We manipulated the distance between the real arm and the virtual arm which was eiher co-located or 30 cm apart. We further manipulated the synchrony of visual and tactile stimulation, which could either be synchoronous or asynchonous, so that we had for each distance (0 or 30 cm) two stimulation conditions.
Results: We found that during co-location between the real and virtual arm the pain threshold was significantly higher than during the 30-cm distance condition.
Conclusions: Looking at a virtual co-located arm has analgesic effects similarly to looking at the own body. However, increasing the distance between the real and the virtual arm eliminates this analgesic effect, what could explain why owning a rubber arm does not induce analgesia.

Supported by EC FET project VERE (257695).

IMPROVING REACHING MOVEMENTS IN PARKINSON'S DISEASE BY MEANS OF A VIRTUAL REALITY TRAINING

Y. Corral Bergantiños, V. Robles García, A. Madrid López, P. Arias, J. Cudeiro
Neuroscience and Motor Control Group (NEUROcom). University of A Coruña, A Coruña.

Introduction and objectives: Reaching movements (RM) are essential in many tasks of daily living, and are impaired in Parkinson's disease (PD) patients. Here we were aimed to evaluate the effects virtual reality (VR) training on the execution of RM in PD.
Material and methods: We have designed and validated a VR system for RM in order to perform a therapeutic protocol. This protocol was performed by two groups of PD patients: experimental (EG) and control (CG). Both groups observed a virtual avatar in 1st person perspective. The structure of study was: *Pre-evaluation*, 8 therapy sessions, *post-evaluation* and *post2-evaluation*. The EG executed RM conditioned by the virtual avatar movements, under a reaction time (RT) protocol. The CG observed their own movements (at real-time) executed by the virtual avatar, while performing the RT tasks. During the training sessions the subjects executed the RM with the dominant arm to one of two targets in front of them; response-signals were always preceded by a warning-cue at two different foreperiods. RT's (lags from response-signal to EMG-onset activities), movement times (MT's; from hand lift to target contact) and cortical excitability parameters before and after the training were studied.
Results: Kinematic variables, such as RT's and MT's showed significant differences when the control and experimental groups were compared after the therapy. Several neurophysiological correlates of those effects were identified.
Conclusions: VR might be a promising tool to correct altered RM in Parkinson's disease.
Support: Ayudas a Grupos Consolidados de Excelencia. Consellería de Educación, Xunta de Galicia.

1º: Cognitive and Behavioral Neuroscience
2º: Disorders and nervous system repair

AFFECT BASED-GAIN CONTROL: DIVISIVE NORMALIZATION AND CONTEXT-DEPENDENT CHOICE BEHAVIOR IN MORAL DILEMMAS.

S. Gomis-Pont[1], M.C. Navarro-Plaza[1], J. Navajas[2,3], M. Mirete-Fructuoso[1], S. Molina-Rodríguez[1], R. Quian Quiroga[2] & L.M. Martinez[1]
[1]. Instituto de Neurociencias de Alicante. CSIC-Universidad Miguel Hernández, San Juan de Alicante, Alicante.
[2]. Centre for Systems Neuroscience. University of Leicester. Leicester, UK.
[3]. Institute of Cognitive Neuroscience. University College London. London, UK.

Morality has traditionally been regarded as a distinct area in cognitive neuroscience; uniquely based on normative or philosophical criteria of what is "right" or "wrong". Current studies suggest that we solve moral dilemmas by using two different strategies linked to two separate cognitive processes: deontology (actions must conform to certain rules to be considered morally acceptable) and consequentialism (actions are considered moral if they produce the best consequences to the maximum number of people). It is often assumed that deontological responses emerge in situations that prompt attention to violation of moral rules, while the opposite is true for dilemmas that highlight utilitarian considerations. However, everyday moral judgments are extremely flexible and they often adapt to different contexts leading to decisions that violate the predictions of these two fundamental moral principles.

We hypothesize here that moral decision making is shaped by the same gain control mechanisms that operate in sensory pathways to represent value in a relative rather than absolute manner. Context should thus strongly influence how people judge actions and actors in a very explicit way that is independent of purely deontological or absolute utilitarian considerations.

To test this hypothesis we have designed 15 new, ecological moral dilemmas in which both the actors and actions are kept constant while context is sequentially altered as more information is added to the moral scenes. Our results show, first, that subjects judge the actor of the moral action encoding a form of “affective” value, likeability, which is directly dependent on the value of the scenes embedding the actions. And, second, that moral acceptability correlates linearly with subjective likeability ($r=0.9719$, $n=35$ subjects).

Finally, this context-dependent modulation of moral decision making is precisely described by divisive normalization, an adaptive form of gain control that may be a general mechanism for sensory and cognitive computations.

1ª: Neurociencia cognitiva y conductual
2ª: Neurociencia teórica y computacional

SAME CHOICE, TWO SYSTEMS: DYNAMICS OF CONTEXT-DEPENDENT CHOICE BEHAVIOR IN MORAL DILEMMAS.

M.C. Navarro-Plaza[1], S. Gomis-Pont[1], J. Navajas[2,3], S. Molina-Rodríguez[1], M. Mirete-Fructuoso[1], R. Quian Quiroga[2] & L.M. Martinez[1]

[1]. Instituto de Neurociencias de Alicante. CSIC-Universidad Miguel Hernández, San Juan de Alicante, Alicante.

[2]. Centre for Systems Neuroscience. University of Leicester. Leicester, UK.

[3]. Institute of Cognitive Neuroscience. University College London. London, UK.

Moral cognition is thought to rely on complex cognitive processes that integrate socio-emotional factors such as situational cues, past experiences and potential future consequences. Despite decades of research, however, we are still lacking a fully mechanistic account of moral decision-making. The most influential model is a dual system theory that contrasts reflective and emotional influences on moral judgment. Thus, fast, automatic responses to moral dilemmas tend to be emotional and correspond to deontological rules. And, subsequently, these system-1 responses are under the control of cognitive, system-2, processes favoring the "best consequences". This model predicts that time pressure or cognitive load should thus decrease the frequency and speed of utilitarian choices, highlighting the role of "affective" value over that of controlled cognition.

Here, we try to shed light on which system, the emotional or the rational, is responsible for making moral decisions in ecological, everyday life situations.

We have designed 15 moral dilemmas in which both the actors and actions are kept constant while context is sequentially altered as more information is added to the moral scenes in four serial steps. 35 subjects made moral decisions in response to these dilemmas while their eye scan paths and computer mouse trajectories were continuously monitored to measure reaction times, response time, response type (deontological vs. contextual/utilitarian) and uncertainty.

Our results failed to demonstrate the emotion-then-deliberation sequence characteristic of current dual-system theories. On the contrary, we have shown that system 1 (automatic) was mainly engaged in decisions that corroborate the acceptance of a previously evaluated moral scene; while the deliberative system 2 was mainly involved in trials when decisions first departed from firm deontological rules. Finally, using signal-detection theory we show that subjects were rarely unaware of their own deliberative process, suggesting that post-hoc mechanisms do not override the conscious perception of the choice.

1ª: Neurociencia cognitiva y conductual
2ª: Neurociencia teórica y computacional

OPTIMIZED MULTIELECTRODE tDCS MODULATES CORTICOLIMBIC NETWORKS: A FUNCTIONAL MRI STUDY

Martínez Ruiz–de Lara, C[1,2,*]; Soriano-Mas, C[2,3]; Martinez-Zalacain, I[2]; Ripolles, O[4]; Subira, M[2]; Via, E[2]; Mitjà, G[4]; Munuera, J[5]; Menchón, JM[2,3] ; Ruffini, CG[4,6], Cardoner, N[2,3]
[1]Centro de Investigación Mente, Cerebro y Comportamiento. Universidad de Granada, Spain
[2] Department of Psychiatry, Bellvitge University Hospital-IDIBELL, Barcelona, Spain
[3]CIBER Salud Mental (CIBERSAM), Instituto de Salud Carlos III, Barcelona, Spain
[4]Neuroelectrics Barcelona SL, Spain
[5]Magnetic Resonance Unit. Hospital Universitari Germans Trias i Pujol
[6]Starlab Barcelona SL, Spain
*Presenting Author

Background
Transcranial direct current stimulation (tDCS) is a noninvasive neuromodulatory technique using weak electrical currents to alter cortical excitability. Currently, studies are exploring distinct stimulation parameters to optimize tDCS effects.
Objectives
Here, we aimed to advance in the characterization of the underlying neural mechanisms of tDCS effects on regional brain activity and connectivity. In addition, we intended to test the differential effect of a novel, computationally–derived electrode montage designed to improve targeting and efficiency of the induced electric field.
Methods
In a randomized, single–blind sham–controlled crossover design, 20 healthy subjects underwent three different tDCS sessions (conventional, multielectrode and sham) in random order, with two weeks of inter–session interval. Stimulation was applied for 20 minutes at 2mA intensity for active conditions, targeting prefrontal regions. Resting state–fMRI scans were acquired immediately after tDCS treatment to identify functional tDCS–induced changes.
We performed a novel and integrated fALFF and seed–based FC analysis. Firstly, we examined fractional amplitude of low–frequency fluctuations (fALFF) to determine between–condition differences on regional spontaneous brain activity. Then, areas exhibiting fALFF differences were selected as regions–of–interest in a seed–based functional connectivity (FC) analysis within areas of the corticolimbic network.
Results
Multielectrode condition resulted in increased fALFF values in frontal and prefrontal relevant brain regions that participate in corticolimbic networks. Concretely, multielectrode montage resulted in significantly higher fALFF values in frontopolar, middle and superior frontal areas. Furthermore, an increase in functional coupling between these regions and right hippocampus was specifically observed in this condition.
Conclusions
Optimized multielectrode tDCS induced a modulation on regional brain activity and FC in corticolimbic networks, thus suggesting this particular montage to be the adequate election when aiming to modulate brain activity in disorders involving corticolimbic network, which opens the possibility for new therapeutic approaches.

1ª Neurociencia Cognitiva y Conductual
2ª Nuevos Métodos y Tecnologías

PROTEIN KINASE-C GAMMA IS INVOLVED IN SHORT-TERM MEMORY PERFORMANCE

M. Gomis-González, A. Busquets-Garcia, R. Maldonado, A. Ozaita
Laboratori de Neurofarmacologia. Departament de Ciències Experimentals i de la Salut. Universitat Pompeu Fabra, Barcelona, Spain.

Memory is a brain function that encodes, stores, and recovers relevant information for the subject. Memories can be classified in short- and long-term depending on the interval between acquisition and retrieval, and it is generally accepted that short-term memories (STM) undergo a consolidation process to form long-term memories (LTM). Protein kinase C (PKC) signaling has been involved in memory performance due to its key control over different components of the synaptic plasticity machinery. We focused our attention in PKC-gamma, a PKC isoform expressed in the hippocampus, which phosphorylates substrates involved in synaptic plasticity. Previous studies detected mild LTM deficits in PKC-gamma KO mice ascribed to spatial and contextual learning. In order to determine whether PKC-gamma signaling is relevant for STM performance, we studied both emotional and non-emotional memory types at short times after training. We found that PKC-gamma KO mice presented a significant STM impairment in all those tests that involved hippocampal function such as novel-object recognition, novel place recognition, context recognition and auditory trace fear conditioning, while no differences were observed in an amygdala-dependent test such as the auditory delayed fear conditioning. Strikingly, all the tasks revealing STM deficits in PKC-gamma KO mice, were performed correctly by these mutant mice when the memory was assessed 24 h after acquisition, demonstrating that the long-term memory trace was patent without a previous short-term memory trace. Interestingly, the molecular signatures of memory retrieval such as extracellular signal-regulated kinase (ERK) signaling were not different when LTM was studied. These results suggest that PKC-gamma signaling is critical for hippocampal-dependent STM function and underline the different molecular mechanisms involved in short-term and long-term memory processes.

1: Cognitive and behavioral neuroscience
2: Disorders and nervous system repair

SEX-DEPENDENT EFFECTS OF CHRONIC UNPREDIACTABLE STRESS ON ALCOHOL CONSUMPTION; ANALYSIS OF NEURAL CORRELATES.

E.M. Marco [1], J.A. Ballesta [1], M.E. Serrano [1], C. Irala[2], M.D. Hernández [2], M. López-Gallardo [2], M.P. Viveros [1].
[1]. Facultad de Biología. Universidad Complutense, Madrid
[2]. Facultad de Medicina, Universidad Complutense, Madrid.

Depression has been frequently associated with alcohol consumption. However, the temporal relationship between these conditions has not yet been elucidated. In the present study we aimed to investigate alcohol consumption in an animal model of depression, analyzing possible neural correlates by western blotting in the frontal cortex (FCx) and the hippocampal formation (Hip). In male and female *Wistar* rats, exposure to 6 weeks of chronic unpredictable mild stress (CUMS) was employed to induce a depressive-like phenotype. As expected, CUMS induced depressive-like responses in the Forced Swimming Test, i.e. increased immobility time, in animals of both sexes, but did not affect behaviour in the Sucrose Preference Test nor in the Open Field. In addition, decreases in body weight gain and food intake were only observed among stressed male animals. Regarding alcohol intake, higher rates of alcohol consumption were exclusively observed in CUMS females when compared to control females, in the absence of differences among males. At least 5 days after the end of the experimental protocol animals were sacrificed. Male and female animals exposed to CUMS showed, in the FCx, an increment in GFAP and CB1R expression, together with a decrease in NCAM expression. In the Hip, these animals showed an increase in PSD95 expression. In addition to these region-dependent effects, effects dependent upon sex were also observed. Thus, a decrease in FCx synaptophysin expression and Hip CB1R expression were found in male animals exposed to CUMS, whereas the opposite effects were observed in females. In turn, hippocampal NCAM expression was increased in males but decreased in females. A decrease in CB2R expression was only observed in the Hip of stressed females. In conclusion, CUMS appears to differently affect male and female animals' behaviour and the neural parameters analyzed show region and sex dependent effects on synaptic plasticity markers.

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

REPEATED THC ADMINISTRATION AFFECTS STRUCTURAL PLASTICITY IN THE HIPPOCAMPUS

V.Salgado-Mendialdúa[1], M. Gomis-González[1], R. Maldonado[1], A. Ozaita[1].
[1].Facultat de Ciències de la Salut i de la Vida, Universitat Pompeu Fabra, Parc de Recerca Biomèdica de Barcelona, Barcelona

Δ9-tetrahydrocannabinol (THC), the main psychoactive component of *Cannabis sativa* plant, causes memory impairment through the activation of CB1 cannabinoid receptors. These amnesic-like effects do not show tolerance after repeated exposure to THC, and are persistent for a few days after THC withdrawal. Memory formation and consolidation trigger alterations at excitatory synapses leading to dendritic spine restructuration in terms of shape and density, a phenomenon called structural plasticity. Structural plasticity depends on local synaptic regulation of protein synthesis and cytoskeleton re-arrangements where actin and its related proteins play an important role. The aim of this study is to analyze whether repeated exposure to THC has any effect on structural plasticity in the hippocampus, a brain area involved in memory formation, by using the Thy1-EGFP transgenic mouse model that expresses enhanced green fluorescent protein (EGFP) in principal neurons of the brain. THC sub-chronic administration under conditions that affect object recognition memory decreased the number of mushroom dendritic spine shape (mature form) and increased the thin and stubby shapes (immature forms) in the hippocampus. In this brain area proteins involved in actin cytoskeleton modulations such as myristoylated alanine-rich C-kinase substrate (MARCKS), neurogranin, cofilin and profilin changed their expression and/or phosphorylation state after THC treatment. Our results show that memory impairment produced by chronic THC treatment is paralleled with hippocampal alterations in structural plasticity and point to the key role played by actin cytoskeleton and its related proteins in this unwanted side-effect of THC.

Supported by BFU2012-33500 (MINECO, Spain). V S-MAN-R was supported by a FPI fellowship (MINECO, Spain).

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

VULNERABILITY TO STRESS: THE ROLE OF INSULIN-LIKE GROWHT FACTOR.

Andrea Santi [1,2], Ignacio Torres-Aleman [1,2]
[1]. Instituto Cajal, Consejo Superior de Investigaciones Científicas, CSIC, 28002 Madrid, Spain
[2]. CIBERNED, Instituto de Salud Carlos III, Madrid, Spain

It has been suggested that there is a vulnerability to develop posttraumatic stress disorder (PTSD) after a mild traumatic brain injury (mTBI), although patient studies are controversial. Circulating insulin-like growth factor 1 (IGF1) levels decrease in late periods of TBI and they are also predictive of cognitive dysfunction. To explore this relationship we investigated the effects of a combined model of mTBI and PTSD on the anxiety behavior of C57BL6 mice and liver IGF1 deficiency (LID) mice. Animals were exposed to a rat two days after they underwent the CCI surgery. Anxiety on the elevated plus maze (EPM) and the startle reflex (ASR) were measured a week after the exposure to account for the late onset of the PTSD and blood samples were collected to measure corticosterone and IGF1 levels. Rotarod, Von Frey filaments and Y maze were used to determine motor, somatosensory and memory function respectively. Preliminary results show that motor behavior was affected only 24h after surgery while somatosensory dysfunction was maintained over time. We also found an increase in corticosterone levels after the predator exposure. In addition, C57BL6 stressed mice exhibited an increased startle response and a reduced time in the open arms compared to control mice. C57BL6 injured-stress mice, however, had a tendency on the ASR, but no effect on the EPM, while LID injured-stress animals had an exacerbated response to the EPM compared to controls. These results suggest that increased anxiety after stressful events was predisposed by the brain insult only when previous circulating IGF-1 levels were low, providing new data that could help understand de comorbidity of PTSD and TBI.

This work was supported by grants from the Spanish Ministerios de Economía y Competitividad and ISCIII, CIBERNED.

1ª: Cognitive and behavioral neuroscience
2ª: System neuroscience

MEASURING PHYSIOLOGICAL EMOTIONAL RESPONSES AND FACIAL EXPRESSION WITH EMOTION INDUCTION USING FILMS

L. Fernández-Aguilar [1], J.M. Latorre [1,2], L. Ros [1,2,], J·P. Serrano[1,2,], J. Ricarte[1,2,], A. Martínez-Rodrigo[3], R. Zangróniz [3,4], J.M. Pastor [3,4], A. Fernández-Caballero [5,6] & J.V. Moncho-Bogani [1,2,]
[1]. Instituto de Investigación en Discapacidades Neurológica, IDINE
[2]. Facultad de Medicina, Albacete, UCLM
[3] Instituto de Tecnologías Audiovisuales de Castilla-La Mancha, ITAV
[4] Escuela Politécnica, Cuenca, UCLM
[5] Instituto de Investigación en Informática, I3A, Albacete
[6] Escuela Superior de Industriales, Albacete

In spite of emotion represent a complex and multicomponent system, previous studies have demonstrated that it is possible to elicit emotional changes artificially. These studies have shown that emotion elicitation using films is one of the best methods for its ecological validity, standardization and ability to induce discrete emotions. Thus, it was used in the project "Improvement of the Elderly Quality of Life and Care through Smart Emotion Regulation", which goal is to have a system of devices with the power to recognize, interpret and modulate the basic emotion states. Although the aim of the study population are the elderly, in the first phase, participants were 25 university students volunteers who were asked to view a set of commercial film clips that try to evoke a set of emotions discriminately: sadness, anger, amusement, affection, disgust, fear and neutral state. Self Assessment Manikin (SAM) was used to evaluate subjective responses in terms to valence, arousal and dominance. Objective responses were given through cameras and body sensors to recognize facial and gestural expressions, activity and behavior. For each film clip, long-term and continuous recording of electro-dermal activity (EDA), heart rate (HR), superficial electromyogram (EMG) and skin temperature (SKT) were measured by a wearable acquisition device, a wristband. This overall system provides different physiological signals, which are ideal indicators of the valence and arousal state of the subjects. Physiological parameters and facial expressions were evaluated while the subjects watched each film clip and subjective emotional response immediately after each film clip. The initial results suggest that it is possible to extract discrete values about positive and negative emotional states and use them as keys to get emotion regulation

1ª: Neurociencia cognitiva y conductual
2ª: Nuevos métodos y tecnologías

Correlation between disease stage and executive functions in SCA36 or 'Costa da Morte' ataxia

R. Martínez-Regueiro[1,2,3], Maria-Jesus Sobrido[1,2,4]; Quintáns, B.[1,2,4]; Pardo, J.[5]; Manuel Arias[5]; García-Sobrino, T.[5]; Vidal, M.P.[5]; María Taboada[6]; García-Murias, M [1,2]; Yáñez T. Zuleima[7]; Cortés, J.[8,9]; Aguiar, P [8,9]; Torcuato Labella-Caballero[10]; José M. Pumar[11]; Banguero Gutiérrez, A.[11]; Carracedo, A. [1,2,4]; Fernández-Prieto, M. [1,2,4]

[1]. Grupo de Genética de Enfermedades Neurológicas, Instituto de Investigación Sanitaria de Santiago (IDIS).
[2]. Fundación Pública Galega de Medicina Xenomica-SERGAS, Santiago de Compostela.
[3]. Facultad de Psicología. Universidad de Santiago de Compostela.
[4]. Grupo U711, Medicina Xenómica, CIBERER.
[5]. Departamento de Neurología, Hospital Clínico Universitario de Santiago de Compostela.
[6]. Facultad de Física. Universidad de Santiago de Compostela.
[7]. Universidad Simón Bolívar de Barranquilla.
[8]. Departamento de Medicina Nuclear, Hospital Clínico Universitario de Santiago de Compostela.
[9]. Grupo de Imagen Molecular, Instituto de Investigación Sanitaria de Santiago (IDIS).
[10]. Departamento de Otorrinolaringología, Hospital Clínico Universitario de Santiago de Compostela.
[11]. Departamento de Neurorradiología, Hospital Clínico Universitario de Santiago de Compostela.

SCA36 is a new type of spinocerebellar ataxia recently described in Galicia and Japan caused by heterozygous expansion of an intronic GGCCTG hexanucleotide repeat in the NOP56 gene on chromosome 20p13. SCA36, relatively frequent among Galician patients with ataxia, is a neurodegenerative disease with a late-onset, slowly progressive cerebellar syndrome, variable eye movement abnormalities and sensorineural hearing loss. Japanese patients with SCA36 show normal global cognitive scores, however they have significant decline in frontal lobe function.
AIM: To evaluate the executive functions involved in abstract reasoning and cognitive flexibility in SCA36 and to correlate these scores with disease severity.
PATIENTS AND METHODS: We evaluated 26 genetically confirmed SCA36 patients (17 women, 9 men) from nearby villages in the 'Costa da Morte' (Northwestern Spain). The mean age at examination was 57.62 ± 14.7 years and the mean length of disease evolution was 14.79 ± 8.96 years, except for seven asymptomatic individuals. The cognitive assessment was carried out with the Wisconsin Card Sorting Test (WCST) and the Scale for Assessment and Rating of Ataxia (SARA). Statistical analyses were performed by nonparametric Spearman's correlation with $P < 0.05$ as significance threshold.
RESULTS: partial correlations between SARA and WSCT scores, adjusted for sex and for years into the course of disease, was found for the presence of perseverative responses ($r = -0,438$, $P < 0,03$) and perseverative errors ($r = -0,432$, $P < 0,03$), but not for non-perseverative errors ($r = -0,05$, $P > 0,79$).
CONCLUSIONS: These results highlight a relationship between disease stage (SARA score) and the decline of frontal executive function. Our data are consistent with previous evidence of prefrontal dysfunction described in other SCA subtypes.

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

BORN TO BE (WILD) MOTHER? MATERNAL AGGRESSION IS HORMONE-DEPENDENT AND MODULATED BY CENTRAL OXYTOCIN IN MICE

Ana Martín-Sánchez[1,2], Guillermo Valera-Marín[2], Marcos Otero-García[1,2], Adoración Hernández-Martínez[1], E. Lanuza[2], F. Martinez-Garcia[1], C. Agustín-Pavón[1]
[1]. Fac. CC de la Salut, Universitat Jaume I, Castelló de la Plana.
[2]. Fac. CC Biològiques, Universitat de València, València.

Virgin adult female mice display nearly spontaneous maternal behaviour towards foster pups. Thus, maternal care is hormone-independent and triggered by pup-derived stimuli. Conversely, the factors influencing maternal aggression are poorly understood. To investigate the neural basis of maternal behaviours, we characterize maternal sensitization in the CD1 strain. To do so, a group of virgin females (godmothers) were exposed to continuous cohabitation with a lactating dam and her pups from the moment of parturition, whereas a second group (pup-sensitized females), were exposed 2h-daily to foster pups. Both groups were tested for maternal behaviour on postnatal days 2-4. Godmothers expressed full maternal behaviour from the first test. Also, they expressed higher levels of crouching than dams. Pup-sensitized females differed from dams in all measures of maternal behaviour in the first test, and expressed full maternal behaviour after 2 sessions of contact with pups. However, both protocols failed to induce maternal aggression toward a male intruder after full onset of maternal behaviour, even in the presence of pups. Our study confirms that adult female mice need a short sensitization period before the onset of maternal behaviours. Further, it shows that pup-oriented and non-pup-oriented components of maternal behaviour are under different physiological control.
To study the physiological and neural bases of the maternal behavioural repertoire, we analysed expression of oxytocin in the brain of virgin females, godmothers and dams. There were no global differences between groups in any of the studied centres. However, a correlation analysis of the expression of oxytocin with maternal aggression reveals positive correlation in different centres of the socio-sexual brain, only in dams. Thus, oxytocin is likely to act as a central modulator of maternal aggression, on top of the effect of hormonal agents related to motherhood, such as prolactin. This study was funded by the Spanish MINECO-FEDER (BFU2010-16656; BFU2013-47688-P) and Government of Castilla-La Mancha/FEDER (PEIC11-0045-4490).

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

INTERMITTENT SUCROSE *DRINKING IN THE DARK* SENSITIZES THE OX SYSTEM AND TRIGGERS ESCALATION IN LOW DRINKERS IN ABSENCE OF ENHANCED ANXIETY

M. Alcaraz-Iborra[1], A. Florido[1], F. Cañadas[1], E. Giménez[1] & I. Cubero[1 & 2].
[1]. Departamento de Neurociencia y Ciencias de la Salud, Universidad de Almería, Almería.
[2]. Carrera de Psicología, Facultad Ciencias Jurídicas y Sociales, Universidad Autónoma de Chile, Santiago de Chile, Chile.

The addiction cycle model holds progressive drug escalation and sensitization of brain stress systems over time. Recent results support the engagement of OXR1 signaling in stress and binge-consumption of sucrose in organisms under no caloric needs. The present study comparatively explores, in High vs Low sucrose drinkers (HD/LD), the contribution of OX to escalation of sucrose intake in an *intermittent Drinking in the Dark* paradigm (iDID). For that aim, following 4 continued or 15 intermittent DID episodes, we comparatively evaluated in adult male C57BL/6J mice spontaneously showing High vs Low sucrose (10%w/v) DID consumption: a) effects of the selective OX-1r antagonist SB-334867 (5 mg/kg, ip) on sucrose binge intake; b) OX mRNA expression in the lateral hypothalamus and c) anxiety responses as measured by the e*levated plus maze procedure* (EPM). *Results*: (A) Following 4 continued episodes of sucrose DID: (a1) HD and LD groups showed similar levels of anxiety-like behavior; (a2) OX mRNA expression was elevated in LD compared to HD when exposed to sucrose or water DID; (a3) systemic SB-334867 did not alter sucrose binge-consumption in HD or LD. (B) Following 12 intermittent episodes of sucrose DID (iDID): (b1) sucrose intake significantly escalated in LD but not in HD; (b2) systemic administration of SB-334867 reduced sucrose consumption in HD and LD but the effect was significantly stronger in LD; (b3) No significant differences were observed in basal anxiety-like responses following iDID exposure. *Conclusions:* Basal differences in OX activity, which might be unrelated to differential anxiety-like responses, might contribute to genetic vulnerability to binge-consumption. Importantly, in non vulnerable organisms, simple exposure to intermittent binge-episodes might trigger OX activity disturbances mediating transition to binge-intake disorders.

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

OX2-R SIGNALING, BUT NOT OX1-R, WITHIN THE NUCLEUS ACCUMBENS SHELL MODULATES SUCROSE AND ETHANOL PALATABILITY IN SPRAGUE-DAWLEY RATS.

A. Florido [1], M. Alcaraz-Iborra [1], F. Carvajal [2], J. M. Lerma-Cabrera [2] and I. Cubero [1 & 2].
[1]. Departamento de Neurociencia y Ciencias de la Salud, Universidad de Almería, Almería.
[2]. Carrera de Psicología, Facultad de Ciencias Jurídicas y Sociales, Universidad Autónoma de Chile, Santiago de Chile.

Feeding is regulated by homeostatic and non-homeostatic factors. Interestingly, peptides known to regulate homeostatic aspects of feeding in the hypothalamus have proven to have a key role in hedonic consumption of rewarding stimulus including ethanol, when acting in the nucleus accumbens (NAc). Thus, recent studies showed that Melanocortins, hypothalamic peptides involved in energy balance and ethanol consumption, do also modulate ethanol palatability as measured by the taste reactivity test (TR) in the NAc. We assess here the contribution of Orexins, hypothalamic peptides modulating homeostatic feeding and ethanol intake, to hedonic processing. To that aim, we evaluate the role of OX1-R and OX2-R signaling within the NAc Shell (NAcSh) to regulate ingestive hedonic and/or aversive responses to sucrose and ethanol in rats, as measured by the TR test. After a period of habituation to the palatable solutions, adult male Sprague-Dawley rats were given an intra-NAcSh bilateral infusion of either SB334867, a selective OX1-R antagonist (1.5 µg/0.5µl/site) or TCS OX2 29, a selective OX2-R antagonist (3 µg/0.5µl/site). 30 min later, the animals received either 1 ml of ethanol (6% w/v) or sucrose (1% w/v) intraoral for 1 minute. Aversive and hedonic behaviors were recorded and quantified. We found that blockade of OX2-R, but not OX1-R, significantly reduced sucrose and ethanol hedonic responses relative to vehicle-infused animals. These data suggest a key role for OX2-R signalling in modulating hedonic aspects of sucrose and ethanol intake, establishing a new promising pharmacological target in therapies aimed to treat eating disorders or ethanol abuse.

1ª: Cognitive and Behavioral Neuroscience.
2ª: Systems Neuroscience.

LANGUAGE-MEDIATED EYE MOVEMENTS IN PARKINSON'S DISEASE

M. Delgado-Alvarado[1], J.A. Duñabeitia[2], C. Caballero[2], L. Garcia-Penton[2], J. Ruiz-Martinez[1,5], A. Bergareche[1,5], J.F. Marti-Masso[1,4,5], M. Carreiras[2,3,4] and M.C. Rodriguez-Oroz[1,3,5]
[1]Instituto de Investigación Sanitaria Biodonostia, San Sebastián, Guipuzcoa.
[2]BCBL, Basque Center on Cognition, Brain and Language, San Sebastián, Guipuzcoa.
[3]Ikerbasque, Basque Foundation for Science, Bilbao, Vizcaya.
[4]UPV/EHU, Universidad del País Vasco, Bilbao, Vizcaya.
[5]Unidad de Trastornos del Movimiento del Hospital Universitario Donostia, San Sebastián, Guipuzcoa.

Objective: To investigate eye-movement patterns of patients with PD with normal cognition (PDNC) and PD with mild cognitive impairment (PD-MCI) when confronted with higher order visual tasks requiring executive control and enhanced visuo-attentional skills
Methods: 15 PDNC, 16 PDMCI patients and 20 healthy controls completed an eye-tracking study in which they had to identify the picture that matched a given auditory input among a set of visual distractors that could be semantically related or unrelated to the target (e.g., hearing "cat" and being presented with the distractor picture of a "dog"; i.e., a test based on the visual-world paradigm). Participants also completed a behavioral flanker task designed to characterize each group's inhibitory skills based on their executive functions.
Results: PDMCI patients showed significantly higher error rates than controls in the general accuracy in the task ($p=0.011$), while PDNC and controls did not differ ($p=0.33$). PDNC and PDMCI patients showed no differences between them and with controls in number and duration of fixations and saccade amplitudes ($p>0.05$). When required to inhibit semantic distractors, PD-MCI patients ($p=0.001$) but no PDNC ($p=0.072$) showed significantly higher fixation probabilities on the competing elements than on unrelated distractors as compared to controls. Accordingly, PD-MCI patients showed significantly larger incongruity effects than controls in the accuracy data from the flanker task ($p=0.036$).
Conclusions: The basic ocular movements are similar in PD patients and controls in an ecologic language-mediated visual task. However, PD-MCI patients show a different pattern of visual performance when the visual analysis of a display requires higher order cognitive mechanisms based on inhibitory skills.

COCAINE-LIKE PROPERTIES OF A NEW DRUG OF ABUSE: MDPV

P. Muñoz-Villegas[1,2], M. Buenrostro[1,2], J. Moreno [1,2], A. Gutierrez[1,2], A.M. Ciudad[1,2], R. López-Arnau[1,2], J. Camarasa, D. Pubill[1,2] & E. Escubedo[1,2].
[1]. Dept Farmacologia i Química Terapèutica, Facultat de Farmàcia.
[2]. Institut de Biomedicina, Universitat de Barcelona.

3,4-methylenedioxypyrovalerone or MDPV, is a new psychostimulant drug that inhibits dopamine and noradrenaline transporter (DAT, NET) with an affinity ten-fold higher than cocaine. The aim of this study was to investigate some MDPV effects that could mimic those of cocaine, in order to assess the abuse liability of this new drug.
Conditioned place preference (CPP), to evaluate rewarding effect, was performed in Sprague-Dawley rats. Behavioural sensitization and also the elevated plus maze (EPM), to evaluate the anxiety-like behaviour, were performed in Swiss-CD1 mice. Changes on DAT were investigated in NGF-differentiated PC12 cells and rat striatal synaptosomes, where binding of [^{3}H]WIN35428 and [^{3}H]dopamine uptake were measured respectively. MDPV (1-5 mg/Kg) induced dose-independent CPP, increasing the time spent in the drug-paired compartment ($P<0.01$). Additionally, MDPV (0.3 mg/Kg) induced a sensitized locomotor response that resulted in an increase of 510.46±51% on the 5th day versus 72.35±5.1% on day 1. After withdrawal, a challenge dose of MDPV, induced an increase of 163.16%, compared with saline-pretreated animals. We demonstrated a significant difference in the EPM between saline and MDPV treated animals ($F3,32 = 3.793$, $p<0.01$). MDPV (but not saline)-treated animals spent more time in the open arms ($p<0.01$) than in the closed arms. Incubation of PC12 cells for 15 or 30 min with 0.1 µM MDPV produced an increase in [^{3}H]WIN 35428 bound of 104.7± 10.9 and 90.3 ± 9.9 %, respectively. In conclusion, animals exposed to MDPV showed cocaine-like behavioral and mechanistic effects, which points to a real abuse liability of this new cathinone.

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

CHRONIC EFFECTS OF JRP2 COMPOUND IN NEURAL PLASTICITY IN A MOUSE MODEL FOR FRAGILE X SYNDROME

J. Albaigès Ràfols [1], S. Molas Casacuberta [2], M. Carbó [3], D. Prous [3], J. Prous [3], M. Dierssen Sotos [1]
[1] Centre de Regulació Genòmica (PRBB), Barcelona
[2]University of Massachusetts Medical School, USA
[3]Prous Institute for Biomedical Research, Barcelona

Fragile X syndrome (FXS) is a common inherited cause of human intellectual disability. It results from the expansion of a CGG repeat region of the Fragile X mental retardation (FMR1) gene located on the X chromosome. In brain, the protein product of FMR1 gene (FMRP) has an important role in synaptic function and synaptic plasticity. Fmr1 Knock-Out (KO) mice recapitulate some of the physical and behavioral characteristics of FXS such as abnormal social behavior, social recognition and abnormal dendritic spine density and morphology. Here, we evaluated JRP2, a small phenyl aminoalkyl ether molecule, designed by Prous Institute for Biomedical Research as a therapeutic drug that could enhance cognitive function by targeting neuronal plasticity. Noteworthy, chronic oral administration of JRP2 completely restored the social recognition abnormalities of Fmr1 KO mice. We also evaluate if JRP2 was able to restore the aberrant morphology and density of dendritic spines in layer III-IV of medial prefrontal cortex (mPFC) neurons by using the Golgi-cox staining. Chronic treatment with JRP2 completely rescued the spine phenotype, by normalizing spine density and mature and immature spine morphology in treated mice. In conclusion, JRP2 could represent a therapeutic strategy to modulate neuroplasticity and treat the social autistic-like behavior associated with FXS.

BRAIN AREAS INVOLVED IN AGGRESSIVE BEHAVIOUR IN *Lemniscomys barbarus*

N. El Mlili[1], R. Boutoual[2], AM Sanchez Perez[3], Errami Mohammed[2], F. Olucha Bordonau[3]
1. Institut Supérieur de Profession Infirmières et Techniques de Santé, Rabat. Morocco
2. Faculté des Sciences, Université Abdelmalek Essaâdi, Tétouan. Morocco
3. Facultad de Ciencias de la Salud, Universidad Jaume I, Castellon de la plana. España

Confrontation between cospecifics sometimes result in aggression or agonistic behavior. Aggression is a kind of behavior directed towards improving the viability of an individual to obtain resources like food and water and also to find mates, especially for males, and defend territories, offspring and maintain social rank. However, aggressive behaviour may be costful in terms of energy expenditure and may lead to fatal consequences, even death. In such conditions, aggression may become pathological. Sometimes when aggressive behavior turns on one subject takes the role of the offender and the other the role of defender. The anatomic areas subserving those roles may be different and this is the goal of this work. We have studied the stripped mouse *Lemniscomys barbarus* which is a north african diurnal rodent well known by its natural aggressiveness toward conspecifics. Each couple of experimental animals was placed in a new neutral cage to be confronted, their behaviors were videtaped and then analysed. One hour after the behavioural test animals were perfused and brains from each experimental group were removed. Then ICC towards c-fos was used as a meassure of neural activity in reklevant brain areas including amygdala, septum, prefrontal cortex, nucleus incertus, anterior hypothalamic area, suprachismatic nucleus and periaqueductal gray. Our results showed that conspecific confrontation induced a general c-fos activation in both offender and defender subjects in all measured areas in comparison with not confronted control. Differences in neural activity between offender and deffender were observed in the lateral, cortical and medial amygddala, suprachiasmatic nucleus and the nucleus incertus. In the amygdala there was a possitive correlation between the freezing time and c-fos activation. The4se results indicate that some brain areas like medial amygdala and the nucleus incertus are differentially activated during the display of offensive and deffensive behaviors.

1. Cognitive and behavioral Neuroscience
2. Systems Neuroscience

THE IMPACT OF PRIOR EXPECTATIONS IN PERCEPTUAL DECISIONS

A. Hermoso-Mendizabal[1], P.E. Rueda-Orozco[2], S. Jaramillo[3], D. Robbe[2] and J. de la Rocha[1]
[1]. IDIBAPS, Barcelona, Spain.
[2]. Institut de Neurobiologie de la Mediterranee, Marseille, France
[3]. University of Oregon, USA

Previous experience plays a critical role in how subjects acquire and interpret sensory information. Little is known however about how the brain circuitry accumulates experience over various time scales to build expectations that combined with sensory information give rise to perception. In order to identify the neural basis of expectation and its impact on perceptual decisions we designed a variant of a standard 2AFC task that required the generation of an unbalanced prior that had to be updated trial-by-trial in order to maximize performance. Acoustic stimuli consisting in the superposition of two amplitude modulated tones of high and low frequency were presented to rats that were trained to associate each frequency to the delivery of water in the Right and Left ports. Stimuli were parameterized by the coherence c that sets the relative weight of each tone. The two stimulus categories, i.e. left or right, were presented randomly using a two-state Markov chain. Transitions were parameterized such that the probability of repeating the previous stimulus category was 0.5+lambda and the probability to switch was 0.5-lambda. The absolute value of the coherence was picked randomly and independently of previous history. We presented randomly blocks of 200 trials with fixed lambda>0 (repetitive environment) and lambda<0 (alternating environment). We found that animals learned the statistics of each environment and made use of them to increase reward rate. Specifically, after error trials animals did not show a systematic history dependent choice bias. After a correct trial however, the behavior showed a bias towards the same or the opposite choice depending on whether they were in the repetitive or in the alternating environments, respectively. Moreover, the magnitude of this choice bias increased with the number of correct past responses following the environment's sequence pattern and were greater in the repeating than in the alternating environment.

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

ROLE OF STRESS IN MORPHINE ASSOCIATED RELAPSE

Teruel Fernandez FJ, Navarro-Zaragoza J, Ribeiro Do Couto BR, Milanés MV, Laorden ML
Research Group of Cellular and Molecular Pharmacology, Faculty of Medicine, University of Murcia, Murcia, Spain.

It has been shown previously that stress has a prominent role in drug addiction existing a loop between noradrenergic system and brain stress system for NA and CRF release. It has been suggested that stress and different factors in daily life could influence an increase in drug-taking and the resulting addiction. This study was based on observing how different physical factors could act as stress factors influencing a future relapse into drug use after a period of abstinence, including withdrawal. This research involved twenty mice divided into two groups: one corresponding to stress stimuli (Tail Pinch), and the other one was the control group. All the animals were administered an increasing dose of morphine (10, 30, 50 and 60 mg/kg) and the last day they were firstly injected with naloxone (1 mg/kg), and secondly introduced in a place-preference box in order to develop CPA. After 5-9 sessions, when the CPA was extinguished in all mice, we proceeded to perform stress tests. Our results, statistically significant, showed that physical-psychic stimulus were able to create the state of aversion which mice had previously faced so it could be said that a physical stimuli like *Tail Pinch* has an effect on substance abuse relapse in opioid-dependent animals.

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

CONSCIOUS PERCEPTION IN PATIENTS WITH INTRACTABLE EPILEPSY: ROLE OF THE HIPPOCAMPUS AND POSSIBLE EFFECTS OF DIRECT ELECTRICAL STIMULATION.

M. Quevedo-Díaz [1,2], T. Gener [2], A. Principe [2], M. Dierssen [1], R. A. Rocamora-Zúñiga [2]
[1]. Centro de Regulación Genómica, Barcelona
[2]. Institut Hospital del Mar de Investigacions Mèdiques, Barcelona

Most of attempts to locate specific parts of the brain involved in conscious processing have been unfruitful. The recent spread of intracranial electroencephalographic recording techniques for pre-surgical evaluation of drug-resistant epileptic patients provides new information on the activity of different brain structures in specific processes. Recent data suggest the involvement of the hippocampus in conscious experience. This work is aimed at understanding local physiological processes in the deep brain during conscious/subconscious perception of emotion-related stimuli. Specifically, we will focus on the activity of the hippocampus using Stereo-electroencephalography (sEEG) in order to find the neurophysiologic correlates of a conscious perception neuropsychological task during subconscious or consciously perceived trials.

Methods: 4 right handed epileptic patients (3 males) with depth electrodes implanted at hippocampal cortices and amygdaline nuclei (1 bilaterally), were asked to solve a forced choice test for discriminate the emotional valence (negative versus neutral) of several words that were shown in two conditions: unmasked (consciously perceived) stimuli and masked (subconscious) stimuli (Significant Word Paradigm, SWP). Event Related Potentials (ERPs) for each condition were obtained. All participants had previously signed an Informed Consent Form and Local Ethics Committee approved all the procedures.

Results: For conscious trials, we have obtained ERPs from hippocampal gyri that show an early response at 800ms after neutral words, and a late response at 850-900ms for negative words. In contrast, ERPs to subconscious trials, were more diffused in time (400-800ms) for neutral words, but for negative words, a more robust response was found at 700-800ms.

Conclusions: We have shown that hippocampus may play a critical role for conscious processing of visual stimuli such as emotional words. Ongoing research applying the same paradigm right after direct electrical stimulation (DES) to hippocampal region could probe this finding as DES may disturb both cognitive performance and its respective electrophysiology readout.

1ª: Neurociencia cognitiva y conductual
2ª: Nuevos métodos y tecnologías

N400 EFFECT IN PROCESSING COMPOSED VISUAL MEANING

P. Álvarez Merino [1], C. Requena Hernández [1], F. Salto Alemany [1,2]
[1]. Facultad de Educación. Universidad de León, León
[2]. Facultad de Filosofía, Universidad de León, León

Objective. While the linguistic meaning of a sentence is a compositional function of its components, the visual meaning of images is not known to be compositional. This research tries to verify N400 elicitation as a measure of compositional semantic processing of images.
Material and Methods. Sample of 27 postgraduate students between 22 and 27 years. A paradigm with four conditions was designed, each including 50 congruent/incongruent tasks. Each task showed 3 images within a 2900 ms interval, where the first acts as context for the other two images, which are presented together. All subjects received previous training and no material used in the training phase was available in the experimental phase. Measured variables were: response times and event-related potential N400 (ERP) registered with electroencephalogram (EEG). EEG was recorded with a 64 channel Neuronic Medicid Equipment using a standard 10–20 electrocap. The impedance of all electrodes was kept below 5 kΩ. N400 potential was analyzed in latencies oscillating between 300 and 550 ms in the centroparietal medial area.
Results. The visual elicitation of N400 in visual complexes is equivalent to the linguistic elicitation in linguistic compounds: more amplitude in more incongruent conditions and less amplitude in more congruent conditions, irrespective of their visual or linguistic nature. Response time increases at the same rate and with the same intensity as incongruency does. It is remarkable the sensitivity of N400 to the order in the presentation of congruent-incongruent images, since ordering relations codify compositional information.
Conclusions. Processing compound visual or linguistic meaning congruent/incongruent with semantic stimulate do elicit N400. However, the relevance of the ordering of compound images is still not explained.

1ª: Neurociencia cognitiva y conductual.
2ª: Historia, Docencia, Divulgación y Ética.

EARLY MOTOR SEQUENCE LEARNING IS ALTERED IN PARKINSON DISEASE: AN fMRI STUDY.

L. Eudave [1], E. Luis [1], M. Martínez[1], M. Aznarez-Sanado[1], M. A. Pastor [1,2]
[1]. Centro de Investigación Médica Aplicada (CIMA). Universidad de Navarra. Pamplona, España.
[2]. Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED). Madrid, España.

Introduction: Parkinson´s Disease (PD) is characterized by a progressive dopaminergic denervation affecting several motor and cognitive domains. Brain activity in early motor learning in healthy subjects has been localized in the motor, prefrontal and pre-SMA cortices, as well as the basal ganglia and the hippocampus. PD patients are known to have a dysfunctional early motor learning, although its neural correlates still remain unclear. Therefore we aimed to explore the early motor learning in PD in three different modalities, its associated brain activity and the effect of task regressors. **Methods**: Ten patients with early phase, right-dominance onset PD in the ON-state, and 18 healthy older adults were recruited and underwent neuropsychological evaluation. Our behavioral paradigm consisted of a simple and complex motor sequence learning test with the right, left and both hands, where accuracy and timing were evaluated. fMRI-BOLD images were acquired and processed using the DARTEL normalization method. Accuracy and timing values were used as regressors. **Results**: PD patients displayed a significant worse cognitive performance across all tasks (lower accuracy, longer inter-tap intervals (ITI) and ITI variation). Bimanual performance was worse in both PD patients and healthy subjects. Neuroimaging results showed hyperactivation in PD of the pre-SMA, DLPFC, prefrontal and sensorimotor cortices, right putamen, posterior cingulate, right supramarginal gyrus, parietal associative areas and cerebellum. High accuracy was correlated with ponto-cerebellar activity only in healthy subjects. Also in the control group, longer ITI variations were correlated with DMPFC, left VLPFC, and right angular gyrus activity; shorter variations were associated with activity in the cortico-basal-cerebellar pathway. No significant differences in hand modality were found. **Conclusions**: Early PD patients develop motor learning deficits even at the ON-state associated while hyperactivating the usual motor sequence learning areas plus parietal associative areas and the posterior cingulate as a possible compensating effect due to dopaminergic denervation.

1ª: Neurociencia cognitiva y conductual
2ª: Nuevos métodos y tecnologías

Topic 5

Theoretical and Computational Neuroscience

DETECTING PHASE TRANSITION PHENOMENA IN THE BRAIN

J. J. Torres[1], J. Marro[1]
[1] Instituto Carlos I, Universidad de Granada, Granada

We describe a theoretical and computational method to detect phase transitions separating different cognitive behaviors in the brain. We consider a brain model set up of a complex network of spiking neurons connected to each other through dynamic synapses involving short-term synaptic plasticity, and stimulate each neuron with a weak signal assuming this is competing with a source of uncorrelated noise. We then monitor the network activity for different levels of the noise and neuron excitability parameters, and compute the correlation among the network activity and the weak stimulus. Our study reveals for well-defined values of the noise strength neat peaks of the signal to noise ratio of the power spectra of the network activity that indicate the occurrence of phase transitions separating regions, in the space of relevant parameters, each associated to a different cognitive behavior. Among others, we found regions in which stable memory and recall emerge, as well as other regions characterized by dynamic memories with various different features. These emergent phenomena happen to be quite robust against wiring topology modification — in fact, we considered from a fully connected network to the Homo sapiens connectome — showing the essential role of synaptic flickering on computations. Our model may be useful to analyze the cognitive and computational properties of different brain connectomes and to detect brain changes in EEG time series, for instance.

Thematic areas:
3ª:Neurociencia de sistemas
4ª:Neurociencia cognitiva y conductual

TASK RELEVANT VARIABLES ARE ENCODED IN OFC

Ramon Nogueira[1], Juan M. Abolafia[2], Jan Drugowitsch[3], MV. Sanchez-Vives[2,4], Rubén Moreno-Bote[1,5]
[1] Fundació Sant Joan de Déu, Esplugues de Llobregat, Barcelona, Spain, 08950
[2] IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer), Barcelona, Spain, 08028
[3] Departement des Neurosciences Fondamentales, Universite de Geneve, Geneve, Switzerland 1211
[4] ICREA (Institució Catalana de Recerca i Estudis Avançats), Barcelona, Spain, 08028
[5] Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Esplugues de Llobregat, Barcelona, Spain

Prefrontal Cortex (PFC) in mammals is well-known for playing a key role in executive function. Orbitofrontal Cortex (OFC), as a part of the PFC, is thought to be mainly involved in reward processing, a fundamental function in goal-directed behavior. In particular it has been proposed that OFC is encoding the expected value of an outcome [1]. However, recent studies in rats have found neuronal correlates with the spatial representation of goals and with decision confidence [2,3].
In our experiment three rats performed an auditory categorization task while populations of neurons were recorded in OFC. The task was designed such that rats could use both sensory information and task history information in order to maximize reward income.
We found that neurons in rats' OFC conveyed a wide variety of task related variables each one with a different time dynamics. Surprisingly, before stimulus onset neuronal activity was predictive of rats' upcoming choice, a neuronal evidence of task-history related behavior. Also before stimulus onset OFC neurons showed significant encoding of previous choice, previous reward and previous stimulus. After stimulus presentation neuronal activity correlated with stimulus difficulty, a proxy for the trial's expected value, as well as with stimulus and reaction time, a well-known proxy for metacognitive processes as confidence.
Our results point in the direction that OFC in rats might not only be involved in reward processing but it also conveys a wide variety of task relevant variables. Our hypothesis is that OFC acts as a hub for decision-making, where information is processed and conveyed to other brain regions.

[1] J.D. Wallis. Annual Review of Neuroscience *30*, 31-56 (2007)
[2] C.E. Feierstein, M.C. Quirk, N. Uchida, D.L. Sosulski, Z.F. Mainen. Neuron *51*, 495-507 (2006)
[3] A. Kepecs, N.Uchida, H.A. Zariwala, Z.F. Mainen. Nature *455*, 227-231 (2008)

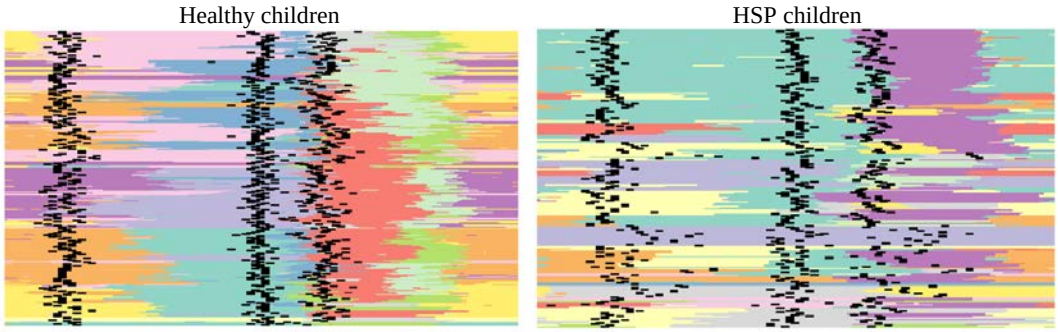
1. Theoretical and Computational Neuroscience
2. Systems Neuroscience

E-mail: rnogueiramanas@gmail.com

KINEMATIC CONFIGURATIONS ALONG GAIT CYCLE IN CHILDREN WITH HEREDITARY SPASTIC PARAPLEGIA: A NEURAL NETWOKS APPROACH.

I. Pulido Valdeolivas[1,2], D. Gómez Andrés[2,4], I. Rodríguez-Andonaegui[3], JA. Martín-Gonzalo[3], J. López-López[4], S.I. Pascual-Pascual[5] E. Rausell[1,2]
[1]Facultad de Medicina. Universidad Autónoma de Madrid, Madrid.
[2]"Trastornos del Desarrollo y Maduración Neurológica" (TRADESMA). IdiPaz. Madrid.
[3]Escuela de Fisioterapia. ONCE-UAM. Madrid.
[4]Hospital Universitario Infanta Sofía. San Sebastián de los Reyes. Madrid.
[5]Hospital Universitario La Paz. Madrid

Objectives: To study kinematic configurations throughout the gait cycle (GC) as a measure of motor control in paediatric patients with hereditary spastic paraplegia (HSP) and to compare them with those of healthy children.
Materials and Methods: Kinematic parameters of 4-5 left and 4-5 right GC from 26 patients with HSP and33 healthy children (HCh) were measured at 201 epochs (Codamotion®). Each GC was represented by 201 kinematic vectors (one vector for each epoch) of 15 elements (frontal, sagittal and rotational parameters from 5 joints: pelvis, hip, knee, ankle and midfoot). An unsupervised 10x10 neurons SOM neural network was trained to classify kinematic vectors. Hierarchical clustering of neurons was used to reduce number of groups and to solve arbitrary selection of network dimension and after GCs were also classified. Colours were assigned to different SOM clusters. Each SOM cluster allocates vectors encoding similar kinematic configurations.
Results: Heatmaps (left: HCh, right: HSP) show the classification of GCs using hierarchical clustering (rows) and 201 epochs in columns (from initial to final foot-strike). Epochs are colored according to the corresponding SOM cluster. In black, observational events: contralateral foot-off, contralateral foot-strike and ipsilateral foot-off. GCs are built as combinations of time-specific kinematic configurations. Observational events along the GC are not coincident with switches of kinematic patterns. HSP kinematic GC structure is altered with respect to healthy subjects. Single support of HSP GC does not show the patterned switch that is normally seen in HCh. Besides, the number of possible configurations is lower. Component planes demonstrate different relationships among joint movements (not shown).

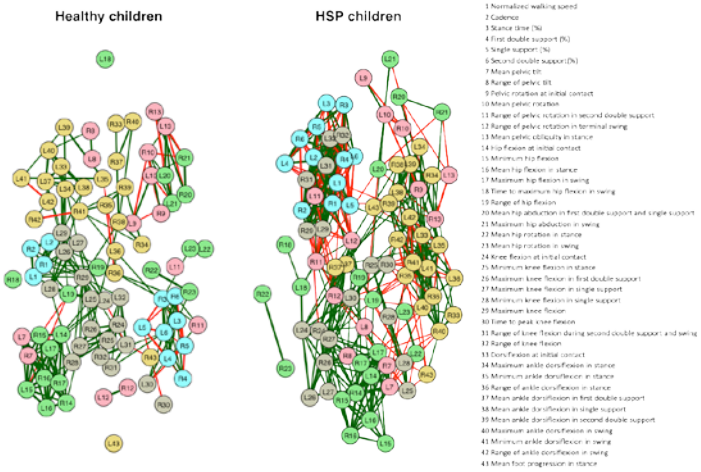


Conclusions: The gait cycle can be studied as an ordered sequence of kinematic patterns featured by well-determined "switching points". Principles that govern switching points are different in HSP children, as a result of the adaptive processes of the CNS to lesion.

GAIT NETWORK PROPERTIES IN HEREDITARY SPASTIC PARAPLEGIA CHILDREN

D. Gómez Andrés[1,4], I. Pulido Valdeolivas[1,2-], I. Rodríguez-Andonaegui[3], JA. Martín-Gonzalo[3], J. López-López[4], S.I. Pascual-Pascual[5] E. Rausell[1,2]
[1]Facultad de Medicina. Universidad Autónoma de Madrid, Madrid.
[2]"Trastornos del Desarrollo y Maduración Neurológica" (TRADESMA). IdiPaz. Madrid.
[3]Escuela de Fisioterapia. ONCE-UAM. Madrid.
[4]Hospital Universitario Infanta Sofía. San Sebastián de los Reyes. Madrid.
[5]Hospital Universitario La Paz. Madrid

Objective: To assess gait system's functioning in hereditary spastic paraplegia (HSP) by analyzing network structure and network statistics.
METHODS: 43 left and right gait parameters (6 spatiotemporal and 37 kinematic) from 33 healthy children (HCh) and 26 HSP children were extracted and averaged from 4-5 left and 4-5 right gait cycles. Gait networks were built for HCh and HSP using gait parameters as nodes and bivariate Spearman's rho correlation coefficient as edges. Integration, segregation, modularity, assortativity, resilience and centrality were assessed in each network.
Results: Both networks behave as exponential complex networks, HSP network is more integrated and less segregated, but has higher modularity. Assortativity is similar in both networks. Although global communicability is similar in both network, node communicability corrected by node strength is higher in HCh. Resilience is higher in HSP network. Spatiotemporal are re-organized in HSP: walking speed and cadence relate with temporal distribution in gait cycle. Knee parameters related to limb clearance joined to spatiotemporal module.



Conclusions: Functioning of HSP gait system is different to healthy children's. The gait of HSP subjects seems to be more rigid that could indicates less adaptation to changes. Gait compensation produced by HSP alteration configures groups of highly correlated gait parameters. HSP gait system has to adapt spatiotemporal parameters to knee constraints. These results are relevant in understanding of gait pathophysiology and mechanisms and planification of treatment.

MODELING THE NON-LINEAR INFLUENCE OF DYRK1A ON ACTIN POLYMERIZATION

GA Patterson, MA Dierssen
Center for Genomic Regulation, Barcelona Biomedical Research Park, Barcelona

Dyrk1A is a member of the dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) family. It plays a significant role in signaling pathways regulating cell proliferation and brain development. Overexpression of Dyrk1A is sufficient to produce dendritic alterations. In particular, it was shown that changes in Dyrk1A gene dosage strongly alter the dendritic arborization processes leading to a reduction of neurite outgrowth and complexity [Martinez, et al., 2012]. Neurons in Dyrk1A+/- mouse, a model lacking one copy of Dyrk1A, and also in TgDyrk1A, overexpressing 1.5 times the kinase, were less branched and less spinous than those of WT. Those results suggest that Dyrk1A is affecting cellular pathways involved in neural development and plasticity in a way that dosage changes lead to similar alteration regardless the direction of this change [Benavides-Piccione, et al., 2005]. Neural Wiskott-Aldrich syndrome protein (N-WASP) is involved in tight regulation of actin polymerization and dynamics and it is a substrate for Dyrk1A [Park, et al., 2012]. Here, we present a simple mathematical model to characterize the nonlinear influence of Dyrk1A in regulation of actin polymerization through N-WASP dynamics. We found that Dyrk1A modulates the concentration of active N-WASP, which is the responsible protein state that promotes the assembly of actin filaments, in a non-monotonic way. These results suggest that there is an optimum amount of Dyrk1A level that maximizes the actin polymerization rendering the experimental evidence of dendritic complexity. We believe that these findings may shed same light on how Dyrk1A affects cellular processes such as morphogenesis and neuronal differentiation.

R. Benavides-Piccione, et al., Neurobiology of Disease (2005)
M. Martinez de Lagran, et al., Cereb. Cortex (2012)

J. Park, et al., J Cell Sci (2012)

DECODING OF PARAMETRIC WORKING MEMORY FROM THE TRANSIENT DYNAMICS OF A CONTROLLED BALANCED NETWORK

M. Ramon Bohigas, A. Compte
Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona

Working memory refers to the ability to briefly store information in memory-guided tasks. Experiments with monkeys have identified the neural correlates of spatial working memory in the spatially tuned persistent activity of prefrontal neurons[1]. The bump attractor computational model has been particularly successful in explaining this sustained activity[2,3]. However, recent experiments on monkeys performing other parametric working memory tasks have found very few prefrontal neurons maintaining persistent activity along the whole delay epoch[4], with stored information often declining and reappearing during the delay[5]. In addition, neuronal tuning could change markedly from stimulus to delay period[4,5,6]. Such dynamics in the mnemonic period are hard to reconcile with the bump attractor model and new theoretical models have been proposed to explain non-sustained neural dynamics in working memory. Here, we address how tuning reversal and tuning emergence can occur during the delay based on such dynamic computational schemes for working memory. We built upon the stability-optimized circuit (SOC) of ref. 7, a recurrent network model where the inhibitory connection weights are tuned to stabilize the spontaneous state while preserving large-amplitude long-lasting transients. We show that a one-dimensional parametric stimulus can be retained in the firing rates of such transient neuronal activations through the delay period, and it can be decoded from the population activity of a ring of neurons receiving converging input from the SOC network units. Interestingly, the collective activity of the SOC and ring-decoding networks displays several of the features observed experimentally: tuning reversal and emergence of tuning at the end of the delay.

1. Funahashi et al. J Neurophysiol 1989
2. Compte et al Cereb Cortex 2000
3. Wimmer et al. Nat Neurosci 2014
4. Hussar and Pasternak. J Neurosci 2012
5. Barak et al. J Neurosci 2010
6. Stokes et al. Neuron 2013
7. Hennequin et al. Neuron 2014

1ª: Neurociencia teórica y computacional
2ª: Neurociencia de sistemas

SCHAFFER-ELICITED GAMMA OSCILLATIONS AND EVOKED RESPONSES IN CA1 ARE ALTERED BY CONCURRENT ACTIVITY IN THE CA3 THROUGH VOLUME CONDUCTION.

O. Herreras[1], A. Korovaichuk[1], V Makarov[2], J. Makarova[1]
[1]Cajal Institute, CSIC, Madrid
[2]Facultad de Matemáticas, Univ. Complutense Madrid

Gamma oscillations are being extensively studied for their alleged participation in perception and in the formation of functional cell assemblies. A main problem in their study is that there are multiple neural sources of gamma oscillations, even in near locations. As extracellular fields, their single or multi-source composition cannot be guaranteed. Hence, the statistical properties and parameters cannot be attributed to specific pathways, weakening severely their physiological meaning. Recently we optimized a spatial discrimination technique (independent component analysis, ICA) to separate the contributions mixed in raw LFPs, and reported several sources of gamma oscillations in the hippocampus of either excitatory or inhibitory character. The separation of these gamma generators depends on non-obvious technical and biological factors that can only be examined using realistic models. We have used real linear recordings of LFPs and a former aggregate (multineuronal) model of the CA1 pyramidal population upscaled with contiguous blocks representing the CA3 region to examine the conditions for ICA separability and identification of nearby sources of gamma generators and the relevant factors. The most relevant findings are: aggressive filtering distorts the spatial profiles of gamma generators and the individual wave characteristics. Selection of electrode groups for analysis has to be made with caution as it may improve or hamper separation. Volume conduction of nearby concurrent CA3 waves is critical to define spatial profiles of spontaneous and evoked fields, affecting the amplitude, polarity, and site of reversal. These results indicate strong effect of technical and biological factors, including concurrent oscillations in nearby regions, which may alter important landmarks employed on the cellular interpretation of LFP oscillations. Financed by MEC: BFU2013-41533-R

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

Topic 6

Disorders and nervous system repair

PATIENTS WITH MINIMAL HEPATIC ENCEPHALOPATHY SHOW INCREASED OXIDATIVE STRESS CORRELATING WITH COGNITIVE AND MOTOR IMPAIRMENT

A. Mangas-Losada[1], R. Garcia-Garcia[2,1], C. Gimenez-Garzó[1,2], A. Agustí[1], A. Urios[1], O. González-López[3], D. Escudero-García[4], A. Escudero-Sanchis[3], M. A. Serra[4], R. Giner-Durán[3], V. Felipo[2] and C. Montoliu[1,5]
1. Fundación Investigación Hospital Clínico de Valencia. Instituto de Investigación Sanitaria-INCLIVA, Valencia, Spain
2. Laboratorio de Neurobiología, Centro Investigación Príncipe Felipe de Valencia, Spain
3. Servicio de Digestivo, Hospital Arnau de Vilanova, Valencia, Spain
4. Unidad de Digestivo, Hospital Clínico de Valencia. Departamento de Medicina, Universidad de Valencia, Spain
5. Departamento de Patología, Facultad de Medicina, Universidad de Valencia, Spain

Cirrhotic patients may suffer minimal hepatic encephalopathy (MHE), with mild cognitive impairment. 3-Nitro-tyrosine levels are a good biomarker for diagnosis of the cognitive impairment and MHE in cirrhotic patients. This suggests that oxidative stress could be involved in the induction of cognitive and motor alterations in MHE. We have observed that patients with MHE show increased oxidative stress in blood compared with cirrhotic patients without MHE, with increased lipid peroxidation, DNA oxidation, protein carbonylation, 3-nitrotyrosine, oxidized glutathione (GSSG)/reduced glutathione (GSH) ratio, and GSH levels. The activities of antioxidant enzymes are enhanced in erythrocytes and mononuclear cells from patients with and without MHE compared with control subjects. Only glutathione peroxidase activity was increased in MHE patients compared with patients without MHE. Oxidative stress markers in blood, especially GSSG/GSH ratio, GSH, malondialdehyde, and 3-nitrotyrosine, correlate with deficits in attention and motor coordination. The increase in antioxidant activities in patients would be an adaptive mechanism to cope with enhanced oxidative stress, although it is not effective enough to normalize it. Our observations lead to the hypothesis that oxidative stress and increased peroxynitrite formation would mediate the synergistic effects of hyperammonemia and inflammation on cognitive and motor impairment in MHE.

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

INCREASED 3-NITROTYROSINE IS ASSOCIATED WITH REDUCED WHITE MATTER MICROSTRUCTURAL INTEGRITY AND COGNITIVE DEFICITS IN MINIMAL HEPATIC ENCEPHALOPATHY

R.Garcia-Garcia[1,2], A. Mangas-Losada[2,], A. Agustí[2], R. Novella[2], A. Urios[2], C. Forn[3], C. Avila[3], O. Gonzalez[4], MA. Serra[5], R.Giner-Durán[4], V. Belloch[6], and V. Felipo[1], C. Montoliu[2,7]
1. Laboratorio de Neurobiología, Centro Investigación Príncipe Felipe de Valencia, Spain
2. Fundación Investigación Hospital Clínico de Valencia. Instituto de Investigación Sanitaria-INCLIVA, Valencia, Spain
3. Departamento Psicología Básica, Clínica y Psicobiologia. Universitat Jaume I. Castellon, Spain
4. Servicio de Digestivo, Hospital Arnau de Vilanova, Valencia, Spain
5. Grupo Hepatología, Servicio Aparato Digestivo, Hospital Clínico de Valencia, Spain
6. ERESA, Unidad de RM, Valencia, Spain
7. Departamento de Patología, Facultad de Medicina, Universidad de Valencia, Spain

Background & aims: Cirrhotic patients with minimal hepatic encephalopathy (MHE) show mild cognitive impairment and psychomotor slowing. Magnetic resonance studies using diffusion tensor imaging (DTI) suggest that these patients may show white matter abnormalities. This work aims were 1) to investigate whole-brain white matter microstructural abnormalities associated with MHE using TBSS; 2) to evaluate the correlations of white matter microstructure changes with performance in psychometric test evaluating different cognitive and motor coordination deficits; 3) assess whether some peripheral biomarker correlates with changes in microstructural integrity of white matter tracts.
Methods: White matter microstructure integrity was analyzed using DTI imaging and Tract-Based Spatial Statistics (TBSS) in 17 controls, 15 cirrhotics without and 15 with MHE. Psychometric tests assessing different functions were performed and several biochemical parameters were measured in blood.
Results: Patients with MHE (but not without MHE) show reduced overall white matter structural integrity, with increased mean diffusivity (MD) and reduced fractional anisotropy (FA). Reduced FA of some tracts correlate with performance in line tracing and serial dotting tests. Increased MD correlate with performance in these same tests and in the symbol digit and number connection A tests and with serum levels of 3-nitrotyrosine. These findings suggest an association between microstructural alterations and reduced performance in attention, mental processing speed, visuospatial and visuomotor coordination tests.
Conclusions: Analysis of white matter microstructural integrity by DTI may provide new, strong, in vivo neuroimaging biomarkers for early diagnosis of MHE and to follow the efficacy of treatments.

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

LEARNING AND MEMORY IMPAIRMENT IN CHRONIC LIVER FAILURE: ROLE OF ALTERED MEMBRANE EXPRESSION OF GLUTAMATE AND GABA RECEPTORS IN HIPPOCAMPUS.

L. Taoro-González[1], A. Cabrera-Pastor[1], A. Agusti[2], V. Hernández-Rabaza[1], M. Llansola[1], V. Felipo[1]
[1]Laboratory of Neurobiology. Centro de Investigación Príncipe Felipe. Valencia
[2]Institute of Health Research, Hospital Clínico de Valencia, INCLIVA

Hepatic encephalopathy (HE) is a neuropsychiatric syndrome which appears in patients with liver diseases (mainly cirrhosis) leading to alterations in cognitive and motor functions. Hyperammonemia and inflammation play synergistic roles in inducing the neurotransmission alterations that cause neurological alterations in HE.
Rats with portacaval shunt (PCS), the main animal model of HE, show learning and memory impairments in the Y-maze and the Morris Water Maze (MWM) and alterations in motor activity and coordination.
Reduced learning and memory in the MWM is associated with altered neurotransmission and impaired long term potentiation (LTP) in hippocampus. Glutamate and $GABA_A$ receptors are the main modulators of LTP and of learning and memory in the MWM. A main mechanism regulating glutamatergic and GABAergic neurotransmission and LTP in hippocampus is the modulation of surface membrane expression of glutamate and GABA receptors, which is tightly controlled mainly through phosphorylation-dephosphorylation.
The aim of the present work was to assess whether membrane expression of the key receptors modulating LTP is altered in hippocampus of PCS rats with HE. We analysed the surface expression of AMPA (AMPAR) and NMDA (NMDAR) glutamate receptors and of $GABA_A$ receptors.
We show that PCS rats show increased p38-MAPK activation in hippocampus, associated with increased surface expression of the α1-containing $GABA_AR$ and GluA2-containing AMPAR. On the other hand, the surface expression of GluA1-containing AMPAR and NMDAR is decreased.
The alterations in hippocampal surface expression of $GABA_A$ and glutamate receptors could be responsible for learning and memory impairment in this animal model of HE.

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

Autor presentador: Lucas Taoro González, ltaoro@cipf.es

NEUROINFLAMMATION AND NEUROLOGICAL ALTERATIONS IN HEPATIC ENCEPHALOPATHY IN CHRONIC LIVER DISEASE

T. Balzano, S. Dadsetan, M. Llansola, A. Agusti, A. Cabrera-Pastor, M. Malaguarnera, V. Hernandez-Rabaza, J. Forteza, V. Felipo.
Laboratory of Neurobiology, Centro de Investigación Príncipe Felipe, Valencia, Spain.

Several million people with chronic liver diseases (cirrhosis, hepatitis) show neurological alterations, named hepatic encephalopathy (HE) with cognitive and motor alterations which impair quality of life and reduce life span. Inflammation acts synergistically with hyperammonemia to induce cognitive and motor alterations in patients with chronic liver disease and minimal HE (MHE). A few studies suggest the presence of neuroinflammation in patients with liver cirrhosis or hepatitis C with HE. A main role of neuroinflammation in the cognitive and motor alterations in MHE is supported by studies in animal models.
The aim of this work was to characterize neuroinflammation by analyzing cytokines and markers of glial cells activation and the relationship between neuroinflammation and cognitive and motor alterations.
Rats were subjected to portacaval anastomosis (PCS) to induce HE. To study spatial learning, working memory and motor coordination, we performed behavioral tests including Morris water maze, radial maze and beam walking. The markers of neuroinflammation were measured using immunohistochemistry and Western blot analysis.
The results show that PCS rats have altered expression of inflammation markers in the brain and these alterations could be related with the neurological impairment showed in the behavioral tests.
In conclusion, these data support the hypothesis that neuroinflammation play an important role in the neurological alterations in MHE. So, identifying new targets to reduce neuroinflammation would serve to develop new therapeutic tools to reverse the cognitive and motor alterations in patients with HE associated to chronic liver diseases.

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

Tiziano Balzano
tbalzano@cipf.es

CHANGES IN TGF-BETA SUPERFAMILY TROPHIC FACTORS, BDNF, ANTIOXIDANT ENZYMES AND FERRITIN IN THE CEREBROSPINAL FLUID OF PARKINSONIAN PATIENTS

A. Martín de Pablos [1, 3], J.M. García Moreno [2], F. Damas Hermoso [2], E. Fernández Espejo [1]
[1] Universidad de Sevilla, Sevilla
[2] UGC Neurociencias & Asociación Neuroinvest, Hospital Macarena-SAS, Sevilla
[3] UGC Cirugía, Hospital Macarena-SAS, Sevilla

Objectives. Alteration of trophic support and anti-oxidant defenses in the CNS along with excess of iron have been affiliated to Parkinson's Disease (PD). Trophic factors of the TGFβ superfamily have been proposed either to support dopaminergic neurons or to mediate neuroinflammation in PD. The objective was to analyze in the cerebrospinal fluid (CSF) of PD patients and controls the levels of: 1) TGFβ superfamily factors (GDNF, persephin, neurturin, $TGF\beta_1$, and $TGF\beta_2$) and BDNF, 2) antioxidant enzymes (catalase, superoxide-dismutases, glutathione-peroxidase, glutathione-reductase, glutathione-S-transferase, peroxiredoxins), and 3) ferritin, iron storage protein.
Material and methods. Molecules were analyzed through ELISA. Clinical stage was evaluated through Hoehn-Yahr scale (Hoehn.Yahr 1 and 2, early PD; Hoehn-Yahr 3 and 4, advanced PD) and UPDRS. Informed consent was obtained from all patients and controls.
Results. The study revealed that, among trophic factors, only $TGF\beta_1$ levels were found to be progressively enhanced in PD patients (early, $p<0.05$; advanced, $p<0.02$), and levels correlated with UPDRS III ($p<0.01$). Among enzymes, activity of glutathione-peroxidase (GPx), catalase and peroxiredoxins (PRDxs) was found to be significantly reduced in patients (GPx, $p<0.001$, 85% mean reduction; catalase, $p<0.03$, 68% reduction; PRDxs, $p<0.01$, 64% reduction). Finally, CSF ferritin was significantly and progressively reduced in patients (early-PD, $p<0.01$, -49%; advanced-PD, $p<0.001$, -80.7%). Ferritin levels correlated with Hoehn-Yahr stage ($p<0.05$).
Conclusions. The levels of potentially "neuroprotectant" ligands such as GDNF, persephin or BDNF are not altered in PD CSF, and since $TGF\beta_1$ is released by reactive microglia, progressively enhanced levels of $TGF\beta_1$ could be related to neuroinflammation. The reliably decrease in catalase, GPx and PRDxs activity reflects a serious reduction of antioxidant enzymatic defenses in the CNS of PD patients, that could be exacerbated by low ferritin levels. Most deficits worsened with the progression of the disease.

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

THE MYELIN MUTANT TAIEP RAT HAS A DISORGANIZED WALKING PATTERN

J. R. Eguibar Cuenca[1,2], C. Cortes Sánchez[2], F. Rocha Reyna[2].
[1]Vicerector of Research and Postgraduate Studies and [2]Institute of Physiology. Benemérita Universidad Autónoma de Puebla. Apdo. Postal 5-66. Puebla, Pue. C.P. 72430. México

The myelin mutant *taiep* rat shows progressively tremor, ataxia, immobility episodes, epilepsy and paralysis, the acronym given its name and it is due to an initial hypomyelination followed by demyelination of the central nervous system, due to an accumulation of microtubules in the olygodendrocytes. The aims of these studies were to analyze the walking patterns and compare them with normal Sprague-Dawley (SD) rats; and the other the role of inhibitory neurotransmitters in lumbar spinal cord. The evaluation of walking pattern were did using the CatWalk system (Noldus, Netherlands). We evaluated during the first six months of age on taiep and SD rats. A catheter was implanted in the subarachnoid space on the lumbar enlargement under ketamine/xylazine anesthesia.
Taiep rats had a significant decreased on the velocity of walking that exacerbates with age and differ from SD rats ($P<0.05$); due to less number of steps with a concomitantly increased in the base of support through wide position of the hind legs in order to compensate tremor and ataxia. Intratechal administration of strychnine, bicuculline or picrotoxin did not affect significantly step frequencies suggesting that spinal cord interneurons are not involved in the alteration of the walking pattern. However, demyelination produced changes in the central pattern generator or in the descending motor commands that control stepping in this myelin mutant rat. Funded by CONACYT grant No. 243247 and VIEP-Health DES 2015 to JRE and MCC. FR was CONACYT fellowship. jose.eguibar@correo.buap.mx

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

THE PROGRESSION OF DEMYELINATION INCREASE ABSENCE SEIZURES IN THE MYELIN MUTANT TAIEP RAT

C. Cortés Sánchez[1], J. Corona Corona [1], J. R. Eguibar Cuenca [2]
[1]Instituto de Fisiología, Benemerita Universidad Autonoma de Puebla. Puebla, Pue. México
[2]Dirección General de Investigación, Vicerrectoría de Investigación y Estudios de Posgrado. Benemerita Universidad Autonoma de Puebla. Puebla, Pue. México

Taiep rat shows an initial hypomyelination, followed by a progressive demyelination in the central nervous system due to an accumulation of microtubules in the olygodendrocytes.
The aim of this study was to analyze the ontogeny of absence seizure from 3 to 12 months in male *taiep* rats using electroencephalographic (EEG) recordings.
Taiep rats anesthetized with ketamine/xylazine were implanted for EEG in frontal, parietal cortices, a bipolar electrode in the hippocampus; two electrodes in neck muscles and one in the right orbit under aseptic conditions. All procedures were approved by the Institutional Animal Care and Use Committee.
EEG recordings showed cortical seizures with a characteristic spike-wave discharge (SWD) pattern characteristic of absence seizures. Fast Fourier Transform during SWD a peak obtained at 6.5 Hz and a significant increase in frequency and duration of SWD with age. Frequency increased from 292.7 ± 50.2 SWD/24h (mean ± EEM) at 3-6 respect to 918.8 ± 61.9 at 9-12 months (P<0.001). Additionally, mean duration of absence seizures also increased significantly with age being less at 3-6 compared to 9-12 months of age (P<0.001). In conclusion, myelin mutant *taiep* rat showed a correlation of the degree of demyelination and the increase in frequency and duration of SWD.
Funded by VIEP-Health DES 2015 to JRE and MCC, and by CONACyT grant No. 243333 to MCC. JCC was fellowship from CONACYT. carmen.cortes@correo.buap.mx

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

7,8 DIHYDROXYFLAVONE AMELIORATES COGNITIVE AND MOTOR DEFICITS IN A HUNTINGON'S DISEASE MOUSE MODEL THROUGH A DIFFERENT ACTIVATION PROFILE FROM BDNF

AG Garcia-Díaz Barriga, A Giralt, M Anglada-Huguet, JM Canals, J Alberch.
Departament de Biologia Cel·lular, Immunologia i Neurociències Facultat de Medicina, Universitat de Barcelona-IDIBAPS, Barcelona, Spain

Huntington's disease (HD) is a fatal neurodegenerative disease caused by an expanded CAG trinucleotide repeat in the gene encoding the protein huntingtin, it presents with abnormal motor coordination, cognitive decline and psychiatric disorders; the pathophysiology includes reduced levels of brain-derived neurotrophic factor (BDNF) in the brain; thus BDNF activation through its high-affinity receptor TrkB has become of interest for its therapeutic implications. 7,8-Dihydroxyflavone (7,8-DHF) has been described as a robust TrkB receptor agonist in a whole variety of in vitro models and tested on in vivo models of neuro-degenerative diseases. Previously it has been described that chronic treatment with 7,8 DHF can ameliorate motor deficits in N171-82Q HD mice; here we tested its effects on cognitive and motor deficits on the R6/1 mouse model of HD. We found chronic treatment with 7,8 DHF is able to delay the onset of motor deficits in R6/1 mice assessed by the Rotarod test; furthermore 7,8 DHF reversed the inability to perform correctly the Novel Object Recognition Test (NORT) at 15 weeks; pathological and biochemical analyses of treated mice revealed improved levels of Enkephalin in striatum, (concordant with a trend on a DARPP32 recovery) and a reduction of striatal volume loss upon treatment. Interestingly in vivo results showed a TrkB Y816 but not TrkB Y515 phosphorylation recovery in striatum with acute and chronic treatment. 7,8 DHF treatment in primary neuronal cultures revealed the same differences in TrkB phosphorylation profile (selective phosphorylation of Y816 residues) in addition to morphological and functional divergent changes from controls with BDNF. Our results suggest 7,8 DHF has therapeutic potential for HD but also that 7,8 DHF has differential effects from BDNF that should be further investigated.
This study was supported by grants from the Ministerio de Economia y Competitividad (SAF2011-29507), CIBERNED (Instituto Carlos III) and Generalitat de Catalunya (2014SGR-968)

T06.- Disorders and nervous system repair
T03.- Systems Neuroscience

LIPID RAFT INVOLVEMENT IN AMYLOID BETA PRODUCTION AND PATHOLOGICAL SIGNALLING IN COGNITIVE BRAIN AREAS

R. Marín[1], N. Fabelo[2], C. Fernández-Echevarría[1], A. Canerina-Amaro[1], M. Díaz[2]
[1]. Sección Medicina. Facultad de Ciencias de la Salud. Universidad de La Laguna, Tenerife
[2]. Sección Biología, Facultad de Ciencias, Universidad de La Laguna, Tenerife

Lipid rafts in neurons have been of increasing interest in recent years. These membrane microstructures, particularly enriched in cholesterol and sphingolipids, are the preferential place of signaling proteins associated with distinct lipids clustered in molecular complexes named signalosomes (Marin R, Front Physiol. 2013;4:188). Raft microdomains are also involved in amyloid beta (Aβ) processing and aggregation, thus reflecting the importance of lipid rafts in Alzheimer's disease (AD) development. In this sense, our previous results have demonstrated that lipid raft impairment is exacerbated with the progression of either AD (Martin et al., J Alzheimers Dis. 2010;19:489) or Parkinson Disease (Fabelo et al., Mol Med. 2011;17:1107).

Here, using raft fractions from human frontal and entorhinal cortical areas and human neuroblastoma cultures, we have investigated by different immunological approaches the potential differences in Aβ processing machinery (amyloid precursor protein, APP, and β/γ secretases) in AD patients at, both, early and late stages of the disease. Equivalent brain areas of age-matched healthy controls, as well as cerebellar lipid rafts were used for comparison controls.

The results indicate that β-secretase accumulates in lipid rafts of early stages of AD, increasing its interaction with APP, thereby suggesting an enhancement of Aβ formation. Furthermore, a voltage dependent anion channel (VDAC) involved in extrinsic apoptosis was observed to interact with, both, APP and Aβ aggregates in raft fractions from cortical areas. This phenomenon induced VDAC dephosphorylation which is related to an enhancement of neuronal death (Fabelo et al., Neurobiol Aging. 2014;35:1801).

Overall, these results indicate that lipid raft alterations observed in AD cortex may be connected to protein rearrangements that promote Aβ formation and activation of toxic intracellular signaling pathways.

Supported by grants SAF2010-22114-C02-01/02 and SAF2014-52582-R.

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: Neurociencia cognitiva y conductual

EPIGENETICS AND BIOMARKERS IN NEUROPSYCHIATRIC DISEASES

Agis-Balboa RC [1], Held T [2], Zeiter S [2], Hennion M [2], Halder R [2], Rivera-Baltanas T [1], Olivares JM [1], Caruncho HJ [3], Kalynchuk LE [3], López M [1], Spuch C [1], Agbemenyah HY [2], Kerimoglu C [2], Benito E [2], Gertig M [2], Bahari-Javan S [2], Burkhardt S [2], Bonn S [2], & Fischer A [2].
[1]. Institute of Biomedical Research of Vigo (IBI), Vigo, Spain
[2]. German Center for Neurodegenerative Diseases (DZNE), Goettingen, Germany
[2]. Department of Medicine, University of Saskatchewan, Canada.

Objectives: We study epigenetic mechanisms associated with psychiatric illness (eg. PTSD, depression & schizophrenia), neurodegenerative (eg. Alzheimer´s disease) and autoimmune (eg. MS) diseases. In addition, we seek biomarkers and therapeutic targets for the diagnosis, prognosis and treatment of these devastating diseases.
Material & Methods: We use human samples (blood, CSF and postmortem tissue) and animals. We perform molecular, histological, biochemical and epigenetic experiments. We also use Omics such as genomics and proteomics.
Results: Several neuropsychiatric disorders have alterations in the IGF2/IGFBP7 signaling and associated epigenetic mechanisms. In addition, clustering of Serotonin receptors in lynphocytes is altered in depression and varies with treatment.
Conclusions: The IGF2/IGFBP7 signaling could be a therapeutic target for the treatment of various neuropsychiatric conditions. Serotonin receptors (and other neurotransmitter receptors) in lymphocytes could be used as diagnostic and prognostic biomarkers for various psychiatric diseases.

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

THE HIGH AFFINITY SUBUNIT OF KAINATE RECEPTORS, GLUK4, A NEW TARGET FOR DEPRESSION

M. Isabel Aller, Valeria Pecoraro, Ana V. Paternain, Santiago Canals, Juan Lerma, Instituto de Neurociencias, CSIC-UMH, 03550 San Juan de Alicante, Spain

Depression is a mood disorder characterized by long periods of sadness, loss of motivation, excessive fatigue and lack of interest in pleasurable activities. Kainate receptors have been linked with this disease, as well as with other mental disorders like schizophrenia, bipolar disorder and more recently with autism spectrum disorders. Several studies demonstrate that many of the brain regions responsible for regulating mood, such as hippocampus, amygdala and prefrontal cortex show disrupted functioning in depression, which can be reversed by antidepressant drugs. No animal models of depression are available, and only chronic stress induces symptoms that are similar to those seen in depressed humans: high levels of anxiety, less social interaction and anhedonia.
Here we developed a new animal model by engineering mice to carry in the forebrain more than one copy of *grik4*, the gene coding for the high affntity kainate receptor subunit, GluK4. In transgenic animals, the expression of GluK4 subunits was higher in the hippocampus (by 30%) and cortex (by 100%), and immunohistochemical studies revealed the expression of this subunit in pre- and postsynaptic sites. In behavior studies, these animals show clear symptoms of depression, high levels of anxiety, less social interaction as well as anhedonia. Some of these symptoms were clearly reversed by treating mice with the antidepressant drug Tianeptine (S 1574, [3-chloro-6-methyl-5,5-dioxo-6,11-dihydro-(c,f)-dibenzo-(1,2-thiazepine)-11-yl) amino]-7 heptanoic acid, sodium salt) but not with Fluoxetine, an inhibitor of the serotonin reuptake. These phenotypes were accompanied by altered synaptic transmission, showing augmented activity propagation through the hippocampal trisynaptic circuit *in vivo*. Together these data indicate that a single variation in the glutamatergic system results in behavioral symptomatology of depression identifying a new target for treatment of this disease.
Supported by the Ministry of Science and Innovation (BFU2011-24084). The IN is a Center of Excellence “Severo Ochoa”.

1. Neurociencia cognitiva y conductual
2. Trastornos y reparación del sistema nervioso

INTRANASAL DELIVERY OF BONE MARROW STEM CELLS IN A MODEL OF SELECTIVE NEURODEGENERATION: THE PCD MOUSE

A. Esteban [1], X.L. Castro [1,2], M. Gil [2,3], F. Franco [2,3], R. Muñoz-Castañeda [1,2], C. del Pilar [1], E. Weruaga [1,2], D. Díaz [1,2].

1. INCyL, Institute for Neuroscience of Castilla y León, Universidad de Salamanca, Salamanca, Spain
2. IBSAL, Institute of Biomedical Research of Salamanca, Salamanca, Spain
3. ORL service, University Hospital. Salamanca, Spain.

The intranasal delivery of cells is a novel technique within the field of cell therapy. Amongst its innovative features, we can point out the simplicity of the procedure itself, and its non-invasive consequences. The method consists of the placement of a drop of cell suspension into each nostril, the animal being able to shuttle the cells towards the brain. The cells may cross the cribriform plate and arrive first to the olfactory bulb, from where they can spread towards the encephalon reaching even so distal places as the cerebellum. This method also avoids some drawbacks of the systemic injections, such as the retention of cells into other different organs.

Our work consists of studying the arrival and integration of bone marrow-derived stem cells into the olfactory bulb of *Purkinje Cell Degeneration* (PCD) mutant mice, employing intranasal delivery. PCD mice suffer from a progressive loss of the mitral cells of the olfactory bulb, among other neuronal populations The animals received 8 million stem cells at postnatal day 70, when the loss of mitral cells is on-going. We have used GFP donor mice in order to follow up the transplanted cells. We have also assessed the possible facilitation of the stem cells arrival into the nervous tissue by means of the administration of hyaluronidase 30 minutes before the transplantation.

Our outcomes verified the effectiveness of the intranasal transplant since we detected transplanted GFP cells into deep layers of the olfactory bulb. According to preliminary results, we can predict a higher arrival of cells in PCD mice compared to wild type animals, which could mean a promising strategy for dealing with specific neuronal degeneration.

Full name: Ada Esteban Sánchez.
Email: aesu12@usal.es; ddiaz@usal.es

Áreas temáticas
6. Trastornos y reparación del sistema nervioso.
8. Nuevos métodos y tecnologías.

THE PLASTICITY OF THE OLFACTORY BULB IS AIMED AT THE MAINTENANCE OF FINE DISCRIMINATIVE TASKS AFTER MASSIVE DAMAGE

D. Díaz [1,2], R. Muñoz-Castañeda [1,2], A. Esteban [1], X.L. Castro [1,2], J.R. Alonso [1,2,3], E. Weruaga [1,2]
1. INCyL, Institute for Neuroscience of Castilla y León, Universidad de Salamanca, Salamanca, Spain
2. IBSAL, Institute of Biomedical Research of Salamanca, Salamanca, Spain.
3. Instituto de Alta Investigación. Universidad de Tarapacá, Arica, Chile.

The olfactory bulb (OB) is widely known to be a plastic structure, their interneurons being subjected to a continuous turnover. Accordingly, neuroblasts are generated in the subventricular zone and travel through the rostral migratory stream towards the OB, where they differentiate into new interneurons. Lethal doses of radiation dramatically decrease neuroblast proliferation, thus impairing such turnover and reducing significantly the size of the OB. Although the effects of radiation are permanent, a residual cell proliferation –and consequently, cell turnover- persists throughout time, the mice behaving normally. Little is known about possible plastic mechanisms aimed at the homeostasis of the OB after such radiation damage.

In this work we have performed both behavioural (olfactometry) and electrophysiological studies to analyse the olfactory capacities and the functioning of the OB, in both control and irradiated mice. Moreover, BrdU injections were performed to determine possible changes in the fate of the newly formed neuroblasts. For this purpose, a wide battery of markers for neuronal populations was combined with BrdU immunolabelling.

Our results corroborated dramatic impairments in OB neurogenesis after radiation, that led to shrinkage in this structure before expected. Mitral cell population was not affected by radiation, but some variations in gamma, beta and theta oscillations appeared, probably because of the reduction of the number of new interneurons. However, only subtle changes in odour discrimination were detected. Accordingly, changes in the distribution of the populations of such interneurons seem to be the responsible of the maintenance of the olfactory capacities after OB damage: our results indicated a preference for the differentiation of neuroblasts to specific types of interneurons, which seem to be essential for fine olfaction.

Full name: David Díaz López
E-mail: ddiaz@usal.es; ewp@usal.es

Áreas temáticas
6. Trastornos y reparación del sistema nervioso
7. Sistemas homeostáticos y neuroendocrino

THE OLFACTORY EPITHELIUM AS INDICATOR OF NEURONAL DAMAGE: STUDY OF HYPOSMIAS / ANOSMIAS WITH DIFFERENT ETIOLOGIC ORIGINS

X.L. Castro [1,2], D. Díaz [1,2], F. Franco [2,3], M. Gil [2,3], R. Muñoz-Castañeda [1,2], C. del Pilar [1], C. Ávila [2,4], E. Weruaga [1,2].

1. INCyL, Institute for Neuroscience of Castilla y León, Universidad de Salamanca, Salamanca, Spain
2. IBSAL, Institute of Biomedical Research of Salamanca, Salamanca, Spain
3. ORL service, University Hospital. Salamanca, Spain.
4. Applied Statistics Group, Department of Statistics, Universidad de Salamanca, Salamanca, Spain.

Hyposmia and anosmia are two olfactory impairments defined as the decreased ability to smell or the absence of olfaction, respectively. Concerning their origin, they can be anterograde (sensory deprivation) or retrograde (neurodegenerative). Changes in the olfactory epithelium have been described in both types of hyposmias or anosmias, although it is unknown whether these alterations run with similar biological changes. One of the early symptoms of neurodegenerative diseases is the loss of olfactory perception; thus an analysis of the olfactory epithelium could reveal specific neurochemical alterations as early indicators of these diseases. Then, our objective was to compare the neurochemical changes of the olfactory epithelium after olfactory deprivation and neurodegeneration animal models.

18 male mice of the C57/DBA strain were used. They were divided in 3 groups: control, PCD (Purkinje Cell Degeneration) and olfactory deprived, 6 animals each. The PCD mouse is a model of retrograde hyposmia/anosmia, which is caused by the loss of mitral cells of the olfactory bulb, from P70 onwards. Deprivation was performed unilaterally at P70 in the right nostril by combining techniques of suture and electrocoagulation. All groups were sacrificed at P110. The olfactory epithelium was dissected and immunostained for a battery of neuronal markers.

Our results demonstrated neurochemical differences in the labelling of olfactory morphogenic protein, neurogranin, calretinin and Fos-B, in the group of deprived mice, compared to control and PCD groups. Therefore, these preliminary observations indicate that the differences between the control, PCD and olfactory deprived groups can provide interesting data regarding differences between anosmias / hyposmias with anterograde or retrograde origin.

Full name: Xavier Lorenzo Castro Ramírez
Email: dr_xlcastro88@usal.es; ewp@usal.es

Áreas temáticas
3. Neurociencia de sistemas.
8. Nuevos métodos y tecnologías.

ARRIVAL OF HUMAN MESENCHYMAL STROMAL CELLS INTO THE MOUSE CEREBELLUM UNDER DIFFERENT HARMFUL CONDITIONS

C. del Pilar [1], D. Díaz [1,2], A. Esteban [1], P. Rubio [1], R. Muñoz-Castañeda [1,2], J.R. Alonso [1,2,3], E. Weruaga [1,2]
1. INCyL, Institute for Neuroscience of Castilla y León, Universidad de Salamanca, Salamanca, Spain
2. IBSAL, Institute of Biomedical Research of Salamanca, Salamanca, Spain
3. Instituto de Alta Investigación. Universidad de Tarapacá, Arica, Chile

Mesenchymal stromal cells (MSCs) have immunomodulatory and neuroprotective properties making them attractive candidates for cell therapy against neuro-inflammatory and neurodegenerative disorders. It is widely accepted that MSCs can migrate and integrate into the central nervous system. However, their rate of integration under different harmful or pathogenic conditions is not well understood. Thereby, the purpose of this research was to evaluate the integration of intravenously injected human MSCs into the cerebellum of mice with different neural damage. For that, different models of neural injury were analyzed: a) no damage (control group), b) low whole-body gamma radiation, c) intraperitoneal administration of lipopolysaccharide (LPS) and d) Purkinje Cell Degeneration (PCD) mice.
Human bone marrow MSCs were intravenously injected into previously described mice groups at postnatal day 25 (P25). LPS-treated and irradiated mice were administered with LPS (2 mg/kg) or exposed to radiation (3 Gy) 24 hours before transplantation (P24), respectively. 24 hours after transplant (P26), animals were perfused transcardially and its encephalons were dissected out. Sagittal sections of the cerebellar vermis were analysed by immunohistochemistry in order to visualize the transplanted cells.
Cells containing human mitochondria were found in all experimental groups. The number of cells in the cerebellum was markedly increased in LPS-treated and PCD mice compared with control animals. Irradiated mice did not present significant differences with regard to controls. These findings indicate that the cerebellar inflammatory microenvironment of PCD mice induces an attractive effect on human MSCs similar to that produced by acute systemic LPS-mediated inflammation.

Full name: Carlos del Pilar Rodríguez
E-mail: karegon@usal.es; ewp@usal.es

Áreas temáticas
6. Trastornos y reparación del sistema nervioso.
8. Nuevos métodos y tecnologías.

EFFECTS OF LEPTIN AND CB2 RECEPTOR ANTAGONIST IN A MURINE MODEL OF TRAUMATIC BRAIN INJURY

A.B. Lopez-Rodriguez[1,2], V. Mela[1], E. Acaz-Fonseca[2], L. M. Garcia-Segura[2], M.P. Viveros[1]
[1]. Departamento de Fisiología Animal (II). Facultad de Biología, Universidad Complutense, Madrid.
[2]. Instituto Cajal (CSIC). Madrid.

In the last few years, many studies have evidenced that leptin exerts neuroprotective effects in different brain injury models (Zhang et al., 2013; Avraham et al., 2010) and that there is a close relationship between this hormone and the endocannabinoid system. Indeed, leptin is a negative regulator of the synthesis of the endocannabinoid 2-arachidonoylglycerol (Di Marzo, 2001) and modifies the levels of cannabinoid receptors, CB1 and CB2, after stroke (Avraham et al., 2010). Since our group has demonstrated that CB1 and CB2 participate in the neuroprotective effects of estradiol (Lopez-Rodriguez et al., 2011) and minocycline (Lopez-Rodriguez et al., 2015) in two models of brain injury, our aim was to assess whether the endocannabinoid system was also involved in the effect of leptin.

For this purpose we used the weight drop model of close-head traumatic brain injury (TBI) in 28-30g male mice (CD1 strain). Forty five minutes before TBI, animals received an injection of CB2 antagonist (AM630, 1mg/kg) or its vehicle. Immediately after lesion, they were administered with leptin (2mg/kg) or its vehicle. Twenty four hours after trauma, animals were subjected to behavioral tests and sacrificed to obtain plasma and brain tissue.

Leptin induced protective effects, recovering to control levels the values of different parameters in TBI animals, such as the neurological score and the mRNA expression of CB1, CB2, BACE-1, TNF-alpha and TGF beta. Moreover leptin increased IL-10 levels, which has a potent inhibitory effect of pro-inflammatory cytokines. CB2 antagonist had an effect *per se* on CB1, TNF-alpha and IL-10 mRNA levels. Some effects of leptin were not detected when animals were co-administered with CB2 antagonist.

The present results point to CB2 receptor as a mechanism underlying the response to TBI and open new avenues for the study of leptin as a potential treatment for these lesions.

GLYCOGEN-INDUCED NEURODEGENERATION

J. Duran[1,2] and J. J. Guinovart[1,2,3]
[1]Institute for Research in Biomedicine (IRB Barcelona) Barcelona, Barcelona.
[2]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Barcelona, Barcelona.
[3]Department of Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Barcelona.

Glycogen is a branched polymer of glucose that constitutes the sole carbohydrate reserve in mammals. It is synthesized by glycogen synthase (GS), the only mammalian enzyme able to polymerize glucose. Within the brain, glycogen is found mainly in astrocytes, while most neurons do not show detectable levels under physiological conditions. However, in some diseases, glycogen is abnormally accumulated in neurons.
Lafora disease (LD) is a fatal neurodegenerative condition characterized by the accumulation of aberrant glycogen in several cell types, including neurons. LD is caused by mutations affecting two enzymes: malin and laforin. Both enzymes interact functionally to promote the degradation of GS and its activator Protein Targeting to Glycogen (PTG). The causal role of glycogen accumulation in neurodegeneration in LD remains controversial, since the malin-laforin complex may have additional functions to that of the regulation of glycogen synthesis, such as the control of autophagy; in fact, KOs of malin and laforin present autophagy impairment.
To study whether the accumulation of glycogen is primarily responsible for the neurodegeneration in LD we have generated several mouse models with altered capacity to accumulate glycogen in neurons. Our findings reveal that glycogen accumulation is indeed responsible for the neurodegeneration of the malin KO model, as well as for the impaired autophagy. These results identify the regulation of glycogen synthesis as a key target for the treatment of LD and other diseases that course with abnormal glycogen accumulation in neurons.

Areas Temáticas:
1ª: Transtornos y reparación del sistema nervioso.
2ª: Excitabilidad neuronal, sinapsis y glia: mecanismos celulares.

PRACTICE GUIDE FOR THE EVALUATION OF IMMUNE SYSTEM IN DRUG-RESISTANT EPILEPSY

L Lorigados Pedre [1], N Pavón Fuentes [1], T Serrano Sánchez [1], MA Robinson Agramonte [1], L Morales Chacón [1], B Estupiñán Díaz [1], A López Martínez [1], S Orozco Suarez [2], L Rocha Arrieta [3]
[1]. Centro Internacional de Restauración Neurológica (CIREN), La Habana, Cuba
[2]. Centro Médico Siglo XXI, Hospital de Especialidades, IMMSS, Distrito Federal, México
[3]. Lab. Farmacobiología CINVESTAV, Sede Sur, Distrito Federal, México

Increasing evidence supports the role of certain immune factors in epilepsy with particular emphasis on drug-resistant epilepsy (DRE). Objective: The purpose of this work was to do some recommendations based on the experience of the Group of Epilepsy Surgery from CIREN on immunological research needed to assess the involvement of immune system in the pathophysiology of DRE. Material and Methods: DRE patients were evaluated by EEG-Video and they were grouped attending to epileptogenic focus localization in: temporals (n=16), lateralizeds (n=6), extratemporals (n=4) and a group with psychogenic epilepsy (n=4). We also evaluated 20 patients with drug-resistant temporal lobe epilepsy (DRTLE) before and after (a month, 6, 12 and 24 months) surgical treatment. The cellular immunity was evaluated by immunocytochemical technique using the following lymphocyte markers: CD3, CD4, CD8, CD25 and HLA-DR. Inflammatory processes at both central and peripheral levels were measured by immunoassay system (IL-1β, IL-6 and TNF-α). Results: Our results evidenced cellular immunological abnormalities with significant increased of CD8+, CD25+ and HLA-DR+ lymphocytes ($p \leq 0.05$) in temporal and lateralized epilepsy but not in extratemporal epileptic patients. The most relevant finding obtained is due to the evolutionary study of these immunological markers in lobectomized DRTLE patients. We found a significant decreased of CD8+, CD25+ and HLA-DR+ cells and de IL-1β, IL-6 and TNF-α one year before surgical treatment ($p \leq 0.05$). Conclusions: These cellular immunity alterations are not related to antiepileptic drugs but it depends of the epileptogenic focus localization. This study confirmed that the immunological and inflammatory alterations disappear at one year after the epileptogenic focus resection. The work provides the necessary rules for the study of DRE and proposed the recommendations for periodicity should be used in the immunological study. Finally, our group evidenced new possible biomarkers for the study of DRE.

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

MICROGLIAL PHAGOCYTOSIS IS IMPAIRED IN CHRONIC MOUSE AND HUMAN MTLE AND CORRELATES WITH INFLAMMATION

Sol Beccari[1,2], Oihane Abiega[1,2], Irune Diaz-Aparicio[1,2], Diego Gómez-Nicola[3], Laura Zaldumbide[4], Lara Galbarriatu[4], Ainhoa Marinas[4], Mirjana Maletic-Savatic[5], Carlos Matute[1,2] , Juan M. Encinas[1,2,6], Amanda Sierra[1,2,6]

[1] . Achucarro Basque Center for Neuroscience, Zamudio, Spain
[2]. University of the Basque Country UPV/EHU, Leioa, Spain
[3]. Southampton University, UK
[4]. University Hospital of Cruces, Bilbao, Spain
[5]. Neurological Research Institute, Baylor College of Medicine, Houston, Texas, USA
[6]. Ikerbasque, the Basque Foundation for Science, Bilbao, Spain

In physiological conditions in the adult hippocampus, apoptotic cells are rapidly and efficiently phagocytosed by microglia. We have observed that during aging, inflammation, and excitotoxicity, microglia responded to the increase in apoptosis by adjusting proportionally their phagocytosis. Conversely, in a mouse model of mesial temporal lobe epilepsy (MTLE) by intrahippocampal administration of kainic acid (KA), microglial phagocytosis was reduced as early as 6 hours after injury, and continued to be impaired in the long term. Importantly, this phagocytic blockade led to the accumulation of non-phagocytosed apoptotic cells, and contributed to the development of an inflammatory response. Unexpectedly, in the subacute phase (3-7 days) of MTLE, microglia showed a hypertrophic, seemingly amoeboid morphology that was related to the cells becoming multinucleated. Further, we also detected some cases of phagoptosis or engulfment or non-apoptotic cells. In later stages (4 months) of MTLE, microglial phagocytosis remained impaired. Importantly, the microglial phagocytosis impairment was observed in human hippocampal tissue from MTLE patients. In the human tissue, we found the same kind of phagocytosis observed in the mouse brain by terminal or en passant branches of microglia. In addition, we observed a unique type of phagocytosis in which several microglial nuclei formed a surrounding the apoptotic cell, in an aster-like structure. These results demonstrate that the impairment of microglial phagocytosis is a novel mechanism contributing to the pathophysiology of epilepsy.

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: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

NEURONAL HYPERACTIVITY UNCOUPLES MICROGLIAL PHAGOCYTOSIS AND LEADS TO DELAYED CELL-CLEARANCE

Irune Diaz-Aparicio[1,2], Oihane Abiega[1,2], Sol Beccari[1,2], Agnes Nadjar[3], Quentin Leyrolle[3], Sophie Layé[3], María Domercq[1,2], Alberto Pérez[1,2], Víctor Sánchez-Zafra[1,2], Iñaki Paris[1,2], María dM Vivanco[4], Mirjana Maletic-Savatic[5], Carlos Matute[1,2], Juan M. Encinas[1,2,6], Amanda Sierra[1,2,6]

[1.] Achucarro Basque Center for Neuroscience, Bizkaia Science and Technology Park, Zamudio, Spain;
[2.] University of the Basque Country, Leioa, Spain
[3.] Université Bordeaux Segalen, Bordeaux, France
[4.] CIC BioGUNE, Derio, Spain
[5.] Baylor College of Medicine, The Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, Houston, TX, USA
[6.] Ikerbasque Foundation, Bilbao, Spain

In physiological conditions, aging, inflammation and excitotoxicity microglial phagocytosis is fast and efficiently coupled to apoptosis in the adult hippocampus, but becomes impaired in a mouse model of epilepsy by intrahippocampal injection of kainic acid (KA). Here we studied the possible mechanisms underlying this uncoupling. To test whether the phagocytic blockade induced by seizures was mediated by the direct effect of KA, we first analyzed the expression of glutamate ionotropic and metabotropic receptor subunits in FACS-sorted microglia. Hippocampal microglia expressed a residual mRNA amount of most subunits, which is unlikely to lead to the formation of functional receptors. In addition, KA had a small effect on microglial phagocytosis in primary cultures and no effect in organotypic cultures, suggesting that the effects of seizures in phagocytosis in vivo are not directly mediated by KA on microglia. Next, we studied the extracellular nucleotide ATP, a well-known "find-me" signal released by apoptotic cells as well as during seizures. We were able to mimic the uncoupling observed in vivo by disrupting ATP gradients in organotypic slices, suggesting that neuronal hyperactivity interferes with "find-me" signals via ATP. Ultimately, microglial phagocytosis impairment leads to the accumulation of non-phagocytosed apoptotic cells and correlates with the development of an inflammatory response in vivo. These results suggest that the impairment of microglial phagocytosis contributes to the early pathophysiology of epilepsy and possibly other neurodegenerative and neurological disorders characterized by neuronal death and inflammation.

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

MICROGLIAL PHAGOCYTOSIS-APOPTOSIS COUPLING: A WIDESPREAD RESPONSE DISTURBED IN EPILEPSY

Oihane Abiega[1,4], Sol Beccari[1,4], Irune Diaz-Aparicio[1,4], Amy L. Brewster[2], Anne E. Anderson[2], Agnes Nadjar[3], Quentin Leyrolle[3], Sophie Layé[3], Mirjana Maletic-Savatic[2], Carlos Matute[1,4], Juan M. Encinas[1,4,5], Amanda Sierra[1,4,5]

[1]. Achucarro Basque Center for Neuroscience, Zamudio, Spain
[2]. Neurological Research Institute, Baylor College of Medicine, Houston, Texas, USA
[3]. Université Bordeaux Segalen, Bordeaux, France
[4]. Department of Neuroscience, University of the Basque Country UPV/EHU, Leioa, Spain
[5]. Ikerbasque, the Basque Foundation for Science, Bilbao, Spain

Phagocytosis is a highly conserved process essential to maintain tissue homeostasis. However, little is known about its dynamics in the adult brain. Using as a model the adult neurogenic cascade, where the majority of the newborn cells undergo apoptosis, we have defined a series of parameters that establish the baseline efficiency of microglial phagocytosis in the adult brain. In physiological conditions, apoptotic cells are rapidly and efficiently phagocytosed by microglia. When subjected to different phagocytic challenges as inflammation *in vivo* (systemic LPS), chronic inflammation *in vivo* (Omega 3 defficient diet), or excitotoxicity *in vitro* (NMDA in organotypic slices), microglia stand up to the increased apoptosis by raising proportionally their phagocytic capacity – hence, phagocytosis remains coupled to apoptosis. In contrast, in an *in vivo* model of epilepsy induced by kainate administration, a major neurological disorder characterized by excitotoxicity and inflammation, microglial phagocytosis is strongly uncoupled from apoptosis. These new parameters we introduce enable us to describe microglial phagocytosis dynamics in both health and disease and represent a very powerful tool to detect disruptions in this physiological process. The consequences and mechanisms underlying this phagocytosis-apoptosis uncoupling will be discussed.

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

AMYLOID-β EFFECTS ON FIRING PATTERNS OF CA3 PYRAMIDAL NEURONS IN RATS

J.D. Navarro-Lopez[1], M.O. Nava-Mesa[2,3], L. Jiménez-Díaz[1], J. Yajeya[3].
[1]. Neurophysiology & Behavior Lab, CRIB, School of Medicine of Ciudad Real, University of Castilla-La Mancha, Ciudad Real, Spain.
[2]. Neuroscience Research Group (Neuros), University of Rosario, Bogotá, Colombia
[3]. Dpt. of Physiology & Pharmacology, University of Salamanca, Salamanca, Spain.

Objectives: Last evidences suggest that, in Alzheimer's disease (AD) early stage, Amyloid-β (Aβ) peptide induces an imbalance between excitatory and inhibitory neurotransmission systems resulting in the functional impairment of neural networks. Such alterations are particularly important in the septohippocampal system where learning and memory processes take place depending on accurate oscillatory activity tuned at fimbria-CA3 synapse. Our previous findings suggest that Aβ, altering channel conductances that control neuron excitability in CA3 pyramidal neurons might have a key role in the septohippocampal activity dysfunction observed in AD.
Material and Methods: Intracellular sharp electrodes recordings from CA3 pyramidal neurons (n = 30) were made in septohippocampal slices, and the action of different concentrations of Aβ demonstrated on their membrane intrinsic properties. To study the firing pattern and input resistance, an increasing 300 ms depolarizing and hyperpolarizing current pulses were elicited. Recorded cells were classified according to its pattern of firing into 2 different groups: single (n = 10) and burst (n = 20).
Results: Our results indicate that Aβ can modulate neuronal excitability in hippocampal CA3 pyramidal neurons changing the firing pattern in a concentration-dependent manner. We also found a different susceptibility of pyramidal CA3 neurons to Aβ since some of them were not significantly affected by the peptide.
Conclusions: In summary, our findings suggest that alteration of firing pattern in CA3 pyramidal neurons by Aβ might explain part of the septohippocampal hyperactivity observed in early AD.

Acknowledgement: Spanish grants from MINECO (SAF2010-14878; BFU2011-22740).

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: Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

LONG-TERM CONTROLLED GDNF OVER-EXPRESSION REDUCES DOPAMINE TRANSPORTER ACTIVITY WITHOUT AFFECTING TYROSINE HYDROXYLASE EXPRESSION IN THE RAT NIGROSTRIATAL SYSTEM

P. Barroso-Chinea[1,2,3], D. Afonso-Oramas[1,2,3], I. Cruz-Muros[1,2,3], J. Salas-Hernández[1], Diego Luis-Ravelo[1,2,3], F. Fumagallo[1,2,3], L. Tenenbaum[4], T. González Hernández[1,2,3,5]

[1]Departamento de Ciencias Médicas Básicas (Anatomía), Facultad de Ciencias de la Salud (Medicina), Universidad de La Laguna, Tenerife, Spain.

[2]Centro de Investigaciones Biomédicas de Canarias (CIBICAN), Tenerife, Spain.

[3]Instituto Universitario de Tecnologías Biomédicas (ITB), Tenerife, Spain.

[4]Clinical Neuroscience Department, Lausanne Hospital University, Switzerland.

[5]Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas, Instituto de Salud Carlos III.

The dopamine (DA) transporter (DAT) is a plasma membrane glycoprotein expressed in dopaminergic (DA-) cells that clears DA after its release to the extracellular space. DAT is involved in DA-cell degeneration in Parkinson's disease (PD) because DA metabolism is the main source of oxidative stress in DA-cells and cytosolic DA levels depend mostly on DAT activity. On the other hand, several studies support a protective effect of the glial cell line-derived neurotrophic factor (GDNF) on DA-cells. However, prolonged GDNF delivery leads to tyrosine hydroxylase (TH, the limiting enzyme in DA synthesis) down-regulation, challenging its therapeutic use in PD.

Subject: Given the role of DAT in DA handling and in the pathogenesis of PD, it would be interesting to know whether DAT is also modulated by prolonged GDNF over-expression, and more important, if DAT may be regulated by GDNF expression levels lower than those required for TH down-regulation.

Material and methods: Adult male rats were injected in the striatum with a tetracycline-inducible adeno-associated viral vector expressing human GDNF cDNA and treated with doxycycline (DOX; 10, 30, 100 and 600µg/ml) in the drinking water during 5 weeks. TH expression and DAT activity, expression, and interactions with members of its proteome were studied at different GDNF over-expression levels.

Results: Prolonged high GDNF over-expression (600µg/ml DOX, 12x basal GDNF striatal levels) induced both DA uptake decrease and TH down-regulation in its native and Ser40 phosphorylated forms, while moderate GDNF over-expression (100µg/ml DOX, 3x basal levels) induces DA uptake decrease but not TH down-regulation. This phenomenon is associated with formation of DAT dimers and an increase in DAT- α-synuclein interactions, without changes in total DAT levels or its compartmental distribution.

Conclusion: we show that at appropriate GDNF transduction levels, DA uptake may be regulated through DAT protein-protein interactions without interfering DA synthesis.

Supported by: BFU 2010-21130, BFU2013-47242-R and IMBRAIN project (FP7-REGPOT-2012-CT2012-31637- IMBRAIN).

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

Pedro Barroso Chinea
Email: pbarrosc@ull.es

TUDCA PROMOTES ACTIVATION OF THE TGFβ PATHWAY IN AN ACUTE NEUROINFLAMMATION MOUSE MODEL

N. Yanguas-Casás[1,2], M. A. de la Barreda-Manso[1], M. Nieto-Sampedro[1,2], L. Romero-Ramírez[2].
[1]. Instituto Cajal, CSIC, Madrid.
[2]. Hospital Nacional de Parapléjicos, Toledo.

Tauroursodeoxycholic acid (TUDCA) is a bile acid conjugate with neuroprotective and anti-inflammatory effects in several neurological diseases. Previous work from our group has shown that the anti-inflammatory effect of TUDCA on glial cells was mediated by NFκβ pathway inhibition in both, glial cell culture and a model of acute neuroinflammation in adult mice. TGFβ has been described as a constitutive signal that keeps glial cells in the resting state. Here, we report on the implication of the TGFβ pathway in the anti-inflammatory activity of TUDCA.

The activation of the TGFβ pathway in the brain was determined with or without intraperitoneal (ip) injection of TUDCA, under two experimental conditions: 1) in an acute model of neuroinflammation, 1 or 3 days after intracerebroventricular (icv) injection of LPS, and 2) after intravenous (iv) injection of LPS, in SBE-luciferase reporter transgenic mice. These mice had been validated previously as a model to study the activation of the TGFβ pathway, by regulating the expression of the firefly luciferase under the control of Smad2 transcription factor. Our results show that LPS activated the Smad2/3-TGFβ pathway in the SBE mouse brain, and that TUDCA increased that activation. Quantitative PCR analysis of the TGFβ isotypes involved in the hippocampus, showed increases in the mRNA for $TGF\beta_2$ and $TGF\beta_3$, 1 day after icv injection of LPS and TUDCA treatement. Furthermore, TUDCA induced an increase in $TGF\beta_3$ in several brain regions, 3 days after LPS injection.

Our results indicate that TUDCA may exert its anti-inflammatory action on glial cells both, by inhibiting the NFκβ pathway and, *in vivo* under neuroinflammatory conditions, by activating the TGFβ pathway.

This work was supported by grants from the FISCAM-Servicio de Salud Castilla-La Mancha (SESCAM PI2009/51) and the Plan Nacional from the Spanish Ministry of Economy and Competitivity (SAF2009-11257 and SAF2012-40126).

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Sistemas homeostáticos y neuroendocrino

EGFR INHIBITION REDUCES ABERRANT CELL PROLIFERATION IN HYPEREXCITATORY KAINATE MODEL OF EPILEPSY

J.R.Pineda[1], S. Martín-Suárez[1,2], J.M. Encinas[1,2,3]
[1]. Laboratory of Neural Stem Cells and Neurogenesis. Achucarro Basque Center for Neuroscience. 48170 Zamudio, Spain.
[2]. Department of Neurosciences, University of the Basque Country, 48940 Leioa, Spain.
[3]. Ikerbasque Foundation, Bilbao, Spain.

The insurmountable and selective blood-brain barrier (BBB) prevents not only harmful substances but also therapeutic drugs and molecules from entering the brain. According to Pardridge[1], more than 98% of small molecules including those with therapeutic use do not cross the BBB. Intranasal route has recently become a center of attention as an alternative way to infuse compounds into the brain. Small molecules are driven through the anatomic connection between the nose and the brain provided by the trigeminal and olfactory nerves. The intranasal route has been previously used to deliver hormones, TGF-beta inhibitors, neuropeptides and viruses into the brain. This route avoids hepatic first-pass metabolism, and has rapid onset of pharmacological action.
Epidermal growth factor receptor (EGFR) is widely expressed by activated neural stem cells and progenitors and its stimulation induces cell proliferation. EGFR inhibition impairs astrocytic differentiation as well as the induction of mitosis. In the present work we used Kainic acid infusion into hippocampus as a model of epilepsy, a hyperexcitatory stimulus recently described as responsible for hyperproliferation and exhaustion of the hippocampal neurogenic niche[2]. We demonstrate that intranasal administration of 10mg/Kg Gefetinib, a reversible inhibitor of the EGFR, twice a day for 3 days reduces aberrant cell hyperproliferation stimulated by neuronal hyperexcitation. We determined the effect of Gefetinib on proliferating cells by BrdU incorporation and KI67 expression, and we checked the induction of arrested cells expression of CCND1 and p21 respectively.
Our results demonstrate that the inhibition of the EGFR pathway is a good candidate to control hyperproliferation in neurogenic niches and that the intranasal route is an effective way to deliver compounds to the brain. This work has been financed by MINECO SAF2012-485 and MINECO “Ramón y Cajal” programs RYC-2012-11137 and RYC-2013-13450.
[1]Pardridge, W.M. *J.Cereb.Blood Flow Metab* (2012) 32(11):1959-1972
[2]Sierra, A. *Cell Stem Cell*. (2015) 7;16(5):488-503

Topics:
1st: Disorders and nervous system repair.
2nd: Neuronal excitability, synapses and glia.

José Ramón Pineda Martí (jr.pineda@achcarro.org)

ACTIVATION OF MICROGLIA BY NEUROMELANIN. ROLE OF CASPASE-8

R.M. de Pablos[1], N. Viceconte[2], M.A. Burguillos[3], A.J. Herrera[1], B. Joseph[4] and J.L. Venero[1]
[1] Facultad de Framacia, Universidad de Sevilla, Sevilla.
[2] Karolinska Institutet, Stockholm, Sweden.
[3] Queen Mary University of London, London, United Kingdom.
[4] Cancer Centrum Karolinska, Stockholm, Sweden

Parkinson´s disease is a neurodegenerative disease that affects approximately 1-3% of the population, and is characterised by a slow and progressive degeneration of dopaminergic neurons in the substantia nigra. Accumulating evidence suggests that inflammation may play a central role in the cell loss seen in Parkinson´s disease. Several studies reveal that damaged dopaminergic neurons release several factors that seem to activate microglia including neuromelanin. Consequently, we tested the ability of synthetic neuromelanin to activate BV2 microglia cells. We found an *in vivo* up-regulation of CD16/32 (M1 marker) in Iba1-immunolabeled microglia. Our results also show that synthetic neuromelanin induced a significant chemotactic response to BV2 microglial cells along with typical morphological features of microglia activation, increased oxidative stress and induction of pattern-recognition receptors including NOD2, Toll-like receptor 2 and CD14. Moreover, we found that caspase-8 is involved in the neuromelanin-induced activation of BV2 microglial cells.

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

MICE CARRYING A DELETION OF THE HINT1 GENE EXHIBIT MOOD-RELATED BEHAVIOURAL ABNORMALITIES

E. Cortés, P. Sánchez-Blázquez, M. Rodríguez-Muñoz, J. Garzón
Neuropharmacology, Instituto Cajal, CSIC, 28002 Madrid.

The *HINT1* gene, located on chromosome 5q31.2, is thought to participate in the neuropathology of schizophrenia and bipolar disorder (1). The consequences of HINT1 deficits have been studied in experimental models, and a mouse model lacking this gene displays anti-depressant and anxiety like behaviour (2, 3). Moreover, the effects of NMDA are enhanced in neurons cultured from HINT1 deficient mice, enhancing its neurotoxicity (4). In this study, we analysed whether the HINT1 deficient mice exhibit a general derangement in reward mechanisms, as seen in bipolar patients when in the manic state. Accordingly, we compared the performance of $HINT1^{+/+}$ and $HINT1^{-/-}$ mice in a battery of tests that assess spontaneous activity, psychostimulant induced hyperactivity and sweet solution preference. Our data indicate that the $HINT1^{-/-}$ mice are more sensitive to the rewarding effects of psychostimulants and that their response to a natural reward, sucrose consumption, was significantly more intense than that of their wild type litter mates. Administration of mood stabilizers (valproate, lithium) attenuated the manic-like behaviour in these assays, whereas antidepressants (imipramine, citalopram) had no such effect, verifying the nature of this behaviour. These mice also had a greater preference for a range of rewarding stimuli, indicative of a hyperhedonic state that resembles the euphoria and increased substance abuse characteristic of human mania. These results suggest that these $HINT1^{-/-}$ mice may be particularly useful to study the pathophysiology of both mania and addiction, and to help explain why these debilitating conditions are often comorbid.
(Supported by MINECO SAF12-034991; Plan Nacional sobre Drogas 2014-0012)

1. Elashoff et al., J Mol Neurosci. 2007;31:221
2. Barbier and Wang, BMC Neurosci. 2009 10:132
3. Varadarajulu et al., Behav Brain Res. 2011 220:305
4. Vicente-Sanchez et al., Mol Brain. 2013 6:42

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

AMYLOID-BETA STAGING IN THE AMYGDALA OF APP/PS1 MICE MODEL OF ALZHEIMER'S DISEASE: CELL TYPES INVOLVED

A. Flores-Cuadrado, I. Ubeda-Banon, D. Saiz-Sanchez, C. De la Rosa-Prieto, A. Martinez-Marcos
Ciudad Real Medical School/CRIB, University of Castilla-La Mancha, Ciudad Real, Spain

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder. Clinically, it is characterized by motor and cognitive impairment. There are two main neuropathological hallmarks: the amyloid beta peptide (Aβ) that forms extracellular aggregates, which constitute the senile plaques and the Tau protein, which form the intracellular neurofibrillary tangles (NFTs). According to the NFTs accumulation, AD is divided into six stages, where the entorhinal cortex and hippocampus are involved in the first stages, and the amygdala is one of the earliest brain areas involved by the pathology.
Calcium homeostasis dysregulation is an early event and induces cell death in AD. A subset of interneuron subpopulations have been analyzed based on their expression of both calcium-binding proteins such as calretinin (CR), calbindin (CB) and parvalbumin (PV) and the neuropeptide somatostatin (SST). This work focuses on the distribution of Aβ in the basolateral, central and cortical amygdaloid complexes in AβPP/PS1 transgenic mouse model of AD of 16, 30, 43 and 56 weeks. Using multiple immunofluorescence techniques and confocal microscopy, we have quantified the differential expression of the interneuron cell populations and their colocalization levels with Aβ. We observed an increase of Aβ expression and a decrease of interneurons expression with age. In addition, the results pointed out a differential vulnerability of the interneuron cell populations with age. These data on the Aβ staging in the different nuclei of amygdaloid complex over time reveal important issues to understand the progression of AD in the brain.
Supported by Spanish Ministry of Economy and Competitiveness-FEDER; grant number: BFU2010-15729 and SAF2014-52300-R; Autonomous Government of Castilla-La Mancha-FEDER; grant: PEIC-2014-006-P.

E-mail: aliciamaria.flores@alu.uclm.es

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

THE *ARG72PRO p53* POLYMORPHISM MODULATES ISCHEMIC PRECONDITIONING-INDUCED NEUROPROTECTION FOLLOWING OXYGEN AND GLUCOSE DEPRIVATION IN CORTICAL NEURONS.

R. Vecino[1,2], ME. Ramos Araque[1,2], C. Rodríguez[1,2], JC. Gomez-Sánchez[1], A. Almeida[1,2] and M. Delgado Esteban[1,2]
[1]. Instituto de Investigación Biomédica de Salamanca (IBSAL), Hospital Universitario de Salamanca
[2]. Instituto de Biología Funcional y Genómica/Universidad de Salamanca (CSIC/USAL) mdesteban@usal.es

Objectives
Cerebral ischemic preconditioning (IPC) is one of the most important endogenous mechanisms responsible for the increased brain resistance after stroke. Although IPC has been associated with the activation of pro-survival signals, the mechanism by which preconditioning confers neuroprotection is not yet fully clarified. The tumor suppressor protein p53 accumulates in the ischemic areas of the brain and contributes to neuronal cell death. Recently, we described that the human *Arg72Pro p53* polymorphism controls susceptibility to ischemia-induced neuronal apoptosis and governs the differential functional outcome of individuals after stroke. Here, we studied the role of p53 and related mitochondrial proteins in IPC using a preconditioning model induced by N-methyl-D-aspartate (NMDA).
Material and Methods
Cortical neurons in primary cultures were obtained from wild type (control), p53 *knockout* (KO) and p53 *knock-in* - expressing human polymorphic Arg and Pro p53 variants - mouse. Neurons were exposed to 20 □M NMDA for 2 hours prior to a subsequent lethal oxygen and glucose deprivation (OGD; 90 min). Neurons were then incubated in culture medium for further 0, 4 and 24 hours of reoxygenation. Thus, we analyze the apoptosis (Annexin-V-staining) and mitochondrial membrane potential (DiIC-staining) by flow cytometry and proteins were analyzed by western blotting.
Results
Our results showed that p53 KO neurons were more resistant to apoptosis induced by OGD than control (normoxia) neurons. In good agreement with our previous results, neurons expressing p53Arg were more vulnerable to the ischemic insult than those expressing p53Pro. Furthermore, NMDA preconditioning failed to protect p53Arg neurons against OGD-induced mitochondrial depolarization and neuronal apoptosis.
Conclusions
Here we conclude that p53 plays an essential role in neuronal apoptosis caused by ischemia. Moreover, *Arg72Pro p53* polymorphism modulates neuronal protection exerted by IPC against a subsequent ischemic insult.

The work was funded by Miguel Servet I (CP0014/00010); FEDER (European regional development fund) and Instituto de Salud Carlos III (PI12/00685 and RD12/0014/0007).

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

MODELING THE BEHAVIORAL PHENOTYPE OF GLUN3A TRANSGENIC AND KNOCKOUT MICE: CLUES TO PATHOLOGIES OF ALTERED GLUN3A EXPRESSION

L.G.Rabaneda[1], R.Martínez-Turrillas[1], R.M.Rodriguiz[2], W.C.Wetsel[2], I.Pérez-Otaño[1].
[1]. Cellular Neurobiology Laboratory, Centro de Investigación Medica Aplicada (CIMA) and Universidad de Navarra, Pamplona, Spain.
[2]. Department of Psychiatry and Behavioral Sciences, Duke University Medical School, Durham, North Carolina, USA

NMDA receptors are heteromeric channels assembled from an obligatory GluN1 subunit and different combinations of four GluN2 (A–D) and two GluN3 (A–B) subunits. The function of GluN2A and GluN2B in brain plasticity, learning and memory has been well established, but little is known about non-conventional GluN3A-subtypes. We have previously shown that alterations in GluN3A expression levels trigger synapse dysfunction and loss, and are associated to CNS pathologies such as Huntington disease (1,2) or cocaine abuse (3). Newer genetic evidence links the *Grin3a* gene (encoding GluN3A) to nicotine or alcohol dependence and depressive states. Importantly, suppressing aberrant expression can restore normal plasticity, connectivity and behavior pointing towards GluN3A as a promising therapeutic target (1).

To define the phenotypic outcomes linked to altered GluN3A expression, we have now performed an extended behavioral study of mice with enhanced GluN3A levels (GluN3A double transgenic mice that express GluN3A under the control of CAMKII promoter), and GluN3A knockout (KO) mice. We found that double transgenic mice showed impairments on a variety of memory tasks, which were associated to high anxiety levels. The behavioral analysis of GluN3A KO mice revealed, on the contrary, an anxiolytic like-phenotype and decreased aversion to risk. We are currently attempting to understand the signaling pathways that underlie the memory deficits associated with enhanced GluN3A expression. To do so, we are analyzing how GluN3A influences the patterns of induction of immediate early genes (IEG) known to have an important role on learning and memory during behavioral tasks.

1. Marco, S., et al. Nature Medicine 19, 1030-1038 (2013).
2. Roberts, A.C., et al. Neuron 63, 342-356 (2009).
3. Yuan, T., et al. Neuron 80, 1-14 (2013).

Work was funded by the UTE project CIMA, a NARSAD Independent Investigator Award (to I.P.O.), and grants from the MINECO (CSD2008-00005, SAF2013-48983-R) (to I.P.O.).

Áreas temáticas:
1ª Trastornos y reparación del Sistema Nervioso
2ª Neurociencia Cognitiva y Conductual

CHRONIC STRESS AND SODIUM AZIDE: A LETHAL COMBINATION FOR THE MOUSE HIPPOCAMPUS

A.M. Espinosa Oliva[1], M.J. Delgado Cortés[1], M. Sarmiento Soto[2], S. Argüelles Castilla[1], A.J. Herrera Carmona[1], R. Mauriño Campanario[1], R. Fernández Villarán[1], J.L. Venero Recio[1], A. Machado de la Quintana[1], R. Martínez de Pablos[1].

[1]. Departamento de Bioquímica y Biología Molecular, Facultad de Farmacia, Universidad de Sevilla, and Instituto de Biomedicina de Sevilla (IBiS)-Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Sevilla.

[2]. Gray Institute for Radiation Oncology and Biology. Department of Oncology. University of Oxford, Oxford.

Objectives: Evidence suggests that chronic stress could be one of the risk factors for Alzheimer's disease. Inhibition of the respiratory electron transport chain complex IV has been observed in post-mortem Alzheimer's disease brains. The aim of this work is to test whether chronic stress is able to induce Alzheimer's disease features after the administration of nontoxic doses of sodium azide, a chemical with a high acute toxicity that inhibits complex IV activity, impairing learning and memory in rats.

Materials and methods: Male C57BL/6 mice (25−30 g) were used for these studies. Mice were anaesthetized with isofluorane and a mini-osmotic pump was surgically implanted subcutaneously in the intrascapular area, filled with either saline solution (200 µl) or NaN_3 (200 µl, 2.5 mg/kg/day). The animals were subjected (or not) to chronic stress. Two groups of animals receiving a subcutaneous dose of corticosterone (20 mg/kg) instead of chronic stress were also included. Two different time points were tested for each of these conditions (1 or 2 months). The effects of the treatments were measured by immunohistochemistry, immunofluorescence and western blot.

Results: Chronic stress increased the levels of several proteins involved in Alzheimer's disease pathogenesis, such as presenilin 1, presenilin 2 and S100β, besides inducing the aggregation of Tau, ubiquitin and β-amyloid proteins in the hippocampus. More important, results show a synergistic effect of stress and sodium azide leading to significant neuronal death in the mice hippocampus.

Conclusions: These results show that chronic stress produced the appearance of Alzheimer's disease features under conditions of experimental mitochondrial dysfunction; these features were not observed when chronic stress and inhibition of complex IV by NaN_3 were applied separately. These results may help to understand the devastating effects of stress on the hippocampus, highlighting the need to reduce the effects of stress on the growing population of elderly people.

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

Autora: Ana María Espinosa Oliva (anaespinosa@us.es)

DIFFERENTIAL INVOLVEMENT OF THE α-SYNUCLEIN IN MONOAMINERGIC TRANSMISSION ON THE MOUSE MODEL OF KNOCKDOWN OR AAV-MEDIATED OVEREXPRESSION

D. Alarcón-Arís [1,2], M. Galofré [1,2,3], E. Ruiz-Bronchal [1,2,3], A. Ferrés-Coy [1,2,3], A. Montefeltro [4], I. Fariñas [5,6], F. Artigas [1,2,3], M. Vila [6,7,8], A. Bortolozzi [1,2,3]

[1]. Instituto de Investigaciones Biomédicas August Pi i Sunyer – IDIBAPS, Barcelona
[2]. Instituto de Investigaciones Biomédicas de Barcelona – CSIC, Barcelona
[3]. Centro Investigación Biomédica en Red de Salud Mental – CIBERSAM, Madrid
[4]. n-Life Therapeutics, S.L., Granada
[5]. Departamento de Biología Celular, Universidad de Valencia, Valencia
[6]. Centro Investigación Biomédica en Red de Enfermedades Neurodegenerativas - CIBERNED, Madrid
[7]. Institut Vall d'Hebron, Barcelona
[8]. Institució Catalana de Recerca i Estudis Avançats, Barcelona

Alpha-synuclein protein is accumulated in patient brain with sporadic Parkinson's disease (PD), and increased gene dosage causes a severe dominantly form of PD, but we know little about the α-synuclein effects on synaptic serotonin (5-HT) and dopamine (DA) transmission. Here, we used two different mouse models with: **a)** down-regulated α-synuclein expression specifically in 5-HT and DA neurons of raphe nuclei (RN) and substantia nigra compacta/ventral tegmental area (SNc/VTA), respectively, using antisense oligonucleotide (ASO) or **b)** over-expressed α-synuclein in RN 5-HT or SNc/VTA DA neurons using AAV5-CBA-human-α-synuclein vector. Alpha-synuclein-ASO was conjugated to indatraline (monoamine transporter inhibitor) to promote its selective delivery into monoamine neurons after intranasal administration. Conjugated indatraline-α-synuclein-ASO (1233ASO) entered into 5-HT and DA cells followed by trafficking to Rab7-marker vesicles resulting in an efficient α-synuclein mRNA knockdown (70.6 and 84.1% of PBS-treated mice). Selective α-synuclein reduction in monoamine cells displayed an enhancement striatal 5-HT and DA tone using intracerebral microdialysis. Veratridine perfusion (50μM) increased extracellular 5-HT and DA levels more efficient in 1233ASO-treated than PBS-treated mice. Similarly, citalopram or nomifensine (1-10-50 μM) showed a marked dose-effect which phenotypic differences. Conversely, we found that a modest human α-synuclein over-expression in 5-HT or DA neurons (244.2 or 228.9% of sham mice, respectively) after four weeks post-infection inhibit 5-HT or DA release in striatum. Moreover, the mice showed depressive-like behaviors and motor impairments. In conclusion, these results confirm that α-synuclein is a negative regulator of monoamine neurotransmission and suggest that ASO targeted α-synuclein in monoamine neurons may lead to new therapies for PD.

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

COMPARATIVE EFFECTS OF MDMA AND METHAMPHETAMINE ON HIPPOCAMPAL CELL GENESIS IN ADOLESCENT AND YOUNG ADULT RATS

R. García-Cabrerizo [1], M.J. García-Fuster [1]
[1]. IUNICS. Universidad de las Islas Baleares, Palma de Mallorca

Adolescence is a period of great vulnerability to the neurotoxic effects of specific drugs of abuse, although some reports agree that during adolescence animals are less susceptible than in adulthood to the neurotoxic effects of amphetamines. This study compared the acute and chronic effects of MDMA and methamphetamine administration on hippocampal cell genesis in adolescent and young adult rats. Male rats were initially pretreated with BrdU pulses (2 x 50 mg/kg, i.p., for 3 days) which acts as a thymidine analogue marking new born cells (adolescent: postnatal day, PND 27-29, young adult: PND 48-50). Then, rats were treated following a binge paradigm (3 pulses per day, i.p., every 2 h, for 1 or 4 days) with either saline (0.9% NaCl, 1 ml/kg), MDMA (5 mg/kg) or methamphetamine (5 mg/kg) (PND 33-36 or PND 54-57). Rats were killed 24 h after the last treatment dose (PND 37 or PND 58) and the left hippocampus was cryostat cut and slide-mounted. Cell genesis markers (i.e., Ki-67 for recent cell proliferation and BrdU for cell survival) were analyzed by immunohistochemistry. The main results showed that in young adult rats (PND 58) chronic, but not acute, MDMA or methamphetamine administration impaired hippocampal cell proliferation (Ki-67+cells/area, MDMA: 21±4%, $p<0.05$; methamphetamine: 26±3%, $p<0.01$) and cell survival (BrdU+cells/area, MDMA: 23±5%, $p<0.05$; methamphetamine: 35±5%, $p<0.05$). Interestingly these effects were not observed in adolescent (PND 37) rats. In summary, the chronic administration of these two amphetamines induce similar neurotoxic effects in the hippocampus of young adult rats by decreasing cell genesis. Moreover, the results support previous data reporting adolescence as a period less susceptible than adulthood to the neurotoxic effects of amphetamines. Current studies are evaluating MDMA and methamphetamine hippocampal neurotoxicity on proteins regulating other neuroplasticity markers (e.g., BDNF, p-ERK) related to cell genesis.

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: Neurociencia de sistemas

Acknowledgments:
'Plan Nacional sobre Drogas' (MSSSI, Spain): Grant 2012/011 (to MJGF)
Fundación Alicia Koplowitz (to MJGF)
RTA-RETICS: RD12/0028/0011 (MINECO, Spain)
MJGF is a 'Ramon y Cajal' Researcher (MINECO, Spain)

LONG TERM CHANGES IN GENE EXPRESSION IN THE INNER EAR AFTER CONDUCTIVE OR CENTRAL DEAFNESS IN THE RAT

M.A. Merchan [1], J.M. Juiz [2], I. Plaza and V. Lamas [3]
[1]. Instituto de Neurociencias de Castilla y León. Lab 4. Auditory Neuroplasticity. University of Salamanca. Spain
[2]. Facultad de Medicina, Instituto de Investigación en Discapacidades Neurológicas (IDINE), Universidad de Castilla La Mancha, Albacete, Spain
[3]. Mass Eye and Ear- Harvard Med. School-USA

The descending corticofugal pathway regulates the response to sound of the inner ear through its direct connections to the brainstem olivocochlear (OC) efferent system. The OC lateral neurons modulates inner hair cells (IHC) activation of auditory nerve fibres. However the synapsis of OC medial system on the outer hair cells (OHC) modulate sound amplification and gain control of the cochlea. In order to compare plastic changes in the inner ear induced by sound or by central nervous system descending regulation, we compare an animal model of unilateral conductive deafness (model 1 - ossicular middle ear chain removal) with another of central deafness (model 2 - auditory cortex – AC- restricted ablation) To do this, we analyze at long term (1, 7 and 15 days post lesion time) by WB and qPCR, changes in the expression of genes or proteins of specific molecules involved in IHC neurotransmission and OHC micromechanic.
Both changes in model 1 or in model 2, triggers a long-term adaptive reorganization in the inner ear molecular machinery. In model 2, a group of specific genes involved in efferent regulation of IHC (glutamate dehydrogenase, subunits of AMPAr – GluR2, GluR3, GluR4, Ach - α 7, Dopamine D2, α1 and γ2) show significant changes both after comparing with controls or the ipsilateral with the contralateral ear, at all postlesion time groups. One of the most significant finding (1 day after lesión) was the upregulation of α7 Ach and α1 GABA A receptors genes, with changes up to 40 fold. These high FC values partially recovers at 15 days pl. We discuss this new data in relationship with previous results of our laboratory on changes in the electromotile protein Prestin, and βactin and α9-10 Ach receptors (Lamas et. Al 2015).

Lamas V, Arevalo JC, Juíz JM, Merchán M. Acoustic input and efferent activity regulate the expression of molecules involved in cochlear micromechanics. Frontiers in System Neuroscience. January 2015 | Volume 8 | Article 253 | Special Issue: : Auditory efferent system: new insights from cortex to cochlea.

1. Systems Neuroscience
2. Disorders and nervous system repair

This research was supported by the grants from the Ministry of Economy and Competitiveness of the Government of Spain,BFU2012-39982-C02andBFU2012-39982-C01.

Miguel Angel Merchán Cifuentes, e-mail: merchan@usal.es

EFFECTS OF 2-AG IN ACUTE TMEV INFECTION: CNS IMMUNOMODULATION AND INHIBITION OF LEUKOCYTE EGRESS FROM THE SPLEEN THROUGH S1P1 RECEPTOR

M. Mecha[1], C. Cordero[1], A. Feliú[1], F.J. Carrillo-Salinas[1], G. Hernández-Torres[2], S. Ortega-Gutiérrez[2], M.L. López-Rodríguez[2] and C. Guaza[1]
[1] Instituto Cajal, CSIC, Spain.
[2] Universidad Complutense de Madrid, Spain

The regulation of the brain-immune axis by cannabinoids provides promising therapeutic implications in a variety of neuroinflammatory conditions. 2-Arachidonoylglycerol (2-AG) has emerged as the chief endocannabinoid neuromodulator, but less is known about its central and peripheral immunomodulatory actions. We have used the intracerebral injection of the TMEV virus in SJL/J mice as a model of acute neuroinflammation. 2-AG and the reversible inhibitor of 2-AG degradation (UCM-03025) were administered in TMEV-infected mice. Immunohistochemistry against Iba1, Arg-1 and MHC-II was performed in coronal sections of the nervous parenchyma at the lesion site. RT-PCR analyses were done to evaluate the effects of 2-AG levels in pro-inflammatory & anti-inflammatory cytokines, chemokines, and ICAM-1. The immune infiltration in the CNS and the number of lymphocytes in lymph nodes and spleen were evaluated by flow cytometry. The results show that the direct administration of 2-AG, or the inhibition of its degradation by the compound UCM-03025, decreased the number of microglial cells at the lesion site and polarized them towards a reparative M2 phenotype. Central immunomodulatory effects of 2-AG also included the decrease of iNOS, TNFα, IL-1β, CCL2, SOCS3 and ICAM-1, whereas it induced an increase of IL-10, CX3CR1 and CCR2/CCL5 expression. Peripheral immunomodulatory effects of 2-AG consisted of a decrease in the immune infiltration into the CNS, and the inhibition of leukocyte egress from the spleen through a mechanism that involves the sphingosine S1P1 receptors. In conclusion, the endocannabinoid 2-AG exerted dual actions in the brain-immune axis to control the inflammatory response in the CNS.
This work was supported by grants from the Ministry of the Economy and Competitiveness MINECO (SAF2013-42784-R, SAF2013-48271), the Comunidad de Madrid (S2010/BMD-2308, S2010/BMD-2353) and the Red Española de Esclerosis Múltiple (REEM) RD12/0032/0008 (CG) sponsored by the Fondo de Investigación Sanitaria (FIS).
Miriam Mecha Rodríguez, miriammecha@cajal.csic.es

Theme
1. Disorders and nervous system repair
2. Homeostatic and neuroendocrine systems

LONG TERM REORGANIZATION OF SENSORY CORTICES AFTER COCHLEAR DAMAGE

M. Pernía [1], C.M. Poveda [2], I. Plaza [1], J.M. Juiz [2] and M.A. Merchán [1]
[1]. Instituto de Neurociencias de Castilla y León, Universidad de Salamanca, Spain
[2]. Facultad de Medicina, Instituto de Investigación de Discapacidades Neurológicas (IDINE), Universidad de Castilla La Mancha, Albacete, Spain

The aim of this study was to analyze in the rat long term (at 15 and 90 days after bilateral cochlear damage) plastic/structural changes in sensory cortices. Bilateral lesions were performed in Wistar rats by middle ear ossicular removal and surgical puncture of the cochlea. Auditory Brainstem Responses did not show sound evoked activity in both lesioned animal groups. Rats were perfused transcardially and the cochleae were serially sectioned and stained with cresyl violet in order to evaluate the percentage of spiral ganglion cell loss. Brains were sectioned serially in the coronal plane and immunostained for GluR2/3 AMPA receptors and for ARC/Arg3.1.
To analyze changes in immunoreactivity, high resolution pictures of 5 equivalent representative rostro-caudal sections (IA: 2.70, 3.20, 3.40, 4.20, 4.70 – Paxinos and Watson coordinates) containing auditory, visual and somatosensory cortices (AC, VC y SSC) per case were taken using a motorized stage. Cytoarchitectural density maps were made after gray level normalization of all sections. Rat brain cortex subdivisions were defined by matching guidelines in previous publications with our own results.
15 days after lesion, we observed an intense loss of immunoreaction density in all layers of the AC. There was also evidence of changes in density, cytoarchitecture and layering in VC and SSC. In GluR2/3 immunostained sections, we found loss of staining in layers II and III of the AC in contrast with an increase in density of immunoreaction in the VC and SSC.
Analysis in course in 90 days lesioned groups showed differences in immunostaining compatible with some recovery of immunoreactivity for both antibodies.

Peripheral deafness induces at long term partially reversible drop of AMPA receptor synthesis and trafficking in the AC and also a crossmodal reorganization in the VC and SSC.

1. Systems Neuroscience
2. Disorders and nervous system repair

Keywords: Rat, Cross-modal plasticity, ARC/Arg3.1, AMPA receptors, densitometry.

This research was supported by the grants from the Ministry of Economy and Competitiveness of the Government of Spain,BFU2012-39982-C02andBFU2012-39982-C01.

Marianny Janiree Pernía Rosales, e-mail: pernia.marianny@gmail.com

CSPGs MODULATION OF THE EXTRACELLULAR MATRIX AS A THERAPEUTICAL APPROACH IN A VIRAL MODEL OF MULTIPLE SCLEROSIS

A. Feliú [1], M. Mecha [1], F.J. Carrillo-Salinas [1], G. Hernández-Torres [2], S. Ortega-Gutiérrez [2], I. Bonilla del Río [3,4], N. Puente [3,4], M.L. López-Rodríguez [2], P. Grandes [3,4] and C. Guaza [1]
[1] Functional and Systems Neurobiology Department, Neuroimmunology Group, Instituto Cajal, CSIC, Madrid, Spain. [2] Universidad Complutense de Madrid. [3] Department of Neurosciences, Faculty of Medicine and Dentistry, University of the Basque Country UPV/EHU, Leioa, Spain.
[4] Achucarro Basque Center for Neuroscience Bizkaia, Science and Technology Park, Zamudio, Spain

The accumulation of extracellular matrix proteins (ECM) and chondroitin sulphate proteoglycans (CSPGs) that provokes scar formation are considered important factors for the failure of regeneration and remyelination in CNS injury and multiple sclerosis (MS). Previous results of our group showed that a combination of phytocannabinoids (CBD: THC) ameliorated symptomatology and modulated the accumulation of CSPGs in a viral model of MS, the Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD).
The aim of the present study was to address whether the pharmacological inhibition of the accumulation of CSPGs by xyloside treatment could affect the course evolution and symptomatology of TMEV-IDD. In addition, we investigated the effects of the endocannabinoid 2-arachidonoylglycerol (2AG) and its hydrolysis inhibition by UCM-03025 on i) the expression of CSPGs at the chronic phases of TMEV-IDD and ii) the production of CSPGs by astrocyte in culture.
Our results show that the upregulation of CSPGs that occurs at the chronic phases of TMEV-IDD were modulated by xyloside and UCM-03025 treatments leading to an amelioration of motor deficits in TMEV-infected mice. Moreover, *in vitro* results confirm that 2AG regulates the production of CSPGs in astrocytes.
As CSPGs are known to be involved in remyelination failure in human MS and in murine demyelinating models, it is suggested that 2-AG, by modulating CSPGs, would be involved in CNS reparative mechanisms.

Key words: Chondroitin sulphate proteoglycans, TMEV-IDD, astrocytes, UCM-03025, 2AG.
Topics: Disorders and nervous system repair

Ana Feliu Martínez (anafelm@cajal.csic.es)

ROLE OF THE SMALL GTPase RhoE IN THE PROCESS OF MYELINATION

M.P. Madrigal[1], B. Ballester-Lurbe[1], O. Gómez [2], R.M. Guasch[3], I. Pérez-Roger[1], J. Terrado[2]
[1]. Facultad de Ciencias de la Salud. Universidad CEU Cardenal Herrera. Valencia
[2]. Facultad de Veterinaria. Universidad CEU Cardenal Herrera. Valencia
[3]. Centro de Investigación Príncipe Felipe. Valencia

Myelination of the central nervous system happens during embryogenesis and first stages of postnatal life. In contrast to the peripheral nervous system, where Schwann cells are responsible for the formation of the myelin sheath, oligodendrocytes produce the myelin sheath surrounding axons in the central nervous system. Each oligodendrocyte can myelinate multiple segments in multiple axons and thus, the damage of a relatively small number of oligodendrocytes may result in alterations in axonal conduction, axonal loss and functional deficits. Demyelinization is implicated in different neurodegenerative disorders, as multiple sclerosis. Rnd3/RhoE is a small GTPase constitutively active with pleiotropic roles in the central nervous system development. Our aim is to study the role of RhoE in myelination/demyelination processes.

To carry out this goal, we have studied the levels and distribution of myelin in the brain of postnatal mice that do not express RhoE ($RhoE^{gt/gt}$) by western blot and immunohistochemistry. Western blot analysis showed that 15-days old $RhoE^{gt/gt}$ mice have lower level of Myelin Basic Protein (MBP) and Myelin Oligodendrocyte Glycoprotein (MOG) in the brain than their wild types and heterozygous littermates. These results were confirmed by MBP immunostaining, which also showed a disorganized arrangement of myelin distribution in the brain, especially in the striatum and a reduction of corpus callosum thickness.

In conclusion, RhoE absence produces a reduction in the levels of myelin as well as disorganization in myelin distribution in the central nervous system. This suggests that RhoE can be involved in important functions in the process of central myelination and that it could play a role in the progression of demyelination and remyelination in the context of demyelinating diseases.

Áreas Temáticas:
6. Disorders and nervous system repair

María del Pilar Madrigal Verdú, pilar.madrigal@uch.ceu.es

INCREASED EXPRESSION OF THE NEUROPROTECTIVE FACTOR VEGF IN THE OCULOMOTOR SYSTEM OF THE RAT

S. Silva-Hucha, R.G. Hernández, B. Benítez-Temiño, R.R. de la Cruz, A.M. Pastor, S. Morcuende.
Facultad de Biología. Universidad de Sevilla, Sevilla

Aims: Recent studies show a relationship between the deficit of vascular endothelial growth factor (VEGF) and motoneuronal degeneration, such as that occurring in amyotrophic lateral sclerosis (ALS). Overexpression of VEGF is involved in neuroprotection of motoneurons and delayed neurodegeneration. Extraocular motoneurons present lesser vulnerability to neurodegeneration in ALS compared to other cranial or spinal motoneurons. Therefore, we were interested in studying possible differences in VEGF dependence between extraocular and non-extraocular brainstem motoneurons, and the possible sources of VEGF to ocular motoneurons, such as astrocytes and extraocular muscles.
Methods: To determine the expression of VEGF in rat motoneurons and astrocytes, we performed immunohistochemistry in the three extraocular motor nuclei (abducens, trochlear and oculomotor) and compared to that observed in two other brainstem nuclei (hypoglossal and facial). The expression of VEGF in rat muscles was also studied by Western blot.
Results: Oculomotor motoneurons expressed higher amounts of the VEGF than others brainstem nuclei, what could be contributing to their higher resistance to neurodegeneration. The expression of VEGF in the astrocytes surrounding brainstem nuclei was not very remarkable, suggesting that astrocytes should not be the main source of VEGF to motoneurons in control situation. Extraocular muscles of the rat expressed VEGF, so it could be acting as a retrograde trophic factor for ocular motoneurons. Autocrine source should be neither discarded.
Conclusions:
1. VEGF expression is higher in extraocular motoneurons, compared to other cranial motoneurons studied.
2. Astrocytes do not seem to be the main source of VEGF for extraocular motoneurons in control situation.
3. Extraocular muscles may act as a retrograde source of VEGF for extraocular motoneurons.
4. Differences in VEGF availability could be then contributing to the different susceptibility of extraocular motoneurons compared with other motoneurons in neurodegenerative diseases.

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

Presenting author: Silvia Silva Hucha, e-mail: silvia_sh88@hotmail.com

NEUROPROTECTIVE ROLE OF LIVER GROWTH FACTOR "LGF" IN EXPERIMENTAL FRIEDREICH'S ATAXIA

[1]D. Reimers, [1]L. Calatrava-Ferreras, [1]R. Gonzalo-Gobernado, [1]A. S. Herranz, [1]M. J. Casarejos, [2]A. Jiménez-Escrig, [1]J. J. Díaz-Gil and [1]E. Bazán
[1]Servicio de Neurobiología-Investigación, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS) y [2]Servicio de Neurología del Hospital Universitario Ramón y Cajal. 28034-Madrid, ESPAÑA

Cerebellar ataxias are a heterogeneous and infrequent group of neurodegenerative diseases characterized by the lack of motor coordination. Friedreich's ataxia (FA) is the most common and studied type of hereditary ataxias. Current therapies focus on the application of chemicals which prevent or reverse some pathogenetic processes, but there still is no effective therapy for this disease. Liver growth factor (LGF) is a hepatic mitogen with notable neuroregenerative activity, which has been recently protected by a family of patents based on **PCT/ES2009/070106**. In addition, LGF has antioxidant properties and a potent activity on cardiovascular system, suggesting that this factor might be effective for FA treatment. In the experimental model of the YG8R mouse, the intraperitoneal administration of LGF (1.6μg/mouse) exerted a neuroprotective effect on neurons of the lumbar spinal cord and improved cardiac hypertrophy. Both events could be the consequence of an increment in frataxin expression induced by the factor in spinal cord (134 ± 5%, $p \leq 0,05$ vs YG8R + vehicle) and in heart (116 ± 6%, $p \leq 0,05$ vs YG8R + vehicle), since degeneration of both structures is directly associated with deficit of this protein. LGF treatment also increased mitochondrial chain complex IV expression in spinal cord (261 ± 30%, $p \leq 0,01$ vs YG8R + vehicle), while in skeletal muscle it significantly reduced the relation oxidized glutation/ reduced glutation, which is an indicator of oxidative stress. Since, in addition, LGF partially restores motor coordination in YG9R mice, these results indicate that the factor might be a potential therapeutic agent for FA treatment.

EFFECTS OF SYNAPTIC DEAFFERENTATION IN EXTRAOCULAR MOTONEURONS

R.G. Hernández[1], B. Benítez-Temiño[1], C.J. Morado-Díaz[1], M.A. Davis-López de Carrizosa[1], R.R. de la Cruz[1], A.M. Pastor[1]
[1]. Facultad de Biología. Universidad de Sevilla, Sevilla

Aims: Horizontal gaze coordination relays on abducens internuclear neurons (ABD INTs), whose axons contact contralateral medial rectus motoneurons (MRMn) through the medial longitudinal fascicle (MLF). Also, MRMn receive a major input from the lateral vestibular nucleus (LVN) through the ascending tract of Deiters (ATD). The effects of MLF transection on horizontal eye movements have been extensively described. However, the consequences of disconnecting MRMn from their afferences on their discharge characteristics remain to be elucidated.
Methods: Stimulating electrodes were implanted in MR muscles and in the IIIrd cranial nerve for MRMn identification. Scleral coils were implanted for eye movement recording and a chamber was drilled on the parietal bone to allow the access of recording electrodes to the midbrain. Control recordings were obtained and then the ATD or the MLF was unilaterally sectioned. Thereafter, recordings were carried out on alternating days for two months. Immunohistochemistry against calretinin, a protein that is expressed by both ABD INTs and LVN neurons, was used to assess the lesion site and the degree of MRMn deafferentation.
Results: MLF axotomy produced a reduction in MRMn sensitivity to eye velocity (r) and position (k), and a diminished firing rate at straight-ahead gaze (F_o). ATD axotomy also reduced r and k, but F_o remained unaltered. Comparison between ATD and MLF lesioned groups revealed that k, r and F_o values after MLF axotomy were lower than those obtained after ATD transection. Immunohistochemistry and confocal analysis revealed that MLF axotomy produced a higher degree of deafferentation when compared with data obtained from ATD-axotomized animals.
Conclusions: ATD and MLF projections on MRMn are not comparable in terms of synaptic coverage and eye movement-related firing coding. According to the present results, ABD INTs seem to have a stronger influence on MRMn firing rate, which might be related with a more extensive innervation.

Presenting author: Rosendo García Hernández, e-mail: rgarciahernandez1@gmail.com

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

DIFFERENTIAL NEUROTROPHIN EXPRESSION IN ADULT RAT CRANIAL MOTONEURONS

R. G. Hernández[1], S. Morcuende[1], S. Ciscares-Velázquez[1], S. Silva-Hucha[1], A.M. Pastor[1], B. Benítez-Temiño[1]
[1]. Facultad de Biología. Universidad de Sevilla, Sevilla

Aims: Extraocular motoneurons exhibit some characteristics that differentiate them from for other motoneuron populations. For instance, their low vulnerability to amyotrophic lateral sclerosis (ALS) make oculomotor system a good target in ALS research. Besides, oculomotor motoneurons are particularly responsive to neurotrophins, as the exogenous administration of these molecules prevents the effects of axotomy. Thus, we aimed to detect possible differences in neurotrophin expression with other cranial motor systems.
Methods: To compare neurotrophin expression in cranial motor systems we performed the following procedures: First, we tested protein expression in extraocular, facial and tongue muscles by Western blot analysis and RT-qPCR. Second, we compared protein presence at oculomotor, trochlear, abducens, facial and hypoglossal nuclei by immunohistochemistry and confocal analysis. Motoneurons were identified with antibodies against choline acetyl transferase. In a different set of experiments, cranial motoneurons from the specified nuclei were isolated using FACS flux cytometry and neurotrophin expression was assessed and compared by RT-qPCR.
Results: Western blots and qPCR showed the presence of the three neurotrophins in all studied muscles, with higher amounts of NGF in the extraocular muscles. Analysis of brainstem nuclei showed that oculomotor motoneurons expressed both BDNF and NT-3, in a higher quantity when compared with other brainstem motoneurons.

Conclusions
1. Extraocular muscles expressed higher amounts of neurotrophins than other cranial muscles.
2. Contrary to other cranial motoneurons, most extraocular motoneurons expressed both BDNF and NT-3.
3. Higher levels of neurotrophin expression and presence in the oculomotor system might explain in part the higher resistance of extraocular motoneurons to ALS.

Presenting author: Beatriz Benítez Temiño, e-mail: bbtmino@us.es

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

LIVER GROWTH FACTOR "LGF" AS A THERAPEUTIC AGENT IN EXPERIMENTAL ALZHEIMER DISEASE

[1]María J. Casarejos, [1]Juan Perucho, [1]Rafael Gonzalo-Gobernado, [1]Diana Reimers, [1]Ana Gómez Soria, [1]Manuela Vallejo-Muñoz, [1]Antonio S. Herranz, [2]Adriano Jiménez-Escrig, [1]María Teresa Montero Vega, [1]Juan José Díaz-Gil and [1]Eulalia Bazán

[1]Servicio de Neurobiología-Investigación, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS) y 2 Servicio de Neurología del Hospital Universitario Ramón y Cajal. 28034-Madrid, ESPAÑA

Alzheimer Disease (AD) is a progressive neurodegenerative disease affecting more than 35 million people older than 65 years, which has currently no effective treatment. Liver Growth Factor (LGF) is a pleiotropic factor, which has antiinflamatory, antioxidative and neuroregenerative properties and could, therefore, be useful for AD treatment. In fact, intraperitoneal administration of LGF in APPswe mice (1.6μg/mouse) significantly restored cognitive damage and reduced the number of beta-amyloid (βA) plaques with a diameter higher than 25 μm in hippocampus (8 ± 1.2 and 5 ± 0,6 plaques/mm^2 in APPswe and APPswe+LGF mice, respectively) and cerebral cortex (9.4 ± 1 and 5 ± 0.8 plaques/mm^2 in APPswe and APPswe+LGF mice, respectively). In addition, LGF treatment reduced βA content and phospho-Tau/Tau ratio in a 60% and a 30%, respectively, in both structures. Parallely, administration of the factor reduced the content of Iba1 and GFAP in hippocampus and cerebral cortex, indicating that LGF reverted microglia and astroglia activation, respectively. These results together with the increase in HSP70 expression in APPswe mice, a chaperone which content is reduced in APPswe mice versus wild mice, strongly indicate that LGF is a potential therapeutic agent for AD.

EFFECTS OF VEGF ADMINISTRATION ON THE DISCHARGE ACTIVITY OF CAT ABDUCENS MOTONEURONS

P.M. Calvo, R.R. de la Cruz, A.M. Pastor
Departamento de Fisiología, Facultad de Biología, Universidad de Sevilla, Sevilla

VEGF was initially characterized by its angiogenic activity but recent evidences indicate that it can also act as a neuroprotective agent, especially on motoneurons. Thus, mutant mice deficient in this factor develop a motoneuronal disease reminiscent of amyotrophic lateral sclerosis. To further explore the neurotrophic potentiality of this factor we evaluated the phsysiological effects of VEGF exogenous administration on the discharge activity of axotomized motoneurons. Our major aim was to determine whether VEGF treatment could prevent the axotomy-induced alterations. For this purpose, adult cats were prepared for chronic single-unit extracellular recordings of abducens motoneurons simultaneously with eye movements under alert conditions.
Axotomy produced drastic changes in the discharge characteristics of these neurons. Thus, they exhibited an overall reduction in firing rate that affected both their tonic discharge during eye fixations (i.e., eye position neuronal sensitivity) as well as their phasic activity for rapid eye movement or saccades (i.e., eye velocity neuronal sensitivity). Other typical signs of axotomy were their inability to maintain their firing during long fixations and the presence of reduced bursts during on-directed saccades.
VEGF was administered through the distal stump of the VIth nerve (0.2 µg/kg of body weight) every other day and immediately after axotomy. Recordings of abducens motoneurons showed a normal discharge pattern both during eye fixations and saccades, yielding eye position and velocity sensitivities statistically similar to control and different to axotomy values. The present data demonstrate that VEGF is able to prevent the axotomy-induced physiological alterations on abducens motoneurons.

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

BONE MARROW-DERIVED MESENCHYMAL STEM CELLS CHARACTERIZATION AND TRANSPLANTATION IN AN ANIMAL MODEL OF CONGENITAL HYDROCEPHALUS

M. García Bonilla, M. Cifuentes, A. Ho Plágaro, K. Shumilov, L. Santos Ruiz, J.M. Pérez Fígares, A.J. Jiménez
Facultad de Ciencias. Universidad de Málaga, Málaga

Congenital hydrocephalus is a disorder presenting a degeneration of the periventricular cerebral parenchyma and the white matter, which causes significant mortality and life-long neurological complications. There are currently no effective therapies for congenital hydrocephalus. Bone marrow-derived mesenchymal stem cells (BM-MSC) are considered as a potential therapeutic tool in neurodegenerative diseases, due to their ability to migrate to degenerated tissues and the production of growth factors. In the present study, using an animal model of congenital hydrocephalus, the hyh mouse, it has been studied the capacity of the BM-MSC to reach the degenerated regions exhibiting glial reactions and their probable neuroprotector effects.
The BM-MSC were isolated from two different sources: a) transgenic mice expressing the monomeric red fluorescent protein (mRFP1); b) wild type mice. In the second case, the BM-MSC were labelled in vitro using bromodeoxyuridine, a fluorescent cell tracker and the lipophilic DiR. Before application, the cells were analysed using flow cytometry and immunofluorescence. The BM-MSC were injected into the retro-orbital sinus or into the lateral ventricle of hyh mice. After 24/96 hours of administration, the BM-MSC were detected under light, confocal and electron microscopes.
The injected BM-MSC reached the degenerated periventricular regions and the disrupted neurogenic niches. They were detected in the periventricular parenchyma, around periventricular blood vessels and in the ventral meninges. Most of the applied BM-MSC expressed the glial cell-derived neurotrophic factor (GDNF), in the same way as the periventricular reactive astrocytes, suggesting a possible neuroprotector effect.
Supported by FIS PI12/0631-FEDER to AJJ.

Áreas Temáticas:
1ª: Trastornos y reparación del sistema nervioso
2ª: Nuevos métodos y tecnologías

Presenting author: María García Bonilla email: mgbonilla@uma.es

ISOLATION AND CHARACTERIZATION OF EXTRACELLULAR VESICLES SECRETED BY GLIAL CELLS IN RESPONSE TO OXIDATIVE STRESS

Raquel Pascua-Maestro [1], Esperanza González[2], Juan Manuel Falcón-Pérez[2,3], M. D. Ganfornina [1], D. Sánchez[1]
[1]IBGM – Universidad de Valladolid / CSIC, Valladolid, Spain. [2]Exosomes group. Metabolomics Unit and Platform, CIC bioGUNE, CIBERehd, Technology Park of Bizkaia. Derio, Bizkaia, Spain; [3]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain.

Understanding aging, particularly brain aging and its associated pathologies, is a challenge for current research and for our society as a whole. The experience in the field demonstrates that direct application of neuroprotectors has important limitations. In recent years, cell-secreted extracellular vesicles (EVs) constitute a promising therapeutic strategy, since EVs have been shown to be able to cross the blood-brain barrier.
Given that EVs are generated both in normal and pathological situations, could EVs show protecting profiles adapted to each particular neurodegenerative condition?
To address this question, our global aim is to study the composition and function of EVs generated by glial cells in response to particular stimuli such as oxidative stress, and to determine if they are enriched in neuroprotective factors that can contribute to improve the viability and the functionality of the affected cells in degenerating brains.
For this purpose, the human astrocyte cell line 1321N1 is used in this study to obtain EVs produced both in control and oxidative stress conditions. The isolated EVs are subjected to analysis of specific EV markers.
This project is the starting point for a wider project in which all components of glial-derived EVs (proteins, microRNA, lipids) will be analyzed with the final goal of producing "EVs *à la carte*" applicable to diverse types of neurodegenerative conditions.

Support: MICINN(BFU2011-23978), GEIVEX.

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

SERPINS PROMOTE CANCER CELL SURVIVAL AND VASCULAR CO-OPTION IN BRAIN METASTASIS

M. Valiente[1,2], A.C. Obenauf[1], X.Jin[1], Q. Chen[1], X.H.-F. Zhang[1,3], D.J. Lee[1], J.E. Chaft[1], M.G. Kris[1], J.T. Huse[1], E. Brogi[1], and J. Massagué[1]
[1].Memorial Sloan Kettering Cancer Center, New York, NY.
[2].Present address: Molecular Oncology Programme, CNIO, Madrid, Madrid.
[8].Present address: Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX.

Objective: Brain metastasis is the most common neurological complication of cancer. These patients frequently suffer from neurocognitive defects that compromise their life quality during the short and invariably fatal course of the disease. In order to generate novel therapeutic opportunities, we intend to dissect the limiting steps for the progression of metastatic cells in the brain using experimental models and human samples.
Results: We have characterized brain colonization from the very initial stages, where a single cancer cell originally generated in the lung or the breast gets exposed to the nervous system, until the moment in which neurological signs appear derived from the growth of established metastatic lesions. A detailed study of this cellular biology allowed us to identify two critical steps:
1. Protection from a strong reactive glial compartment.
2. Ability to physically interact with brain capillaries.
We characterized molecularly both mechanisms allowing a few metastatic cells to avoid the selective pressure imposed by the brain microenvironment. Metastatic cells get protected from anti-protease members of the Serpin family Neuroserpin and SerpinB2, which are also necessary to develop an L1CAM dependent cell-adhesion program to grow around capillaries. All these mechanisms were validated in human samples and their presence in lung primary tumors correlates with an increased risk of developing brain metastasis.
Conclusion: These findings shed light on the long recognized inefficiency of the metastatic cascade during brain colonization, where 90% of the cancer cells that initially arrive to this organ finally perish. By studying the biology behind multiple models of brain metastasis we have identified new mediators of critical steps during brain colonization and generated novel therapeutic opportunities. Our work also presents a new clinically relevant model to study the function and molecular signatures of reactive astrocytes and the provoking possibility of including these cell type to target brain metastasis.

Areas of interest (in order of preference):
- Trastornos y reparación del sistema nervioso
- Excitabilidad neuronal, sinapsis y glía: mecanismos celulares

Manuel Valiente: mvaliente@cnio.es

MICROGLIA AND INFILTRATED MACROPHAGES ABUNDATLY POPULATE PSEUDO-PALISADES OF GLIOBLASTOMA MULTIFORME

E. Saavedra-López[1], G.P. Cribaro[1], A. Pérez-Vallés[2], J.M. Gallego-Sánchez[2], C. Barcia, Sr.[2], C. Barcia, Jr.[1]
[1]. Institut de Neurociències, Departament of Biochemistry and Molecular Biology, School of Medicine, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona.
[2]. Department of Neurosurgery & Department of Pathology, Hospital General Universitari de València, Valencia.

Glial tumors or gliomas are the most common type of primary brain tumors, they have a very poor prognosis and remain incurable. Glioblastoma multiforme (GBM), the most aggressive form of astrocyte-derived glioma, presents a series of histopathological hallmarks, namely glomeruloid vessels, necrotic areas, gemistocytic formations, aberrant mitoses and pseudo-palisades. Precisely, these pseudo-palisades are weakly understood, although they are increasingly often regarded. Reports hypothesize that when a blood vessel collapses, tumor cells escape the harsh environment created by the lack of nutrients and oxygen, therefore creating this palisade-like structure. Moreover, references state that the inflammatory component in these structures is very low.
We, however, have found by studying pseudo-palisades in six human GBM samples that these in-comprehended regions have a vast amount of immune cells. More specifically, tumor-associated microglia and macrophages (TAM/Ms) were strikingly conspicuous. In addition, when quantifying at these immune cells, we saw that their density does not depend on the aggressiveness of the tumor, meaning that they are always present in similar amounts. Furthermore, by means of confocal microscopy, we observed evidence of TAM/Ms directionality and potential organized motility within these structures.
This finding sheds light on the understanding of GBM microenvironments, infers the versatility of TAM/Ms and it therefore brings the possibility of manipulating TAM/Ms to eradicate the harmful tumor cells, what could elucidate new therapies against this fatal disease.

This work was supported through grants from the Spanish Ministry of Economy and Competitiveness (RYC2010-06729, SAF2010-21274 and SAF2013-45178-P).

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

GENETIC REDUCTION OF CDK5 IN Hhd$^{Q7/Q111}$ MICE AMELIORATES COGNITIVE DYSFUNCTION IN HUNTINGTON'S DISEASE

E. Alvarez Periel[1,2,3], M. Puigdellívol Cañadell[1,2,3], M. Cherubini[1,2,3], J. Alberch Vié[1,2,3], S. Ginés Padrós[1,2, 3]
[1]. Facultad de Medicina, Universidad de Barcelona, Barcelona
[2]. Institut d´Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona
[3]. CIBERNED

Cognitive impairment is an early clinical feature of Huntington's disease (HD) with increasing relevance over the classical motor symptoms of the pathology. Unfortunately, molecular mechanisms underlying these defects remain unclear. Cdk5 is a serine/threonine kinase whose activity is primarily restricted to the nervous system. In recent years Cdk5 has emerged as a key regulator of synaptic plasticity and cognition and it has been involved in many neurodegenerative diseases such as Huntington, Alzheimer or Parkinson´s Disease. Importantly, our group has demonstrated aberrant Cdk5 activity in the striatum of different HD models and in HD human brain. This data highlights Cdk5 as an important modulator of neuronal dysfunction and points out therapeutic strategies aimed to inhibit Cdk5 activity as prospect means to delay or prevent HD progression. To determine whether altered Cdk5 activity could contribute to cognitive decline in HD we generated a new transgenic mouse model expressing mutant huntingtin and heterozygous for Cdk5 (Hdh$^{Q7/Q111}$; Cdk5$^{+/-}$). The genetic modulation of Cdk5 levels in Hdh$^{Q7/Q111}$ mutant mice restored corticostriatal learning deficits and improved performance in spatial and memory learning tasks, which suggests that alterations in both corticostriatal and hippocampal functions in HD could involve aberrant Cdk5 activity. Moreover, our data shows that restoration of cognitive functions is paralleled by a recovery of GluNR2B surface levels in the striatum and cortex of Hdh$^{Q7/Q111}$; Cdk5$^{+/-}$ mice. This recovery correlates with a restoration of total pTyr1472-GluN2B and pTyr416-Src levels in the cortical region, which suggests that Cdk5 might be regulating GluNR2B surface levels through this pathway. Altogether, these findings demonstrate that modulation of Cdk5 activity or signalling in HD may contribute to restore synaptic plasticity and learning defects in this devastating disorder.

Funding: CHDI foundation (A-6146); Ministerio de Ciencia e Innovación [SAF 2012-39142, integrated in Plan Nacional de I+D and Fondo Europeo de Desarrollo Regional (FEDER)].

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

Presenting Author:
Elena Alvarez Periel – ealvarezp@ub.edu

CHRONIC INHIBITION OF HDAC3 IMPROVES LEARNING AND MEMORY DEFICITS IN A MOUSE MODEL OF HUNTINGTON'S DISEASE

N. Suelves Caballol[1,2,3], J. Alberch Vié[1,2,3], R.S. Lahue[4,5], S. Ginés Padrós[1,2,3]
1. Facultad de Medicina, Universidad de Barcelona, Barcelona
2. Institut d´Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), Barcelona
3. Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED)
4. Centre for Chromosome Biology, School of Natural Sciences, National University of Ireland Galway, Galway.
5. NCBES Galway Neuroscience Centre, National University of Ireland Galway, Galway

Huntington's disease (HD) is a fatal neurodegenerative disorder characterized by motor, cognitive and psychiatric symptomatology. Although motor features are the most obvious in HD, cognitive deficits such as learning and memory problems precede the overt motor symptoms by years in HD patients and animal models. In recent studies, transcriptional dysregulation has emerged as an important underlying pathogenic process that appears early in disease progression and is observed across multiple HD models. Therapeutic development has focused on targeting HDACs with small molecule inhibitors in order to alter chromatin structure and thereby raise gene transcription. Although broad acting inhibitors have shown promising results, they can elicit toxic side effects particularly in long-term treatment. Therefore, selective HDAC inhibitors are predicted to show better therapeutic outcome. Since HDAC3 has shown to play a role in memory formation and in HD pathology we want to test if selective-HDAC3 inhibition prevents or ameliorates motor learning and long-term memory deficits in the $Hdh^{Q7/Q111}$ knock-in (KI) mouse model of HD.
Behavioral assessment revealed that chronic treatment with HDAC3 inhibitor 966 completely reversed the altered acquisition of new motor skills and the impaired recognition and spatial memories in KI mice. Biochemical and molecular analysis in mouse brain samples showed that systemic administration of HDAC3 inhibitor significantly increased histone H3 acetylation and upregulated the expression of genes related to memory formation.
Taken together, the results demonstrate a critical role for HDAC3 in learning and memory impairments and reveal a good pharmacotherapeutic approach for early cognitive deficits in HD.

Funding: Ministerio de Ciencia e Innovación (SAF2012-39142), integrated in Plan Nacional de I+D+I and Fondo Europeo de Desarrollo Regional (FEDER). European Huntington's Disease Network (EHDN).

Presenting author
Nuria Suelves Caballol. email: nuriasuelves@ub.edu.

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

EFFECT OF TRANSCRANIAL MAGNETIC STIMULATION ON A MULTIPLE SCLEROSIS RAT MODEL

M. Aguilar-Luque[1], E. Luque[1], A.I. Giraldo[1], M. LaTorre-Luque[1], E. Agüera[2], F. Sánchez-López[2], B. Escribano[3], I. Túnez[1].
[1] Facultad de Medicina, Universidad de Córdoba. Córdoba
[2] Hospital Universitario Reina Sofía. Córdoba
[3] Facultad de Veterinaria, Universidad de Córdoba. Córdoba

Aim
The main aim was studied the transcranial magnetic stimulation (TMS) effects in a myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE) model, similar to reproduce human multiple sclerosis (MS). We was analyzed the changes in clinical score of rat, oxidative stress biomarkers and histological changes in brain.
Material and Methods:
For this study, 30 male *Dark agouti* rats 8 weeks old at the beginning of the study were used. They were divided into 3 groups of 10 as follow: i) control; ii) EAE; and iii) EAE+TMS. Rats were injected with an emulsion containing myelin oligodendrocyte glycoprotein (MOG) supplemented with heat-inactivated *Mycobactirium tuberculosis* H37Ra. TMS was applied during 21 days, starting 14 days after inoculation with MOG. On day 35, all rats were anesthetized and sacrificed, and the brain were rapidly removed and blood were collected.
Results:
The results of our study showed a significant decrease score as well as a reversion towards to normality in the oxidative stress biomarkers. Histological study revealed that in EAE group decreased cellularity and increase the astrocytes number, while in EAE + TMS increased the cellularity and decreased the astrocytes.
Conclusions:
The TMS application of TMS show positive and protective effects in EAE animals. However, more experimental and clinical studies in this line are required

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

A COMBINATION OF ANTIOXIDANTS AND MAGNESIUM AMELIORATES THE THRESHOLD SHIFT FOLLOWING ACUTE NOISE-INDUCED HEARING LOSS

J.C. Alvarado[1, 2], V. Fuentes-Santamaría[1, 2], P. Melgar-Rojas[1, 2], M.C. Gabaldón-Ull[1, 2], J.M. Miller[3, 4], J.M. Juiz[1, 2].
1. Instituto de Investigación en Discapacidades Neurológicas (IDINE), Albacete, Spain.
2. Facultad de Medicina, Universidad de Castilla-La Mancha, Albacete, Spain.
3. Karolinska Institutet, Stockholm, Sweden.
4. University of Michigan, Ann Arbor, MI, USA.

With increasing exposure to work-related and recreational noise, our population is increasingly at risk to developing noise-induced hearing loss (NIHL). The aim of the present study was to determine if a combination of free radical scavengers (vitamins A, E and C) plus the natural vasodilator magnesium (ACEMg) could prevent noise-induced hearing loss (NIHL). Three month-old Wistar rats were distributed into two groups: one fed a normal diet (ND), the other an ACEMg enriched diet (ED). The ED began 10 days before the noise stimulation. All rats were then exposed to a continuous white noise at 118 dB SPL for 4h for 4 consecutive days. ABR recordings at 0.5, 1, 2, 4, 8, 16 and 32 kHz were performed prior (control condition) and at 1 day (1d) and 10 days (10d) after noise overexposure. The results demonstrated that in both ND and ED animals, there was a significant NIHL at 1d and 10d following exposure at all frequencies tested. However, the threshold shift observed in the ED rats was significantly lower than that observed in ND rats at 1d as well as at 10d after the noise overexposure. These findings, extending previous study (LePrell et al, 2007) to multiple noise-exposures in a different species, indicate that the combined use of free radical scavengers and magnesium before and during the noise overexposure is indeed effective in reducing the risk of NIHL and therefore, should be considered a potential therapy for the treatment of this condition in humans.
This study was supported by the MINECO (BFU2012-39982-C02-02) and the PROHEARING project of the 7th Framework Programme (FP7-HEALTH-2012-INNOVATON 304925).

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

AUDITORY THRESHOLD SHIFT IN AGE-RELATED HEARING LOSS IS REDUCED BY A COMBINATION OF MICRONUTRIENTS

J.C. Alvarado[1, 2], V. Fuentes-Santamaría[1, 2], P. Melgar-Rojas[1, 2], M.C. Gabaldón-Ull[1, 2], J.M. Miller[3, 4], J.M. Juiz[1, 2].
1. Instituto de Investigación en Discapacidades Neurológicas (IDINE), Albacete, Spain.
2. Facultad de Medicina, Universidad de Castilla-La Mancha, Albacete, Spain.
3. Karolinska Institutet, Stockholm, Sweden.
4. University of Michigan, Ann Arbor, MI, USA.

The increasing rate of age-related hearing loss (ARHL) in the population, with its subsequent reduction in quality of life and increase in health care costs require new therapeutic strategies to prevent or reduce ARHL. The goal of this study was to determine if presbycusis could be reduced in adult animals by administering a combination of free radical scavengers (vitamins A, E and C) plus the natural vasodilator magnesium (ACEMg). Three month-old Wistar rats were divided into two groups: one fed a normal diet (ND), the other an ACEMg enriched diet (ED). The ED began 10 days before the noise stimulation. Then, in order to accelerate presbycusis, ND and ED rats were exposed to 1 h continuous white noise at 118 dB SPL for 5 days a week until the animals reached the age of 12-14 months. ABR recordings were performed at 0.5, 1, 2, 4, 8, 16 and 32 kHz at 3 and 12-14 months of age. The results showed that in both ND and ED animals of 12-14 months there was a significant increase in auditory thresholds at all frequencies tested. No statistical differences were found in the higher and lower frequencies; however, the threshold shift observed in ED rats was significantly reduced by at least 10 dB (3 fold) when compared to that seen in ND rats at 2 and 4 kHz. These findings indicate that ACEMg may provide an effective therapeutic intervention for the treatment of ARHL.
This study was supported by the MINECO (BFU2012-39982-C02-02) and the PROHEARING project of the 7th Framework Programme (FP7-HEALTH-2012-INNOVATON 304925).

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

SHORT-DURATION AND LONG-TERM NOISE OVERSTIMULATION COULD INCREASE HEARING LOSS IN AGING WISTAR RATS.

J.C. Alvarado[1, 2], V. Fuentes-Santamaría[1, 2], P. Melgar-Rojas[1, 2], M.C. Gabaldón-Ull[1, 2], J.M. Miller[3, 4], J.M. Juiz[1, 2].
1. Instituto de Investigación en Discapacidades Neurológicas (IDINE), Albacete, Spain.
2. Facultad de Medicina, Universidad de Castilla-La Mancha, Albacete, Spain.
3. Karolinska Institutet, Stockholm, Sweden.
4. University of Michigan, Ann Arbor, MI, USA.

Despite the fact that there is a great deal of information about the impact of noise on hearing, there is no consensus on the long-term functional effects of noise exposure on age-related hearing loss (ARHL). The aim of the present study was to determine if a short-duration, long-term noise overexposure increased auditory thresholds in an animal model of ARHL. Three month-old Wistar rats were divided into two groups: one exposed (EXP), the other not exposed (NOT-EXP) to noise overstimulation. The protocol of stimulation consisted of 1 h continuous white noise at 118 dB SPL, 5 days a week until the animals reached an age of 12-14 months. ABR recordings at 0.5, 1, 2, 4, 8, 16 and 32 kHz were performed at 3**,** 6-8 and 12-14 months of age. The results demonstrated that in the EXP group at 6 months of age, and in both EXP and NOT-EXP animals at 12-14 months of age there were significant increases in the auditory thresholds at all frequencies tested. However, the threshold shifts observed in the EXP group was significantly higher than that observed in the NOT-EXP at the same ages. The thresholds observed in the EXP animals at 6-8 months were similar to those observed in NOT-EXP rats at 12 -14 months. These findings suggest that long-term noise overstimulation in short-duration episodes accelerates the time-course of hearing loss in this animal model of presbycusis and may represent a significant burden of ARHL in humans.

This study was supported by the MINECO (BFU2012-39982-C02-02) and the PROHEARING project of the 7th Framework Programme (FP7-HEALTH-2012-INNOVATON 304925).

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

TEMPORAL ELECTROPHYSIOLOGICAL PROFILE OF SOMATOSENSORY CORTEX AFTER SPINAL CORD INJURY.

E. Alonso-Calviño[1], E. Fernández-López[1], E. Goiriena[2]. A Higuero[2] J Abad-Rodríguez[2] J. Aguilar[1].
[1] Neurofisiología Experimental. Hospital Nacional de Parapléjicos. Toledo.
[2] Biología de Membranas. Hospital Nacional de Parapléjicos. Toledo

The somatosensory cortex suffers a massive deafferentation and functional reorganization after a spinal cord injury (SCI). Cortical reorganization has been described as an increased magnitude of the somatosensory evoked potentials (SEP) to peripheral stimulation in the body regions located above the lesion level. But this increased magnitude of SEP has been observed at fixed time windows after spinal lesion in different studies. A longitudinal study is required to fully understand the temporal evolution of cortical changes after SCI. The main objective of this study is to create an electrophysiological profile of temporal evolution of cortical responses in the corresponding deafferented region. Experiments were performed in 2 groups of Wistar rats with a chronic thoracic SCI (transection and severe contusion) and in a control group. Five small screws were fixed in the skull in order to record extra-cranial EEG and SEP from the hindpaw somatosensory cortex. Animals were anesthetized (isoflurane) to maintain under slow-wave activity the state of EEG. Electrical stimulation was applied (0.5ms; 0.5Hz) at two intensities (5V and 50V) in the four extremities. The average of SEPs was used to study the cortical reorganization and the cortical excitability after SCI. Our results show an increased magnitude of SEPs during the first two weeks after SCI. However, a decrease in magnitudes of SEPs occurs in the contusion group (3th and 4th week) at both intensities stimulation while the transected group remains at high magnitude of responses. A measure of cortical excitability was obtained by quantification of activated states triggered by peripherals inputs. Our data show a similar increased profile of SEPs for experimental SCI groups vs no changes in control group. Our results indicate that the cortical excitability of deafferented cortex by SCI has a different temporal profile depending on the type of lesion and the time window of observation.

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

LAMIN B1 PROTEIN LEVELS AND DISTRIBUTION ARE HIGHLY ALTERED IN HUNTINGTON'S DISEASE BRAIN

R. Alcalá Vida[1,2,3], J. Creus Muncunill[1,2,3], M. Garcia Forn[1,2,3], J. Alberch[1,2,3] and E. Pérez Navarro[1,2,3].
[1]. Departament de Biologia Cel·lular, Immunologia i Neurociències, Facultat de Medicina, Universitat de Barcelona, Catalunya.
[2]. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Catalunya.
[3]. Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), España

Huntington's disease (HD), a hereditary neurodegenerative disease caused by a CAG repeat expansion in the exon-1 of the huntingtin gene, is characterized by motor and cognitive impairment. Nowadays, it is not clear how mutant huntingtin induces selective neuronal dysfunction. Lamins, the major structural proteins inside the nuclear lamina, are crucial for maintaining the homeostasis and functionality of the cellular nuclei and their alterations are involved in relatively few diseases, classified as laminopathies. Previous results from our group showed that lamin B levels are increased in the putamen of HD patients, as well as in the striatum of R6/1 mouse model of HD (Rue et al., 2014). Here, we extend our study by analyzing lamin B protein isoforms, B1 and B2, and also lamins A and C in R6/1 mice at different stages of the disease. We found that R6/1 mice displayed increased lamin B1 and B2 levels in the striatum and cortex from early stages of the disease while in the hippocampus their levels were only augmented at late stages. Lamin A and C levels were also enhanced in the striatum and hippocampus at late stages, but they were not altered in the cortex. Immunohistochemical analysis showed that lamin B1 was clearly redistributed inside the nucleus, which presented altered morphology in R6/1 mice neurons when compared with wild-type mice. Interestingly, treatment of R6/1 mice with betulinic acid reduced lamin B1 levels in the hippocampus and improved recognition and spatial memories. Due to the role of lamins in the chromatin organization, gene transcription and oxidative stress responses, our results suggest that these alterations could have important implications in the pathophysiology of HD. Thus, normalizing lamin protein levels could be a potential therapeutic strategy in the fight against this devastating neurodegenerative disease.

This work was supported by the project PI13/01250 *integrado en el Plan Nacional de I+D+I y cofinanciado por el ISCIII-Subdirección General de Evaluación y el Fondo Europeo de Desarrollo Regional (FEDER)* and Fundación Ramón Areces to E. P.-N. R.A. is a fellow of Ministerio de Economia y Competitividad, Spain.

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

EFFECT OF SELECTIVE ABLATION OF STRIOSOMES ON DOPAMINERGIC NIGROESTRIATAL INERVATION

Kirill Shumilov, José Medina-Luque, Alejandra Valderrama-Carvajal, Ruth Roales-Buján, Adelaida de la Calle, Alicia Rivera
Facultad de Ciencias. Universidad de Málaga, Málaga

Dopamine neurons of the substantia nigra pars compacta (SNc), that originate nigrostriatal dopamine projections to the caudate putamen (CPu), are tonically inhibited by midbrain GABAergic interneurons expressing μ opioid receptors (MOR). However, neurons in substancia nigra are more diverse than previously assumed, and the organization of their local circuit and projections arising from other brain regions is more complex than initially expected. In the SNc, the function of dopaminergic neurons is regulated not only by local GABAergic interneurons, but also by GABAergic striatonigral neurons of the patch/striosome compartments of the CPu, also enriched in MOR. The specific functions of these compartments are not completely understood, but a recent study has suggested a crucial role in opiate reward-driven behaviours (Cui *et al*., 2014).

Here we used intrastriatal microinjections of the toxin dermorphin-saporin (MOR-SAP) to selectively ablate MOR-rich striosomal cells. We have analyzed the effect of specific striosomal ablation on nigrostriatal dopamine pathway and striatal interneurons populations using immunohistochemical and image analysis techniques.

Intrastriatal MOR-SAP microinjections affect the dopamine pathway markers tyrosine hydroxylase (TH) and dopamine transporter (DAT) by increasing its density in the striosomal compartment and producing a reduction in the surrounding matrix. In addition, acetylcholine transferase and somatostatin interneuron populations are declined. The present results suggest that MOR-rich striosomal cells are necessary for the optimal nigrostriatal dopamine pathway function.

1ª: Neurociencia de sistemas

INCREASED RICTOR LEVELS CONTRIBUTE TO SUSTAINED ACTIVATION OF THE mTORC2 PRO-SURVIVAL PATHWAY IN THE STRIATUM OF HUNTINGTON'S DISEASE

J. Creus-Muncunill [1-3†], L. Rué [1-3†,‡], R. Alcalá-Vida [1-3], J. Romaní [4], S. Marco [5], J. Alberch [1-3], I. Perez-Otaño [5], C. Malagelada [4], E. Pérez-Navarro [1-3,*]

[1]. Departament de Biologia Cel·lular, Immunologia i Neurociències, Facultat de Medicina, Universitat de Barcelona, Barcelona.

[2]. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona.

[3]. Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED).

[4]. Departament d'Anatomia Patològica, Farmacologia i Microbiologia, Facultat de Medicina, Universitat de Barcelona, Barcelona.

[5]. Laboratorio de Neurobiología Celular, Centro de Investigación Médica Aplicada y Universidad de Navarra, Pamplona.

[†]. These authors contributed equally to this work.

[‡]. Present address: VIB Vesalius Research Center, KU Leuven, Campus Gasthuisberg, 3000 Leuven.

Huntington's disease (HD) is a hereditary neurodegenerative disease caused by a CAG repeat expansion in the exon-1 of the huntingtin gene that gives rise to a mutant huntingtin (mhtt) protein. Striatal projection neurons are the most affected, but altered molecular mechanisms accounting for this degeneration have not been elucidated yet. mTOR is a serine/threonine kinase, which is an energetic imbalance sensor and forms the catalytic core of two complexes, the mTOR complex 1 (mTORC1) and 2 (mTORC2). To form these complexes mTOR binds to specific regulatory accessory proteins, Raptor and Rictor, respectively. Here, we have analyzed whether mTOR activity could be altered in HD brain. Our results show an increase in mTORC2 activity in the striatum, but not in the cortex, of several HD mouse models and patients. In contrast, mTORC1 activity was not altered in the presence of mutant huntingtin. Striatal cells expressing mutant huntingtin displayed enhanced Rictor levels, which resulted into more rictor-mTOR complexes formation giving a possible explanation to the enhancement in mTORC2 activity. Through the phosphorylation of its main substrate, Akt, mTORC2 plays an anti-apoptotic role within the cell. Interestingly, acute down-regulation of Rictor in cells expressing mhtt reduced pSer473 Akt levels and increased mutant huntingtin-induced cell death *in vitro* whereas overexpression of Rictor counteracted it. Finally, normalization of endogenous Rictor levels in R6/1 mice striatum by injection of adeno-associated viruses expressing a shRNA against Rictor, enhanced cleaved caspase-3 levels and TUNEL-positive cells along with reduced DARPP-32 levels. In conclusion, our results provide clear evidences that over-activation of mTORC2 signaling in HD striatum plays a neuroprotective role against mutant huntingtin-induced cell death.

This work was supported by the project PI13/01250 *integrado en el Plan Nacional de I+D+I y cofinanciado por el ISCIII-Subdirección General de Evaluación y el Fondo Europeo de Desarrollo Regional (FEDER)*, Ministerio de Economia y Competitividad (grants SAF2010-21058; CSD2008-00005, SAF2013-48983-R and SAF2013-45888R), European Commission with a Marie Curie International Reintegration Grant (PIRG08-GA-2010-276957), Spain, funds obtained via the crowdfunding platform Goteo.org and sponsored by "Mememtum: early detection of neurological disorders", Portal d'Avall SL, the UTE project CIMA and a NARSAD Independent Investigator Award. R.A. is a fellow of Ministerio de Economia y Competitividad, Spain.

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

THE MICRONEUROTROPHIN BNN27 PROVIDES NEUROPROTECTION TO RETINAL NEURONS THROUGH NGF TrkA RECEPTOR, WHEN IT IS ADMINISTERED EITHER INTRAPERITONEALLY OR AS EYE-DROPS

R. Ibán Arias, S. Lisa, S. Poulaki, N. Mastrodimou, D. Kokona, M. Boumpouli, A. Gravanis, I. Charalampopoulos, K. Thermos
Department of Pharmacology, School of Medicine, University of Crete, Heraklion, Crete, Greece

Purpose: Diabetic Retinopathy (DR) is an important cause of blindness in people who suffer chronically from diabetes. The eye endures proliferative formation of new blood vessels and impairment of retinal neurons such as retinal ganglion and amacrine cells, leading to apoptotic processes (Lieth et al., 2000). The neovascularization component of DR is currently being treated, but no therapies exist to attenuate the retinal neurodegenerative processes. Dehydroepiandrosterone (DHEA) exerts antiapoptotic actions through the NGF-TrkA receptor (Lazaridis et al., 2011). It affords neuroprotective effects in the retina (Kokona et al., 2012). A DHEA spiro-analogue, BNN27, deprived of endocrine actions, has been designed as a neuroprotective treatment against various diseases, including DR (Calogeropoulou et al, 2009). The aim of this study was to elucidate the mechanisms involved in the neuroprotective properties of BNN27 in the diabetic retina.
Materials and Methods: The streptozotocin (STZ)-model of DR was used in Sprague-Dawley rats. Four weeks after its injection, BNN27 (2, 10, 50mg/kg) was administered intraperitoneally (i.p) or by eye-drops for one week.
Results: Immunohistochemical studies revealed that BNN27 (i.p and eye drop administration), protected the ganglion cell axons and cholinergic, dopaminergic and nitric oxide amacrine cells. The presence of a TrkA inhibitor reversed the neuroprotective potential of BNN27 (10mg/kg; i.p). Western blot analysis showed that BNN27 (i.p and eye drop administration) increased the phosphorylation of NGF-TrkA receptor in a dose-dependent manner resulting in the activation of the prosurvival signaling pathway ERK1/2 kinases and a decrease in the activity of the cell death SAPK/JNK kinase.
Conclusion: BNN27 provides neuroprotective effects on damaged retinal cells in the STZ-model of DR through NGF-TrkA receptor and its prosurvival down-stream signaling. Studies are in progress examining further the pharmacokinetic and pharmacodynamic properties of BNN27 and its importance in the treatment of diabetic retinopathy. [Study funded by GGET ARISTEIAII to K.T.]

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

EXPERIMENTAL MYOFASCIAL TRIGGER POINTS CREATION IN MICE

R. Margalef[1], M. Sisquella[1], M. Bosque[1], C Romeu[1], O. Mayoral[2], S. Monterde[3], M. Priego[1] N. Ortiz[4], J. Tomàs[1], MM Santafe[1]

[1]Unidad de Histología y Neurobiología, Departamento de Ciencias Médicas Básicas, Facultad de Medicina y Ciencias de la Salud, Universidad Rovira i Virgili, Calle St. Lorenzo 21, 43201 Reus. [2]Unidad de terapia física, Hospital Provincial de Toledo, Cerro de San Servando s / n, 45006 Toledo. [3]Unidad de Fisioterapia, Departamento de Medicina y Cirugía, Facultad de Medicina y Ciencias de la Salud, Universidad Rovira i Virgili, Calle St. Lorenzo 21, 43201 Reus. [4] Sección de Neurología, Departamento de Medicina, Hospital Universitario de San Juan. Reus.

An abnormal release of acetylcholine (ACh) in the synaptic cleft seems responsible for localized increase in diameter of muscle fibers located below the synapse. There are called "contraction knots" and the existence of a sufficient number of them is considered a myofascial trigger point identifiable by palpation and electromyography in humans.

Objective: The increase of acetylcholine in the synaptic cleft into muscles of mice generates contraction knots comparable to those described in humans.

Methodology: We increase ACh in the synaptic cleft by the anticholinesterase neostigmine both *in vivo* and *ex vivo* samples. For *in vivo* experiments, neostigmine is injected subcutaneously in adult male Swiss mice.

- Electrophysiology study: we use the levator auris longus (LAL) and diaphragm. Intracellular recordings are performed to evaluate the spontaneous release of ACh. In adition, the end plate noise is evaluated with an EMG.
- Morphological experiments: to evaluate the morphology of synaptic contacts the AChR are stained with α-bungarotoxin rhodaminated in the LAL muscle. Moreover, the presence of glycosaminoglycans (GAGs) is assessed with the PAS- Alcian technique.

Results:

- Electrophysiology study: When neostigmine is applied subcutaneously (in vivo, in toto): spontaneous ACh release is strongly increased in the LAL muscle but not in the diaphragm. When neostigmine is added in the bath of the recording chamber (ex vivo), spontaneous ACh release is increased moderately in the LAL muscle and highly in the diaphragm. Noise plate is also increased.
- Morphological experiments: With α-bungarotoxin rhodaminated we observe abundant contracted synaptic contacts. With PAS-Alcian technique we have obtained images of contraction nodes rich in GAGs.

Conclusions. Mice treated with a single subcutaneous injection of neostigmine can be a good model for the study of myofascial trigger points because they showed an increase in spontaneous ACh release, noise plate and contraction knots.

This work is funded by a grant from FISS (PI13 / 02084)

1. Disorders and nervous system repair

FINGOLIMOD (FTY720) ENHANCES HIPPOCAMPAL SYNAPTIC PLASTICITY AND MEMORY IN HUNTINGTON'S DISEASE BY PREVENTING P75^{NTR} UP-REGULATION AND ASTROCYTE-MEDIATED INFLAMMATION

A. Miguez[1,2,3], G. García Díaz-Barriga[1,2,3], V. Brito[1,2,3], M. Straccia[1,2,3], A. Giralt[1,2,3], S. Ginés[1,2,3], J. M. Canals[1,2,3], J. Alberch[1,2,3]

[1] Facultat de Medicina, Universitat de Barcelona, 08036 Barcelona

[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), 08036 Barcelona

[3] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED)

Huntington's disease (HD) is a hereditary neurodegenerative disorder characterized by motor, cognitive and behavioral impairments. The primary regions of degeneration in HD have long been considered the striatum and cerebral cortex, but other structures involved in cognition, particularly the hippocampus, are affected in early stages of the disease. Synaptic and memory dysfunction in HD mouse models have been related to low levels of brain-derived neurotrophic factor (BDNF) and imbalance between TrkB and p75^{NTR} receptors. In addition, astrocyte over-activation has also been suggested to contribute to HD memory deficits. Fingolimod (FTY720), a modulator of sphingosine-1 phosphate receptors commonly used in Multiple Sclerosis patients, has recently been shown to increase BDNF levels and to reduce astrogliosis, proving its potential to regulate trophic support and inflammatory response. In this view, we have investigated whether FTY720 improves synaptic plasticity and memory in the R6/1 mouse model of HD, through regulation of BDNF signaling and astroglial reactivity. Chronic administration of FTY720 from pre-symptomatic stages ameliorated long-term memory deficits and dendritic spine loss in CA1 hippocampal neurons from R6/1 mice. Furthermore, FTY720 delivery prevented astrogliosis and over-activation of nuclear factor kappa beta (NF-κB) signaling in the R6/1 hippocampus, reducing tumor necrosis factor alpha (TNFα) and induced nitric oxide synthase (iNOS) levels. TNFα decrease correlated with the normalization of p75^{NTR} expression in the hippocampus of FTY720-treated R6/1 mice, thus preventing p75^{NTR}/TrkB imbalance. In addition, FTY720 increased cAMP levels and promoted phosphorylation of CREB and RhoA in the hippocampus of R6/1 mice, further supporting its role in the enhancement of synaptic plasticity. Our findings provide new insight into the mechanism of action of FTY720 and reveal a novel therapeutic strategy to treat memory deficits in HD.

Funding: IDIBAPS Postdoctoral Fellowship-BIOTRACK (EC FP7/2007-2013), MINECO, ISCIII-Subdirección General de Evaluación and European Regional Development Fund (RETICS and CIBERNED), CHDI Foundation (USA).

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

GSK3 INHIBITION BY P38 MAPK IN NEURAL PRECURSOR CELLS: A NOVEL REGULATORY PATHWAY

M. Fernández [1], T.M. Thornton [2], M. Rincon [2], and P. Tranque [1].
[1]. Instituto de Investigación en Discapacidades Neurológicas (IDINE), Universidad de Castilla-La Mancha, Albacete
[2]. Department of Medicine/Immunobiology, Vermont University, USA

GSK3 is a constitutively-active kinase involved in multiple cell functions including apoptosis, neuronal growth and differentiation. GSK3 is the most-abundant isoform in brain, and has an active role in neuranl death in Alzheimer disease. GSK3 is controlled by inhibition rather than by specific activation. In this regard, the PI3K-Akt pathway is well known for its role in GSK3 inhibition. In contrast to Akt, which phosphorylates Ser9 in all GSK3 isoforms, p38 MAPK has emerged as a GSK3 -specific inhibitory pathway that phosphorylates Ser389. Although p38 MAPK has a role in cell survival in response to DNA damage, the consequences of GSK3 inhibition by p38 MAPK for brain physiology and pathology have not been defined yet. Here we show by Western blot analysis that phospho-Ser389 of GSK3 is highly expressed in all brain regions examined, including hippocampus and cortex of adult mice. Moreover, neural precursors cells (NPCs) cultured from the subventricular zone of newborn mouse brain show induction of phospho-Ser389 of GSK3 in response to agents that induce double strand breaks in DNA such as oxidative stress inducers, UV radiation and chemotherapy agents. Immunofluorescent microscopy revealed that phospho-Ser389 of GSK3 is mostly localized in the nuclear compartment, in contrast to phospho-Ser9 of GSK3 whose localization is cytosolic. These findings uncover a new mechanism of GSK3 regulation in the brain. The possible consequences for neuronal survival in neuropathology are discussed.

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

MOLECULAR AND BEHAVIOURAL EVIDENCE FOR AMYLOID-*β* EFFECTS ON GIRK CHANNELS IN THE RODENT HIPPOCAMPUS

J. Mayordomo-Cava[1], JD. Navarro-López[1], A. Gruart[2], JM. Delgado-Garcia[2], J. Yajeya[3], L. Jiménez-Díaz[1].
1) Neurophysiology & Behavioral Lab, CRIB, School of Medicine of Ciudad Real, University of Castilla-La Mancha, Ciudad Real, Spain.
2) Division of Neurosciences, Pablo de Olavide University, Seville, Spain.
3) Dpt. of Physiology & Pharmacology, University of Salamanca, Salamanca, Spain.

During early stages of Alzheimer's disease (AD), synaptic dysfunction induced by toxic amyloid-*β* (A*β*) is present before accumulation of histopathological hallmarks of the disease. This scenario produces impaired functioning of neuronal networks, altered patterns of synchronous activity and severe functional and behavioural deficits mainly due to hyperexcitability of hippocampal networks. The molecular mechanisms underlying these alterations remain unclear but functional evidence point to the involvement of receptors/channels which modulate neuronal excitability, playing a pivotal role in early **Aβ-induced AD pathogenesis**. We have recently proposed a novel Aβ-mediated mechanism of *loss-of-function* of G-protein-coupled activated inwardly-rectifying potassium channel (GIRK), which controls neuronal excitability, to contribute to the alteration of hippocampal inhibitory neurotransmission, and subsequent network hyperactivity and hypersynchrony in AD. In the present work, our two main goals were to determine the effect of 1) A*β* on the **transcriptional expression** pattern of 17 genes encoding neurotransmitter receptors and **GIRK and other associated channels** which maintain excitatory-inhibitory neurotransmission balance in hippocampal circuits, and 2) **GirK channel blocker**, Tertiapin-Q, on learning and memory capabilities in and ***in vivo*** **non-transgenic model of AD.** For objective 1, we analyzed mRNA expression by RT-qPCR in hippocampal slices incubated with $A\beta_{25\text{-}35}$. For objective 2, hippocampal intracerebroventricular (icv) injections of $A\beta_{25\text{-}35}$ were performed to generate a local early AD pathology in mice. Injections of Tertiapin-Q (icv) were evaluated using **two behavioral learning tests (new object recognition and open field habituation tasks**). Our results indicate that A*β* modulates, among others, the gene expression of GirK channels in the hippocampus. In addition, data collected from behavioral experiments showed significant differences in both tests between controls and Aβ/Tertiapin-Q groups. Taken together, our results point out that **GirK channels could contribute to the imbalance in excitatory/inhibitory neurotransmission in the hippocampus** that causes aberrant network activity and early cognitive impairment in AD models.

Acknowledgement: Spanish MINECO (BFU2011-22740, MHE2011-00118, DIPUCR-6).

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

CHANGES IN THE EXPRESSION OF POTASSIUM CHANNELS Kv1.1 AND Kv3.1b AFTER AUDITORY DEPRIVATION SUGGEST ALTERED CENTRAL EXCITABILITY WITH DEAFNESS

C.M. Poveda[1], M. Valero[1], M.A. Merchán[2] and J.M. Juiz[1]
1.- Instituto de Investigación en Discapacidades Neurológicas, Universidad de Castilla-La Mancha, Albacete
2.- Instituto de Neurociencias de Castilla y León, Universidad de Salamanca, Salamanca.

Lack of activity from afferent inputs may have profound consequences on the structure and function of postsynaptic neurons and circuits. Relatively simple and reliable peripheral afferent 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 in order to shed light on possible reactive/plastic mechanisms to altered auditory experience.
The soma and axons of auditory brainstem neurons are heavily invested with ion channels containing the low-threshold potassium channel subunit Kv1.1 and the high-threshold potassium channel Kv3.1b, which previous in vitro and in vivo studies suggest are important for regulating high input-output correspondence and temporal synchrony. In this work, we examined by immunohistochemistry and Western blot Kv1.1 and Kv3.1b protein changes in different nuclei of the auditory brainstem (anteroventral cochlear nucleus, olivary complex and inferior colliculus) at three time points after mechanical (surgical) lesions of the cochlear receptor of adult rats: 1 day, 15 days and 3 months.
Downregulation of both voltage-dependent potassium channel proteins occurs in the anteroventral cochlear nucleus, with its lowest level at 15 days for both channels. Nuclei in the superior olivary complex show a down regulation as well, with the lowest level in Kv1.1 seen at 90 days and in Kv3.1b at 15 days postlesion. The inferior colliculus also undergoes a slight downregulation in Kv1.1 and Kv3.1b levels.
These results show deafness-associated regulation in the expression of potassium channels in the auditory brainstem suggesting that changes in intrinsic membrane properties could contribute to altered central neuronal excitability after deafness.

Supported by MINECO BFU2012-39982-C02-02.

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

ARC/ARG 3.1 AND AMPA RECEPTOR GluR2/3 AND GLUR4 SUBUNIT EXPRESSION CHANGE IN THE AUDITORY BRAINSTEM OF THE RAT AFTER DEAFFERENTATION.

C.M. Poveda[1], M. Valero[1], M.A. Merchán[2] and J.M. Juiz[1]
1.- Instituto de Investigación en Discapacidades Neurológicas, Universidad de Castilla-La Mancha, Albacete
2.- Instituto de Neurociencias de Castilla y León, Universidad de Salamanca, Salamanca.

Changes in the central nervous system after afferent deprivation are not completely understood. Relatively simple input manipulation makes central auditory circuits privileged models to unravel neuronal adaptations to normal or pathological changes in sensory experience. One of the aims of our current research program is to look at changes at the cellular and molecular level in central auditory neurons after interfering with inputs, in order to shed light on possible reactive/plastic mechanisms to altered auditory experience. Of particular interest are activity-dependent cytoskeletal protein Arc/Arg3.1 and AMPA glutamate receptor subunits as their expression and function seems to be affected after stimulation or pathological alterations of the auditory pathway.
After experimental deafness by surgical cochlear ablation in the adult rat, immunocytochemistry and Western blotting were applied in order to detect possible changes in ARC/ARG3.1, and AMPA GluR2/3 and GluR4 protein expression after short (one day), or more prolonged time (15 and 90 days) in brainstem and midbrain auditory nuclei.
The cochlear nucleus showed long-term down regulation of the three proteins. Major changes were found in the superior olivary complex, with a large reduction in immunolabeling. In the inferior colliculus, ARC/ARG3.1 and GluR2/3 changed and ARC/ARG3.1 tended to recover control levels at 90 days whereas GluR2/3 did not. GluR4 immunolabeling increased even at 90 days postlesion by immunohistochemistry, although no changes were observed by western blot.
In conclusion, neurons in the brainstem are capable of modulating their synaptic strength by regulating Arc/Arg3.1 levels and the number of AMPA receptors at the synapse, playing a selective role in regulating homeostatic plasticity of excitatory synaptic transmission in vivo.

Supported by MINECO BFU2012-39982-C02-02.

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

EFFECT OF TATTOOS INKS ON PERIPHERAL NERVES

Manel M Santafe[1], Helena Irina Cazacu[1], Aida Marcotti[1], Fabiola Medina[1], Nicolau Ortiz[2], Josep Tomàs[1]
[1]Facultad de Medicina y Ciencias de la Salud, Universidad Rovira i Virgili. Tarragona
[2] Sección de Neurología, Hospital Universitario de San Juan. Reus. Tarragona.

Introduction: The composition of tattoos inks, their kinetics in the human body and potential risks of inoculation of the inks are unknown. Clinical interventions with a potential risk of neurotoxicity are lumbar puncture and dural anesthesia when tattoos are on the lower back. There is a risk of dragging these dyes with the needle into the body. Because of the uncertainty of the neurotoxic potential of these procedures, some anesthetists choose to cut the skin to avoid dragging.
Objetive: Determine the neurotoxicity of tattoos inks.
Material and methods: Sciatic nerves from Sprague-Dawley rat were used. Records of nerve conduction were made. Different concentrations of chemical compounds of the majority of inks and commercial inks (Intenze and Millenium) where tested. Incubations lasted 60-90minuts. We evaluated amplitude of compound action potentials (peack to peack).
Results: The conduction of sciatic nerves were blocked with commercial inks. The chemical components analyzed are not as toxic as the inks. Moreover, the effect of the inks is obtained very late or without effect. Control records were made and shown a maximum oscillation of 20% at the end of the 90 minutes.
Conclusions: The nerve conduction is blocked by the dyes used in tattoos. However, each chemical compound that gives color to the inks studied solely has low effect on nerve conduction. The inks of tattoos are a mix of a lot of these chemical compounds. Then, we conclude that the action of the inks on nerve conduction is the result of synergic interactions between these chemicals compounds.

This work has been supported by a grant from FISS (PI13 / 02084)

1ª: Trastornos y reparación del sistema nervioso
2ª: Nuevos métodos y tecnologías

ROLE OF KAINATE RECEPTORS ON THE ESTABLISHMENT OF MOSSY FIBER SPROUTING IN TEMPORAL LOBE EPILEPSY

S. Valbuena, J. Lerma
Instituto de Neurociencias CSIC-UMH, San Juan de Alicante, España

Temporal lobe epilepsy, the most common type of seizure disorder, is characterized by a plethora of cellular, morphological and functional changes. Among them, sprouting of the hippocampal mossy fibers, the axons of the granular cells in the dentate gyrus, has been repeatedly proposed as an important alteration, as it can produce the overactivation of the hippocampal circuit, favoring the appearance of seizures. In the past decades, different proteins have been described to be involved in the generation of mossy fiber sprouting. One of them is CRMP2, which belongs to the collapsin response mediator protein family, originally described to play important roles in neuronal polarity and axon growth. Recently, it was shown that CRMP2 inhibition reduces mossy fiber sprouting in rodent models of epilepsy, suggesting that its modulation could prevent or reduce the degree of sprouting.

Previous studies carried out in our laboratory have described CRMP2 as important targets of kainate receptors' (KARs) signaling, resulting in changes of CRMP2 phosphorylation state during development. In this study we assessed the possible role of KARs in triggering mossy fiber sprouting through the modulation of CRMP2 in an organotypic slice culture model of epilepsy. We evaluated mossy fiber sprouting by measuring responses of recurrent collaterals antidromically activated by stimulating mossy fibers at the hilus while recording granular cells. Results showed that a treatment with 100 μM UBP310, a KAR antagonist, induced a reduction in the amplitude of these responses, which was concomitant with an increase in the phosphorylation of the residue T514 of CRMP2, which has been shown to be involved in axon growth and mossy fiber sprouting. Our results indicate that kainate receptors' activation, likely by excess of extracellular glutamate during acute epileptic activity, triggers mossy fiber sprouting, pointing these receptors as important antiepileptic targets.

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

RNAi-BASED MODULATION OF TASK3 POTASSIUM CHANNEL IN MONOAMINERGIC NEURONS AS A NEW ANTIDEPRESSANT STRATEGY

A. Ferrés-Coy [1,2,3], E. Ruiz-Bronchal [1,2,3], V. Paz [1,2,3], M. Galofré [1,2,3], F. Artigas [1,2,3], A. Bortolozzi [1,2,3]
[1]. Institut d'Investigacions Biomèdiques de Barcelona (IIBB-CSIC), Barcelona
[2]. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona
[3]. Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), ISCIII, Madrid

Major depressive disorder (MDD) is a chronic, recurring and potentially life-threatening mental illness. Current pharmacology therapies remain inadequate. Therefore, there is still a need for development of faster-acting and more effective treatments. Recently, TASK3 protein (KCNK9), member of two-pore domain (K2P) potassium channel family, has been identified as a potential target in depression. Deletion of TASK3 in mice markedly reduced REM sleep and evoked antidepressant-like effects, suggesting that TASK3 channel blockers may be a new antidepressant drug class. Here, we examined the effects of the selective reduction of TASK3 expression in serotonin (5-HT) and norepinephrine (NE) neurons in dorsal raphe (DR) and locus coeruleus (LC) nucleus, respectively. We assessed the ability of a small interfering RNA (siRNA) sequence against TASK3 (TASK3-siRNA) to down-regulate TASK3 expression by local stereotactic infusion into DR or LC (10 µg/day for 2-day) of C57Bl/6J mice. TASK3 mRNA levels were reduced to 69±4% (DR) and 84±4% (LC) of control groups (vehicle and nonsense-siRNA). Further, TASK3-siRNA was conjugated to cell-specific ligands, sertraline-S (5-HT-transporter inhibitor) or reboxetine-R (NE-transporter inhibitor) to promote their selective delivery to monoaminergic neurons. Intranasal administration of a fluorescence-labeled conjugated siRNAs (30 µg/day for 4-day) were identified in $TPH2^+$ (DR) and TH^+ (LC) cells but not in other brain regions. Moreover, intranasal delivery of S-TASK3-siRNA or R-TASK3-siRNA (7-day treatment) selective reduced TASK3 mRNA density in 5-HT or NE neurons. Specifically, TASK3 knockdown in DR i) evoked antidepressant-like effects in the tail suspension and novelty suppressed feeding tests, ii) desensitized $5\text{-}HT_{1A}$ autoreceptors, iii) enhanced the effect of fluoxetine on forebrain extracellular 5-HT, and iiii) increased plasticity-associated gene expression. Overall, these results support that loss of function of TASK3 channels in 5-HT neurons markedly enhances serotonergic activity and evokes antidepressant-like effects in mice, supporting the validity of TASK3 as a new target in antidepressant drug development.

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

Grants*: Instituto de Salud Carlos III (ISCIII) Grant PI10/00290; and NARSAD Independent Investigator Grant from the Brain & Behavior Research Foundation Grant 20003. Some of these grants are co-financed by the European Regional Development Fund "A way to build Europe".*

ANTIOXIDANT ENZYME AND APOPTOSIS GENE EXPRESSION IN THE AUDITORY RECEPTOR FOLLOWING NOISE EXPOSURE

P. Melgar–Rojas[1, 2], J.C. Alvarado[1, 2], V. Fuentes–Santamaría[1, 2], M.C. Gabaldón–Ull[1, 2], J.M. Miller[3, 4], J.M. Juiz[1, 2].
[1] Instituto de Investigación en Discapacidades Neurológicas (IDINE), Albacete, Spain.
[2] Facultad de Medicina de Albacete. Universidad de Castilla–La Mancha, Albacete, Spain.
[3] Karolinska Institutet, Stockholm, Sweden.
[4] University of Michigan, Ann Arbor, MI, USA.

Noise exposure is a common cause of hearing loss. Recent studies have demonstrated that intense noise causes an excessive production of free radicals in cochlear cells leading to oxidative stress and subsequent cell damage. In the present study, we have tested the involvement of both cellular mechanisms in noise–induced hearing loss by analyzing the mRNA levels of key genes by reverse transcriptase–quantitative polymerase chain reaction (RT–qPCR). Three month–old Wistar rats were exposed to continuous white noise (0.5–32 kHz, 118 dB SPL) for 4h/day on four consecutive days. Auditory brainstem responses (ABR) were evaluated prior to exposure (Ctrl group), after 2 consecutive days of noise exposure (Dur−Exp group) and at 1 (1d–post Exp), 10 (10d–post Exp) and 30 (30d–post Exp) days post–exposure. Cochleae from animals sacrificed at each time point were micro–dissected and the expression of key genes related to oxidative stress and apoptosis was evaluated. Permanent auditory threshold shifts were demonstrated by the increase in the auditory thresholds up to 30d post–exposure. RT–qPCR revealed a progressive up–regulation of the main antioxidant enzyme genes (*Sod1, Cat* and *GPx1*) reaching a maximum expression level at 10d post–Exp coincident with the previously reported peak of free radical formation. A coincident, parallel expression pattern was observed for the apoptosis-regulatory genes (*Bax*, *Bcl–2* and *Casp3*). *Sod2* and *Bad* genes did not show significant expression changes. Therefore, RT–qPCR analysis revealed an endogenous antioxidant response and coincident apoptosis regulation in the cochlea of Wistar rats after noise exposure.

Acknowledgements: This study was supported by the MINECO (BFU2012–39982–C02–02) and the PROHEARING project of the 7th Framework Programme (FP7–HEALTH–2012–INNOVATON 304925).

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

NOISE OVERSTIMULATION INDUCES UPREGULATION OF ANTIOXIDANT AND APOPTOTIC PATHWAYS IN THE RAT COCHLEA

V. Fuentes–Santamaría[1, 2], J.C. Alvarado[1, 2], P. Melgar–Rojas[1, 2], M.C. Gabaldón–Ull[1, 2], J.J. Cabanes[1, 2], J.M. Miller[3, 4], J.M. Juiz[1, 2].
[1] Instituto de Investigación en Discapacidades Neurológicas (IDINE), Albacete, Spain.
[2] Facultad de Medicina de Albacete. Universidad de Castilla–La Mancha, Albacete, Spain.
[3] Karolinska Institutet, Stockholm, Sweden.
[4] University of Michigan, Ann Arbor, MI, USA.

High-intensity noise overstimulation has been shown to induce functional and structural cochlear alterations that may result in permanent sensorineural hearing loss. Evidence indicates that an excess of free radical formation and blood flow reduction are key factors that contribute to cellular dysfunction in the cochlea. The goal of this study was to evaluate oxidative stress- and apoptotic-induced histological changes in the noise damaged cochlea. To do so, Wistar rats were exposed to broadband noise (0.5-32 kHz, 118 dB SPL), for 4h/day for 4 consecutive days to induce a permanent threshold shift. Auditory brainstem responses were recorded at 1, 10 and 30 days post-exposure and the results compared to those of unexposed rats. At 1,10 and 30 days post-exposure groups of, animals were perfused and cochleae were micro-dissected, decalcified and processed for immunocytochemistry, using markers of oxidative stress and apoptosis. The results demonstrate significant increases in the immunostaining of both the anti-apoptotic marker B-cell lymphoma 2 (Bcl-2) and the antioxidant marker superoxide dismutase (Sod1) and catalase (Cat) at 1 and 10 days post-exposure in the outer hair cells, spiral ligament and spiral limbus. By 30 days post-exposure, the expression of these markers decreased to normal values. These findings provide evidence of an early and immediate response of antioxidants and anti-apoptotic mechanisms to reduce cochlear oxidative damage following acoustic trauma.

Acknowledgements: This study was supported by the MINECO (BFU2012–39982–C02–02) and the PROHEARING project of the 7th Framework Programme (FP7–HEALTH–2012–INNOVATON 304925).

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

ORAL ADMINISTRATION OF ANTIOXIDANT VITAMINS AND MG^{2+} MODIFIES GENE EXPRESSION IN THE AUDITORY RECEPTOR IN RESPONSE TO INTENSE NOISE EXPOSURE

P. Melgar–Rojas[1, 2], J.C. Alvarado[1, 2], V. Fuentes–Santamaría[1, 2], M.C. Gabaldón–Ull[1, 2], J.M. Miller[3, 4], J.M. Juiz[1, 2].
[1] Instituto de Investigación en Discapacidades Neurológicas (IDINE), Albacete, Spain.
[2] Facultad de Medicina de Albacete. Universidad de Castilla–La Mancha, Albacete, Spain.
[3] Karolinska Institutet, Stockholm, Sweden.
[4] University of Michigan, Ann Arbor, MI, USA.

Noise–induced hearing loss (NIHL) is the main cause of acquired hearing loss in the population between 20–69 years. The involvement of the oxidative stress and the causal relationship between inhibition of oxidative stress and reduction in hearing impairment has been well established. The aim of the present study was to determine by reverse transcriptase–quantitative polymerase chain reaction (RT–qPCR) if ACEMg (a combination of the antioxidants Vitamins A, E and C plus the natural vasodilator magnesium) and candidate to prevent NIHL modifies the expression of antioxidant and apoptotic genes in the cochlea.
Three month–old Wistar rats were fed with either normal diet (ND group) or an ACEMg enriched diet (ED group). The ED started 10 days before noise exposure. All rats were exposed to continuous white noise (0.5–32kHz, 118dB SPL) for 4h/day during four consecutive days. Auditory brainstem responses (ABR) were evaluated prior to exposure (Ctrl group), after 2 consecutive days of noise exposure (Dur−Exp group) and at 1 (1d–post Exp), 10 (10d–post Exp) and 30 (30d–post Exp) days post–exposure. Cochleae were collected following the ABR at each time and RT–qPCR analysis was performed. Permanent threshold shift was confirmed by an increase in the auditory thresholds up to 30d post–exposure. RT–qPCR revealed that ACEMg enhanced the early antioxidant cell response by increasing the expression level of *Sod1*, *Cat* and *GPx1* genes (at 1 day post–exposure). This was accompanied by a decreased apoptosis activation (*Casp3* expression) relative to ND over-exposed animals. Therefore, RT–qPCR analysis allowed us to conclude that ACEMg blocks oxidative stress in cochlear cells by enhancing the antioxidant response.

Acknowledgements: This study was supported by the MINECO (BFU2012–39982–C02–02) and the PROHEARING project of the 7th Framework Programme (FP7–HEALTH–2012–INNOVATON 304925).

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

CELL AND MITHOCONDRIAL MEMBRANE FLUIDITY IN THE BRAIN OF SENESCENCE-ACCELERATED MICE

P. Almeida-Souza, L. López- Pingarrón, E. Esteban-Zubero, J.M. Ramirez, M.C. Reyes-Gonzales, J.J. García

Department of Pharmacology and Physiology, University of Zaragoza, Spain.

ABSTRACT

Senescence-accelerated mouse (SAM) is an experimental murine model of accelerated aging that consists in two strains: senescence-accelerated prone mouse (SAMP) and senescence-accelerated resistant (SAMR). Progressive and irreversible functional decline is a characteristic of all organisms late in life. Physiological aging has been associated with lipid peroxidation and oxidative stress of several biological membranes. The aim of this study was to evaluate membrane fluidity levels in synaptosomes and mitochondria obtained from $SAMP_8$ and $SAMR_1$ mice of 5 and 10 months old. Membranes were isolated from the brain by differential centrifugation. Fluidity levels were monitored using fluorescence spectroscopy, and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene-*p*-toluene sulfonate (TMA-DPH) as probe. Excitation and emission wavelengths of 360 and 430 nm were used, respectively. The emission intensity of vertically polarized light was detected by an analyzer oriented parallel or perpendicular to the excitation plane. Since an inverse relationship exists between membrane fluidity and polarization of emitted light, membrane fluidity was expressed as 1/polarization. In $SAMP_8$ at 5 months of age compared to control $SAMR_1$, we observed a significant decrease in the fluidity of synaptosomal and mitochondrial membranes ($p=0.04$ and 0.01, respectively). Synaptosomes isolated from $SAMP_8$ and $SAMR_1$ at 10 months of age showed similar fluidity levels. However, we detected a several decrease in both groups when they were compared to animals at 5 months of age ($p\leq0.001$). Mitochondrial membrane from $SAMR_1$ at 10 months of age were more fluid than those isolated from $SAMP_8$ animal at the same age ($p=0.04$), moreover, we detected no difference between $SAMP_8$ and $SAMR_1$ at 5 and 10 months of age. In conclusion, our findings are in agreement with previous observations using other animal physiological models in which aging causes rigidity in biological membranes.

1ª: Trastornos y reparación del sistema nervioso
2ª: Sistemas homeostáticos y neuroendocrinos

SONIC HEDGEHOG AND ITS NON CANONICAL RECEPTOR MEGALIN ARE SPECIFICALLY UP-REGULATED IN ASTROCYTES ASSOCIATED TO DEMYELINATED PLAQUES OF MULTIPLE SCLEROSIS PATIENTS

M.C. Ortega[1], D. Clemente[1], A. González de la Aleja[1] and F. de Castro[1]
[1] Grupo de Neurobiología del Desarrollo-GNDe, Hospital Nacional de Parapléjicos. Toledo. Spain.

Sonic hedgehog (Shh) is involved in oligodendrogliogenesis and oligodendrocyte precursor cell (OPC) mobilisation during development. In multiple sclerosis (MS), other important cue involved in the biology of OPCs, the fibroblast growth factor type 2 (FGF2), is exclusively present in areas where remyelination spontaneously occurs. Different reports point to lipoprotein receptor-related proteins (LRPs) as alternative receptors for different FGFs and Shh. We have shown that Megalin (LRP-2) is an essential actor in the Shh-mediated effects on OPC biology during CNS development, probably by the establishment of a proper biological gradient of the secreted cue. Although previous works have described the presence of LRPs in active demyelinating plaques of MS patients, the exhaustive description of Megalin and its ligand Shh have not been described to date. In our current study, we analyse the distribution and the cellular characterisation of Megalin and its biological active ligand Shh in MS tissue to elucidate its role in the pathogenesis of demyelination.
Both Megalin and Shh are up-regulated in activated astrocytes within active plaques and in the periplaque of chronic active MS lesions, two regions where remyelimation spontaneously occurs. This observation represents another example of the re-expression of developmental molecular cues (and their receptors) in the context of a demyelinating disease, as a spontaneous reaction for myelin repair. In addition, whereas Megalin is restricted to the abovementioned areas, Shh is also present in the normal appearing white matter immediately adjacent to the inflammatory focus. Indeed, Shh-expressing astrocytes in the inflamed area show a higher intensity of staining than those detected in its close vicinity. Within these two areas, two astrocytic subpopulations can be observed after mixing both markers: $Shh^{high}Megalin^{+}$ astrocytes, restricted to the demyelinating areas, and $Shh^{low}Megalin^{-}$ astrocytes which are detected both in the demyelinating area and in the adjacent NAWM. Interestingly, $Shh^{-}Megalin^{+}$ astrocytes are not present in any of the analyzed lesions. Together, this suggests that reactive astrocytes (and indirectly the inflammatory environment) induce a Shh gradient close to demyelinating plaques. Both aspects strongly suggest that therapeutic strategies to modulate specific LRPs and its ligands would be useful to effectively repair MS.

This work was supported by the Spanish *Ministerio de Economía y Competitividad (*SAF2012-40023; RD07-0060-2007 and RD12-0032/0012, and partially cofinanced by F.E.D.E.R., European Union, *"Una manera de hacer Europa").* DC and FdC are employees of SESCAM. CO was employed with funds from ARSEP Foundation and PAION Deutschland GmbH.

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

TRANSCRIPTION FACTOR EB OVEREXPRESSION IN SUBSTANTIA NIGRA DOPAMINERGIC NEURONS TRIGGERS CELL GROWTH AND RENDERS NEUROPROTECTION IN THE MPTP PARKINSON'S DISEASE MOUSE MODEL

A. Torra [1], B. Rodríguez [1], M. Vila [1,2,3], J. Bové [1]
[1]. Neurodegenerative Diseases Research Group, Vall d'Hebron Research Institute – Center for Networked Biomedical Research on Neurodegenerative Diseases (CIBERNED), Barcelona, Spain
[2]. Department of Biochemistry and Molecular Biology, Autonomous University of Barcelona, Barcelona, Spain.
[3]. Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain.

Parkinson's disease (PD) is a common neurodegenerative disorder mainly characterized by the loss of substantia nigra pars compacta (SNpc) dopaminergic neurons. Although the cause of PD remains unknown, we have previously demonstrated that in the MPTP mouse model there is a disruption of the lysosomal integrity that leads to a decreased number of lysosomes and a defective lysosomal-mediated clearance of autophagosomes, which precedes cell death. Our results with PD postmortem brains, suggest that this may be also occurring in PD.
Therefore, we hypothesized that compensating this lysosomal impairment will be a potential neuroprotective strategy suitable for PD. To test this hypothesis, we overexpressed the transcription factor EB (TFEB) in the dopaminergic neurons of the SNpc by means of recombinant adeno-associated viral vector and assessed its neuroprotective effect in the MPTP model. TFEB is a master gene for lysosomal biogenesis that encodes a transcription factor which coordinates expression of lysosomal hydrolases, membrane proteins and genes involved in autophagy.
Our results demonstrate that TFEB overexpression not only prevents completely MPTP-induced neuronal death but triggers neuronal growth that implies an increase of cell size and higher levels of tyrosine hydroxylase. Even though TFEB overexpression is indeed able to compensate lysosomal impairment, its neuroprotective effect goes beyond boosting lisosomal-mediated degradation. TFEB promotes activation of AKT and mTOR-dependent downstream targets S6K1 and 4EBP1/eIF4E that explain both the cellular growth and neuroprotective effects. Overall, our results confirm TFEB as a relevant target in PD to develop disease modifying strategies.

CHANGES IN THE TERMINAL PATTERN OF PRIMARY AFFERENTS IN THE SPINAL TRIGEMINAL NUCLEUS AFTER CHRONIC SINGLE VIBRISSA TRIMMING IN ADULT RAT

Y. B. Martin [1], J. Fernández-Montoya [2], P. Negredo [2], C. Avendaño [2]
[1] Dept. Anatomy, Francisco de Vitoria University, Madrid, Spain
[2] Dept. Anatomy, Histology & Neuroscience, Medical School, Autonoma University of Madrid, Spain

The manipulation of sensory input through the vibrissal system in adult rats induce alterations of dendritic arbors in the trigemino-thalamic neurons in the principal trigeminal nucleus (Pr5) and in the neurons of the caudal nucleus (Sp5c) that project to Pr5 through intersubnuclear connections. These changes could result from, or at least be correlated with structural alterations in the central terminal arbors of primary sensory neurons innervating the whisker pad. Here we report an unexpected finding that not only proves the existence of the proposed primary afferent changes, but also connects these changes to other models of altered sensory input in the spinal cord following nerve lesions.

We used three groups of adult male rats. One group was deprived unilaterally from active touch by whisker clipping during 7-8 weeks; a second group sustained a similar manipulation, but restricted to vibrissa C1; a third group was untouched. All animals received an intraneural deposit of cholera-toxin B (CTB) and the lectin IB4 in the deep vibrissal nerve of C1, using a procedure that achieved the labeling of all axons. After the survival period, the terminal labeling was studied on horizontal sections of the brainstem. In controls, and after global whisker pad deprivation, there is a clear-cut laminar segregation of terminals in Sp5c, whereby IB4-labeled terminals (specific to unmyelinated and finely myelinated fibers) distribute in lamina II, and CTB-labeled terminals (taken up by larger myelinated fibers) are restricted to laminae III-IV. In contrast, CTB-labeled terminals also appear in lamina II after isolated deprivation of C1.

A comparable phenomenon described in the spinal cord, followed a peripheral nerve lesion, and was succesively interpreted as sprouting of thick fibers in superficial laminae, or uptake specificity changes in thin fibers. Our results show, however, that severe local haptic deprivation is sufficient for this change to occur.

Supported by Grant BFU 2012-39960 from the Plan Nacional I+D

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

OLEOYLETHANOLAMIDE PREVENTS HMGB1/TLR4 DANGER SIGNALING ASSOCIATED WITH NF-kB PROINFLAMMATORY CASCADE AND CASPASE-3 ACTIVITY IN FRONTAL CORTEX AND BLOOD CORTICOSTERONE RISE INDUCED BY ETHANOL BINGE ADMINISTRATION IN RATS.

M. Antón[1], F. Alén[1], R. Gómez de Heras[1], FJ. Pavón[3], JC. Leza[2], B. García-Bueno[2], F. Rodríguez de Fonseca[3,1], L. Orio[1]

[1]*Department of Psychobiology, Faculty of Psychology, Complutense University, Madrid (UCM), Spain;* [2]*Department of Pharmacology, Faculty of Medicine, UCM, and Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Spain;* [3]*Instituto de Investigación Biomédica (IBIMA), Málaga, and Red de Trastornos Adictivos (RTA), Spain.*

Alcohol abuse is frequently characterized by a specific pattern of intake in binge drinking episodes, inducing neuroinflammation and brain damage. Here, we characterized the temporal profile of neuroinflammation in rats exposed to intragastric binge ethanol administrations and tested the anti-inflammatory/neuroprotectant properties of the satiety factor oleoylethanolamide (OEA). Pre-treatment with OEA blocked the expression of High Mobility Group Box 1 (HMGB1) danger signal and the innate immunity Toll-like receptors 4 (TLR4), inhibiting the Nuclear factor-*kappa* B (NF-kB) proinflammatory cascade induced by alcohol binge in frontal cortex. OEA reduced the levels of Interleukin-1beta (IL-1β), the monocyte chemoattractant protein-1 (MCP-1), and the enzymes cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in ethanol binged animals. Elevations in plasma Tumor necrosis factor alpha (TNF-α) and IL-1β after ethanol were also inhibited by OEA. Additionally, OEA prevented ethanol-induced lipid peroxidation, caspase-8 and pro-apoptotic caspase-3 activation in frontal cortex. Finally, OEA blocked the rise in blood corticosterone levels after ethanol with no alteration in blood ethanol levels. Altogether, results highlight a beneficial profile of OEA as a potent anti-inflammatory/neuroprotectant compound to treat alcohol abuse.

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

P2Y RECEPTOR FAMILY REGULATES MICROGLIAL PHAGOCYTOSIS IN VIVO

V. Sánchez-Zafra*[1,2], I. Paris*[1,2], A. Sierra[1,2,3]
*Equal contributors
[1] Achucarro Basque Center for Neuroscience, Bizkaia Science and Technology Park, Zamudio, Biscay
[2] University of the Basque Country, Leioa, Biscay
[3] Ikerbasque Foundation, Bilbao, Biscay

Phagocytosis of cellular debris is an essential process in the response to damage, because it prevents the spillover of intracellular contents and is actively anti-inflammatory. In the brain, is performed by microglia, the resident macrophages. Microglia detect and recognize dying cells via several signalling molecules, such as ATP, recognized by receptors of the P2 family such as P2X7, P2Y12 and the P2Y12-like receptor GPR34, at least in vitro. To test their involvement in phagocytosis in vivo, we analyzed the efficiency of microglial phagocytosis in adult wild type (WT; C57BL/6), and P2X7, P2Y12 and GRP34 knock-out mice. We focused on the adult hippocampal neurogenic cascade, located in the dentate gyrus, where newborn cells undergo apoptosis and are rapidly phagocytosed by "resting" microglia throughout adulthood in physiological conditions. We observed an increased proportion of non-phagocytosed apoptotic cells in P2Y12 and GPR34 KO mice, which did not occur in P2X7 KO mice, suggesting a partial impairment of microglial phagocytosis due to the absence of P2Y12 and GPR34. While similar numbers of microglia were found in WT and KO mice, KO animals showed a decreased number of phagocytic microglia leading to a decreased phagocytic capacity compared to WT mice. As a consequence of the phagocytosis impairment, the coupling between apoptosis and phagocytosis that occurs in physiological conditions was lost. The level of impairment of microglial phagocytosis was similar in P2Y12 and GPR34 KO mice (65-70%), leading us to speculate that these receptors may regulate the same downstream pathway. In summary, we conclude that P2Y12 and GRP34 are regulators of microglial phagocytosis in vivo in physiological conditions. In the future, we will utilize these KO mice to test the involvement of microglial phagocytosis in the regulation of inflammation, neurogenesis and other relevant processes.

EFFECTS OF KAINIC ACID ADMINISTRATION ON THE BLOOD-BRAIN BARRIER OF C57BL/6 MICE

S.Vidal-Lletjós[1], I.Cabezón[1], A.Camins[2], M.Pallàs[2], C.Auladell[3], C.Pelegrí[1], J.Vilaplana[1], G.Manich[1]
[1]. Departament Fisiologia, Facultat de Farmàcia, Universitat de Barcelona.
[2]. Unitat Farmacologia i Farmacognòsia, Facultat de Farmàcia, Universitat de Barcelona.
[3]. Departament de Biologia Cel·lular, Facultat de Biologia, Universitat de Barcelona.

In rodents, intraperitoneal injection of kainic acid (KA), a potent agonist of the glutamate receptors, results in hippocampal cell death and provides a suitable model to study some human neurodegenerative diseases as epilepsy. This work aims to examine the blood–brain barrier (BBB) integrity on the hippocampus of C57BL/6 mice after KA-induced *status epilepticus*. Three-month-old C57BL/6 mice were intraperitoneally administered with KA (30 mg/kg, n=11) or PBS (n=7). 24h post-injection animals were sacrificed, their brains were removed and hippocampal slices were obtained and analyzed by conventional immunohistochemistry techniques. KA-treated animals showed an important decrease on their body weight 24h after the KA administration, and their motor behaviour progressed through the different stages of the Racine scale reaching stage three (repetitive head bobbing and forepaw shaking) in all cases during the 2h post-injection. In hippocampal regions of KA-treated animals, an increase of cell damage was observed on CA1 and CA3 regions using Nissl staining. Moreover, positive Fluoro-Jade neurons were also observed and a significant increase of activated microglial cells was detected respect to the control mice. This reactivity was accompanied by an overexpression of GFAP in astrocytes. A general extravasation of IgG and an important disruption of laminin, a basal protein membrane of blood vessels, were also observed. No differences were detected between control and treated groups regarding MMP-2 presence. However, the MMP-9 immunofluorescent stainings suggested an increase of this gelatinase in KA-treated mice. We conclude that, in C57BL/6 mice, the glutamate excitotoxicity induced by KA administered intraperitoneally produces several neuronal changes and an increase in glial reactivity. These changes are accompanied by a BBB disruption, which can be produced by the increase of the MMP-9, and may contribute to the spread of neuronal loss and glial reactivity.

1ª: Trastornos y reparación del sistema nervioso
2ª: Sistemas homeostáticos y neuroendocrino

DISRUPTED IN SCHIZOPHRENIA (DISC1) ASSOCIATES WITH MITOFILIN IN A MULTIPROTEIN COMPLEX ESSENTIAL FOR OXIDATIVE PHOSPHORYLATION IN MITOCHONDRIA

E. Piñero-Martos[1], E. Cisneros-Barroso[1], B. Ortega-Vila[1], D. Heine-Suñer[1,2], J. Lladó[1,3], G. Olmos[1,3], C. Vives-Bauzá[1]

[1]. Instituto de Investigación Sanitaria de Palma (IdISPa), Palma de Mallorca, Baleares

[2]. Servicio de Genética, Hospital Universitario Son Espases, Palma de Mallorca, Baleares

[3]. Departamento de Biología, Universidad de las Islas Baleares, Palma de Mallorca, Baleares

Disrupted in Schizophrenia-1 (DISC1) is a multi-compartmentalized protein found in the cytoplasm, centrosome, nuclei and mostly enriched in mitochondria that has been associated with a broad spectrum of mental diseases including bipolar disorders, autism spectrum disorders and schizophrenia. The mitochondrial function of DISC1 is unknown. In order to shed light on the role of DISC1 in mitochondria, we studied its sub-mitochondrial localization and determined whether DISC1 is recruited in a protein complex. Proteinase K protection assay and alkaline extraction experiments showed that DISC1 is anchored in the inner mitochondrial membrane facing the intermembrane space. Blue-Native PAGE analysis followed by 2D-western blotting demonstrated that DISC1 forms part of a multiprotein complex containing the inner mitochondrial membrane proteins mitofilin and CHCHD3. DISC1 knockdown was performed in SH-SY5Y cells by shRNA against human DISC1. In these cells we observed a partial disassembly of the DISC1/mitofilin/CHCHD3 complex, which resulted in oxidative phosphorylation (OXPHOS) dysfunction, evidenced by impaired O_2 consumption, ATP synthesis and mitochondrial membrane potential, measured by tetramethylrhodamine methyl ester (TMRM) fluorescence. Transfection of recombinant full-length human DISC1 restored DISC1/mitofilin/CHCHD3 complex assembly and rescued OXPHOS function, meanwhile DISC1 truncated form Δ597-854, known to be pathogenic, failed to rescue. These results should contribute to reveal DISC1 normal physiological function and potential pathogenic role in severe mental illnesses.

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

INTRACISTERNAL *GTF2I* GENE THERAPY AMELIORATES THE DEFICITS IN COGNITION AND SYNAPTIC PLASTICITY OF A MOUSE MODEL OF WILLIAMS-BEUREN SYNDROME

C. Borralleras Fumaña[1,2,3], I. Sahun Abizanda[4], LA. Pérez Jurado[1,2,3], V. Campuzano Uceda[1,2,3]
1. Neurosciences Program, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona
2. Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona
3. Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), ISCIII, Spain
4. PCB-PRBB Animal Facility Alliance, Barcelona

Objectives: Williams-Beuren Syndrome (WBS) is a neurodevelopmental disorder caused by a heterozygous deletion of 26-28 genes at chromosome band 7q11.23. Haploinsufficiency at *GTF2I* has been shown to play a major role in the neurobehavioral phenotype. The objective of this study was to clarify the involvement of *Gtf2*i in the neurocognitive features of WBS and to attempt a phenotypic rescue by restoring *Gtf2i* expression in the brain.
Material and Methods: We characterize the neuronal architecture and we performed some behavioral tests in four animal models with intragenic partial and complete deletions of the WBS critical interval (*ΔGtf2i*$^{+/-}$, *ΔGtf2i*$^{-/-}$, PD and CD). The expression levels in hippocampus of two synaptic plasticity markers were analyzed. Finally, a *Gtf2i*-gene therapy using adeno-associated virus was performed in CD mice.
Results: Dendritic length was decreased in the CA1 area of *ΔGtf2i*$^{+/-}$, *ΔGtf2i*$^{-/-}$ and CD mice. Spine density was reduced and spines were shorter in *ΔGtf2i*$^{-/-}$, PD and CD mice. Overexpression of *Pik3r1* and downregulation of *Bdnf* were observed in *ΔGtf2i*$^{+/-}$, and CD mice. Intracisternal *Gtf2i*-gene therapy in CD mice resulted in increased m*Gtf2i* expression and normalization of *Bdnf* levels, along with beneficial effects in motor coordination, sociability and anxiety, despite no significant changes in neuronal architecture.
Conclusions: Our findings further indicate that *Gtf2i* haploinsufficiency plays an important role in the neurodevelopmental and cognitive abnormalities of WBS and that it is possible to rescue part of this neurocognitive phenotype by restoring *Gtf2i* expression levels in specific brain areas.

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

MYELOID-DERIVED SUPPRESSOR CELL POPULATION CONTRIBUTES TO A LESSER SEVERITY OF THE CLINICAL COURSE IN A MURINE MODEL OF MULTIPLE SCLEROSIS

C. Melero-Jerez, F. de Castro * and D. Clemente *.
Grupo de Neurobiología del Desarrollo-GNDe, *Hospital Nacional de Parapléjicos, Toledo, Spain.*

Multiple Sclerosis (MS) is the most frequent neurological disease in the Western world. MS disease course is highly heterogeneous from primary progressive, in which neurological disability grows continuously, to the most frequent form, the relapsing remitting, in which periods of neurological symptoms are followed by phases of total or partial recovery. In addition, MS develops with a variable degree of aggressiveness, especially in the case of the RR-MS. However, the reason for this high clinical variability is still unknown.

Myeloid-derived suppressor cells (MDSCs) form a heterogeneous population of immature myeloid cells that participate in the suppression of the inflammatory response in the most common model of MS, the experimental autoimmune encephalomyelitis (EAE). Our group described that MDSCs are present in the spinal cord of EAE mice, contributing to the resolution of the inflammatory insult. In fact, the density of MDSCs is directly proportional to the clinical score and to the percentage of apoptotic T cells within the damaged tissue. These data point to a clear relationship between MDSC enrichment in the CNS and the disease course evolution.

In this study, we demonstrate that EAE mice experiment different clinical courses, ranging from mild to severe, which can be assessed by a relation between the maximal clinical score and the time elapsed since onset to then (AI: aggressiveness index). We confirm a significant negative correlation between the splenic content of MDSCs and the AI, and other clinical parameters. Regarding the CNS, EAE mice suffering from a milder clinical course showed lesser demyelination compared to those suffering from more severe EAE. Furthermore, the spinal cords of mice with severe EAE clinical course were enriched in neutrophils and presented Arg-I^+ cells in the ventral horns of the grey matter, while mild EAE mice showed more defined infiltrated areas, always restricted to the white matter and with an enriched density of MDSCs.

In sum, our current findings offer new perspectives about the putative use of MDSCs as biomarker for MS severity.

This work was supported by the Spanish *Ministerio de Economía y Competitividad (*SAF2012-40023; RD07-0060-2007 and RD12-0032/0012, and partially cofinanced by F.E.D.E.R., European Union, *"Una manera de hacer Europa")* and ARSEP Foundation (France). DC and FdC are employees of SESCAM. CM-J holds a Research Training fellowship by the Spanish *Ministerio de Economía y Competitividad* (BES-2013-062630).

1ª: Trastornos y reparación del sistema nervioso
2ª: Sistemas homeostáticos y neuroendocrino

AMYLOID-β, NEWBORN CELLS AND INTERNEURONS IN THE OLFACTORY BULB OF APP/PS1 MICE MODEL OF ALZHEIMER´S DISEASE

C. De la Rosa-Prieto[1], D. Saiz-Sánchez[1], A. Flores-Cuadrado[1], I. Ubeda-Banon[1], A. Martinez-Marcos[1].
[1] Facultad de Medicina de Ciudad Real, Universidad de Castilla-La Mancha, Ciudad Real, Spain.

Alzheimer´s disease is the most prevalent neurodegenerative disorder. This disease is characterized by cognitive and motor deficits. In the extracellular space, amyloid-β fragments accumulate in the diseased brains affecting several areas. Impaired olfaction has been described as an early symptom of the disease. Previous studies describe that some populations of interneurons in the olfactory bulb show earlier vulnerability to β-amyloid than other populations. On the other hand, it is widely accepted that neurogenesis in rodents provides a high number of new interneurons to the olfactory bulbs. In fact, the amyloid precursor protein is related with this neurogenic process. Interestingly, neurogenesis is impaired in most of the transgenic mice model that express amyloid-β. However, the relationship between the involvement of these newborn cells and the decrease of the interneuron protein expression is poorly known.
The aim of this work is to describe alterations in the number of newborn cells, marked with Bromodeoxyuridin (BrdU), in the olfactory bulb over time and their involvement by the proteinopathy. By using multiple immunofluorescence techniques, the co-expression with calretinin, somatostatin and parvalbumin interneuronal markers, and the colocalization levels with amyloid-β under confocal microscopy have been quantified.
The results show a decreased number of BrdU cells over time and vs. controls. In the same line, we observed BrdU cells coexpressing interneuron markers fenced by the missfolded amyloid-β peptide. Some populations of interneurons are decreased presumably due to pathology. These results evidence a correlation between the amyloid-β progression within the olfactory bulb and the reduced levels of both newborn cells and interneuron populations.

Supported by Spanish Ministry of Economy and Competitiveness-FEDER; grant number: BFU2010-15729 and SAF2014-52300-R; Autonomous Government of Castilla-La Mancha-FEDER; grant: PEIC-2014-006-P.

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

GLUCOCEREBOSIDASE GENE IN LEWYS BODY DISEASES

A. Gámez-Valero[1, 2], P. Prada-Dacasa[2], C. Adame-Castillo[2], K. Beyer[1,]
[1] Servicio de Anatomía Patológica, Fundació Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol
[2] Universidad Autónoma de Barcelona

Abstract

Parkinson disease (PD) and dementia with Lewy body (DLB) are considered as Lewy body diseases (LBD), being characterized by Lewy bodies deposition. Although they are different disorders, pathological characteristics are often identical. Moreover, about 20%-50% of PD patients develop dementia (PDD) after no less than 10 to 15 years following PD diagnosis.

Glucocerebrosidase (GCase) is a lysosomal enzyme responsible for the breakdown of glucocerebroside into glucose and ceramide. In the last years, several studies have shown a possible relation between alterations in the GCase gene, GBA, and the development of LBD. Both, gain and loss of function of the enzyme have been considered to explain the role of these mutations in LBD. Nevertheless, in Spanish population, there is only one study about GBA mutations in PD but their frequency in DLB has not been analyzed so far.

In the present study, we performed the analysis of GBA mRNA sequence, comparing sequences from PD (n=58), DLB (n=88) and controls (n=44) derived from blood and brains and looking for possible mutations. After Trizol based mRNA extraction, reverse-transcription was performed to obtain the corresponding cDNA. cDNA was used for PCR amplification and sequencing reactions. Sequencing was carried out dividing GBA mRNA in three fragments; one fragment comprising from exon 2 to exon 5; the second one from exon 5 to exon 8 and a last fragment from exon 8 to exon 11. Most of the mutations found were located in exons 8-11, included mutations such as N370S, E326K or L444P that have been previously described. We report as well frequency differences of these mutations between PD and DLB, and between clinical and pathological cases. In conclusion, our results suggest that GBA mutations are associated with DLB in Spanish population as has been previously shown for PD.

1ª: Disorders and nervous system repair
2ª: Cognitive and Behavioural Neuroscience

DELETION OF P75^{NTR} IN SAMP8 MOUSE STRAIN INCREASES THE NUMBER OF BASAL FOREBRAIN CHOLINERGIC NEURONS

P. Dolz-Gaitón[1], H. Anka-Idrissi[1], M. Vilar[1]
[1].UFIEC-Neurodegeneración, Instituto de Salud Carlos III, Majadahonda, Madrid.

Degeneration of the basal forebrain cholinergic neurons (BFCN) is one of the earliest pathological events in the brain of patients suffering from Alzheimer's disease (AD). The mechanism of BFCN degeneration in AD is not known but several data suggest that the neurotrophin receptor p75 (p75^{NTR}) is playing a role. The Senescence Accelerated Mice Prone 8 (SAMP8) display several age- related pathologies associated with AD and have been proposed as a model for sporadic AD. To gain some insight we generated a new SAMP8 mouse with a p75^{NTR} third exon knockout (SAMP8/ p75^{KO}). We show that at 2 months both the number of choline-acetyltransferase (ChAT) and p75^{NTR}-inmmunoreactive neurons was greater in SAMP8/p75^{KO} mice than their respective SAMP8 wild-type littermates. These results are in accordance with previous reports, and strongly suggest that the absence of a neurotrophin-dependent p75^{NTR} signalling causes an increase in the cholinergic population in the basal forebrain also in the SAMP8 strain. We think that this new mouse model will help to elucidate the role of p75^{NTR} in BFCN degeneration.

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

UBIQUINOL EFFECTS ON CEREBRAL MICROVASCULATURE IN THE 3XTG-AD MOUSE MODEL OF ALZHEIMER DISEASE.

F.J. Sancho-Bielsa[1*], J. Frontiñan[1*], R. Santiago-Mora[1], J.R. Peinado[1], L. Gimenez-Llort[2], M. Durán-Prado[1] and F.J. Alcain[1].
[1]Faculty of Medicine of Ciudad Real, University of Castilla-La Mancha, Ciudad Real, Spain
[2]Institut of Neurosciences, University Autonoma of Barcelona, Spain
*Both authors equally contributed.

Numerous abnormalities in the cerebral microvasculature associated to a disturbance of the oxidative balance to pro-oxidant conditions have been described in animal models of Alzheimer disease (AD). Soluble circulating amyloid β-peptide (Aβ) damages endothelial cells in asymptomatic Alzheimer's stages preceding Aβ deposition. The goal of our work is to analyze the role of ubiquinol in the homeostasis of the cerebrovascular system. Pretreatment with CoQ prevented Aβ-induced damage due to the inhibition of Aβ entry and accumulation into endothelial cell mitochondria. The 3xTg-AD mice mimic critical hallmarks of the human disease with a temporal and regional specific profile. Mice were orally supplemented with ubiquinol (stabilized with the Kaneka QH P30 powder) whose concentration in plasma increased from 0.2-0.3 to 2.5-3.3 pmoles/μg protein. In this model, 12 months-old mice presented high level of oxidative stress in plasma compared to age-matched no-transgenic (NTg) mice. Ubiquinol treated 3xTg-AD mice showed levels of carbonyl groups and lipid peroxidation in plasma, similar to NTg animals. No change was detected in the level of oxLDL. Oxidative stress in leucocytes, increased in 3xTg-AD mice compared to NTg animals but in ubiquinol-treated animals decreased to values close to NTg mice. Kaneka QH P30 powder also reduced these parameters but a lesser extent than ubiquinol. 3xTg-AD females displayed higher levels of Aβ-plaques in the hippocampal area than males. Areas with higher Aβ burden also showed hypoxic regions that colocalize with Aβ plaques. In ubiquinol-treated females, the burden of Aβ were reduced significantly and almost non-hypoxic regions were detected. A greater collagen-IV deposition occurred around vessels in both, 3xTg-AD males and females, independently of the Aβ burden. In ubiquinol-treated animals collagen-IV deposition in basal membrane was similar to NTg animals. All these parameters were also reduced in 3xTg-AD mice treated with Kaneka QH P30 powder but at a lesser extent. Overall, the present results in 3xTg-AD mice reveal disruption of the homeostasis of hippocampal microvasculature and the preventive benefits of ubiquinol. In addition, preliminary results show differences in cognitive and non-cognitive behavioral profiles of 3xTg-AD mice treated with ubiquinol.

1. Disorders and nervous system repair
2. Cognitive and Behavioral Neuroscience

REGULATION OF SIGNALLING THROUGH CELL-DEATH RECEPTORS BY MIRNAS AS A NEUROPROTECTIVE STRATEGY IN BRAIN ISCHEMIA

A. Otxoa de Amezaga[1], D. Siedlecki Wullich[1], J. Català Solsona[1], L. Gallardo Alfaro[1], A.J. Miñano-Molina[1, 2], J. Rodríguez-Álvarez[1, 2]
[1.] Institut de Neurociències and Dpt. Bioquímica and Biología Molecular, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain.
[2.] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain.

Stroke is a major cause of death and disability in developed societies. Despite its importance, there are no appropriate therapeutic approaches to limit ischemic damage. The disease occurs by a decrease in blood supply to a certain area of the brain causing a necrotic death in the core of the injury and an apoptotic death in the penumbra. Due to its slow development, apoptosis seems to be the target for therapeutic approaches when patients arrive at the hospital's stroke units.
Numerous experimental evidences indicate that receptors with death domain such as TNFR or Fas would be involved in apoptosis associated with cerebral ischemia. That is the reason why the regulation of the functionality of these receptors can be considered a potential neuroprotective strategy against ischemic damage. In this study we explore the modulation of these receptors through by the regulation of miRNAs (miRs).
It has recently been described the existence of non-coding RNAs regulating the stability of mRNAs or their translation on ribosomes. Nowadays, the role of miRs in gene regulation is becoming increasingly important. Nevertheless, very few data exist on their regulatory function in stroke, and there is no information about the role they can play in regulating death receptors in ischemic damage. In this project we explore the function of some miRs (miR331, miR125b, miR181c) in a widely used in vitro model of ischemia, OGD (Oxygen-glucose deprivation). It has been previously described that these miRs regulate the biological function of TNF-a.
Preliminary results show an over-expression of levels in miR331 at 2h of reoxygenation after 90 minutes of OGD in mixed cortical cells of rat embryos. Little but no significant changes were also observed in expression levels of miR125b and miR181c. Therefore, miR331 could be a potential biomarker candidate in stroke.

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

ULTRASTRUCTURAL STUDY OF THE PLAQUE-GLIA INTERFACE IN THE HIPPOCAMPUS OF APP/PS1 ALZHEIMER MICE

A. Gomez-Arboledas[1], R. Sanchez-Varo[1], J. Vitorica[2], A. Gutierrez[1] and J.C. Davila[1].
1 Dpto. Biología Celular, Genética y Fisiología. Facultad de Ciencias, Universidad de Málaga. IBIMA. CIBERNED. Málaga.
2 Dpto Bioquímica y Biología Molecular, Facultad de Farmacia, Universidad de Sevilla. IBIS. CIBERNED. Sevilla

The accumulation of amyloid-beta (Aβ) into diffuse and compact plaques is a major histopathological feature of Alzheimer's disease (AD). Activated glia (microglia and astroglia) surround and infiltrate compact Aβ plaques in AD patients and APP-based transgenic models, however whether these reactive glial cells are actively contributing to ongoing neurodegenerative processes or play a neuroprotective role is highly debated. Here, we examined the plaque-glia interactions in the hippocampus of PS1M146L/APP751SL transgenic mouse by immunohistochemistry at both light and electron microscopy. This AD model develops Aβ plaques at early ages (3-4 months), and these plaques are typically surrounded by axonal/synaptic dystrophies intermixed with astroglial and microglial processes. Activated microglial processes completely surrounded all examined Aβ plaques, independently of their size or location. Ultrastructural analysis revealed that the microglial cell membrane was in intimate contact with the Aβ fibrils, and that their processes showed frequently numerous cisternae of endoplasmic reticulum in the contact area. Reactive astrocytes also associated with the Aβ plaques, especially the larger ones. The astrocytic processes contacting the Aβ plaques were characterized by a virtual lack of glial filaments and other organelles; however these processes were immunoreactive for typical astrocytic markers as ALDH1L1 or AQP4. Near the Aβ plaques, astrocytic processes were observed contacting microglial cells, and also surrounding dystrophic neurites. Our results highlight the close relationship of the activated glial cells with Aβ plaques, and suggest a major contribution of these cells in the dynamic of amyloid plaque development/degradation and/or forming a protective barrier around amyloid plaques and therefore modulating plaque toxicity. *Supported by FIS PI12/01431, FIS PI12/01439, CIBERNED and La Marató TV3.*

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

IL-4 DELETION EXACERBATES AMYLOID PATHOLOGY IN ALZHEIMER APP TRANSGENIC MICE

L. Trujillo-Estrada[1], V. Navarro[2], MV. Sanchez-Mico[2], E. Sanchez-Mejias[1], R. Sanchez-Varo[1], M. Vizuete[2], JC Davila[1], J. Vitorica[2] and A. Gutierrez[1].
[1]. Dept. Cell Biology, Faculty of Sciences. University of Malaga. IBIMA. CIBERNED. Malaga.
[2]. Dept. Biochemistry&Molecular Biology. University of Seville. IBIS. CIBERNED. Sevilla.

Over the past decade neuroinflammation has been a focus of increasing interest in the Alzheimer's disease (AD) field not only for its potential role in neuronal degeneration but also as a promising target for new therapies. We have previously demonstrated that microglial cells change their activation phenotype, from neuroprotective (M2) to cytotoxic (M1), during disease progression in a transgenic APP/PS1 model. In this sense, in AD there is an increase in pro-inflammatory cytokines with age which is accompanied by a decrease in hippocampal interleukin-4 (IL-4) level. Therefore, modulating the inflammatory pathways in animal models of AD has offered the possibility to investigate the contribution of this process to disease. Here, we have generated an APP mouse knockout for IL-4 (APP/IL4$^{-/-}$) in order to determinate the implication of this anti-inflammatory cytokine in the disease progression. Accumulating evidences indicate that IL-4 plays a critical role in memory and learning, and it is implicated in tissue reparation and homeostasis through "alternative" macrophage activation. APP and APP/IL4$^{-/-}$ transgenic mice were examined at 6, 9, 12 and 18 months of age. In these animals we have investigated the Abeta pathology by immunohistochemical and molecular techniques. Moreover, we have evaluated the microglial phenotype in both genotypes as well as the implication of the deletion of IL-4 in the microglial morphology. We found a significant increase in the amyloid deposition in APP/IL4$^{-/-}$ animals compared to aged-matched APP mice. APP/IL4$^{-/-}$ microglial cells surrounding plaques showed an overactivated phenotype characterized by spherical appearance and poorly ramified processes compared to APP microglia. Besides, regarding to neuronal survival, we detected an increase in the axonal dystrophic pathology in APP/IL4$^{-/-}$ animals, with higher accumulation of APP, ubiquitin and phosphorylated tau dystrophies. Our data demonstrate a deleterious effect of overactivated microglial cells on Abeta pathology in APP/IL4$^{-/-}$.
Supported by grants FIS-PI12/01431(AG),FIS-PI12/01439(JV).

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

IMMUNOSUPRESSION ACCELERATES ALZHEIMER'S DISEASE RELATED PATHOLOGY IN APP/PS1 TRANSGENIC MICE

R. Gomez-Gutierrez[1], R. Sanchez-Varo[1], S. Jimenez[2], Navarro V, Vizuete M[2], JC Davila[1], J. Vitorica[2] and A. Gutierrez[1]
[1]Dept. Cell Biology, Faculty of Sciences, University of Malaga. CIBERNED. IBIMA, Malaga, Spain
[2]Dept. Biochemistry&Molecular Biology, Faculty of Pharmacy, University of Seville. CIBERNED. IBIS., Seville, Spain

OBJECTIVES: Initial cognitive and memory decline in Alzheimer's disease (AD) is highly likely caused by synaptic dysfunction prior to neuronal loss. Previous works have shown that neuroinflammatory response could be implicated in the AD progression. Microglial cells are the main cell type of the innate immune system in the brain. In fact, patients and animal models display abundant microglial activation; however, it is still unknown whether it is a cause or a consequence of the pathology. An inefficient immunologic response could be involved in the increase of amyloid-beta levels with disease progression in aged patients, so we aimed to investigate the effect of immunosuppression over the pathological progression in the hippocampus of APP/PS1dE9 transgenic mouse model.
METHODS: Cyclosporine (15 mg/kg) and prednisone (20 mg/kg) were intraperitoneally administered to 9 month-old APP/PS1dE9 mice for 3 months. Untreated mice were used as controls. Hippocampal amyloid burden and glial cells (GFAP, Iba-1) were investigated by immunohistochemistry over free-floating sections and quantified by image analysis. Moreover, Abeta levels, glial activation, cytokines production and GABAergic neurodegeneration were assessed by molecular techniques (Western-blot and RT-PCR).
RESULTS: A strong decrease in the microglial marker Iba1 and some proinflammatory cytokines was detected by RT-PCR in the immunosuppressed mice compared to controls. Conversely, a significant increase in Abeta levels and astroglial marker (GFAP) was found. Moreover, SOM and NPY subpopulations were also negatively affected due to the treatment.
CONCLUSIONS: Immunosuppression treatment downregulated microglial population activity and accelerated amyloid pathology and neuronal degeneration in this APP/PS1 model. Deficiencies in the innate immune system with age could promote AD pathology and cognitive decline in patients. Therefore, regulating microglial activation signalling pathways in the brain might have therapeutic benefits for AD.

This work was supported by FIS PI12/01431 and PI12/01439 grants.

1ª: Disorders and nervous system repair
2ª: Neuronal excitability, synapses and glia: cellular mechanisms

MICROGLIA AND ABETA PLAQUE ASSOCIATION IN THE HUMAN ALZHEIMER'S DISEASE HIPPOCAMPUS

E. Sanchez Mejias[1], L. Trujillo Estrada[1], C. Nuñez-Diaz[1], V. Navarro[2], S. Jimenez[2] , R. Sanchez-Varo[1], A. Gomez-Arboledas[1], M. Vizuete 2, J.C. Davila[1], J. Vitorica[2] A. Gutierrez[1].
[1]. Dept. Biología Celular. Facultad de Ciencias, Universidad de Málaga. IBIMA. CIBERNED. Málaga
[2]. Dept. Bioquimica & Biología Molecular. Facultad de Farmacia, Universidad de Sevilla. IBIS. CIBERNED. Sevilla.

Alzheimer's disease (AD) is characterized by extracellular amyloid-beta (Abeta) deposition and glial activation. Despite the relationship between activated microglia and senile plaques is widely known, the role of microglial cells in AD and their preferentially association with some amyloid deposit types remains unclear. Here we examined the spatial distribution pattern of different plaques (fibrillar, dense-core with non-fibrillar halo and diffuse) and the distinct response of associated microglia in the hippocampus of AD patients and APP/PS1 mice. Immunostainings for light and confocal microscopy, and RT-PCR, were performed in hippocampal samples from human autopsies of demented Braak V-VI patients and 6 month-old APP751SL/PS1M146L mice. Two types of Abeta deposits were identified in AD hippocampus: 1) Thioflavin S-positive fibrillar plaques, restricted to dentate gyrus with surrounding positive-Iba1 cells; 2) dense-core plaques, with fibrillar core and diffuse halo, restricted to CA1, with infiltrating activated/phagocytic microglia. Diffuse plaques did not contain associated microglia and were confined to parahippocampal gyrus. Abeta deposits of APP/PS1 hippocampus showed similar fibrillar amyloid composition and microglia association to those found in Braak V-VI dentate gyrus. Moreover, it has been noted numerous and huge AT8 (phospho-tau) and APP-positive axonal dystrophies restricted to the periphery of fibrillar plaques or within dense-core deposits. Finally, clear microglial activation was identified on BraakV-VI samples with a classic differentiation state by expression of M1 markers. In conclusion, this plaque type-specific spatial pattern with different microglia association suggests distinct etiologies among amyloid deposits in human AD brain. Although it remains unclear whether inflammation represents a cause or consequence of AD, the spatial relationship between activated microglia and extracellular Abeta indicates a role in plaque formation and disease progression. We are now trying to elucidate if microglia contributes to amyloid fibrillization for Abeta plaque formation.

Supported by FIS-PI12/01431(AG), FIS-PI12/01439(JV).

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

DOWN SYNDROME SLEEP DISTURBANCES ARE ASSOCIATED WITH THE SHORT ALLELE HOMOZYGOTE OF SEROTONIN TRANSPORTER PROMOTER POLYMORPHISM (5-HTTLPR).

L del Hoyo[1,3], L Xicota[1,2,4], K Langohr[3], S de Sola[1,2], A Cuenca-Royo[1], G Sánchez- Benavides[1], J Rodriguez[1], M Farre[3], M Dierssen[2,4], R de la Torre[1,4] and the TESDAD STUDY GROUP.
[1]. Integrated Pharmacology and Systems Neurociencies, FINS, Neurosciences Research Program, IMIM (Hospital Del Mar Medical Research Institute) Barcelona, Spain
[2]. Cellular & Systems Neurobiology, CRG, Barcelona, Spain
[3]. Universitat Autònoma de Barcelona, Barcelona, Spain
[4]. Universitat Pompeu Fabra, Barcelona, Spain
[5]. Universitat Politècnica de Catalunya, Barcelona, Spain

Several studies have documented sleep problems in Down syndrome (DS) such as bedtime resistance, anxiety, breathing disorders, settling, co-sleeping, and daytime sleepiness. Serotonin is involved in sleep regulation, and a serotoninergic syndrome is reported in DS population. Furthermore, there is an actual association between carrying the short allele of serotonin transporter promoter polymorphism (5-HTTLPR) and worse sleep quality in typically developing population. Having all these data, we propose to examine whether DS sleep disturbances are associated with 5-HTTLPR polymorphism. **Material & methods:** 78 DS young adults (16 to 34) of both genders were enrolled in our analysis. Sleep quality assessment: The Pittsburgh Sleep Quality Index (PSQI) questionnaire (parental report) was self-administered. Parents were also asked about children poor sleep quality and bad postures while sleeping as dichotomy yes/no questions. Genotyping analysis: extraction was made using Flexi Gene DNA kit and genotyping analysis using polymerase chain reaction (RT-PCR).Statistical analysis: The Chi-Square fisher test was calculated for dichotomy variables (PSQI good/bad quality compute, poor sleep quality and bad postures during sleeping) and “Linear by linear Association” for the rest PSQI ordinal subscales fitted as rows, and polymorphism grouping (L carriers vs. S/S) as columns. **Results:** Results showed significant differences between polymorphisms in the following subscales: Drowsiness during activity (p=0.041), Diurnal dysfunction (p=0.041), Sleep disturbances (p= 0.013), and also in interview reports of bad quality of sleep (p=0.023) and bad postures while sleeping (p=0.008) in the S/S group. We also find a trend pointing to S/S bad sleep quality in the PSQI compute (p=0.079) and larger sleep onset latency (p=0.097). **Conclusions:** Our findings show that 5HTTPLR polymorphisms influence quality of sleep in DS population, pointing to higher sleep problems in S homozygote group. These results may be taken in consideration when assessing sleep disturbance treatment efficacy in DS clinical trials.

1. Trastornos y reparación del sistema nervioso.
2. Neurociencia cognitiva y conductual.

YEARS OF EDUCATION INVERSELY CORRELATES WITH ALZHEIMER DISEASE PERIPHERAL BIOMARKER IN A YOUNG DS POPULATION.

L. Xicota [1,2,3], L. del Hoyo [1,4], K. Langohr [1,5], S de Sola[1,3], A Cuenca-Royo[1], G Sánchez-Benavides[1], J Rodriguez[1], M Farré[4], M Dierssen[2,3], R de la Torre[1,2] and the TESDAD STUDY GROUP.
[1]. Grupo de Farmacología Integrada y Neurociencia de Sistemas (FINS), IMIM (Hospital del Mar Res. Institute), Barcelona.
[2]. Universitat Pompeu Fabra, Barcelona.
[3]. Cellular & Systems Neurobiology, CRG (Centre for Genomic Regulation), Barcelona.
[4]. Universitat Autònoma de Barcelona, Barcelona.
[5]. Universitat Politècnica de Barcelona, Barcelona.

Introduction: Down syndrome (DS) patients have increased risk of developing Alzheimer disease (AD) than control population due to the overexpression of *APP*. This overexpression leads to elevated Aβ production in the brain and higher Aβ plasma concentrations from a young age, which provides an easy readout of the state of Aβ production.
Objectives: The aim of this study is to investigate the possible effects of cognitive load, as measured through Years of Education (YoE), in the plasma concentrations of AD biomarkers in a young DS population.
Material and Methods: Aβ plasma concentrations were analyzed in 54 DS young adult subjects using the Inno-bia Plasma Aβ forms assay (Innogenetics, Fujirebio). The variable "Years of education" (YoE) was recollected through a caregiver interview, taking into account regular school attendance in specialized or non-specialized educational centers.
Results: We found an association between $A\beta_{1-40}$ plasma concentrations and YoE, corrected for both IQ and age. No association was found with YeO for $A\beta_{1-42}$ or for $A\beta_{42}/A\beta_{40}$ ratio. No effect of age, IQ or gender was observed in $A\beta_{1-40,}$ and $A\beta_{1-42}$ plasma concentrations or in the $A\beta_{42}/A\beta_{40}$ ratio. When analyzing the truncated forms $A\beta_{N-40}$ and $A\beta_{N-42}$ similar results were observed with an association between $A\beta_{N-40}$ and YoE, and no effect of YoE seen on the plasma concentrations of $A\beta_{N-42}$. These later results were expected due to the correlation found between the full and the truncated forms of the peptides.
Conclusions: In our study population we have observed that longer educational periods inversely correlated with lower concentrations of all the forms of $A\beta_{40}$. No correlation was observed, however on $A\beta_{42}$ concentrations or the $A\beta_{42}/A\beta_{40}$ ratio and YoE. These results point to the possibility that the cognitive load could have an effect on AD plasma biomarkers, although further studies would be needed to confirm it.

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

VARIATIONS IN GENE AND PROTEIN EXPRESSION IN THE INFERIOR COLLICULUS OF THE GASH:SAL HAMSTER.

D. Sánchez-Benito[1,2,3], E. García-Calzado[1], JR. del Bosque[1], R. Gómez-Nieto[1,2,3], C. Sancho[1,2,4], D. López-López[1,2], D. E. López[1,2,3]

[1]. Neuroscience Institute of Castilla y León, University of Salamanca, Salamanca
[2]. Institute of Biomedical Research of Salamanca (IBSAL), Salamanca
[3]. Department of Cellular Biology and Pathology, University of Salamanca, Salamanca, Spain.
[4]. Department of Physiology and Pharmacology, University of Salamanca, Salamanca, Spain.

Aims: This study aims to determine differences in gene and protein expression between the control Syrian hamster (*Mesocricetus auratus)* and the strain GASH:Sal (genetic audiogenic seizure hamster, inbred at the University of Salamanca). We focused on the inferior colliculus (IC), the auditory nucleus involved in epileptogeneis, with the goal of explaining the etiology of audiogenic epilepsy in the GASH:Sal.

Materials and Methods: We performed two different approaches: the proteomic analysis of the IC, and the comparative study of gene expression in control and GASH:Sal hamsters at basal conditions. The collected ICs were processed for two-dimensional gel electrophoresis in SDS-polyacrylamide gels. The protein spots were visualized with silver staining, and the spots of interest were manually excised, destained, digested with trypsin, and analyzed by reversed-phase LC-MS/MS.-For gene expression analyses, RNA isolation, and subsequent microarray and data analysis were performed using mouse Affimetrix arrays (GeneChip® Mouse Gene ST Arrays).

Results: The IC of the GASH:Sal showed less protein levels than the control. The differential analysis of spots and identification of the MS/MS spectra indicate greater concentration of metabolism-related proteins in the GASH:Sal. Comparison of gene expression profiles between the control and GASH:Sal hamsters resulted in up-regulation of five genes and down-regulation of eight genes, most of them were regulation factors. Both methods showed increased Glutathione S-transferase P (GST) protein in the IC of the GASH:Sal. This protein is abundantly expressed in mammalian tissues associated with malignancies, acting as a negative regulator of some stress kinases.

Conclusions: The IC of the GASH:Sal exhibited variations in gene and protein expression that might contribute to better understanding the etiology of audiogenic epilepsy.

Acknowledgments: University of Salamanca Research Support Grant 2015 to D.E. López**,** and the Proteomics facility of Centro de Investigación del Cáncer that belongs to ProteoRed, PRB2-ISCIII, supported by grant PT13/0001.

1. Neurociencia cognitiva y conductual
2. Trastornos y reparación del sistema nervioso

THE TRANSCRIPTOME OF THE INFERIOR COLLICULUS AFTER ACOUSTIC INDUCED SEIZURE IN THE GASH:SAL HAMSTER.

D. Sánchez-Benito[1,2,3], D. López-López[1,2], S. Hernández-Noriega[1,2,4], M.J. Herrero-Turrión[1], R. Gómez-Nieto[1,2,3], C. Sancho[1,2,4], O. Castellano[1,2,3], L.J.Muñoz, D.[2] E. López[1,2,3]
[1]. Neuroscience Institute of Castilla y León, University of Salamanca, Salamanca
[2]. Institute of Biomedical Research of Salamanca (IBSAL), Salamanca
[3]. Department of Cellular Biology and Pathology, University of Salamanca, Salamanca, Spain.
[4]. Department of Physiology and Pharmacology, University of Salamanca, Salamanca, Spain.

Aims: This work aims to study the transcriptome of the inferior collicullus (IC) in the Control Syrian hamster (*Mesocricetus auratus)* and the strain GASH:Sal (genetic audiogenic seizure hamster, inbred at the University of Salamanca) after acoustic stimulation. The goal is to determine genes associated with epileptic phenotypes in the GASH:Sal after acoustic induced seizure.
Materials and Methods: RNA from 15 biosamples from the IC of the GASH:Sal and Control hamsters. We created the RNASeq libraries through the Truseq RNA Sample prep kit v2 (Illumina), deleting the ribosomal RNA. Both libraries were validated on the 2100 Agilent Bioanalyzer and quantified with qPCR. Sequencing of the libraries was done using a genome Analyzer IIx (Illumina) using single read format. Analysis of different systems, pathways, relations, and specific searches, were performed to reveal genes that may be relevant to the audiogenic epilepsy.
Results: The IC of the GASH:Sal express genes involved in cellular response to drugs and those related to synaptic transmission (Npy5r, Ptgs2, Chrna2, Drd3, Gdnf and Adipoq). The most over-expressed gene is Depdc5 that is related to intracellular signal transduction, suggesting that the GASH:Sal have more neuronal activation.
One of the most interesting genes under-expressed is GPR98 that encodes for VLGR1 or Mass1. This protein coding gene is associated with sensory perception of sound, detection of mechanical stimulus involved in sound perception and inner ear receptor stereocilium organization. This suggests that audiogenic seizures might cause some sort of sensory shutdown. The data is available in the data base https://submit.ncbi.nlm.nih.gov/ft/byid/bq7rg36z/gashsubmission.sqn, under the project number PRJNA230618
Conclusions: It's necessary to keep working on establishing genic correlations and implications of the deregulated genes in the stimulated GASH:Sal for deepening the role of the proteins encoded by the more important genes that we have shown in this study. One example is GPR98 case.
Acknowledgments: University of Salamanca Research Support Grant 2015 to D.E. López

1. Neurociencia cognitiva y conductual
2. Trastornos y reparación del sistema nervioso

ASSOCIATION OF GRIN2B GENE OF NMDA RECEPTOR WITH IMPULSE CONTROL DISORDER IN PARKINSON DISEASE

J. Pol[2], B. Vives[1,2], B. Ortega[2], E. Piñero[2], M.J. Torres[1,2], I. Legarda[1,2], G. Olmos[2,3], J. Lladó[2,3], C. Vives-Bauzà[2]

[1]. Servicio de Neurología, Hospital Universitari Son Espases

[2]. Institut d'Investigació Sanitaria de Palma (IdISPa), Palma, Illes Balears

[3]. Facultat de Ciències, Universitat de les Illes Balears, Palma, Illes Balears

Impulse Control Disorder (ICD) has been identified in Parkinson's disease (PD) as a medication side effect derived of the use of dopaminergic drug therapies. Although almost all Parkinson's disease patients are treated with dopaminergic agonists, only 14% of them develop ICD. Parkinson's disease age of onset, the type of dopaminergic agonist used and the therapeutic dose, as well as the sex and genetic factors might contribute to the variability between individual responses to medication.

This work was aimed to identify common genetic variants associated to ICD in Parkinson's disease patients treated with dopaminergic medication.

Parkinson's disease patients subjected to dopaminergic therapy for over two years under clinical follow-up, and classified upon the Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease-Rating Scale (QUIP-RS) were genotyped to analyze common genetic variants of genes involved in different pathways such as dopamine, catecholamine, serotonin, glutamate, opioid, and monoamine-oxidase signaling pathways. Non-parametric analyses were used to compare allelic and genotypic frequencies for each genetic variant. The possible interaction between genetic variants was also determined by logistic regression.

We have identified two genetic variants, rs7301328 and rs1806191, in the GRIN2B gene, which encodes the subunit 2 of the NMDA ionotropic glutamate receptor, associated to ICD in PD patients treated with dopaminergic agonists.

Our results should contribute to predict the adverse consequences of dopaminergic therapy, advancing towards a personalized therapy.

1ª: Trastornos y reparación del sistema nervioso

2ª: Neurociencia cognitiva y conductual

STUDY OF CELL PROLIFERATION AND/OR NEUROGENESIS IN THE MOUSE MODEL OF FRAGILE X SYNDROME

M.A. Real , E. Frías, M.N. Ávila, M. Pérez-Martín, S. Guirado
Faculty of Science. University of Málaga, Málaga

Fragile X Syndrome (FXS), the most common form of inherited mental retardation, is caused by the lack of FMRP (fragile X mental retardation protein) as a result of the transcriptional silencing of the *FMR1* gene. FMRP is thought to repress the synthesis of proteins required for protein synthesis-dependent synaptic plasticity. Previous studies have shown that lack of FMRP also causes alterations in adult hippocampal neurogenesis in null mice for *Fmr1* gene (*Fmr1*-KO), causing changes in patterns of cell proliferation and differentiation, dendritic development defects, and learning and behavior deficits (Luo et al., 2010). Recently, a novel neurogenic hypothalamic area in the parenchyma surrounding the third ventricle has been described (Pierce and Xu, 2010; Perez-Martín et al, 2010.). In this regard, hypothalamic neurogenesis seems to be involved in the regulation of energy balance (Pierce and Xu 2010).
The aim of this study is to analyze possible alterations in cell proliferation and/or hypothalamic neurogenesis in *Fmr1*-KO mice compared to WT mice. Immunohistochemical techniques were carried out using a phosphohistone H3 antibody (anti-php3) as a marker for cells undergoing division. To study proliferation and cell survival, bromodeoxyuridine (BrdU) was injected in vivo and posteriorly detected by immunocytochemistry. Besides, double immunohistochemistry for BrdU/βIII-tubulin and BrdU/GFAP was performed to determine whether new cells are neurons or astrocytes. The two classical neurogenic regions, the dentate gyrus of the hippocampus and the subventricular zone of the striatal wall of the lateral ventricle were also analyzed. Quantitative studies of BrdU marked cells show that the number of these marked cells is significantly lower in the hypothalamus of *Fmr1*-KO mice.

THE ENDOPLASMIC RETICULUM STRESS AND THE HIF-1 SIGNALLING PATHWAYS ARE INVOLVED IN THE NEURONAL DAMAGE CAUSED BY CHEMICAL HIPOXIA

B. López-Hernández1, V. Ceña[1,2] and I. Posadas[1,2].
[1]Unidad Asociada Neurodeath CSIC-Universidad de Castilla-La Mancha. Albacete, Spain; [2]CIBERNED, Instituto de Salud Carlos III, Madrid, Spain.

In the Central Nervous System (CNS), hypoxia inducible factor-1 (HIF-1) is induced in response to hypoxia. HIF-1 seems to promote transitory neuronal survival, suggesting that additional mechanisms might be involved in determining neuron survival/death at later stages. The endoplasmic reticulum (ER) is a sensor capable of triggering both adaptive and pathological signaling, which makes it a good candidate for mediation of neuronal death/survival at later stages of hypoxia.
In this study, we exposed cortical neurons to chemical hypoxia, induced by $CoCl_2$, to explore a possible relationship between ER stress and the HIF-1 pathway. We found that chemical hypoxia activated the PRK-like endoplasmic reticulum kinase (PERK) pathway of the ER stress response and increased the expression of the C/EBP homologous protein (CHOP) and the activity of caspase 12, suggesting the participation of ER stress PERK-dependent pathway in $CoCl_2$-mediated neuronal death. In addition, we found that $CoCl_2$ reduced the half-life of HIF-1α mRNA leading to a reduction in HIF-1α mRNA and protein content at later stages coinciding with ER stress-PERK-dependent pathway activation. Moreover, salubrinal, a selective pharmacological inhibitor of phospho-eIF2α phosphatase, protected the neurons from chemical hypoxia by reducing CHOP levels and caspase 12 activity, and increasing the half-life of HIF-1α mRNA and the levels of HIF-1a protein.
In summary, our data indicate that chemical hypoxia-induced neuronal apoptosis is a process regulated by HIF-1α stabilization early on and by ER stress activation at later stages. In addition, the neuroprotective effect of salubrinal seems to be related to HIF-1α levels.

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

ALZHEIMER AMYLOID-BETA INTERNALIZATION BY ASTROCYTES REQUIRES CLATHRIN-MEDIATED ENDOCYTOSIS.

Domínguez-Prieto M., Velasco A. & Medina J.M.
Instituto de Neurociencias de Castilla y León (INCYL), Universidad de Salamanca, Salamanca, España.

Alzheimer's Disease is characterized by the accumulation and deposition of amyloid-beta (Aβ) within the brain. Aβ accumulation depends not only on the rate of its synthesis but also on the rate of clearance. Since astrocytes have being proposed to participate in Aβ clearance from the brain, we decided to study the effects of different Aβ peptides (Aβ 25-35, Aβ 40, Aβ 42) on rat astrocytes in primary culture. Our results showed that all the peptides assayed significantly decreased astrocyte viability while increasing the production of reactive oxygen species (ROS). In order to examine the localization of Aβ within the cells we carried out immunocitochemistry against Aβ. Unexpectedly, we observed that all the Aβ assayed were avidly internalized by astrocytes. To get inside the molecular mechanisms involved in Aβ internalization, we have challenged the astrocytes with inhibitors of endocytosis showing that both phenylarsine oxide (PAO) and chlorpromazine prevented Aβ internalization, a fact consistent with the idea that Aβ is internalized by clathrin-mediated endocytosis.

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

STUDY OF HUMAN PRIMARY GLIOMA STEM CELLS INVASION IN THE PRESENCE OF CELL-PENETRATING PEPTIDES BASED ON THE INTERACTION BETWEEN CONNEXIN43 AND C-SRC

M. Jaraíz-Rodríguez[1], M.D. Tabernero[2], M. González-Tablas[3], A. Otero[4], A. Orfao[3], J.M. Medina[1] and A. Tabernero[1]

[1]. Instituto de Neurociencias de Castilla y León. Universidad de Salamanca, Salamanca. Spain.
[2]. Instituto de Estudios de Ciencias de la Salud de Castilla y León (IECSCYL) and Research Laboratory of the University Hospital of Salamanca, Instituto de Investigación Biomedicina de Salamanca (IBSAL) and Centre for Cancer Research (CIC-IBMCC; CSIC/USAL; IBSAL). Salamanca. Spain.
[3]. Centre for Cancer Research (CIC-IBMCC; CSIC/USAL; IBSAL) and Department of Medicine, Universidad de Salamanca, Salamanca. Spain.
[4]. Neurosurgery Service of the University Hospital of Salamanca and IBSAL, Salamanca. Spain.

Connexin43 (Cx43) is the main gap junction channel-forming protein in astrocytes. This protein is downregulated in brain tumours called gliomas. Tumour initiation, relapse, and therapeutic resistance in gliomas is attributed to Glioma Stem Cells (GSCs). Interestingly, several cell-penetrating peptides (CPPs) containing different regions of Cx43 involved in c-Src interaction reverse Glioma Stem Cells (GSCs) phenotype and reduce the rate of cell growth. Considering the controversial Cx43 migration properties and the infiltrative nature of these tumours, we have investigated the role of these CPPs in human primary GSC migration and invasion.

Human primary GSCs were obtained from fresh tumour biopsies and were treated with CPPs. Human GSCs G166 were treated with CPPs. Migration was studied using tiny-tumour cultures, Time-Lapse live-cell Imaging and Immunocytochemistry. Invasion was studied using 8.0 µM pore transwell inserts with or without Matrigel. The mechanism involved in migration was studied by Western blot, evaluating the activity of Focal Adhesion Kinase (FAK).

Our findings indicate that our CPPs reduced the rate of human primary GSCs and G166 GSCs migration and invasion. In addition, CPPs inhibited c-Src activity in these cells and consequently decreased FAK phosphorylation necessary to establish adequate focal adhesions in order to migrate. It should be mentioned that FAK is activated by Src-mediated phosphorylation.

In conclusion, our results show that c-Src plays an essential role in the effects of Cx43 on migration and suggest these CPPs by inhibiting migration and invasion could be the basis for promising therapies.

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

REDUCED I_1-IMIDAZOLINE RECEPTOR CONTENT IN POSTMORTEM PREFRONTAL CORTEX OF COCAINE ADDICTS

B. Keller [1], M. Álvaro-Bartolomé [1], M.J. García-Fuster [1], J.A. García-Sevilla [1]
[1] Laboratory of Neuropharmacology, IUNICS and RETICS-RTA, Universitat de les Illes Balears (UIB), Palma de Mallorca

Cocaine addiction is a major medical syndrome involving a dysregulation of dopamine neurotransmission in the brain. Recent studies suggested that I_1-imidazoline receptors (IR) participate in the prevention of cue-induced cocaine relapse in rats. To test the hypothesis that human cocaine addiction is also associated with malfunction of I_1-IR, the content of two I_1-related proteins were quantified in the prefrontal cortex (PFC) of well characterized cohorts (all addicts: 10 male/9 female; 38±3 yr; 38±6 h PMI, postmortem interval) of pure cocaine addicts (n=8), mixed cocaine/opiate addicts (n=11) and matched controls (13 male/6 female; 41±3 yr; 39±6 h PMI) with specific and validated antibodies (anti-IRBP antibody for 85 kDa I_1-IR, and anti-NISCH antibody for 167 kDa I_1-IR). Blood samples of these cocaine abusers revealed high concentrations of cocaine (3-13 microg/ml) and benzoylecgonine (0.6-10 microg/ml), the main active metabolite. Cocaine and benzoylecgonine were also detected in hair samples (8-310 ng/mg), indicating chronic exposure to cocaine over the last 6-12 months. As a marker of cocaine addiction, the density of dopamine D_2-like receptors (anti-D_2/D_3/D_4 receptor antibody) in the PFC of pure cocaine addicts was reduced (–29%, n=8, P=0.05) when compared with that quantified in age-, gender, and PMI-matched controls. The content of 85 kDa I_1-IR was also significantly reduced (one-sample t-test) in the PFC of cocaine addicts (all cocaine abusers: 27±5%, n=17, P=0.0002; pure cocaine abusers: 19±6%, n=8, P=0.02; mixed cocaine/opiate abusers: 33±8%, n=9, P=0.005). In contrast, the content of 167 kDa related to I_1-IR (nischarin/IRAS**)** was not altered (all cocaine abusers: 97±8%, n=17; pure cocaine abusers: 113±15%, n=8; mixed cocaine/opiate abusers: 87±9%, n=9). These results suggest impaired I_1-IR-mediated functions (e.g. regulation of neuroplasticity or apoptosis inhibition) in the brain of cocaine addicts. Supported by RETICS-RTA RD12/0028/0011 (MINECO-FEDER) and Plan Nacional sobre Drogas Grant 2012/0011(MSSSI-FEDER). MJGF is a ‘Ramón y Cajal’ Researcher (MINECO).

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

ALZHEIMER TRANSGENIC $APP_{751SL}/PS1_{M146L}$ MICE REPRODUCE THE EARLY SYNAPTOPATHOLOGY OF THE DISEASE

R. Sanchez-Varo[1], S. Jimenez[2], V. De Castro[1], V. Navarro[2], E. Sanchez-Mejias[1], L. Trujillo-Estrada[1], M. Vizuete[2], JC. Davila[1], J. Vitorica[2], A. Gutierrez[1].
[1]Dept. Cell Biology, Faculty of Sciences, University of Malaga. CIBERNED. IBIMA, Malaga, Spain.
[2]Dept. Biochemistry&Molecular Biology, Faculty of Pharmacy, University of Seville. CIBERNED. IBIS. Seville, Spain.

Objectives: Cognitive failure in Alzheimer's disease (AD) is likely caused by synaptic dysfunction prior to neuronal loss. We have previously characterized the neuronal and axonal vulnerability in the hippocampus of the APP751SL/PS1M146L mouse model of AD. Plaque-associated abnormal neurites/synapses represent an early indicator of pathology and might compromise synaptic function. Here, we examined whether this AD model reproduces the early synaptic pathology seen in patients.
Methods: Several presynaptic markers (synaptophysin, VGLUT-1, VGAT and ChAT) and the post-synaptic marker PSD-95 were analyzed by quantitative Western blots in the hippocampus of 2, 4, 6 and 12 month-old APP/PS1 and wildtype mice. Moreover, all these presynaptic markers were studied by immunohistochemistry and quantified using image analysis software. Animals were tested on Morris water maze for memory performance at 6 months of age.
Results: Synaptic changes occurred from very early ages in the PS1/APP hippocampus. Most synaptic proteins assessed by Western blot were significantly decreased from 4 months of age, except VGLUT-1 which decline started at 6 months. ChAT exhibited a tendency to decrease at 12 months of age. Immunohistochemistry showed that the loss of synapses was strongly associated to plaques. Some regions, like subiculum and some specific layers of dentate gyrus were specially affected. Additionally, periplaque swollen synaptic processes were detected with all these markers. These changes correlated with early hippocampus-associated cognitive impairment.
Conclusions: Our findings are consistent with the existence of an early and progressive loss of synaptic markers in the hippocampus of this PS1/APP mouse model, along with spatial memory deficiencies. Therefore, this AD model reproduces the early pathology reported in patients and might be a very valuable tool for testing new synaptotherapies aimed to halt or slow down the cognitive impairment at the earliest stages of this disease.
This work was supported by FIS PI12/01431 and PI12/01439 grants.

1ª. Disorders and nervous system repair
2ª. Neuronal excitability, synapses and glia: cellular mechanisms

AMYLOID-PLAQUES TOXICITY PROGRESSION IN A TRANSGENIC APP/PS1 MODEL OF ALZHEIMER'S DISEASE

C. Nuñez-Diaz[1], R. Sanchez-Varo[1], L. Trujillo-Estrada[1], E. Sanchez-Mejias[1], A. Gomez-Arboledas[1], J.C. Davila[1], J. Vitorica[2] and A. Gutierrez[1].
[1] Dept. Cell Biology, Faculty of Sciences, University of Malaga. IBIMA. CIBERNED. Malaga, Spain.
[2] Dept. Biochemistry & Molecular Biology, University of Seville. IBIS. CIBERNED. Seville, Spain.

Objectives: Amyloid plaques, which are composed of beta-amyloid peptides (Aβ), constitute one of the main pathological hallmarks of Alzheimer's disease (AD). In this disease, Aβ progressively accumulates and aggregates giving rise to toxic oligomers, fibrillar structures and, eventually, amyloid plaques. In turn, plaques may release soluble oligomeric forms of Aβ, contributing to the progression of the pathology. Here we aimed to characterize amyloid plaques properties during the pathological progression in the hippocampus of the APP751SL/ PS1M146L mouse model of AD.
Methods: 4, 6, 12 and 18 month-old APP/PS1 mice were analyzed to determine changes in the number, size and toxicity of plaques during this model aging. Tioflavin-S and immunohistochemical stainings with different anti-Aβ antibodies were performed in order to identify different amyloid conformations. Afterwards, quantifications were done by densitometry and stereology.
Results: Both the number and size of amyloid plaques increased with age in the hippocampus proper and subiculum, especially in the latter. At early ages, amyloid plaques were mainly of non-fibrillar nature, however, as they grew they turned into a fibrillar conformation. On the other hand, the compaction of plaques diminished with the progression of the pathology, which might be related to an increase in their toxicity. Importantly, the ring of oligomeric Aβ surrounding the plaque core increased in size during the disease progression.
Conclusion: Plaques may play a relevant role in the pathological progression of AD by acting as reservoirs of soluble Aβ oligomers that, as the disease progresses, could release these toxic amyloid species to the brain parenchyma, causing synaptic/neuronal damage. Therefore, regulating plaque quality properties might be a promising therapeutic approach.
This work was supported by FIS PI12/01431 (to AG) and PI12/01439 (to JV) grants.

1ª: Trastornos y reparación del sistema nervioso.

MICROTUBULE STABILIZATION IMPROVES MEMORY AND REDUCES PATHOLOGY IN APP/PS1 ALZHEIMER'S DISEASE MICE

Fernandez-Valenzuela JJ.[1], Sanchez-Varo R.[1], De Castro V.[1], Moyano FJ[2], Vizuete M[2], Davila JC[1], Vitorica J.[2] and Gutierrez A.[1]

[1]Dept. Cell Biology, Faculty of Sciences, University of Malaga/CIBERNED/IBIMA, Malaga, Spain.

[2]Dept. Biochemistry and Molecular Biology, Faculty of Pharmacy, University of Seville/CIBERNED/IBIS, Seville, Spain.

OBJECTIVES: Cognitive and memory decline in Alzheimer's disease (AD) is highly related to synaptic dysfunction and subsequent neuronal loss. In AD patients, the hiperphosphorylation of the microtubule associated protein tau leads to the destabilization of microtubules and axonal transport failure, with the consequent accumulation of autophagic/vesicular material, generation of dystrophic neurites, and thus contributing to synaptic dysfunction. The aim of this study was to analyze the effect of a treatment with a microtubule stabilizing agent in the progression of the disease in the APP_{751SL}/$PS1_{M146L}$ transgenic model of AD.

METHODS: APP/PS1 mice (3 month-old) were treated with a weekly intraperitoneal injection of 2 mg/kg epothilone D (Epo-D) for 3 months. Vehicle-injected animals were used as controls. At the end of treatment mice were tested on the Morris water maze, Y-maze and object recognition task for memory performance. Levels of Aβ, AT8 (hyperphosphorylated tau), ubiquitin and synaptic markers (PSD95, VGAT) were analyzed by Western-blot. Hippocampal plaque loading and dystrophic area were quantified by image analysis after immunohistochemical staining of free-floating sections with Aβ42, APP or ubiquitin antibodies.

RESULTS: Epo-D treated transgenic mice showed a significant improvement in the performance of hippocampus-associated cognitive tests. The recovery of spatial/episodic-like memory correlated with a reduction in the AD-like hippocampal pathology. The levels of Aβ, APP and ubiquitin significantly decreased in treated animals at both histological and molecular levels. However, phospho-tau (AT8) levels did not change. Synaptic markers (PSD95 and VGAT) were found to be increased in treated animals compared to controls.

CONCLUSION: Our results indicated that Epo-D treatment promotes synaptic and cognitive improvement, reduces the accumulation of extracellular Aβ and the associated dystrophic pathology in the hippocampus of APP/PS1 transgenic model. Therefore, microtubule stabilizing drugs could be considered as therapeutical candidates to slow AD progression.

This work was supported by FIS PI12/01431 and PI12/01439 grants.

1ª. Disorders and nervous system repair

2ª. Cognitive and Behavioral Neuroscience

SYNAPTOGENESIS PREVENTS Aβ-42 NEURODEGENERATION

M. Arnés [1], A. Acebes [2], S. Casas-Tintó [1] A. Ferrús [1]
[1]. Instituto Cajal, Av. Doctor Arce 37, Madrid
[2]. Centro de Investigaciones Biomédicas de Canarias, Universidad de La Laguna, Tenerife

Synaptic loss is one of the first steps leading to neurodegeneration in Alzheimer's disease (AD). The accumulation of β-amyloid peptides from the APP (Amyloid Precursor Protein) has been related to the onset of the pathology, but little is known about the underlying mechanisms of AD, and to date, no cure is available.
In our study, we induced the expression of the human Aβ42 peptide to produce Alzheimer like phenotypes in *Drosophila* using the binary Gal4-UAS expression system, this strategy allows space and temporal control of expression in the central nervous system. Our group has previously demonstrated that elements of the PI3K/AKT pathway control synapse number in addition to their well-known roles on cell survival and proliferation. In our work, flies expressing the human Aβ42 peptide showed progressive decrease in the number of synapses in the neuromuscular junctions of adult flies. These morphological features had a functional correlation as we demonstrate in survival and locomotion assays, where Aβ42 expressing flies exhibited severe defects.
Further, we tested whether the overexpression of PI3K was able to prevent this early synaptic loss, and its behavioral consequences. In all cases, the co-expression of PI3K was able to rescue the defects caused by Aβ42. In particular, the co-expression of PI3K prevented the degeneration of the active zones in adult NMJ's, by recovering from the toxic effects of Aβ in microtubule dynamics. Also, PI3K expression delayed the locomotor defects and the early mortality shown by Aβ42 expressing flies.
Hence, synaptogenic tools, such as PI3K overexpression, could act as neuroprotective agents in the treatment of neurodegenerative diseases, AD in particular.

1ª: Trastornos y reparación del sistema nervioso

PRESENILIN-1 REGULATES AXONAL GROWTH THROUGH RHOA IN HIPPOCAMPAL NEURONS

M. Javier Torrent [1], S. Marco [1], P. W. Janes [2], M. Lackmann [2], C.A. Saura [1].
[1]. Institut de Neurociències, Departament de Bioquímica i Biologia Molecular, Centro de Investigación Biomédica en Red Enfermedades Neurodegenerativas (CIBERNED), Universitat Autònoma de Barcelona, Barcelona, Spain.
[2]. Department of Biochemistry and Molecular Biology, Monash University, Victoria 3800, Australia.

Autosomal dominant mutations in the presenilin (*PS*: PS1 and PS2) genes account for the majority of familial Alzheimer's disease (AD) cases. Presenilins are the catalytic subunits of γ-secretase, an enzymatic complex that cleaves membrane domains of type I transmembrane proteins, including the β-amyloid precursor protein (APP), Notch and ephrin receptors (Eph). Recent evidence indicates that familial AD-linked mutations in PS cause reduced γ-secretase cleavage, suggesting a loss-of-function pathogenic mechanism. In this study, we analysed the biological role of PS on regulating molecular mechanisms mediating axon growth during development of hippocampal neurons. We performed immunofluorescence and quantitative imaging analyses using neurofilament (SMI), intermediate neurofilament (nestin) and dendritic/growth cone (β-actin) markers to quantify axonal growth in embryonic brains and cultured hippocampal neurons of control $PS1^{+/+}$ and $PS1^{-/-}$ mice. Our results show brain hemorrhages and reduced staining and length of neurofilament- and nestin-stained axons in cells of the outer layer of the hippocampus and ventricular zone of $PS1^{-/-}$ embryos. In the hippocampus, neurofilament staining is restricted to the cytoplasm but not processes of doublecortin-positive immature neurons in $PS1^{-/-}$ brains compared to $PS1^{+/+}$ embryos. Similar reduction of axon length is observed in cultured hippocampal neurons from $PS1^{-/-}$ embryos. γ-secretase and TACE inhibitors reduce similarly axon growth in hippocampal neurons, suggesting that PS/γ-secretase function is required for axon growth of hippocampal neurons. Since RhoGTPases are key players in actin cytoskeleton reorganization during axon development, we examined whether RhoA was involved on PS-dependent regulation of axon growth. Both, a dominant negative RhoA mutant and an inhibitor of ROCK, the effector of RhoA during the reorganization of actin cytoskeleton, reversed axon collapse in $PS1^{-/-}$ hippocampal neurons. These results indicate that PS/γ-secretase plays an essential role on axon growth and elongation by inhibiting RhoA signaling and regulating the cleavage of an unknown substrate during hippocampal development.

THE HUMAN TP53 ARG72PRO POLYMORPHISM MODULATES ENDOTHELIAL PROGENITOR CELL MOBILIZATION AND FUNCTIONAL OUTCOME AFTER INTRACEREBRAL HEMORRHAGE

C. Rodríguez[1,2], J. Agulla[1,2], T. Sobrino[3], V. Bobo-Jiménez[1,2], J.C. Gómez Sánchez[1], J. Castillo[3] and A. Almeida[1,2]

[1]. Instituto de Investigación Biomédica de Salamanca, Hospital Universitario de Salamanca-Universidad de Salamanca.

[2]. Instituto de Biología Funcional y Genómica, Universidad de Salamanca-CSIC.

[3]. Instituto de Investigación Sanitaria de Santiago de Compostela, Servicio de Neurología - Laboratorio de Investigación de Neurociencias Clínicas, Hospital Clínico Universitario, Universidad de Santiago de Compostela.

Objectives: Differences in genetic susceptibility to apoptosis account for the different functional recovery on stroke patients. Recently, we described that *Tp53 Arg72Pro* single nucleotide polymorphism (SNP) is associated with functional prognosis in patients after intracerebral hemorrhage (ICH). There is also evidence of a beneficial effect of endothelial progenitor cell (EPC) mobilization and neovascularization through growth factor signaling, such as VEGF and SDF-1α, in brain repair after ischemia. Here, we analyze the possible effect of this p53 SNP on circulating $CD34^+$ progenitor cells and its impact on functional prognosis after ICH.

Materials and methods: To elucidate the mechanisms underlying this phenomenon, we have used the collagenase ICH model in two groups of *knock-in* (KI) mice each one carrying a humanized allele of the *Tp53* gene (*Arg72* and *Pro72*). We performed immunohistochemistry analysis at different days after experimental ICH to analyze cell death. VEGF and SDF-1α serum levels and the percentage of $CD34^+/VEGFR2^+$ cells were determined by ELISA and flow cytometry, respectively.

Results: We found that neuronal apoptosis was higher in Arg72 KI mice, than in the Pro72, from 24 hours up to day seven, as revealed by NeuN-TUNEL co-staining. Furthermore, mice carrying the Pro72 polymorphic variant showed higher VEGF and SDF-1α serum levels and enhanced number of circulating EPCs after ICH, than those with the Arg72 variant.

Conclusions: Our results indicate that the Arg72 polymorphic variant is accountable for a higher level of apoptosis in the brain of rodents under experimental ICH. *Arg72Pro* SNP also modulates VEGF and SDF-1α release and, subsequently, EPC mobilization after ICH, which may account for the different brain repair. In conclusion, the human *Tp53 Arg72Pro* polymorphism controls functional outcome and brain recovery after ICH.

This work was funded by FEDER and Instituto de Salud Carlos III (PI12/0685; INVICTUS RD12/0014/0001 and RD12/0014/0007).

1. Trastornos y reparación del sistema nervioso

IFN-ß AMELERIORATES THE CLINICAL COURSE OF EAE THROUGH THE ENHANCEMENT OF MDSC IMMUNOSUPPRESSIVE ACTIVITY

Melero-Jerez C[1], Suardíaz M[2], Marín-Bañasco, C[2], Fernández Ó[2], de Castro F[1*] and Clemente D[1*].
[1]Grupo de Neurobiología del Desarrollo-GNDe, Hospital Nacional de Parapléjicos, Toledo.
[2]Unidad de Gestión Clínica de Neurociencias, Hospital Regional Universitario Carlos Haya- FIMABIS, Málaga.

Multiple sclerosis (MS) is the most frequent autoimmune demyelinating disease of the human central nervous system (CNS). The most frequent clinical form of the disease is the relapsing-remitting (RR) variant, characterized by phases with increasing neurological symptoms (relapses) followed by periods of partial recovery (remissions). This implies the existence of immunomodulatory agents that promote the relapsing-to-remitting transition. Among others, IFN-ß remains as the most widely prescribed treatment for RRMS, although its mechanism of action and its effect on modulation of myeloid cell activity and its impact on myelin preservation or promotion of remyelination are still poorly understood. In the last years, our group unravelled the role of a heterogeneous population of immature myeloid cells, namely the myeloid-derived suppressor cells (MDSCs), during the clinical course of the most used MS model, experimental autoimmune encephalomyelitis (EAE). MDSCs are important players on T cell suppression and immune response control in EAE. In all cases, the preservation of MDSC undifferentiated state is crucial for MDSCs to be active. Given the fact of the unknown mechanism of the IFN-ß as treatment for MS, we explored whether this effect could be due to the potentiation of the immunosuppressive role of the MDSCs. Our current results show a decrease in the severity of the clinical course of the EAE when treated with IFN-ß, an increase in the presence and immature phenotypic features of the MDSCs both in the periphery and within the CNS and an enhancement of their immunosuppressive function *in vitro* by increasing activated T cell apoptosis. Altogether, IFN-ß, in addition of potentiating MDSC suppressive activity, is shown to be an important factor to prevent MDSC maturation, something to have into account for the design of future cell-based therapies.

This work was supported by the Spanish *Ministerio de Economía y Competitividad (*SAF2012-40023; RD07-0060-2007 and RD12-0032/0012, and partially cofinanced by F.E.D.E.R., European Union, *"Una manera de hacer Europa")* to FdC and ARSEP Foundation (France) to DC. DC and FdC are hired by SESCAM. CM-J holds a Research Training fellowship by the Spanish *Ministerio de Economía y Competitividad* (BES-2013-062630).

1ª: Trastornos y reparación del sistema nervioso
2ª: Sistemas homeostáticos y neuroendocrino

ROLE OF CANNABINOID CB2 RECEPTORS IN BRAIN DAMAGE FOLLOWING HYPOXIA-ISCHEMIA IN ADULT MICE

E. Kossatz[1], P. Robledo[1, 2], R. Maldonado[1]
[1]Laboratory of Neuropharmacology, University Pompeu Fabra, Barcelona
[2] IMIM-Hospital del Mar Research Institute, Barcelona

The endogenous cannabinoid system seems to play an important role in the neuropathology associated with brain ischemia. The aim of this study was to evaluate the neuroprotective effects of cannabinoid CB2 receptors in the behavioural and biochemical alterations induced following hypoxia-ischemia. Mice lacking CB2 receptors (KO) and control littermates (WT) were anesthetized, and the left common carotid artery was permanently ligated. Following recovery, mice were placed in a hypoxia chamber with 10% oxygen for 60 min. Behavioural measurements in the rotarod, beam walking, object recognition, open field, and Irwin tests were carried out 24 h, 72 h and 7 days after this procedure. After testing, brains were prepared for histological and immunohistochemistry analysis. Hypoxia-ischemia induced brain damage ipsilateral to the carotid ligation, although significant differences in lesion size were observed in both genotypes. WT mice showed small damage in the hippocampus and cortex, while KO mice exhibited larger lesions in hippocampus, striatum, cortex and amygdala. Behavioural alterations were observed in both genotypes. However, WT mice progressively recovered motor functionality, while KO mice showed persistent deficits in motor learning, coordination and balance. A significantly higher expression of astrocytes and microglia in the hippocampus, striatum and cortex was observed in KO with respect to WT mice, consistent with the greater lesion size observed in these animals. Memory deficits in the object recognition test were observed 72 h following hypoxia-ischemia in KO and WT mice. However, no recovery in this function was observed in either genotype, suggesting that CB2 receptors may not exert neuroprotective effects on memory dysfunction. Our results indicate that CB2 receptors may have a specific neuroprotective role in motor learning, coordination and balance deficits following hypoxia-ischemia insult, and strongly suggest that they may be important new targets to accelerate the recovery of these brain functions after brain damage occurs.

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

CHARACTERIZATION OF OLIGODENTROCYTE PROGENITOR CELLS IN THE ZEBRAFISH VISUAL SYSTEM

F. León Lobera [1,2], J. Lara Pradas[1, 2], R. Arévalo Arévalo[1, 2], J. Aijón Noguera[1, 2], A. Velasco Arranz [1, 2],
[1]. Instituto de Neurociencias de Castilla y León, Universidad de Salamanca, Salamanca.
[2]. Instituto de Investigación Biomédica de Salamanca, IBSAL, Salamanca.

Zebrafish visual system constitutes a powerful tool to study axonal regeneration and remyelination. Oligodendrocytes (OLs) are found not only in the Optic Nerve (ON) but also in the retina. Oligodendrocyte Progenitor Cells (OPCs) are the OLs precursors. The aim of our study is the characterization of Oligodendrocyte Progenitor Cells (OPCs) in the visual pathway, and their behaviour in axonal regeneration processes.
Adult zebrafish (D. rerio) were kept in 28,5ºC in our fish facility. Fish were deeply anesthetized in tricaine and their optic nerves and retinas were fixed in PFA. Tissue was further cryoprotected in sucrose 30% and frozen in OCT freezing medium. Then, sections were obtained in a cryostat. We performed immunohistochemical techniques to detect sox10, sox2, olig2, pax2, cytokeratin, PCNA and glutamine synthetase (GS).
We found $sox10^{+}$ and $olig2^{+}$ cells in the ganglion cell layer in the retina and also throughout the optic nerve. Retinal OLs were arranged in rows. ON oligodendrocytes were also labelled with the GS antibody. However, pax2 reticular astrocytes were GS^{-} but $cytokeratin^{+}$. $Sox2^{+}$ cells were colabelled with cytokeratin and only a few of them were GS^{+}. These $sox2^{+}/GS^{+}$ were identified as potential OPCs in the ON.
Zebrafish oligodendrocytes are found all along the visual system. They not only express olig2 and sox10 markers but also GS. GS labelling was not found in the other main cell population in the optic nerve, the $pax2^{+}$ reticular astrocytes. Sox2 OPCs, which also express GS, remain to be functionally characterized with degeneration/regeneration experiments.

Fund: FIS PI12/00939 and Junta de Castilla y León (GR 183).

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

NEW INSIGHTS IN ANKRD55, A MULTIPLE SCLEROSIS SUSCEPTIBILITY GENE WITH UNKNOWN FUNCTION

N. Ugidos[1,2], A. Feliú[3], A. Lopez de Lapuente[1,2], M. Mecha[3], I. Astobiza[1,2], J. Riera[1,2], M. Comabella[5], X. Montalban[5], I. Alloza[1,2,4], C. Guaza[3], K. Vandenbroeck[1,2,4]

[1] Neurogenomiks Laboratory, University of Basque Country (UPV/ EHU), Zamudio, Spain,

[2] Achucarro Basque Center for Neuroscience, Zamudio, Spain,

[3] Neuroimmunology Group, Functional and System Neurobiology Department, Instituto Cajal, Madrid, Spain,

[4] IKERBASQUE, Basque Foundation for Science, Bilbao, Spain,

[5] Servei de Neurologia-Neuroimmunologia. Centre d'Esclerosi Múltiple de Catalunya (Cemcat), Institut de Receca Vall d'Hebron (VHIR), Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Spain.

An intronic variant in *ANKRD55*, rs6859219, has been established as risk variant for multiple sclerosis (MS) but the biological reasons behind this association, as well as the function of this gene and its protein product, are unknown. We characterized the expression of *ANKRD55* in human peripheral blood mononuclear cells (PBMCs) and in human immune (U937 and Jurkat) and nervous (SH-SY5Y) cell lines by RT-PCR. Three *ANKRD55* transcript variants (i.e. Ensembl protein coding isoforms 001, 005 and non-coding RNA 007), could be detected in PBMCs, and the risk (C) allele of rs6859219 was significantly associated with higher expression of each of the three splice variants in both MS patients and healthy controls. Immunocytochemistry and western blot in Jurkat and SH-SY5Y cells showed that the ANKRD55 protein, especially the Ensembl isoform 001, is mainly located in the nucleus. Moreover, experiments in mice revealed that ANKRD55 is produced by primary cultures of hippocampal neurons and microglia and by the murine microglial cell line BV2, and that the expression of the protein is induced by inflammatory stimuli. Importantly, ANRKD55 protein expression was increased in a mouse model of experimental autoimmune encephalomyelitis. Analysis of diverse stimuli (cytokines, TLR ligands etc) on ANKRD55 expression is being studied, and ANKRD55 interactome analysis is being performed. Together, our data support an important role of ANKRD55 in multiple sclerosis and highlight the relevance for further studies of this gene in neuroinflammation.

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

IDENTIFICATION OF CAUSATIVE AND SUSCEPTIBILITY VARIANTS IN THE NEUREXIN-NEUROLIGIN PATHWAY IN PATIENTS WITH ALZHEIMER'S DISEASE. THE ROLE OF A TRUNCATING MUTATION IN NEUROLIGIN 1

E. Tristán-Clavijo[1,2], R J. Camacho-Garcia[1], E. Robles-Lanuza[1,2], A. Ruiz[3], I. Hernandez[3], A. Martinez-Mir[1], F. G. Scholl[1,2]

1. Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla. Sevilla, Spain
2. Departamento de Fisiología Médica y Biofísica, Facultad de Medicina, Universidad de Sevilla. Sevilla, Spain
3. Research Center and Memory Clinic. Fundació ACE. Institut Català de Neurociències Aplicades. Barcelona, Spain

Neurexins (NRXN) and neuroligins (NLGN) are synaptic cell-adhesion proteins involved in neurodevelopmental disorders, as autism and intellectual disability. Previously, we have postulated a role of NRNX-NLGN in Alzheimer's disease (AD). Here, we present genetic and functional approaches to identify variants of the NRNX-NLGN pathway in AD patients.

Using next-generation sequencing, we have studied a panel of 36 genes of the NRNX-NLGN synaptic pathway in 192 AD patients. Potential causal variants were studied by *in silico* analysis and validated by Sanger sequencing. Susceptibility variants were analyzed by manual inspection in IGV (Integrative Genomics Viewer). Functional studies of selected variants were performed in N2A and COS cells and cultured hipocampal neurons.

To identify susceptibility alleles, we selected SNPs whose allelic frequency differed among the genotyped AD patients and the control databases. These SNPs were then genotyped in a geographically-matched control population. On the other hand, potential causative mutations were selected among novel variants with a predicted pathogenic effect. We present a two base-pair insertion in *NLGN1* (p.Thr271fs) in a patient with familial history of AD. The frameshift mutation in *NLGN1* predicts a premature STOP codon that truncates the extracellular domain. We generated expression vectors for the p.Thr271fs mutation. In western blot experiments we detected a band of ~130 kD corresponding to wild type NLGN1. In contrast, the p.Thr271fs mutation resulted in truncated proteins of ~30 kDa. Localization studies in COS cells showed that p.Thr271fs NLGN1 failed to reach the plasma membrane and accumulated in the ER. In neurons, NLGN1 induces the formation of glutamatergic synapses. We showed that p.Thr271fs NLGN1 failed to induce the formation of glutamatergic synapses and accumulated in the soma of transfected neurons. Thus, the synaptic activity of NLGN1 was abolished by the AD-associated p.Thr271fs mutation. Our data report the first inactivating mutation in the *NLGN1* gene in AD patients and support a role for the NRXN-NLGN pathway in the etiology of AD.

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

CHRONIC FLUOXETINE TREATMENT ALTERS THE STRUCTURE, CONNECTIVITY AND PLASTICITY OF CORTICAL INTERNEURONS

M. Perez-Rando[1], R. Guirado[2], M. Belles[1], P. Corbin-Agusti[1], E. Castrén[2], J. Nacher[1]
[1]. Facultad de Ciencias Biológicas, Universitat de València, Valencia
[2]. Neuroscience Center, University of Helsinki, Finland

Novel hypotheses suggest that antidepressants, such as the selective serotonin reuptake inhibitor Fluoxetine, induce neuronal structural plasticity, resembling that of the juvenile brain, although the underlying mechanisms of this reopening of the critical periods still remain unclear. However, recent studies suggest that inhibitory networks play an important role in this structural plasticity induced by Fluoxetine. For this reason we have analyzed the effects of a chronic Fluoxetine treatment in the hippocampus and medial prefrontal cortex (mPFC) of transgenic mice displaying eGFP labeled interneurons. We have found an increase in the expression of molecules related to critical period plasticity, such as the polysialylated form of the neural cell adhesion molecule (PSA-NCAM), GAD67/65 and synaptophysin, as well as a reduction in the number of parvalbumin expressing interneurons surrounded by perineuronal nets. We have also described alterations in the perisomatic inhibitory puncta on pyramidal neurons and on eGFP interneurons in the mPFC. Finally, we have found that chronic Fluoxetine treatment affects the structure of interneurons in the mPFC, increasing their dendritic spine density. The present study provides evidence indicating that Fluoxetine promotes structural changes in the inhibitory neurons of the adult cerebral cortex, probably through alterations in plasticity-related molecules of neurons or the extracellular matrix surrounding them, which are present in interneurons and are known to be crucial for the development of the critical periods of plasticity in the juvenile brain.

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

Spanish Ministry of Economy and Competitiveness BFU2012-32512, Generalitat Valenciana Prometeo Excellence Program PROMETEO2013/069.

ROLE OF CB1 RECEPTOR IN TEH SENSITIVITY OF DIRECT VS INDIRECT CORTICOSTRIATAL PATHWAYS TO MUTANT HUNTINGTIN TOXICITY

Ruiz-Calvo, A.*, Bellocchio, L.*, Chiarlone, A., Bajo-Grañeras, R., Resel, E., Galve-Roperh, I., Guzmán, M.

Centro de Investigación Biomédica en Red Sobre Enfermedades Neurodegenerativas, Instituto Ramón y Cajal de Investigación Sanitaria, Instituto Universitario de Investigación Neuroquímica, and Department of Biochemistry and Molecular Biology I, Complutense University, Madrid. *Co-first authors.

The CB_1 receptor exerts a protective role in many different animal models of acute brain damage and chronic neurodegeneration, which has raised hope about the possible clinical use of cannabinoids as neuroprotective drugs. However, the assessment of the physiological relevance and therapeutic potential of CB_1R in neurological diseases is hampered, at least in part, by the lack of knowledge of the cell-population specificity of CB_1R action.

Aim: In order to study the potential neuroprotective role of different CB_1R pools in the cortico-striatal circuitry we used an adenoviral-vector delivery strategy based on the expression of CFP-tagged mutant huntingtin harboring a pathogenic polyQ repeat of 94 residues under the control of specific promoters.

Methods: In a first serie of experiments, we expressed mutant huntingtin under a minimal neuronal promoter (CaMKIIa), in motor cortex or dorsal striatum, in order to achieve how cell selective toxicity accounts for striatal and motor impairment. Going further, to determine the differential sensitivity of the major two striatal neuron populations (D_1R and D_2R MSNs), we expressed mutant huntingtin in the dorsal striatum of BAC trangenic mice, which harbour GFP and tdTomato fluorescence reporters in D_2R or D_1R medium-sized spiny neurons (MSNs), respectively. Finally, to study the role of cortical CB_1R pool in mutant huntingtin-derived toxicity, we infected the motor cortex of conditional knock out mice lacking CB_1R in cortico-striatal projections (Nex-$CB_1^{-/-}$).

Results: Striatal but not cortical mutant huntingtin expression induced DARPP-32 (striatal marker) loss and motor impairment. However, expression of mutant huntingtin in dorsal striatum did not account for any differences in neuronal and DARPP-32 loss between D_1R and D_2R populations. Instead, cortical expression of mutant huntingtin resulted in striatal damage and motor disfunction when CB_1R is absent from cortical-striatal projections, showing greater D_1R expression loss.

Conclusions: While mutant huntingtin expression in dorsal striatum is sufficient for striatal damage and motor impairment, cell specific sensitivity within striatum is a non-autonomous effect of mutant huntingtin in which CB_1R seems to be a key player.

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

Key words: CB1 receptor, huntingtin, corticostriatal circuitry

CANNABIS USE AND SCHIZOPHRENIA: WHAT IS HAPPENING IN THE MICE BRAIN AFTER CANNABIS EXPOSITION DURING THE ADOLESCENCE?

C. García-Mompó, A. Mencher-Beltrán, A. Company-Torres Y. Curto-Sastre and J. Nácher
Neurobiology Unit, Cell Biology Department, Universitat de València, Burjassot, València, Spain.

Several studies have linked cannabis use and psychotic symptoms. In particular, it appears to exist a relationship between the risk of developing schizophrenia and cannabis use during adolescence. However, the cellular and molecular bases of these alterations are still unclear. In this line, the generation of animal models reproducing some of the core features of schizophrenia, constitute valuable tools to investigate these alterations. The main objective of this study was to evaluate the effects of cannabis use during adolescence on the development of schizophrenia, especially its impact on the inhibitory cortical networks. We generated a double developmental/environmental mice model of schizophrenia in a transgenic strain displaying fluorescent interneurons (GINs, Tg (GadGFP) 45704Swn). We sought to mimic a wider range of features of this disorder and to find alterations in the structural plasticity of inhibitory circuits. Mice were subjected to a perinatal injection of a N-methyl-D-aspartate receptor (NMDA-R) antagonist, MK-801, and were socially isolated from postweaning to adulthood. The effects of cannabis were studied through peripubertal treatment with THC (Δ9-tetrahydrocannabinol), the main psychoactive substance of the cannabis. Additionally, the animals were treated with AM251, an antagonist of CB1 receptors during adulthood, in order to prevent or revert the effects of the THC. Prepulse inhibition of Startle Reflex (PPI) response was assessed to evaluate attentional deficits and prefrontal cortex function. Different structural parameters were also evaluated in the prefrontal cortex. Our results show that the exposure to cannabis during adolescence influences behavioral and structural parameters in our double hit model of schizophrenia.

Acknowledgements: Spanish Ministry of Economy and Competitiveness BFU2012-32512, Generalitat Valenciana Prometeo Excellence Program PROMETEO2013/069 and the Fundación Alicia Koplowitz.

1st Disorders and nervous system repair.
2nd Developmental Neurobiology.

APOMORPHINE-INDUCED DYSKINESIAS IN PARKINSONIAN RATS: PET AND HISTOLOGICAL STUDIES

B. Gago [1,2], D. Garcia-Garcia [3], C. Marin [4], H. Jimenez-Urbieta [1,2], Oregui A [1,2], V. Gómez-Vallejo [5], J. Llop [5], G. Mengod [2,6], R. Cortés [2,6], M. Delgado-Alvarado [1,2], M.C. Rodríguez-Oroz [1,2,7,8]
[1]. Instituto de Investigación Sanitaria Biodonostia, San Sebastián
[2]. CIBERNED, Madrid
[3]. Universidad Carlos III de Madrid, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid
[4]. IDIBAPS, Barcelona
[5]. CICbiomaGUNE, San Sebastián
[6]. Institut d'Investigacions Biomèdiques de Barcelona (IIBB-CSIC), Barcelona
[7]. Ikerbasque, Basque Foundation for Science, Bilbao
[8]. Hospital Universitario Donostia, San Sebastián

Dyskinesias induced by dopaminergic drugs complicate the long-term treatment of Parkinson's disease (PD). Animal models of levodopa-induced dyskinesias have provided evidence supporting a relevant role of dopamine and serotonin receptors as well as opioid peptides in their pathophysiology. We have analyzed whether the induction of dyskinesias by apomorphine (APO) in the unilateral 6-hydroxydopamine (6-OHDA)-lesioned rat model alters the expression of serotonin receptors both postmortem, by *in situ* hybridization, and *in vivo* using the [^{18}F]Altanserin binding to 5-HT_{2A} receptor by PET. Besides, we have studied the *in vivo* cerebral metabolism by [^{18}F]FDG PET and the postmortem striatal levels of mRNA of D_1R and D_2R dopamine receptors, encephalin and dynorphin. A 15-d dose-increase protocol of APO administration followed by 2 weeks of the highest dose administration was used to induce dyskinesias, which were evaluated with the abnormal involuntary movements (AIMs) scale. APO induced significant rotational behavior, proving its antiparkinsonian effect, and dyskinesias (total AIMs score = 50). No significant changes in the 5-HT_{1A} and 5-HT_{2A} mRNA levels in the prefrontal cortex and striatum in both hemispheres were observed in comparison with control animals. [^{18}F]Altanserin binding was neither different between dyskinetic and control animals in any cerebral region. However, dyskinetic animals showed glucose hypometabolism in the anterior striatum of the non-lesioned hemisphere and hypermetabolism in the posterior striatum and globus pallidum in the lesioned hemisphere. This last finding was also present in hemiparkinsonian rats without APO treatment. No differences in the D_1R and D_2R mRNA striatal levels were found. However, in the lesioned striatum, the 6-OHDA lesion reduced dynorphine mRNA and increased encephalin mRNA while APO treatment increased both opioid peptides mRNA expression. Thus, we have shown that APO-induced dyskinesias are associated with striatal changes in the glucose metabolism and opioid peptides but not with alterations in serotoninergic and dopaminergic receptors (DFG11/019, PI11/02109).

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

SYNAPTIC REMODELING OF MSNS IN BILATERAL GENETIC MODEL OF PARKINSON´S DISEASE: THE PITX3-/- MOUSE

S Alberquilla[1], LM Suarez[1,2], JR García-Montes[1,2] and R Moratalla[1,2].
[1] Instituto Cajal, Consejo Superior de Investigaciones Científicas, CSIC, Madrid
[2] CIBERNED, Instituto de Salud Carlos III, Madrid

Postmortem studies in patients with Parkinson´s disease (PD) have demonstrated that striatal projection neurons (MSNs) present a marked atrophy of dendritic arbor and loss of spines. These results have also been shown in animal studies with lesions of the dopaminergic pathway using MPTP or 6-OHDA-injections. L-DOPA is the best non-invasive treatment of PD, however chronic treatment with L-DOPA produces dyskinesia. Recent experiments have demonstrated that L-DOPA restores spine-pruning selectively in D2-MSNs in hemiparkinsonian mice. However, striatal rewiring and excitability has been only studied in 6-OHDA-models of PD where the use of the toxin might cause compensatory mechanisms. Therefore, to conclude that this effect is due to L-DOPA-treatment we used the Pitx3-/- or aphakia mouse which represent genetic model of PD and thus we avoid the use of neurotoxins.
We crossed Pitx3-/- mice with bacterial artificial chromosome (BAC)-mice to identify D1- and D2-MSNs to study the synaptic and structural plasticity changes induced by L-DOPA.
Our results demonstrated that in dorsolateral striatum, both D1- and D2-MSNs have shorter dendritic tree, lower spine density and higher number of action potential compared to those in wild type (WT) mice. After chronic L-DOPA-induced dyskinesia treatment, D2-MSNs which do not reestablish the length of their dendritic arbor selectively restore their spine-density and their firing rate. By contrast, in D1-MSNs, coinciding with the supersensitization of D1-receptors, the length of the dendrites does not recover and the loss of spines became more evidence in dyskinetic mice. Moreover, the firing rate remains enhanced at higher intensities.
These results demonstrated that Pitx3-/- mice develop the same alterations described in patients of PD and thus, are a good model to study the dendritic remodeling of MSNs underlyied to PD and dyskinesia.

This work was supported by grants from the Spanish MINECO, ISCIII (SAF2013-48532-R), PNSD (PNSD2012I071) CIBERNED (CB06/05/0055) and CAM (S2011/BMD-2336).

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

AUTOPHAGY ALTERATIONS IN PARKINSON´S DISEASE: ROLE OF MUTATIONS IN THE GBA GENE

L Orgaz*[1,2], G Bueno-Gil*[1,2], P García-Sanz [1,2], I Espadas[1,2], E Rodríguez-Traver[1,2], J Kulisevsky[2,3], RA González-Polo[2,4], JM Fuentes[2,4], A Gutierrez[2,5], C Vicario[1,2] and R Moratalla[1,2]

[1]. Instituto Cajal, CSIC, Madrid

[2]. CIBERNED, Madrid

[3]. Servicio de Neurología, Hospital de la Santa Creu i Sant Pau, Universitat Autónoma de Barcelona, Institut d'Investigacions Biosanitáries Sant Pau, Barcelona

[4]. Departamento de Bioquímica y Biología Molecular y Genética, F. Enfermería y T.O., Universidad de Extremadura, Cáceres

[5]. Departamento de Biología Celular, Genética y Fisiología, Facultad de Ciencias, Universidad de Málaga, Málaga.

* Orgaz and Bueno-Gil contributed equally to this work.

Parkinson's disease (PD) is one of the most common neurodegenerative disorders. The major motor features of PD are the result of the loss of dopamine neurons in the substantia nigra pars compacta (SNc) of the midbrain. Despite extensive experimental effort, the cause of neuronal degeneration in PD is not fully understood. Mitochondrial dysfunction, oxidative stress, abnormal protein handling, excitotoxicity, and apoptotic and inflammatory processes have been implicated in the cascade of events leading to loss of dopaminergic neurons in the SNc.

A growing body of evidence show that mutations in the glucocerebrosidase (*GBA*) gene, which encodes the lysosomal enzyme that is deficient in Gaucher's disease (GD), are important and common risk factors for PD. However, the mechanisms by these mutations predispose to cell death remain unclear. Fibroblasts share the genetic complexity of neurons and represent an easily accessible source of proliferating cells, making them a unique patient-specific cellular model of different neurodegenerative diseases. To study the role of heterozygous GBA mutations in the pathology of PD, we generated fibroblast lines from skin biopsies of five PD patients with heterozygous GBA mutation carriers (N370S and L444P) and four controls.

We found that both GBA mutations present significantly reduced level of GCase protein and enzyme activity as well as an altered autophagy flux and lysosomal function due to an accumulation of misfolded proteins in endoplasmic reticulum.

This work was supported by grants: CIBERNED-ISCIII (CB06/05/0055 and collaborative project), MINECO (SAF2013-48532-R) and CAM (S2011/BMD-2336).

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

THE ACCUMULATION IN GLUTAMINASE CONTRIBUTES TO EXCITOTOXICITY IN ALZHEIMER'S DISEASE

T. Fuchsberger, A. Lloret, V. Teruel-Marti, S. Martinez-Bellver, P. Monllor, E. Giraldo, J. Vina
Facultad de Medicina. Universidad de Valencia, Valencia

Background

A growing body of evidence suggests that excitotoxicity plays a major role in Alzheimer's disease (AD), but the molecular mechanisms of this phenomenon are still not fully understood. Glutaminase is one of the major sources for glutamate in neurons. Here we describe a new pathway by which amyloid beta (Aβ) causes an elevated generation of glutamate in neurons, mediated by the down-regulation of E3 ubiquitin ligase anaphase-promoting complex/cyclosome (APC/C) and its activator cdh1, which leads to accumulation of glutaminase.

Material and Methods

Neurons were isolated from fetal Wistar rats (14 dpf) and cells were seeded (2.5*10^5 cells/cm²) on polylysine covered plates in DMEM (Sigma) supplemented with 10% (v/v) fetal bovine serum and incubated at 37°C in a 5% CO²-containing atmosphere. Neurons were used by day 7 for treatments with Aβ (5 μM for 20 h), glutamate (100 – 500 μM for 20 hours), compound 963 (100 μM) for glutaminase inhibition. The samples were analysed by immunoblotting, enzymatic assay for glutamate determination, confocal microscopy, immunohistology and flow cytometry.

Results

Aβ treatment or APC/C inhibition using proTAME leads to accumulation of glutaminase and this leads to an elevation of glutamate concentration in the extracellular medium. The increase of glutamate caused by Aβ or proTAME can be attenuated by inhibition of glutaminase and is completely abolished when using glutamine-free medium. Moreover, Aβ or proTAME-induced apoptosis can be reduced by inhibition of glutaminase.

Conclusions

These results indicate that glutaminase accumulates in Alzheimer's disease due to the inactivation of the cell cycle related ubiquitin ligase APC/C-Cdh1. We suggest that this pathway might explain the slow excitotoxicity shown in the disease.

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

SIRT1 OVEREXPRESSION IS NEUROPROTECTIVE IN 3xTg-AD MICE

R. Corpas[1], S. Revilla[1], S. Ursulet[2], L Giménez-Llort[3], C. Sarkis[2], M. Pallàs[4], C. Sanfeliu[1]
[1] Institute of Biomedical Research of Barcelona (IIBB), CSIC-IDIBAPS, Barcelona, Spain
[2] NewVectys, Villebon-sur-Yvette, France.
[3] Department of Psychiatry and Forensic Medicine, Autonomous University of Barcelona, Bellaterra, Spain.
[4] Department of Pharmacology and Therapeutic Chemistry, Faculty of Pharmacy, IBUB, University of Barcelona and CIBERNED, Barcelona, Spain.

Background: SIRT1 pathway is involved in longevity and cell survival through regulating the acetylation homeostasis of key proteins. The expression level of this deacetylase enzyme is decreased in the brain tissue of 3xTg-AD, a mouse model of Alzheimer's disease (AD) that shows cognitive decline and increased amyloid β (Aß) and phosphorylated tau (p-tau). We had previously shown that a neuroprotective therapy of physical exercise recovered SIRT1 expression levels in the hippocampus of 3xTg-AD mice. Therefore, SIRT1 might be a target for neuroprotection against AD.
Objectives: We aimed to study the effects of SIRT1 enhancement through SIRT1 overexpression in hippocampal neurons of 3xTg-AD, and to study the underlying neuroprotective mechanisms in neuronal cultures from these mice.
Material & methods: Bilateral infusions of a recombinant lentiviral vector encoding mouse SIRT1 or GFP were injected into the dorsal CA1 hippocampal area of 3xTg-AD and non-transgenic (NoTg) mice of 4-month-old. Neuron cultures obtained from 3xTg-AD and NoTg mouse embryos were transduced with lentiviral vectors at 4 DIV and analyzed at 11 DIV.
Results: Six-month chronic overexpression of SIRT1 induced a significant protective effect against AD pathology, leading to preserved learning and memory capacities in 10-month-old 3xTg-AD mice. Analysis showed a decrease of Aβ and p-tau in brain tissue of SIRT1-injected mice and in SIRT1-transduced neurons cultures. Furthermore, SIRT1 induced higher expression of ADAM10, IDE, neurotrophic factors and synaptic plasticity markers.
Conclusions: Increase of ADAM10 and IDE expression suggests an up-regulation of the non-amyloidogenic and the Aß degradation pathway, respectively. Reduction of acetylated tau suggests that prevention of tau tangle formation is produced through p-tau deacetylation and thus allows tau degradation. The neuroprotective effects of SIRT1 demonstrated the modulatory power of this molecule to prevent age-related neurodegenerative disorders.
Acknowledgements: Supported by SAF2012-39852 and CSD2010-00045, MINECO, Spain, and the European Regional Development Fund.

1ª: Nuevos métodos y tecnologías
2ª: Neurociencia cognitiva y conductual

ROLE OF APC/C-CDH1 IN IN THE PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE

T. Fuchsberger, A. Lloret, V. Teruel-Marti, S. Martinez-Bellver, P. Monllor, E. Giraldo, J. Vina
Facultad de Medicina. Universidad de Valencia, Valencia

Background

The anaphase promoting complex/cyclosome (APC/C)-Cdh1 is a large protein complex forming an E3 RING finger ubiquitin ligase that has a major role as cell cycle regulator. A new role of APC/C-Cdh1 in post-mitotic neurons has been discovered, and it was shown that it controls neuronal cell cycle exit, axonal growth, differentiation and synaptic development. Dysregulation of APC/-Cdh1 have been related to neurodegenerative diseases, but its implications in Alzheimer's disease remains unclear.

Material and Methods

Neurons were isolated from fetal Wistar rats (14 dpf) and cells were seeded (2.5*10^5 cells/cm²) on polylysine covered plates in DMEM (Sigma) supplemented with 10% (v/v) fetal bovine serum and incubated at 37°C in a 5% CO^2-containing atmosphere. Neurons were used by day 7 for treatments with Aβ (5 μM for 20 h), APC/C inhibition by proTAME (16 μM for 20 hours), roscovitine (15 μM for 20 hours), or cdh1-silencing. The samples were analysed by immunoblotting, confocal microscopy, immunohistology and flow cytometry.

Results

Our results show that the Alzheimer's Disease-related peptide $A\beta_{(1-42)}$ decreases the protein level of nuclear cdh1 in neurons in culture. These treatments increase intracellular Ca^{2+} levels, which stabilizes Cdk5-p25 levels. Inhibition of the cdk5 inhibits Aβ-induced cdh1 degradation. Decreased APC/C-Cdh1 activity was measured by accumulation of degradation targets of the ubiquitin ligase and we observed accumulation of cyclin B1 and glutaminase after Aβ treatment. The inhibition of APC/C-Cdh1 caused apoptosis in neurons.

Conclusions

The decrease of cdh1 caused by Aβ exhibits a direct implication of APC/C-Cdh1 in Alzheimer's disease. APC/C-Cdh1 controls the degradation of several proteins which are implicated in important processes of neurons. The ubiquitin ligase might therefore be an interesting target in Alzheimer's disease research and treatment.

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

DISMANTLEMENT AND REBUILDING OF CALYCEAL SEPTATE JUNCTIONS IN A MOUSE MODEL OF CHRONIC VESTIBULAR TOXICITY.

J. Llorens [1,2], E. Greguske [1], M. Bosch-Mola [1], M. Deulofeu [3], B. Álvarez-Pérez [3], M. Carreres-Pons [1], S. Prades[1], E. Verdú [3], L. Sedó-Cabezón [1], P. Jedynak [1], J. Homs [3], P. Boadas-Vaello [3].
[1]. Facultat de Medicina. Universitat de Barcelona, L'Hospitalet de Llobregat, Barcelona
[2]. IDIBELL, L'Hospitalet de Llobregat, Barcelona
[3]. Facultat de Medicina. Universitat de Girona, Girona

Ototoxic compounds cause degeneration of the mechanotransducer hair cells in the vestibular and auditory sensory epithelia. However, increasing evidence indicate that other pathogenic mechanisms may also be relevant to ototoxicity. Recently, we have demonstrated that chronic ototoxic exposure may cause significant loss of vestibular function before degeneration of the hair cells begins. This has been observed in rats exposed to 3,3'-iminodipropionitrile (IDPN) through drinking water, which suffered a reversible dismantlement of the septate junction that characterizes the contact between the type I hair cells and their afferents. These afferents have a calyx shape and encase the amphora-shaped hair cell up to its neck. In this study, we have developed a mouse model to study this synaptic pathology preceding hair cell loss during chronic ototoxicity. We tested both sexes of two strains of mice, 129S1/SvImJ and RjOrl:Swiss/CD-1, at several different IDPN concentrations in the drinking water, and found that male 129/S1mice exposed to 30 mM offer the desired model. In this model, vestibular dysfunction, assessed weekly by a test battery, appeared progressively and reverted after the intoxication was terminated at 5 weeks of exposure. We compared the distribution of septate junction proteins in control mice, exposed mice, and mice allowed recovery for 5 weeks after being exposed for 5 weeks. Immunohistochemical confocal microscopy analysis showed loss of the tenascin-C and caspr1 labeling, and mislocalization of the KCNQ4 labeling, denoting the dismantlement of the septate junctions. In the recovery animals, the distribution of these proteins revealed an almost complete rebuilding of the septate junction, although some differences with the control situation persisted. This model shall be useful to understand the role of the septate junction in vestibular physiology and the relevance of afferent pathology in chronic vestibular dysfunction.

Supported by grants BFU2012-31164 (MINECO-FEDER) and 2014SGR943 (AGAUR).

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

A CASE STUDY OF THE FUNCTIONAL IMPACT OF A *DE NOVO* NMDA RECEPTOR MISSENSE MUTATION IN GENETIC INTELLECTUAL DISABILITY

D. Soto [1], M. Olivella [2], C. Grau [1], M. Gómez de Salazar [1], À. Bayés [3], C. Sindreu [4], I. Ferrer [1], J. Armstrong [5,7], À. García-Cazorla [6,7], X. Altafaj [1]

[1]. Bellvitge Biomedical Research Institute (IDIBELL), Barcelona

[2]. Universitat de Vic, Barcelona

[3]. Molecular Physiology of the Synapse Laboratory, Biomedical Research Institute Sant Pau (IIB Sant Pau), Barcelona

[4]. Universitat de Barcelona, Barcelona

[5]. Genetics and Molecular Medicine Service, Hospital Sant Joan de Déu, Barcelona

[6]. Department of Neurology and Laboratory of Synaptic Metabolism, Hospital Sant Joan de Déu, Barcelona

[7]. Centro de Investigación Biomédica para el Estudio de las Enfermedades Raras, CIBERER

Autosomal dominant non-syndromic intellectual disability (NSID) is a rare disease with a multigenic origin. A growing number of genetic studies have identified *de novo* NMDA receptor (NMDAR) mutations associated with NSID. However, the underlying molecular mechanisms linking these mutations with neurological impairment remain elusive. The present study is based on a clinical case of a 4 years-old patient with a severe encephalopathy with epileptic seizures and intellectual disability. Genetic analysis by WES and Sanger sequencing showed the presence of a *de novo* missense mutation of *GRIN2B*, affecting a single amino acid position (P553T) located in the close vicinity of the M1 transmembrane domain. Because GluN2B plays a pivotal role in synaptic development and plasticity, and considering the location of the mutation close to the pore of the channel, we hypothesized that *GRIN2B* missense mutation could perturb NMDAR channel activity, ultimately leading to neuronal dysfunction and neurodevelopmental defects. To address this question, we expressed a GluN2B(P553T) mutant construct in cultured cells and evaluated the potential biochemical, trafficking and electrophysiological consequences on receptor activity. Biochemical studies showed that mutant GluN2B conserved its ability to physically interact with the obligatory GluN1 subunit, as well as with GluN2A. In addition, GFP-GluN2B(P553T) showed normal surface expression in transfected primary cortical neurons. In contrast, electrophysiological studies showed that although functional, mutant receptors have a significantly reduced channel conductance, together with a strong reduction of NMDA-evoked current density. These data are in agreement with our structural molecular model, and strongly suggest that this *de novo* mutation causes a loss-of-function of NMDARs. Currently, we are evaluating whether the *in vitro* administration of positive allosteric modulators of GluN2B subunit-containing NMDARs could increase the activity of mutant receptors, to therapeutically correct their hypo-functionality.

1ª: Trastornos y reparación del sistema nervioso

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

PERIPHERAL NEURODEGENERATIVE CHANGES OBSERVED IN THE CORNEA FROM A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

Bech Federico[1], Alcalde Ignacio[1], Íñigo-Portugués Almudena[1], González-González Omar[1], Artime Enol[1], Braga Paola[1], Merayo-Lloves Jesús[1,2]
[1]. Superficie Ocular. Instituto Universitario Fernández-Vega. Universidad de Oviedo, Oviedo, Asturias.
[2]. Departamento de Oftalmología. Universidad de Oviedo, Oviedo, Asturias.

Objectives
Some neurodegenerative diseases present morpho-functional alterations in the peripheral nervous system and can be detected analysing the density of intraepithelial sensory nerve fibers (IENFD index) on skin biopsies. As the cornea is the most innervated tissue of the body we established as our purpose to describe degenerative events in the cornea using a transgenic mouse model of Alzheimer's disease.
Methods
Plantar glabrous skin and corneas were obtained from transgenic APP/PSE1 double knock (KO) out and wild type (WT) C56BL/6J mice. IENFD was set as the number of intraepithelial terminals per linear mm. First we compared IENFD index in transversal 10 µm sections of plantar skin and cornea using immunohistochemical techniques to β-Tubulin III (Covance). Then we performed whole mount immunohistochemical preparations of cornea to adapt the measurements to the whole organ and to characterize the changes in the morphology of the fibers and terminals of transgenic mice. We used confocal microscopy (Leica TCS-SP2-AOBS) and 3D image reconstruction to study differences in terminal morphology between WT and KO mice.
Results
We adapted the IENFD index to transversal sections of cornea finding direct correlation with the results of plantar skin biopsies. Alterations in the morphology of corneal nerve terminals were described in Alzheimer transgenic mice. In flat whole mount preparations of corneal subbasal nerve fibers and terminals showed signs of degeneration as fibers ending without emitting any terminal and changes in density and ramification of the fibers.
Conclusions
Whole mount preparations of cornea allow to better quantify and describe peripheral degenerative changes in density and morphology of subbasal sensory nerve fibers and terminals in diseased mice. Once understood the peripheral neuropathies in the cornea associated with Alzheimer's disease, neurologists and ophthalmologists could use *in vivo* confocal microscopy on patients to follow the progression of the disease using a non invasive method.

1ª: Trastornos y reparación del sistema nervioso
2ª: Nuevos métodos y tecnologías

FRONTOBASAL GRAY MATTER LOSS IS ASSOCIATED WITH THE TREM2 P.R47H VARIANT.

E.O.Luis[1], S. Ortega-Cubero[2], I. Lamet[3], C. Razquin[4], C. Cruchaga[5], BA. Benitez[5], E. Lorenzo [4], J. Irigoyen[2]; Alzheimer's Disease Neuroimaging Initiative (ADNI), M. A. Pastor[6], P. Pastor[7].

[1]Neuroimaging Laboratory, Division of Neurosciences, Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain.

[2]Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research, University of Navarra, Pamplona, Spain; Department of Neurology, Clínica Universidad de Navarra, University of Navarra School of Medicine, Pamplona, Spain.

[3]Department of Neurology, Clínica Universidad de Navarra, University of Navarra School of Medicine, Pamplona, Spain.

[4]Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research, University of Navarra, Pamplona, Spain.

[5]Hope Center for Neurological Disorders, Department of Psychiatry, Washington University School of Medicine, St Louis, MO, USA.

[6]Neuroimaging Laboratory, Division of Neurosciences, Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain; Department of Neurology, Clínica Universidad de Navarra, University of Navarra School of Medicine, Pamplona, Spain; Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain.

[7]Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research, University of Navarra, Pamplona, Spain; Department of Neurology, Clínica Universidad de Navarra, University of Navarra School of Medicine, Pamplona, Spain; Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain.

A rare heterozygous TREM2 variant p.R47H (rs75932628) has been associated with an increased risk for Alzheimer's disease (AD). We aimed to investigate the clinical presentation, neuropsychological profile, and regional pattern of gray matter and white matter loss associated with the TREM2 variant p.R47H, and to establish which regions best differentiate p.R47H carriers from noncarriers in 2 sample sets (Spanish and Alzheimer's Disease Neuroimaging Initiative, ADNI1). This was a cross-sectional study including a total number of 16 TREM2 p.R47H carriers diagnosed with AD or mild cognitive impairment, 75 AD p.R47H noncarriers and 75 cognitively intact TREM2 p.R47H noncarriers. Spanish AD TREM2 p.R47H carriers showed apraxia (9 of 9) and psychiatric symptoms such as personality changes, anxiety, paranoia, or fears more frequently than in AD noncarriers (corrected p = 0.039). For gray matter and white matter volumetric brain magnetic resonance imaging voxelwise analyses, we used statistical parametric mapping (SPM8) based on the General Linear Model. We used 3 different design matrices with a full factorial design. Voxel-based morphometry analyses were performed separately in the 2 sample sets. The absence of interset statistical differences allowed us to perform joint and conjunction analyses. Independent voxel-based morphometry analysis of the Spanish set as well as conjunction and joint analyses revealed substantial gray matter loss in orbitofrontal cortex and anterior cingulate cortex with relative preservation of parietal lobes in AD and/or mild cognitive impairment TREM2 p.R47H carriers, suggesting that TREM2 p.R47H variant is associated with certain clinical and neuroimaging AD features in addition to the increased TREM2 p.R47H atrophy in temporal lobes as described previously. The high frequency of pathologic behavioral symptoms, combined with a preferential frontobasal gray matter cortical loss, suggests that frontobasal and temporal regions could be more susceptible to the deleterious biological effects of the TREM2 variant p.R47H.

1ª: Trastornos y reparación del sistema nervioso

ASSESSMENT OF DISTRIBUTION AND INTERACTIONS OF ALPHA SYNUCLEIN IN NEURONAL MEMBRANE DOMAINS AS A CONSEQUENCE OF PARKINSON DISEASE PATHOLOGY.

A. Canerina-Amaro[1], D. Rodríguez-Barreto[2], N. Fabelo[2], M. Díaz[2], R. Marín[1].
[1]Facultad de Ciencias de la Salud; [2]Facultad de Ciencias, Universidad de La Laguna, Tenerife, Spain.

Our previous work has demonstrated that structural lipids of lipid raft microdomains are altered in cortical areas of Parkinson's disease (PD), even at early preclinical stages. Here, we aim to elucidate whether these alterations may be related to changes in the proteins associated with these microstructures and, consequently, with cognitive deterioration during neuropathological progression.
Here, using a murine model of PD treated with MPTP as neurotoxic, we have purified lipid rafts from different brain areas, and analyzed their lipid composition. Furthermore, in order to determine whether the observed raft lipid alterations may be involved in PD pathological events, we have analyzed by immunoblotting and immunoprecipitation whether the neurotoxin treatment affects raft lipid composition and also if these potential alterations correlate with redistribution and interactions of α-synuclein, a main hallmark of this disease which is integrated in these microstructures. Different brain areas (septum, frontal and posterior cortices and cerebellum) of mice at different ages were investigated. The potential associations of α-synuclein with other raft protein markers related to toxic pathways have also been analyzed.
Our data suggest that, structural alterations of lipid rafts as a consequence of neuronal injury may also affect the behaviour of proteins interacting within these structures, altering their normal behavior and dynamics, and ultimately contributing to neuronal dysfunctions.

Supported by SAF2010-22114-C02-01/02. A. Canerina-Amaro holds a fellowship from Fundación CajaCanarias-La Caixa. We also acknowledge the support of Agencia Canaria de Investigación, Innovación y Sociedad de la Información (ACIISI)

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

MELATONIN RESTORES MITOCHONDRIAL REGULATORY REGULATORY GENE EXPRESSION AND FUNCTION IN PARKINSONIAN ZEBRAFISH

ME. Díaz-Casado[1,2], E. Lima[1], JA. García[1,2], H. Volt[1,2], C. Doerrier [1,2], G. Escames[1,2], D. Acuña-Castroviejo[1,2,3]

[1]Instituto de Biotecnología, Centro de Investigación Biomédica, Parque Tecnológico de Ciencias de la Salud, Universidad de Granada, Avda. del Conocimiento s/n, 18100 Armilla, Granada, Spain.

[2]Departamento de Fisiología, Facultad de Medicina, Universidad de Granada, Avenida de Madrid 11, 18012 Granada, Spain.

[3]Unidad de Gestión Clínica de Laboratorios Clínicos, Hospital Universitario San Cecilio, Avenida Dr. Olóriz s/n, 18012 Granada, Spain.

Introduction: Parkinson's disease (PD) is a neurological disorder characterized by the loss of dopaminergic neurons, oxidative stress and inflammation. Both mechanisms are related to mitochondrial dysfunction and bioenergetic failure, which promotes neuronal death. The neurotoxin MPTP, which specifically inhibits mitochondrial complex I activity, is usually used to yied parkinsonism in roedents and zebrafish. The association between mitochondrial dysfunction and neurodegeneration is gaining experimental support in PD, which can be associated with mutations in mitochondria-associated genes including PARK2 (parkin) PARK6 (pink1), PARK7 (dj-1), alpha-synuclein, and MUL1. Because melatonin has neuroprotective effects in mouse model of PD (1), we evaluated here whether melatonin affect the regulation of these genes in the zebrafish model of PD.

Methods: Zebrafish embryos of the AB line were used at 24 hpf. The embryos were randomly divided in: control group (C); MPTP group treated with 600 µM MPTP; aMT1 and aMT2 groups, comprising groups treated with MPTP plus 0.2 µM or 1 µM melatonin, respectively. Embryos were treated for 48 h and analyzed at 72 hpf. Complex I activity and analysis of gene expression (qRT-PCR, immunohistochemistry and Western Blot) of THase, PARK7, alpha-synuclein, PARK2, PARK6, MUL1 and iNOS, were preformed.

Results: Complex I activity decreased by 55% in MPTP-treated embryos, whereas melatonin counterated this effect. MPTP also caused a significant decrease in the mRNA levels and protein content of TH, PARK2, PARK6 and PARK7, as well as a significant increase in the mRNA and protein content of iNOS and alpha-synuclein versus control. The administration of melatonin counteracted these changes dose-dependently, recovering the normal mRNA expression and protein content of the studied genes.

Conclusion: These results suggest that 72 hpf embryos treated with MPTP showed a PD-like feature, consisting in a significant damage of their mitochondria, accompanied by mitochondria-related genes impairment. These effects were antagonized by melatonin treatment, supporting a functional role of this indoleamine in controlling mitochondrial homeostasis even in the presence of a strong neurodegenerative process.

Supported by grants P10-CTS-75784 and CTS-101 from the Consejería de Economía, Innovación y Ciencia (Junta de Andalucía, Spain).

1.- Khaldy, H., et al. Neurobiol Aging; 2003 24:491-500.

MELATONIN RESTORES MITOCHONDRIAL FUNCTION AND LOCOMOTOR ACTIVITY IN PARKINSONIAN ZEBRAFISH

ME. Díaz-Casado[1,2], JA. García[1,2], JA. Guerrero-Martínez[1,2], A. López[1,2], I. Rusanova[1], G. Escames[1,2], D. Acuña-Castroviejo[1,2,3]

[1]Instituto de Biotecnología, Centro de Investigación Biomédica, Parque Tecnológico de Ciencias de la Salud, Universidad de Granada, Avda. del Conocimiento s/n, 18100 Armilla, Granada, Spain.

[2]Departamento de Fisiología, Facultad de Medicina, Universidad de Granada, Avenida de Madrid 11, 18012 Granada, Spain.

[3]Unidad de Gestión Clínica de Laboratorios Clínicos, Hospital Universitario San Cecilio, Avenida Dr. Olóriz s/n, 18012 Granada, Spain.

Melatonin is an first-rate antioxidant able to maintain mitochondrial homeostasis under conditions of strong oxidative stress and bioenergetic failure such as that produced by MPTP (1). This study was designed to evaluate whether melatonin protects mitochondria and restores locomotor activity in parkinsonian zebrafish embryos. 24 hpf zebrafish embryos (AB) were incubated with MPTP (600 μM) and/or melatonin (200 nM -1 μM) for 2 days and studied at 72 hpf. Complex I activity, SOD, glutathione cycle enzymes (PCR and activity), *in vivo* mitochondrial respiration (Seahorse), and macroscopic malformations, were analyzed. Another group of zebrafish embryos was followed up to 120 hpf to evaluate motility (Smart videotracking system). In the group of 72 hpf, the results show that MPTP significantly reduced complex I activity and affected mRNA expression of SOD and glutatione enzymes, yielding an hyperoxidative status. Here, basal and maximal respiration was blunted by MPTP, increasing proton leak and RCR, reducing the ATP production. Melatonin treatment was able to counteract the effects of MPTP, restoring the normal physiology of the zebrafish embryos. In the group of 120 hpf, iNOS and α-synuclein expression remained elevated, and both the distance travelled and maximal speed were strongly reduced compared to untreated embryos. Melatonin also prevented the deleterious effects of MPTP, maintaining a normal locomotor activity. Finally, MPTP induced morphological malformations of the embryos, including oedema, alterations in yolk differentiation and tail malformations, which were mostly prevented by melatonin. The results support that the MPTP model of zebrafish parkinsonism shared similar degenerative features that mammal models, in terms of oxidative stress, complex I inhibition, and mitochondrial bioenergetic impairment, leading zebrafish embryos to malformations and impaired locomotor activity. Melatonin, which is known by its specific effects on mitochondria, where it was able to absolutely prevent the MPTP injury, restoring the normal status of the zebrafish embryos. We can conclude that parkinsonian zebrafish embryos are an excellent model to study the neuroprotective properties of melatonin.

Supported by grants P10-CTS-75784 and CTS-101 from the Consejería de Economía, Innovación y Ciencia (Junta de Andalucía, Spain).

1. Khaldy, H., et al. Neurobiol Aging 2003; 24:491-500.

ELECTROPHYSISIOLOGICAL STUDY BY mfERG OF A CASE OF RETINAL HYDROXYCHLOROQUINE TOXICITY

J. M. Rodríguez-Ferrer [1], J. A. Sáez Moreno [1]
[1] Laboratorio de Neurociencia Visual y Cognitiva, Instituto de Neurociencias. Universidad de Granada, Granada

Background: Hydroxychloroquine (HCQ) that is widely used for the treatment of rheumatic and connective tissue diseases can produce permanent retinal toxicity with visual loss and interruption of its administration is the only management of the toxicity. Therefore, early detection of retinopathy, when the retinal alterations can be reversed, is essential and a current challenge of medicine. Multifocal electroretinography (mfERG) is the most advanced method to study the electrophysiological activity of the retina in humans and it is considered the most sensitive technique to detect retinal functional alterations induced by HCQ.
Purpose: To study the effect on the retina of HCQ treatment cessation in a patient of a connective tissue disease with suspicion of HCQ retinal toxicity.
Methods: mfERG recordings were performed in a woman of 49 years of age before and 4 months after cessation of the treatment with HCQ. The stimulus matrix consisted of 61 hexagons elements that independently alternated between black and white at a rate of 75Hz. Response P1 density (nV/deg^2) was obtained from the 61 responses grouped in five concentric rings, corresponding ring 1 to the fovea.
Results: In a first mfERG the patient showed low P1 density in the peripheral retina of both eyes, rings 2-5, and in the central area of the left eye, ring 1, probably due to an early HCQ toxicity. In a second mfERG, 4 months after drug withdrawal, it was observed the normalization of the response P1 densities obtained in both eyes, with marked improvement in the macular region of the left eye.
Conclusions: These results show an uncommon case of rapid recovery of retinal toxicity from HCQ, as well as the usefulness of mfERG for the safe handling of this drug treatment in the prevention of its retinal toxicity.

1ª: Disorders and nervous system repair
2ª: Systems Neuroscience

MAJOR ROLE OF AURORA B IN THE REGULATION OF TUNNELING NANOTUBES (TNT) FORMED BY GLIOMA STEM CELLS

J.R. Pineda [1,2], M. Jeitany [1], T. Kortulewsky [1], L. Gauthier [1], M.P. Junier [3,4,5,*], H. Chneiweiss [3,4,5,*], A. Andrieux [6], F.D. Boussin[1]

[1]. CEA DSV iRCM SCSR, INSERM, Laboratoire de Radiopathologie, UMR967, F-92265 Fontenay-aux-Roses, France

[2]. Achucarro Basque Center for Neuroscience, Parque Tecnológico de Bizkaia, E-48170 Zamudio, Vizcaya, Spain. Department of Neurosciences, University of the Basque Country, 48940 Leioa, Spain.

[3]. CNRS UMR8246 Neuroscience Paris Seine - IBPS; 7 quai Saint-Bernard 75005 Paris, France

[4]. INSERM U1130, Neuroscience Paris Seine - IBPS; 7 quai Saint-Bernard 75005 Paris, France

[5]. University Pierre and Marie Curie UMCR18, Neuroscience Paris Seine - IBPS; Team GP; 7 quai Saint-Bernard 75005 Paris, France

[6]. CEA, IRTSV-GPC, INSERM, U836-GIN Site Santé La Tronche, BP170, 38042 Grenoble, Cedex 9, France

*Contributed equally to this work

Glioblastoma multiforme is the latest stage of malignant tumor of the adult central nervous system. Actual palliative treatments extend the median of survival to less than one year. Surgical resection fails to completely extirpate the tumor given the high infiltrative nature of invading cells with high stemness characteristics identified as glioma stem-like cells (GSC). GSC share characteristics of neural stem cells (NSC) phenotypes such as the capacity of long-term proliferation and self-renewal.

One of the key aspects of glioma dispersion is the ability to coordinate cell activities to integrate and synchronize tasks such as migration and invasion. Intercellular bridges, also known as tunneling nanotubes (TNT) were first described in cells of the immune system as an intimate form of long-range communication coordinating cell-division and migration. TNT are *de novo* formation of a thin membrane channels with F-Actin cytoskeleton that permits direct transfer and share of cytoplasm content.

In this work we found that GSC are able to create TNT structures in which Aurora B kinase plays a major role for TNT scission. Contrary to mid-bodies, TNT structures are devoid of INCENP and accumulation of Aurora B is asymmetrical between cells. Its genetical or pharmacological inhibition by siRNA or using a specific inhibitor Hesperadin impairs TNT scission promoting a progressive cell clustering. Our results show that upon inactivation of Aurora B the molecular scissor FIP3 is lost from the TNT and a progressive reduction of focal adhesion kinases is observed. Both mechanisms block GSC dispersion independently of cell viability or cell cycle status, leading to progressive GSC aggregation into a cellular mass where no cell can escape.

1st: Disorders and nervous system repair.
2nd: New methods and technologies.

SELECTIVE siRNA-MEDIATED SUPPRESSION OF ASTROGLIAL GLUTAMATE TRANSPORTERS INDUCES DEPRESSIVE-LIKE BEHAVIORS IN MICE

Neus Fullana [1,2,3], A. Bortolozzi [1,2,3], F. Artigas [1,2,3]
[1]. Instituto de Investigaciones Biomédicas de Barcelona – CSIC, Barcelona
[2]. Instituto de Investigaciones Biomédicas August Pi i Sunyer – IDIBAPS, Barcelona
[3]. Centro Investigación Biomédica en Red de Salud Mental – CIBERSAM, Madrid

Classically, major depression (MD) has been associated with an abnormal monoaminergic function. However, alterations of the energy metabolism and reduced glial populations in ventral cingulate regions of depressive patients have also been reported. Further, deep brain stimulation of this area evokes immediate antidepressant effects in treatment-resistant patients. These observations suggest that an abnormal excitatory neurotransmission in ventral cingulate may be involved in the pathophysiology of MD. In an attempt to mimic these alterations in rodents, we knocked-down the expression of the astrocytic glutamate transporters GLAST and GLT-1, which remove synaptic glutamate, in order to evoke an increase of excitatory neurotransmission in the infralimbic (IL) subdivision of the prefrontal cortex (PFC), the rodent equivalent of ventral cingulate. We microinjected small interfering RNA (siRNA) molecules directed against GLAST or GLT-1 (GLAST-siRNA or GLT-1-siRNA, respectively) unilaterally into prelimbic (PrL) or infralimbic (IL) subdivisions of the mouse PFC and examined the cellular and behavioral effects. Local GLAST-siRNA infusion in the IL (4,2 nmol) reduced GLAST mRNA levels 24 h post-administration to ~20% of control mice and evoked a depressive-like behavior (increased immobility) in the tail suspension test (TST). Likewise, intra-IL infusion of GLT-1-siRNA (4,2 nmol) reduced GLT-1 expression and evoked an increased immobility time in the TST compared to control mice. However, unilateral infusion of GLAST-siRNA or GLT-1-siRNA in the PrL did not affect mouse behavior in the TST, consistent with the predominant involvement of IL in affective functions. Overall, the present results suggest that down-regulation of astroglial GLAST and GLT-1 in the IL (but not PrL) impairs the physiological clearance of synaptically released glutamate and disrupts the functional connectivity between IL and cortio-limbic areas involved in MD. These findings also help to identify regional and pharmacological targets for the development of more rapid and effective antidepressant treatments.

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

Grants: *Spanish Ministry of Economy and Competitiveness, SAF2012-35183 (FA); Instituto de Salud Carlos III PI13/01390 (AB) co-financed by the European Regional Development Fund "A way to build Europe".*

THE EPISODIC MEMORY, RETROACTIVE INTERFERENCE AND VOLUME OF THE TEMPORO MESIAL STRUCTURE

ME García Navarro
L. Morales Chacon
Centro Internacional de Restauración Neurológica. CIREN.
Ciudad de la Habana, Cuba.

The relation between the volume of mesial temporal structures (MTS) with the episodic memory, and the high vulnerability to the retroactive interference in the patients with affectation in this subtype of memory is recognized. We studied the influence of the volume of the MTS in the episodic memory and the sensibility to the retroactive inhibition.
Volumetry´s study were made and was used a paradigm of verbal memory, formed by two conditions, one with interference and another without. Both studies were applied to 11 healthy controls and 11 patients with temporal lobe epilepsy (TLE) who had undergone temporal lobectomy. A norm of the volume of the structures in the normal population was constructed. The patients, although showed a greater vulnerability to the retroactive interference, were like the controls, beneficiaries in their memory when this one was diminished. The pole of temporary superior is an important
structure for the process of episodic memory showing to greater volume of resections, the greater effect of the interference. In the rest of the MTS significant correlations were not obtained, supposing that the compensation effect can be in the base of this behavior. The patients with TLE with surgical treatment present the high sensitivity to the retroactive inhibition and the pole of temporary left plays an important role in the episodic memory.

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

FROM UNILATERAL TO BILATERAL PARKINSONISM: EFFECTS OF LATERALIZATION AND ASSOCIATED MOLECULAR MECHANISMS.

C. Marin[1,*], M. Bonastre[1], G. Mengod[2,4], R. Cortés[2,4], M.C. Rodríguez-Oroz[3,4,*]
[1] IRCE, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona
[2] Institut d'Investigacions Biomèdiques de Barcelona, CSIC-IDIBAPS, Barcelona
[3]Instituto de Investigación Sanitaria Biodonostia, San Sebastian
[4]CIBERNED, Madrid
[*]C. Marin and M.C. Rodríguez-Oroz act as equivalent co-senior authors

Parkinson's disease (PD) is a neurodegenerative disorder that usually has a unilateral motor onset, either the right or left side, becoming bilateral as disease progresses. The mechanisms underlying lateralization and progression of motor symptoms from unilateral to bilateral in PD remain to be elucidated. In addition, the molecular mechanisms involved in levodopa-induced dyskinesias (LIDs) depending on lateralization and disease progression from unilaterally to bilateral have not been described yet.

We investigated motor symptoms, LIDs and associated striatal molecular markers expression after unilateral left or right, and after a sequential bilateral 6-hydroxydopamine (6-OHDA)-induced nigrostriatal lesions in rats.

Sequentially bilateral lesioned animals showed a bilateral increase in striatal preproenkephalin (PPE) mRNA ($p<0.01$) without changes in pre-prodynorphin (PDyn) mRNA expression. The increase in dyskinesias when parkinsonism becomes bilateral was mostly due to an increase in orolingual dyskinesias ($p<0.01$) associated to a increase in PDyn mRNA expression ($p<0.01$). Right lesion induces, or facilitates when first-done, a greater level of LIDs ($p<0.05$) and an increase in striatal PPE and PDyn mRNAs in the second lesioned side ($p<0.01$).

Our results describe a new sequential bilateral model of parkinsonism in rats that allowed us to show the new striatal molecular pattern that appears when parkinsonism becomes bilateral. In addition, our results show the relevance of the lateralization for the development of higher levels of dyskinesia and point out a relevant role for striatal PDyn mRNA expression in the development of dyskinesias when parkinsonism becomes bilateral.

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

NMDA-INDUCED OLFACTORY BULB EXCITOTOXICITY AND OLFACTORY DYSFUNCTION AS A MODEL FOR SECONDARY NEURONAL DEGENERATION IN TRAUMATIC BRAIN INJURY: ROLE OF ADULT NEUROGENESIS.

I. Tejero[1], I. Alobid[2], J. Berenguer[2], M. Bernabeu[3], S. Centellas[2], S. Laxe[3], E. Lehrer[2], F. Mariño-Sánchez[2], J. Mullol[1,2], C. Marin[1]
[1]IRCE, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona
[2]Rhinology Unit & Smell Unit, ENT, and CDI Departments, Hospital Clínic, Barcelona
[3]Institut Guttmann, Barcelona

Traumatic brain injury (TBI) constitutes one of the main causes of olfactory dysfunction. One event related to TBI is the secondary neuronal degeneration (SND), a downstream cascade of events promoting further damage. Excitotoxicity is a key factor in SND since during the TBI acute phase, a massive release of glutamate occurs. The role of excitotoxicity on TBI olfactory dysfunction is still unknown.
Our goal was to examine the olfactory dysfunction and to investigate the changes in neurogenesis markers induced by the bilateral administration of the glutamate agonist N-methyl-D-aspartate (NMDA) in the rat olfactory bulbs (OB), as an experimental model of SND.
Sprague-Dawley rats were maintained in a food-deprivation schedule. Olfactory discrimination tests were performed before, 1 and 2 weeks after NMDA-lesion. The dish in which rats dug first and the spent time were recorded. NMDA or vehicle was bilaterally injected into OB (1, 2, or 3 injections of 1.5 µl). Nissl staining, NeuN, tyrosine hydroxylase, and glial fibrillary acidic protein immunohistochemistry were performed in OB. Doublecortin, polysialylated-neural cell adhesion molecules, and proliferating cell nuclear antigen immunohistochemistry were performed in the subventricular zone.
One week after NMDA lesions animals showed a significant 70% ($p<0.01$) decrease in correct trials when 3, but not 1 and 2, injections were administered ($p<0.01$) and an increase on the time spent to achieve the correct odour ($p<0.05$). A recovery of olfactory function ($p<0.01$) and changes in neurogenesis markers were observed two weeks after lesion. NMDA lesions resulted in neural injury through all bulb layers.
The present results indicate that bilateral OB NMDA lesion is a useful tool to investigate excitotoxicity in the SND after TBI and to study the pathophysiology and repair mechanisms of the olfactory dysfunction.
This study was sponsored by a grant from La Marató TV3 (274/U/2011)

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

ANALYSIS OF MOLECULAR PATHWAYS IN THE REVERSION OF AUTISM-ASSOCIATED SYMPTOMS IN MUTANT β-NEUREXIN-1 MOUSE MODEL BY TRANSCRIPTOME PROFILING

E. Robles- Lanuza[1,2], F. G. Scholl[1,2]
1. Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla. Sevilla, Spain
2. Departamento de Fisiología Médica y Biofísica, Facultad de Medicina, Universidad de Sevilla. Sevilla, Spain

Autism spectrum disorders (ASD) comprise a group of clinical phenotypes characterized by repetitive behaviours and social and communication deficits. ASD is viewed as a neurodevelopmental disorder that appears in early infancy but persists during the whole lifetime. The identification of mutations in the *NRXN1* gene that reduce the synaptic levels of the mutant proteins has suggested a role for synaptic dysfunction of neurexin-1 in the etiology of autism. Our previous results demonstrated that the inducible expression of a mutant β-neurexin protein in postnatal neurons resulted in ASD-like symptoms in βNrx1ΔC mice. The symptoms associated with autism can be reversed even in old mice when normal neurexin function is resumed. Our aim is to characterize molecular pathways responsible for the onset and reversion of autism behavioural deficits.

We have analysed mRNA expression profile in βNrx1ΔC mice, a validated animal model for ASD. RNA was extracted from βNrx1ΔC and control littermate mice and hybridized with GeneChip® Mouse Transcriptome Assay 1.0. Statistical analysis was performed with LIMMA (Linear Models for Microarray Analysis) using oneChannelGUI package (R-based). Main statistically significant changes in RNA expression were validated and replicated by RT-PCR in another group of mice. Functional reversion of these changes was analysed in Doxycycline (DOX)-treated mice (Tet-off system).

βNrx1ΔC mice express a dominant negative β-neurexin protein under the control of the tet-off system. Thus, the molecular signature associated with the onset and reversion of the autism-related phenotype can be studied by comparing βNrx1ΔC mice OFF DOX with βNrx1ΔC mice fed with DOX. We show a differential RNA expression pattern in βNrx1ΔC mice in brain areas associated with the onset of autism-like behaviour. Genes with a potential role in the reversion of symptoms were analysed using βNrx1ΔC mice ON DOX. We observed that functional transcriptional changes associated with autism are grouped within GO categories related to synaptic transmission and that they can be reverted to control levels when autism-like behaviour is rescued.

These data show that dysfunction of β-neurexin-1 causes a global change in the synaptic program. Molecular analysis of the reversion of autistic-like phenotype may lead to the identification of new candidates for future interventions in ASD treatment.

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

NEURON-MICROGLIA COMMUNICATION IMPAIRMENT IN HUNTINGTON'S DISEASE

M. Straccia[1], Jordi Carrere[1], Andrea Comella Bolla[1], Andres Miguez[1], Monica Pardo[1], Georgina Bombau[1], Phil Sanders[1], Carme Solà Subirana[2], Jordi Alberch[1], Josep M. Canals[1].
[1]. Department of Cell Biology, Immunology and Neuroscience, Faculty of Medicine, IDIBAPS, University of Barcelona, Barcelona and Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), SPAIN.
[2]. Department of Cerebral Ischemia and Neurodegeneration, Institut d'Investigacions Biomediques de Barcelona-Consejo Superior de Investigaciones Científicas (CSIC), Institut d'Investigacions Biomediques August-Pi i Sunyer (IDIBAPS), Barcelona, SPAIN.

Huntington's disease (HD) is a monogenic autosomal dominant disease where all cells of the affected individuals are carrying the CAG mutant expansion in the Huntingtin gene (HTT). Years before neurologic symptoms, HD pre-manifest patients show microglial activation, which correlates with the disease progression. Although these data suggest an important role of innate immune system in HD pathogenesis, the neuro-immune communication between neurons and microglia has not been investigated so far.
We focus our study on CX3CL1/CX3CR1 and CD200/CD200R1 systems that have been involved in synaptic plasticity, neurogenesis and control of microglial-mediated neurotoxicity in several neurodegenerative disorders. The presence of the ligands on neuronal membranes is generally considered an "off" signal for microglial activation.
Here, we analyzed the CX3CL1 and CD200 expression during human and mouse striatal development. Then we analyzed their expression at mRNA and protein levels in R6/1 HD mouse model and in human induced pluripotent stem cell (iPSC)-derived neurons from control and affected individuals. Once we detected the CX3CL1 and CD200 impairment along the HD pathogenesis, we studied the functional effects of these changes in an *in vitro* neuron-microglia chimeric system. We co-culture human iPSC-derived neurons from control and affected individuals with wild-type or R6/1 mouse microglia. Thus, we induced microglial activation and compared control and human HD iPSC-derived neuronal death. Neurons derived from affected human patient iPSCs showed an altered response to microglial-mediated toxicity.
In conclusion, the altered levels of CX3CL1 and CD200 play a key role in the HD pathogenesis by inducing neuron-microglia communication dysfunction, which contributes to neuronal cell death triggered by microglia.

This study was supported by grants from the Ministerio de Economia y Competitividad and from the ISCIII-Subdirección General de Evaluación and European Regional Development Fund (ERDF) [RETICS and CIBERNED], Spain; and CHDI Foundation, USA.

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

ISOLATION, CULTURE AND EXPRESSION OF MYOGENIC MARKERS IN PROGENITOR CELLS OBTAINED FROM EXTRAOCULAR MUSCLES BY THE PREPLATE TECHNIQUE.

M.A. Davis López-Carrizosa, G. Carrero Rojas, A. González González, T. Rodríguez Chinchilla, A. M. Pastor
Facultad de Biología. Universidad de Sevilla, Sevilla

Extraocular muscles (EOMs) are distinct from other skeletal muscles. These highly specialized muscles are intriguingly spared in some dystrophies like Duchenne muscular dystrophy (DMD). Contrary to the severe damage to other muscles, DMD patients present normal eye movements even at the final stages of the disease. Along the last decade, several explanations were postulated to explain their resistance, but until the discovery of specific properties of satellite cells (SCs) in EOMs, none were sufficiently convincing. These cells have high proliferative potential, a superior expansion capacity and contribute significantly more to the renewal of the SCs pool than their limb counterparts. These traits made them a plausible candidate in cell-based therapies to combat muscle disease. However, the location of EOMs within the orbit and their small size has resulted in the lack of appropriate harvesting techniques and cell culture methods to study their properties.

Therefore, an optimized protocol of the standardized *preplate technique* was developed for SCs EOM and by means of immunocitochemistry the number of myogenic cells obtained on successive plates were compared.

The results show that our isolating and harvesting techniques were valid for different species. EOM myogenic precursors and miotubes were obtained from cats, mice and rats. In the latter, while the percentage of $Pax7^+$ cells was similar on plates PP3 to PP6 (above 85%), the number of cells/plate was about a hundred times higher on PP3 than in the successive plates. The expression of MyoD was significantly higher on the first plates (PP2 to PP4) than in plates PP5-6 (75% and 45%, respectively).

Overall, these results demonstrate the protocol used here may be suitable for isolating and culturing EOM SCs in sufficient number for cell-therapy purposes and shows that seeding on plate PP4 and further does not significantly enrich the sample on myogenic precursors as previously thought.

1ª: Trastornos y reparación del sistema nervioso

TRANSCRANIAL STATIC MAGNETIC FIELD STIMULATION (tSMS) DECREASES CORTICAL EXCITABILITY IN PARKINSON'S DISEASE

M. Dileone[1,2], M.C. Carrasco-López[2], J. C. Segundo-Rodriguez[3], L. Mordillo-Mateos[2],N. López-Ariztegui[3], F. Alonso-Frech[1,4,] A. Oliviero[2], J.A. Obeso[1], G. Foffani[1,2].
1. CINAC, HM Puerta del Sur, Hospitales de Madrid and CEU San Pablo University, Madrid, Spain;
2. Hospital Nacional de Parapléjicos, SESCAM, Toledo, Spain;
3. Servicio de Neurología, Hospital Virgen de la Salud, SESCAM, Toledo, Spain;
4. Hospital Clínico San Carlos, Madrid, Spain.

Objective: Reducing cortical excitability in patients with Parkinson's disease – using other non-invasive neuromodulation techniques (i.e. TMS) – already showed promising results for improving levodopa-induced dyskinesias and motor symptoms. We recently demonstrated that transcranial static magnetic field stimulation (tSMS), a novel non-invasive, DBS-compatible, low cost neuromodulation technique, is able to decrease cortical excitability in healthy subjects. In the present study we aimed to test the hypothesis that tSMS reduces motor cortex excitability also in patients with Parkinson's disease (PD). This study is the first step of a Michael J. Fox Foundation project that aims to test the therapeutic potential of tSMS to manage levodopa-induced dyskinesias.
Materials and Methods: A randomized double-blind sham-controlled cross-over study was performed to assess cortical excitability in 10 L-DOPA responders PD patients (H&Y: 2-3). Each patient underwent 4 experimental sessions: tSMS-OFF, tSMS-ON, sham-OFF, sham-ON. These 4 sessions were performed in two separate days, at least one week apart. Both days the patients were studied after overnight withdrawal of dopaminergic medication and, after one experimental session OFF medication received their levodopa medication (150% of effective morning dose) to undergo another experimental session ON medication (if the OFF session is tSMS, the ON session is sham, and viceversa). Cortical excitability was quantified by the amplitude of motor evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS). MEPs were measured in the first dorsal interosseus (FDI) of the more affected side.
Results: In OFF condition ANOVA revealed a significant "Stimulation x Condition" interaction (P=0.017): MEP amplitudes were significantly reduced by 31% between 2 and 4 min and 22% between 4 and 6 min after tSMS compared to sham (paired t-test p=0.034, p=0.037). In ON condition ANOVA revealed no significant interactions (P=0.16)
Conclusions: tSMS is able to decrease cortical excitability in OFF-medication PD patients.

1. Disorders and nervous system repair
2. New methods and technologies

EPIGENETIC REGULATION OF CAROTID BODY GDNF EXPRESSION BY AGE: IMPLICATIONS FOR ANTIPARKINSONIAN CELL THERAPY

J. Villadiego[1,2], R. Ramirez-Lorca[1,2], N. Suárez-Luna[1,2], A. Bermejo-Navas[1,2], M. Olivares-Blanco[1], M. Oliver[1], M. Echevarría[1,2], J. López-Barneo[1,2] and J.J Toledo-Aral[1,2].
1. Instituto de Biomedicina de Sevilla-IBiS, HUVR/Universidad de Sevilla/CSIC. Sevilla, Spain.
2. Departamento de Fisiología Médica y Biofísica. Universidad de Sevilla. Sevilla, Spain.

Intrastriatal carotid body (CB) grafts induce trophic protection and restoration of the dopaminergic nigrostriatal pathway in rodent and primate models of Parkinson's disease (PD), which seems to be mediated by the high levels of glial cell line-derived neurotrophic factor (GDNF) produced by the CB implants. Pilot clinical trials have also demonstrated that CB autotrasplantation can improve motor symptoms in PD patients although less efficiently than in experimental models. One of the main factors that influence the clinical outcome of CB cell therapy is patient age. In order to discern limiting factors that could affect the clinical efficacy of CB transplants, we have studied how aging and the chronic hypoxia present on intracerebral grafts, influence CB GDNF expression. Chronic hypoxia, induced an up-regulation of CB GDNF expression in young mice (2-3 months old), while the same treatment resulted in decreased CB GDNF expression in aged mice (>14 months old). Interestingly, this differential regulation of GDNF expression along ageing is also present in the intraestriatal graft and affects the efficacy of mice antiparkinsonian CB cell therapy. We found that human CB xenografts from young donors induced an important protection of the nigrostriatal dopaminergic neurons of immunosuppressed MPTP treated mice, while human CB implants from aged donors failed to produce a significant effect. Finally, we performed an study of the methylation status of the human and murine GDNF promoter from young and aged CBs. In this analysis, we identified regions of the GDNF promoter that was differentially methylated on aged CB and could alter the regulation of GDNF expression. These findings provide a molecular explanation of previous clinical trials data showing that the efficacy of CB autotransplantation is inversely related to patient age, and could offer new insights about age related epigenetic regulation of GDNF expression.

1ª: Trastornos y reparación del sistema nervioso

THE NEUROPEPTIDE PACAP ENHANCES HIPPOCAMPAL SYNAPTIC PLASTICITY IN HUNTINGTON'S DISEASE

N. Cabezas-Llobet[1], R. Díaz-Hernandez[1], A. Fournier[2], J. Alberch[3,4,5], D. Vaudry[6], X. Xifró[1,3,4,5]

1 New Therapeutic Targets Group, Medical Sciences Department, School of Medicine, Universitat de Girona. c/ Emili Grahit, nº77, 17071, Girona, Spain.

2 INRS - Institut Armand-Frappier, 531 boul. des Prairies, Laval, QC, Canada H7V 1B7; Laboratoire International Associé Samuel de Champlain.

3. Departament de Biologia Cel·lular, Immunologia i Neurociències, Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain.

4 Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

5 Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain.

6 INSERM-U982, Laboratory of Neuronal and Neuroendocrine Differentiation and Communication, IRIB, Université de Rouen, 76821 Mont-Saint-Aignan, France. Laboratoire International Associé Samuel de Champlain.

Huntington's disease (HD) is a hereditary neurodegenerative disorder characterized by early cognitive impairment. Deficits in memory and learning are due to hippocampal dysfunction through alteration of synaptic plasticity. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a pleiotropic and multifunctional peptide that promotes synaptic plasticity, memory, hippocampal neurogenesis and neuroprotection, among others. PACAP exerts its effects via three main receptors, called PAC_1, $VPAC_1$ and $VPAC_2$. Therefore, the aim of this study was to analyze PACAP as a possible therapeutic agent in order to block cognitive deficits in Huntington's disease. We first studied the protein levels of PACAP receptors in the hippocampus of two HD mice models, the R6/1 an exon-1 mice model, and a full-length mice model, the knock-in mice (Hdh^{111}). We observed *in vivo* a decrease of PACAP receptors in both models from the onset of cognitive dysfunction. The transfection of huntingtin-94-Q-GFP in neural cells ($STHdh^{Q7/Q7}$ cells) also resulted in a loss of PACAP receptors indicating that the decrease of the PACAP receptors seems to be related to the expression of htt mutants. Interestingly, the analysis of post-mortem hippocampal human samples revealed a specific reduction of PAC_1, without changes in $VPAC_1$ and $VPAC_2$ compared to control samples. The addition of PACAP in hippocampal cultures from wild-type and R6/1 mice showed an important increase in the number of dendrites and its length. Moreover, the treatment of neuronal cultures with PACAP restored the expression of genes related to synaptic plasticity, such as c-fos or egr1. These effects are not observed by the addition of VIP (vasoactive intestinal peptide), a peptide that stimulates $VPAC_1$ and $VPAC_2$. In addition the transfection of $siPAC_1$ abolishes the PACAP-related effects. Those results indicate that the effects of PACAP seem to be mediated trough PAC_1. Our findings suggest that PACAP could be a suitable candidate to reestablish the synaptic plasticity in HD.

Fungings: *Supported by Ministerio de Economia y Competitividad (SAF2011-29507), CIBERNED (Instituto Carlos III), Generalitat de Catalunya(2014SGR-968) and the PeReNE interreg project.*

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

A MODEL OF INCREASED IMPULSIVITY IN RATS WITH BILATERAL PARKINSONISM TREATED WITH PRAMIPEXOLE

H. Jiménez-Urbieta [1,2], B. Gago [1,2], C. Marin [3], M. Delgado-Alvarado [1,2], M.C. Rodríguez-Oroz [1,2,4,5]
[1]. Instituto de investigación Sanitaria Biodonostia, Donostia-San Sebastián
[2]. Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Madrid
[3]. IDIBAPS, Barcelona
[4]. Ikerbasque, Basque Foundation for Science, Bilbao
[5]. Hospital Universitario Donostia, San Sebastian

Impulse control disorders (ICD) is a common side effect of the dopaminergic treatment in patients with Parkinson's disease (PD), which is more associated with dopamine agonists than with levodopa. To understand its pathophysiology, reliable animal models are essential. We have developed a model of increased impulsivity in bilateral parkinsonian rats treated with pramipexole (PPX). Impulsivity was evaluated with the variable delay-to-signal (VDS) paradigm. In this test, rats have to introduce the snout into a nose poke that is signaled by a light (presented at variable delays) triggering the delivery of a food reward when the response is correct. As rats reach a stable baseline performance, a partial bilateral dopaminergic lesion was induced with the injection of 6-OHDA in the dorsolateral region of the striatum (AP: +1mm and L: ±3.4mm from Bregma, V:-4.7 mm). Two weeks after the dopaminergic lesion, rats were acutely treated with two doses of PPX (0,25mg/kg and 3mg/kg; Latin-square design) in two different days. Rats undertook the VDS test under 5 different conditions: basal state, after 6-OHDA injection, under the effect of the two doses of PPX, and the day after the last dose of PPX. Only the acute administration of 3 mg/kg of PPX significantly increased the number of premature responses, indicating an increase of the impulsive behavior, in parkinsonian but not in sham rats. Both doses of PPX significantly decreased the accuracy of responding (correct/total number of responses) and increased the incorrect and perseverative (compulsive behavior) responses in both parkinsonian and sham treated groups when compared with saline-treated groups. In conclusion, PPX induced attention deficit, reflected by the lack of accuracy, as well as compulsive behavior in control and parkinsonian rats, but increased impulsivity only in the parkinsonian animals. This model could constitute a valid tool to investigate the pathophysiology of ICD (DFG11/019, PI11/02109).

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

SFRP1 MODULATES NEURO-INFLAMMATORY RESPONSE.

J. Rueda-Carrasco[1,2], A. Borroto[1], M.J. Martin-Bermejo[1,2], M.T. Heneka[3,4], B. Alarcón[1], P. Bovolenta[1,2] and P. Esteve[1,2]

[1]. Centro de Biología Molecular "Severo Ochoa", Consejo Superior de Investigaciones Científicas – Universidad Autónoma de Madrid, Madrid, Spain

[2]. Centro de Investigación Biomédica en Red de Enfermedades Raras, Madrid, Spain

[3]. German Centre for Neurodegenerative Diseases (DZNE) Bonn, Bonn, Germany

[4]. Department of Neurology, Clinical Neuroscience, University of Bonn, Bonn, Germany

Secreted-Frizzled-Related-Protein 1 (Sfrp1) is a multifunctional regulator of cell-to-cell communication, which modulates Wnt signaling and acts as an inhibitor of the metalloprotease ADAM10. ADAM10 sheds multiple substrates, including the Amyloid Precursor Protein (APP), a molecule involved in the pathogenesis of Alzheimer Disease (AD) and a variety of proteins that act as mediators or receptors of inflammatory events. In a mouse model for AD (APP;PS1), in which Sfrp1 has been genetically inactivated (APP;PS1;*Sfrp1*$^{-/-}$), brain inflammation characteristic of the APP;PS1 mice is nearly absent, suggesting that Sfrp1 could directly modulate brain inflammation. To test this hypothesis, we treated mixed glial cultures from postnatal wild type (*wt*) cortex with pro-inflammatory compounds such as LPS and IL6. In their presence, both astrocytes and microglial cells up-regulate the levels of Sfrp1 expression. Consistent with this observation, similar cultures from *Sfrp1*$^{-/-}$ mice treated with LPS present a poor inflammatory response, measured by the production of pro and anti-inflammatory cytokines, compared to cultures derived from *wt* mice. Similar results were obtained after intra-cortical infusion of LPS in *wt* and *Sfrp1*$^{-/-}$ mice. Remarkably, lentiviral derived *Sfrp1* gene addition in the brain of *wt* mice induces a strong and time-sustained inflammatory response characterized by the presence of activated microglial cells and myeloid cell infiltration. To further corroborate that Sfrp1 regulates CNS inflammatory response, we investigated how the absence of Sfrp1 influences the pathological traits of Experimental Autoimmune Encephalitis (EAE), a model for Multiple Sclerosis, which is a degenerative autoimmune disease with a strong inflammatory component. Our preliminary results indicate that *Sfrp1*$^{-/-}$ mice are more resistant to EAE than *wt*. Altogether these data suggests that Sfrp1 act as pro-inflammatory molecule in the CNS, opening the possibility that its pharmacological targeting could be of help in neurodegenerative processes.

Supported by MINECO BFU2013-43213-P, EMBO ASTF 452–2014 and Fundación Tatiana de Pérez de Guzmán el Bueno.

1. Disorders and nervous system repair
2. Neuronal excitability, synapses and glia: cellular mechanisms

ALTERED PHB2 PROTEIN EXPRESSION IN SCHIZOPHRENIA AND REGULATION BY CALCIUM-DEPENDENT SIGNALING

E.Vila [1], R. Pinacho [1], I. Ferrer [2], B. Ramos [1]
[1] Unitat de recerca. Parc Sanitari Sant Joan de Déu, Fundació Sant Joan de Déu, CIBERSAM, Universitat de Barcelona, Barcelona, Spain.
[2] Instituto de Neuropatología, IDIBELL-Hospital Universitario de Bellvitge, CIBERNED, Universidad de Barcelona, Hospitalet de LLobregat, Barcelona.

Schizophrenia is a severe complex psychiatric disorder where changes in gene expression programs may play an important role. Altered circuits distributed in different brain regions, as the cortico-cerebellar-thalamic-cortical circuit, and evidences for alteration of calcium signaling pathways have been described in the pathology. Preliminary findings of our group using a proteomic approach suggested altered levels of Prohibitin2 (PHB2), a gene expression regulator, in the postmortem cerebellum of schizophrenia subjects. However, confirmation of altered human brain PHB2 protein in schizophrenia and its possible regulation by calcium signaling in neurons is still unknown. The aim of this study was to investigate whether PHB2 protein levels were altered in postmortem cerebellum in subjects with chronic schizophrenia compared to matched control individuals (n=14 per group). Moreover, we investigated the modulation of PHB2 in dissociated cerebellar granule neurons and organotypic cerebellar slice culture by different calcium signaling pathways: membrane depolarization, and inhibition of N-methyl-D-Aspartate receptor activity. PHB2 protein levels were analyzed by immunoblot.
We found a reduction of PHB2 protein levels in cerebellum in chronic schizophrenia. We found an increase of PHB2 levels upon serum withdrawal in immature neurons at Day In Vitro 4 (DIV4) and in hyperpolarized mature neurons at DIV7 in rat cerebellar granular neurons. We also observed an increase of PHB2 levels with dizocilpine treatment in cerebellar slices. These results show dysregulation of PHB2 protein in the cerebellum providing evidences of a new possible altered candidate involved in the disease. In addition, our data show that calcium-dependent pathways modulate PHB2 levels in neurons suggesting that altered calcium signaling in schizophrenia could impact on PHB2 abundance.

Acknowledgments: Financed by Marie Curie Program IRG RTD REG/T.2(2007)D/530573 and the Plan Nacional de Investigación BFU2008-01103 (MCINN) to B.R.

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

PHOSPHODIESTERASE-7 AND GSK-3 DUAL INHIBITION PROMOTES REMYELINATION AFTER INJURY IN THE CENTRAL NERVOUS SYSTEM.

EM. Medina-Rodríguez[1], A. Bribián[1,2], R. Lebrón[1], I. Sánchez-Raya[1], I. Machín-Díaz[1], A. Boyd[3], A. Williams[3], C. Gil[4], A. Martínez[4], F. de Castro[1].
[1]. Grupo de Neurobiología del Desarrollo GNDe, Hospital Nacional de Parapléjicos, Toledo, Spain.
[2]. Departamento de Neurobiología Molecular, Celular y del Desarrollo. Instituto Cajal-CSIC, Madrid, Spain.
[3]. MRC Centre for Regenerative Medicine, Edinburgh, United Kingdom.
[4]. Instituto de Química Médica, CSIC, Madrid, Spain.

Oligodendrocyte precursor cells (OPCs) are present in the adult CNS and they are able to remyelinate the injured areas, although that not always occurs efficiently in demyelinating diseases such as Multiple Sclerosis (MS). In order to find factors which could favor this process, two newly developed phosphodiesterase-7 (PDE7) and GSK-3 dual inhibitors (VP1.15 and VP3.15), or a GSK-3 inhibitor (TDZD8) have been used. The anti-inflammatory effect of these inhibitors has been previously showed *in vitro* and in animal models of spinal cord injury or MS where a reversion of the clinical symptoms was observed. However, their implication on remyelination remains unknown. In the present study, first, remyelination was studied in response to these inhibitors in an *ex vivo* model of demyelination by lysolecithin on cerebellar slices. VP1.15 and VP3.15 enhanced remyelination 3 days after injury, while TDZD8 did not induce any effect. After that, the improvement on remyelination by VP1.15 and VP3.15 was ascertained by using two *in vivo* murine models of demyelination induced by cuprizone or lysolecithin. The corpus callosum of mice fed with cuprizone during 5 weeks increased the amount of MBP, after cuprizone withdrawal, with three intraperitoneal injections of VP1.15 or VP3.15. Finally, mice whose corpus callosum was demyelinated by lysolecithin showed an increase of the percentage of myelinated axons and the myelin thickness when two dose of VP1.15 or VP3.15 were injected. With these interesting results, we could conclude that remyelination in the damaged brain can be improved with the treatment with PDE7 and GSK-3 dual inhibitors and this observation, joined to the good pharmacokinetic, pharmakodynamic, and safety profile properties of VP1.15 and VP3.15, allows us to propose them as a potential treatment for MS not only able to reduce the immune response but also to induce the repair, mediated by endogenous OPCs, of demyelinated areas.

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

SINGLE NUCLEOTIDE POLYMORPHISMS AND PARKINSON'S DISEASE IN ANDALUSIA: AN ASSOCIATION STUDY

R. Durán[1], S Bandrés[1], F.J. Barrero[2], F. Escamilla-Sevilla[3], J. Pelegrina[2], J.A. Medina[1], B. Gutiérrez[4], A. Ching [4], M. Ramírez[5], F. Vives[1].

[1]Department of Physiology and Institute of Neurosciences Federico Olóriz, Centro de Investigaciones Biomedicas (CIBM), University of Granada, Granada, Spain.

[2]Movement Disorders Unit, University Hospital San Cecilio, Granada, Spain.

[3]Movement Disorders Unit, Department of Neurology, Instituto de Investigación Biosanitaria (IBS), University Hospital Virgen de las Nieves, Granada, Spain.

[4]Department of Psychiatry and Institute of Neurosciences Federico Olóriz, Centro de Investigaciones Biomédicas (CIBM), University of Granada, Granada, Spain.

[5]Department of Psychiatry and Institute of Neurosciences Federico Olóriz, Centro de Investigaciones Biomédicas (CIBM), University of Granada, Granada, Spain Departament of Health Sciences, University of Jaén, Spain.

OBJECTIVE: Increasing evidence supports an extensive and complex genetic contribution to Parkinson's disease (PD) etiology. Our aim was to evaluate the association between certain genetic variants and the risk to develop Parkinson's disease (PD) in a cohort of Andalusian population.

METHODS: We performed a case-control association study by genotyping 64 single nucleotide polymorphisms (SNPs) in 117 patients diagnosed with PD and 408 controls from southern Spain. SNPs were selected from PD related genes (SNCA, LRRK2, PARK2, DJ-1, VPS37) and also some described in genome wide association studies (GWAS) as MAPT, GBA, HLA-DOA, STK39, ACMSD and GAK. Genotyping was carried out using Taqman assays in an Open Array Real-Time PCR platform. Data analysis included logistic regression adjusted by multiple testing.

RESULTS: Significant differences were observed in the allele frequencies between PD patients and controls after multiple testing adjustment for the following SNPs: HLA rs206769 (p= 1.187x 10-10; OR= 0.2135), SNCA rs2736990 (p= 0.009268; OR= 1.75), SNCA rs356204 (p= 0.022; OR= 1.673), SNCA rs356219 (p= 0.03002; OR= 1.752), LRRK2 rs34637584 (p= 0.001774; 5.87x 109), LRRK2 rs28903073 (p= 0.0181; OR= 11.09). No significant differences were found in allele distribution between cases and controls for the rest of the SNPs analyzed.

CONCLUSIONS: Our findings suggest that SNPs SNCA rs2736990, SNCA rs356204, SNCA rs356219 and LRRK2 rs34637584, LRRK2 rs28903073 likely contribute to PD susceptibility in Andalusian population whereas HLA rs206769 might be protective against neurodegeneration.

1ª: Trastornos y reparación del sistema nervioso
2ª: Nuevos métodos y tecnologías

NOVEL MUTATION AND GENETIC VARIABILITY IN PARKINSON'S DISEASE PATIENTS FROM SOUTHERN SPAIN

R. Durán[1], S. Bandrés[1], F. Barrero[2], F. Escamilla-Sevilla[3], J. Pelegrina[2], J.A. Medina[1], B. Gutiérrez[4], A. Ching[4], M. Ramírez[5], F. Vives[1].

[1]Department of Physiology and Institute of Neurosciences Federico Olóriz, Centro de Investigaciones Biomedicas (CIBM), University of Granada, Granada, Spain.

[2]Movement Disorders Unit, University Hospital San Cecilio, Granada, Spain.

[3]Movement Disorders Unit, Department of Neurology, Instituto de Investigación Biosanitaria (IBS), University Hospital Virgen de las Nieves, Granada, Spain.

[4]Department of Psychiatry and Institute of Neurosciences Federico Olóriz, Centro de Investigaciones Biomédicas (CIBM), University of Granada, Granada, Spain.

[5]Departament of Health Sciences, University of Jaén, Spain.

OBJECTIVE: To date, a large spectrum of genetic variants has been related to familial and sporadic Parkinson's disease (PD) in diverse populations worldwide. However, very little is known about the molecular features of PD in Southern Spain, despite its noteworthy and continuous interchange of population with other Mediterranean countries. We aimed to screen for pathogenic mutations in the PD related-genes LRRK2, SNCA, PARKIN, PINK1, DJ-1, VPS35, GBA and GCH1.

METHODS: 134 patients were included in the study of which 97 individuals were diagnosed with late-onset sporadic PD and 37 with familial or early-onset sporadic PD. The genetic analysis was performed by Sanger sequencing and next-generation sequencing respectively.

RESULTS: An early-onset sporadic patient carried a novel heterozygous mutation of unknown significance in VPS35 (p.R32S). Additionally, we identified the following 11 known pathogenic mutations (GBA p.D448H, p.L483P, p.N409S, p.E365K; LRRK2 p.G2019S and p.R1441G; PARK2 c.154delA, p.R402C, p.V56E and p.C212Y; PINK1 p.G309D) by next-generation sequencing among 15 patients (40.5%). Moreover, 7 known pathogenic mutations (GBA p.D448H, p.L483P, p.N409S, p.T408M and p.E365K; LRRK2 p.G2019S and p.R1441G) were identified by Sanger sequencing in 17 patients (17.5%).

CONCLUSIONS: This study suggests that southern Spain population might have the potential of harbouring novel mutations. Further research is needed to study the contribution of the novel found mutation p.R32S in VPS35 to the pathogenesis of PD. A sizable number of known pathogenic mutations related to PD have been identified. GBA and LRRK2 mutations appear to be considerably frequent in our population.

1ª: Trastornos y reparación del sistema nervioso
2ª: Nuevos métodos y tecnologías

ANALYSIS OF SYNAPTIC-RELATED MICRORNAS EXPRESSION IN ALZHEIMER'S DISEASE.

D.J. Siedlecki[1], A. Otxoa de Amezaga[1], J. Català[1], C. A. Saura[1,2], J. Rodríguez-Alvarez[1, 2] & A. J. Miñano Molina[1,2].

[1]Institut de Neurociencies and Dpt. Bioquímica i Biología Molecular, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain.

[2]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain.

Abstract: MicroRNAs are small non-coding RNAs that regulate gene expression post-transcriptionally. Recent studies have shown that deregulation of specific microRNAs could be involved in the development of Alzheimer's disease (AD). However, few studies exploring the relationship between microRNAs deregulation in AD and synaptic plasticity exist despite the involvement of some microRNAs in synaptic plasticity. Since it is believed that alterations in synaptic function are related to mild cognitive impairment, it is feasible to hypothesize that alterations in plasticity-related microRNAs could underlie AD progression.

Here, levels of a small number of microRNAs involved in the regulation of AMPA receptors function were examined in mice hippocampal cultures, an AD mice model, where we reported previously changes in AMPA receptors regulation related with early deficits in learning and memory processes, and in human samples. We found increases in miR-181c-5p (~40%), miR-210-3p (>60%) and miR-92a-3p (~25%) expression after oAβ treatment in cultures. Furthermore, some changes in miR-181c-5p and miR-92a-3p were found in entorhinal cortex and hippocampus of $APP_{Sw,Ind}$ six months transgenic mice. However, a compensatory mechanism (such as synaptic scaling) could occur in AD early stages since mice are still able to learn. It remains to be determined what happens later, when these mechanisms would no longer be enough.

Moreover, the analysis of hippocampal human samples at different Braak stages, show an increase in miR-181c-5p and miR-92a-3p levels during AD progression. These findings indicate a possible relationship between these microRNAs and the reported changes in glutamate receptor levels and early learning and memory deficits in the AD animal model. Our results suggest that microRNAs involved in synaptic plasticity might be important factors that contribute to AD neuropathology progress.

Support: This work was supported by grants from Ministerio de Economia y Competitividad (SAF2014-59697-R), CIBERNED (CB06/05/0042), Fundació Marato TV3 (2014- 3610) and Generalitat de Catalunya (SGR2009-1231)

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

EXTRA-STRIATAL DOPAMINERGIC ACTIVITY AND COMPENSATORY MECHANISMS IN PARKINSONIAN MONKEYS

I. Trigo-Damas[1,2], H. Iwamuro[3], A Vian-Lains[4] , J. Blesa[1] , C. Cavada[4], J. A. Obeso [1,2]
[1]. CINAC. Hospital Puerta del Sur y Universidad San Pablo CEU, Madrid
[2]. Centro de Investigación Biomédica en Red Enfermedades Neurodegenerativas CIBERNED
[3]. Department of Neurosurgery, Tokyo Metropolitan Neu, Fuchu, Japan
[4]. Facultad de Medicina, Universidad Autónoma de Madrid, Madrid

Traditionally, the major pathophysiological emphasis in Parkinson's disease (PD) has focused on striatal dopamine (DA) deficit. However, the extra-striatal compensatory mechanisms may also be relevant to explain the delay at the onset of motor features.
To investigate the impact of dopaminergic activity on extra-striatal basal ganglia nuclei we infused the DA agonist Quinpirole (5 μg/μl) and the antagonist Sulpiride (10 μg/μl) into the somatomotor region of the subthalamic nucleus (STN) and the external segment of the globus pallidus (GPe) of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys (*Macaca fascicularis*) at three different motor states (asymptomatic, recovered, and parkinsonian). The somatomotor regions of the STN and GPe were identified by extra-cellular neuronal recording and transient dyskinetic movements induced by local infusion of muscimol. Post-mortem histological assessment of dopaminergic lesion and recording tracks were carried out.
Infusion of Quinpirole in the STN increased parkinsonism whereas Sulpiride improved motor signs. In both circumstances the effect was more pronounced in the parkinsonian state. Both drugs had the inverse effect when administered into the GPe. Measurement of total axonal length of dopaminergic STN terminals revealed significant loss in asymptomatic and recovered monkeys indicating early extra-striatal denervation. These results suggest that dopaminergic activity of the GPe and STN can modulate motor activity in the presence of nigro-striatal DA deficit of various degrees. DA appears to exert a differential and opposite effect onto GPe/STN. These results are in keeping with our primary hypothesis that early dopaminergic denervation in the STN plays an important compensatory role in the pre-symptomatic state of PD.

Supported by CIBERNED and by the Chair UAM - Fundación Tatiana Pérez de Guzmán el Bueno.

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

COENZYME Q 10 REDUCES OXIDATIVE STRESS-INDUCED INJURY BY B-AMYLOID IN ENDOTHELIAL CELLS.

J.Frontiñán [1], R.l Santiago-Mora [1] J. R. Peinado [1], J. A. López-Domínguez [2], J. M.Villalba [2], F. J. Alcaín [1], M. Durán-Prado [1]*

1Regional Centre for Biomedical Research, Faculty of Medicine, University of Castilla-la Mancha, Ciudad Real

2 Faculty of Sciences, University of Córdoba, Córdoba

*Mario Durán Prado (mario.duran@uclm.es). Faculty of Medicine, University of Castilla-La Mancha. Camino de Moledores s/n, 13071, Ciudad Real, Spain.

Neuropathological symptoms of Alzheimer's disease appear in advances stages, once neuronal damage arises. Nevertheless, recent studies demonstrate that in early asymptomatic stages, ß-amyloid peptide damages the cerebral microvasculature through mechanisms that involve an increase in reactive oxygen species and calcium, which induces necrosis and apoptosis of endothelial cells, leading to cerebrovascular dysfunction. The goal of our work is to study the potential preventive effect of the lipophilic antioxidant coenzyme Q (CoQ) against ß-amyloid-induced damage on human endothelial cells. We analyzed the protective effect of CoQ against Ab-induced injury in human umbilical vein endothelial cells (HUVECs) using fluorescence and confocal microscopy and biochemical techniques. Our results show that CoQ pretreatment of HUVECs delayed Ab incorporation into the plasma membrane and mitochondria. Moreover, CoQ reduced the influx of extracellular $Ca^{2+,}$ and Ca^{2+} release from mitochondria due to opening the mitochondrial transition pore after b-amyloid administration, in addition to decreasing $O_2^{\cdot 2}$ and H_2O_2 levels. Pretreatment with CoQ also prevented ß-amyloid induced HUVECs necrosis and apoptosis, restored their ability to proliferate, migrate and form tube-like structures in vitro. We also are probing the effect of CoQ against Ab-induced injury in mouse endothelial brain cells and in 3xTg-AD mice model. CoQ protected endothelial cells from Ab induced injury at physiological concentrations in human plasma after oral CoQ supplementation and thus could be a promising molecule to protect endothelial cells against amyloid angiopathy.

1ª: Trastornos y reparación del sistema nervioso
2ª: Sistemas homeostáticos y neuroendocrino

EXPRESSION OF NFκB RELATED GENES CORRELATES WITH SP TRANSCRIPTION FACTORS AND NEGATIVE SYMPTOM SEVERITY IN THE POSTMORTEM CEREBELLUM IN SCHIZOPHRENIA

R. Pinacho [1], K. MacDowell [2], I. Moreno [2], B. García Bueno [2], J. M. Haro [1], B. Ramos [1]
[1] Unitat de recerca. Parc Sanitari Sant Joan de Déu, Fundació Sant Joan de Déu, CIBERSAM, Universitat de Barcelona, Barcelona.
[2] Departamento de Farmacología. CIBERSAM. Facultad de Medicina, Universidad Complutense, Madrid.

Alterations in transcriptional regulation in pathological conditions such as psychotic disorders can lead to gene expression reprogramming that could affect a variety of signalling pathways in the brain. We have previously reported that Specificity Protein (SP) 1 and 4 transcription factors are reduced in the cerebellum of schizophrenia subjects with more severe negative symptoms. SP factors have been described to be part of the neuronal NFκB binding factors. Indeed SP1 binds to NFκB binding sites in neurons, suggesting that an unbalance between SP factors and activated NFκB could lead to more pronounced changes in the expression of inflammatory NFκB-dependent genes in schizophrenia. The aim of this study was to investigate whether SP protein abundance in brain could be linked to changes in the activation of NFκB pathway. We evaluated the activity of NFκB pathway indirectly through the gene expression of p65 NFκB subunit and NFκB inhibitory protein *(IκBα)* measured by RT-qPCR in the *postmortem* cerebellum of subjects with schizophrenia (n=16), and if these genes correlate with negative symptom severity measured *premortem* with the Positive and Negative Syndrome Scale in schizophrenia (n=16). We found that IκBα cerebellar expression shows a significantly inverse correlation with SP1 and SP4 protein levels and a direct correlation with the severity of negative symptoms in schizophrenia subjects. Moreover, we also found that the expression of p65 NFκB subunit shows a trend direct correlation with the severity of negative symptoms in this region in schizophrenia subjects. The findings of this pilot study suggest an increase of the inflammatory pathway mediated by NFκB linked to SP protein abundance and negative symptoms in schizophrenia. Thus, a possible crosstalk between SP transcription factors and NFκB signalling pathway in psychotic disorders could be occurring in the cerebellum and might contribute to a deeper pro-inflammatory response in the context of negative symptoms in schizophrenia.

1ª: Trastornos y reparación del sistema nervioso
2ª: Sistemas homeostáticos y neuroendocrino

PATTERN OF STRIATAL DOPAMINE LOSS IN ASYMPTOMATIC AND SYMPTOMATIC 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE-TREATED MONKEYS

J. Blesa [1], C. Cavada [2], C. Pifl [3], O. Hornykievicwz [3], J.A. Obeso [1]
[1] Centro Integral de Neurociencias (CINAC), Hospital Puerta del Sur-HM Hospitales de Madrid, Mostoles , Universidad, CEU San Pablo and CIBERNED, Instituto Carlos III, Madrid
[2] Facultad de Medicina, Universidad Autonoma de Madrid, Madrid
[3] Center for Brain Research, Medical University of Vienna, Vienna

Administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to non-human primates produces an excellent behavioral and histochemical model of nigro-striatal lesion as in Parkinson's disease. We have administered MPTP to a large number of monkeys using a slow intoxication protocol to produce a more gradual development of nigral lesion than earlier models. Regional differences in the dopamine (DA) innervation of the striatum were examined in four different MPTP-treated groups and compared to a control group.

The MPTP-treated groups included monkeys who were always asymptomatic, monkeys who recovered after showing mild parkinsonian signs, and monkeys with stable parkinsonism, either moderate or severe. Sections of the caudate nucleus and putamen at pre-commisural and post-commisural levels were immunostained for tyrosine hydroxilase (TH), a marker of DA innervation, and analyzed quantitatively using Image J software to measure optical densities.

Overall, there was a loss of TH immunostaining in the MPTP-treated monkeys which was consistent with the severity of the condition. The decrease in TH immunostaining followed rostrocaudal and dorsoventral patterns, such that the earliest and most denervated regions were the dorsal caudate and putamen (>60%). The precommisural ventral striatum was the least affected. The dorsoventral denervation pattern was evident just in the precommisural striatum. Likewise, dopamine striatal concentration fall earlier and preferentially in the dorsal and caudal putamen. Indeed, the difference between the asymptomatic and symptomatic parkinsonian state is given by a striatal dopaminergic threshold in the caudal (motor) putamen.

These data indicate that the regional and temporal patterns of striatal DA loss characteristic of Parkinson's disease can be partially reproduced in a model of Parkinson’s disease based on slow MPTP-intoxication in monkeys. Moreover, this model serves to study compensatory mechanisms taking place early in PD evolution (asymptomatic) and after the diagnosis has been made (symptomatic).

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

SFRP1 AND NEUROINFLAMMATION IN NEURODEGENERATIVE DISEASES AND AGING.

M.I. Mateo[1,2], J. Rueda-Carrasco[1,2], M.J. Martin-Bermejo[1,2], P. Bovolenta[1,2] and P. Esteve[1,2]
[1]. Centro de Biología Molecular "Severo Ochoa", Consejo Superior de Investigaciones Científicas – Universidad Autónoma de Madrid, Madrid, Spain
[2]. Centro de Investigación Biomédica en Red de Enfermedades Raras, Madrid, Spain

Secreted frizzled-related proteins (Sfrps) compose a family of relatively small, secreted proteins with five members in mammals, which act as important regulators of cell-cell communication. Studies of our laboratory demonstrated that Sfrp1 binds and negatively modulates the activity of ADAM10, a metalloproteinase that cleaves, among others, the amyloid precursor protein (APP). Because ADAM10 has been involved in the pathogenesis of Alzheimer Disease (AD), we have investigated the possible involvement of Sfrp1 in this disease. Our studies indicate that genetic inactivation of Sfrp1 in a mouse model of AD prevents the formation of amyloid plaques and strongly decreases the associated neuroinflammation. As shown in the abstract by Rueda-Carrasco et al, Sfrp1 directly modulates neuroinflammatory events. To further corroborate this hypothesis, we have generated an inducible transgenic mouse model over-expressing Sfrp1 in astrocytes. We will present data aimed at elucidating whether and how Sfrp1 modulate neuroinflammation and whether peripheral inflammatory stimuli alter Sfrp1 expression/distribution thereby exacerbating brain neurodegeneration and aging.

1. Disorders and nervous system repair
2. Neuronal excitability, synapses and glia: cellular mechanisms

EXTRACELLULAR MATRIX PROTEIN ANOSMIN-1-OVEREXPRESSION INDUCES A SELECTIVE POTENTIATION OF DOPAMINERGIC NEUROGENESIS: IMPLICATIONS FOR PARKINSON´S DISEASE

Villadiego, J.[1], García-González, D.[2#], Lebrón[2], R., Sánchez-Raya[2], I, Suárez-Luna[1], M., Toledo-Aral[1]*, J.J., de Castro[2]*, F.

[1]: Dept. de Fisiología Médica y Biofísica, Universidad de Sevilla, e Instituto de Biomedicina de Sevilla-IBiS, Sevilla, Spain

[2]: Grupo de Neurobiología del Desarrollo-GNDe, Hospital Nacional de Parapléjicos, Toledo, Spain

[#]: Current address: Dept. of Clinical Neurobiology, German Cancer Research Center-DKFZ, Heidelberg, Germany

Parkinson´s disease (PD) is one of the most prototypical among neurodegenerative diseases and it is characterised by the massive loss of dopaminergic neurons (TH^+) in the substantia nigra and subsequent loss in the secretion of dopamine in the striatum. Therapeutic strategies for PD are manily foccused on increase the liberation of the neurotranmitter by TH^+-neurons at the striatum. The composition of a neurogenic niche determines the number ant type of neurons generated there. Recently, we have demonstrated how the over-expression of human anosmin-1 gives rise to a selective significant increase in the number of TH+-interneurons at the olfactory bulb of adult mice. In our current work, we show that the over-expression of the cited protein results in an increased number of TH^+-neurons at the substantia nigra as well as of dopaminergic terminals within the striatum in the adult brain of mouse. We have characterised these changes and we have also studied the induced changes in TH+-neurons from different neural crest-derived structures from the PNS (carotid body, adrenal medulla and superior cervical granglion) both during development and in the adult. A better comprehension of the role of components of the extracelular matrix, including anosmin-1, on the neurogenesis of TH^+-neurons should be very valuable for the design of future therapeutic options to treat PD, either by modifying the physiology of endogenous neurogenic niches or via cell therapy.

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

POSITIVE AND NEGATIVE SYMPTOMS IN A NEURODEVELOPMENTAL MODEL OF SCHIZOPHRENIA DO NOT TRANSLATE INTO ALTERED COCAINE CONSUMPTION.

R. Capellán [1], R. Santos-Toscano[1], J. Orihuel [1], E. Borcel[2], M. Ucha[1], D. Roura-Martínez[1], A. Contreras[1], E. Ambrosio[1] y A. Higuera-Matas [1]
[1]Facultad de Psicología. UNED, Madrid
[2]Brain and Mind Institute, École Polytechnique Fédéral de Lausanne, Suiza.

Objectives: Epidemiological data suggest that infections during pregnancy might render the developing fetus more susceptible to schizophrenic disorders in adulthood. In addition, there is a higher incidence of addictive disorders among schizophrenic patients. The aim of this study was to use a prenatal infection model of schizophrenia to study in male and female rats the relationship between cognitive, negative and positive symptoms and the propensity consume cocaine later in life.
Material and Methods: Pregnant female Sprague-Dawley rats were injected with the lipopolysaccharide –LPS- (GD15,16; 100 µg/kg i.p.) or saline and upon reaching adulthood, cognitive (working memory in a T maze), negative (deficits in social interaction) and positive symptoms (deficits in sensory-motor gating) were assessed. Cocaine self-administration, dose-response patterns and extinction were then examined.
Results: LPS administration resulted in higher levels of plasma TNFα, decreased weight gain and hypothermia in the mothers. As adults, LPS-exposed offspring showed impaired working memory and a deficit in sensory-motor gating (regardless of the sex). No alterations were observed in the social interaction test. Both LPS- and saline-exposed rats self-administered cocaine with no differences between groups. Similar dose-response curves were observed in the four groups.
Conclusions: These results do not support the association between schizophrenic symptoms and the higher prevalence of cocaine use disorders.

Supported by: SAF2013-47520-P); RTA-RD12/028/0020 PNSD Grant nº: 2012I057; Grant nº: S-2011/BMD-2308; Programa de Actividades I+D+I CANNAB-CM); UNED (Plan de Promoción de la Investigación); and European Union (Grant nº: JUST/2013/DPIP/AG/4823-EU MADNESS).

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

GENERATION OF A NEW TRANSGENIC MOUSE LINE FOR CONDITIONAL OVEREXPRESSION OF THE TRANSCRIPTION FACTOR ATF5 IN ADULT BRAIN.

I. Hernández Hernández [1,2], J.J. Lucas [1]
[1] Centro de Biología Molecular Severo Ochoa (CBM"SO") CSIC/UAM, Madrid
[2] Facultad de Ciencias. Universidad Autónoma de Madrid, Madrid

Activating transcription factor 5 (ATF5) is a basic-leucine-zipper transcription factor of the ATF/CREB family. The Atf5 gene generates two transcripts, Atf5α and Atf5β, of which Atf5α is selectively translated in response to different stresses like aminoacid limitation, oxidative stress, viral infection and endoplasmic reticulum (ER) stress in non-neuronal cells. ATF5 has been implicated in tumor cell survival, apoptosis and cell differentiation among other processes. This transcription factor is widely expressed during development and in the adult. In the developing brain neuroprogenitor cells must have ATF5 levels downregulated to undergo differentiation into mature neurons and glial cells. This has led to the extended notion that differentiated neural cells do not express ATF5 unless they suffer tumorigenic transformation. However, our group recently described a wide, neuronal-patterned ATF5 expression in the adult encephalon that is further induced upon ER stress as a pro-survival mechanism in *status epilepticus*. Interestingly ER stress condition is believed to contribute to the pathogenesis of many neurodegenerative diseases, like Alzheimer's disease, Parkinson's disease and Huntington's disease. Combined, these pieces of evidence suggest that ATF5 holds important roles in the physiology of adult brain. However, little is known about ATF5 transcriptional targets in the nervous tissue. To study neuronal ATF5-responsive genes *in vivo* we generated a novel tetracycline-responsive, conditional transgenic mouse line overexpressing mouse ATF5 and the reporter β-galactosidase, designated TREmATF5. To achieve specific induction of ATF5 overexpression in adult neurons we used transgenic mice with a tetracycline-regulated transcriptional transactivator linked to the CamKIIα promoter (CamKIIα-tTA). As expected, the reporter was induced in adult neurons of the double transgenic animals (CamKIIα-tTA; TREmATF5) and we are currently exploring the levels of overexpression of ATF5. These mice will constitute a new tool for the understanding of brain physiology and open a new field for neuroprotective strategies focused on ATF5 modulation.

[60]FULLERENE DERIVATIVE IS NEUROPROTECTIVE AGAINST AMYLOID-β PEPTIDE TOXICITY THROUGH MODULATION OF ADENOSINE AND METABOTROPIC GLUTAMATE RECEPTORS

Giust D [1], T. Da Ros[3], M. Martín[1,2], J.L. Albasanz[1,2]
[1]Facultad de Ciencias y Tecnologías Químicas. Universidad de Castilla-La Mancha. Ciudad Real, Spain.
[2]Facultad de Medicina. Universidad de Castilla-La Mancha. Ciudad Real, Spain.
[3]Universitá di Trieste, Dipartimento di Scienze Chimiche e Farmaceutiche, Trieste, Italy.

Amyloid-beta peptide (Aβ) aggregation is widely known as an important hallmark of Alzheimer's disease (AD). It is aggregated into senile plaques which are responsible for neurodegeneration and cell death characteristic of AD. Although the mechanisms for its formation and toxicity are still unclear, Aβ is considered as a potential target for therapeutic agents. [60]Fullerenes have been observed to be neuroprotective due to its antioxidant and radical scavenger properties. These effects have been associated to neurotransmitter receptors modulation. Some of these receptors, as adenosine and metabotropic glutamate receptors, have been shown to be altered in AD. Metabotropic glutamate receptors are significantly decreased in the frontal cortex from AD human brain in parallel to progression of disease. On the contrary, adenosine receptors are significantly increased in the same area even since early stages. The aim of the present work was to determine the possible neuroprotective effect of t3ss, a fullerene hydrosoluble derivative, and the role of adenosine and mGlu receptors in this neuroprotection. To this end, SH-SY5Y and SK-N-MC cells were treated with Aβ at different concentration and time exposure. Cell viability, radioligand binding and quantitative real time PCR assays were performed with treated and control cells. Results showed that Aβ caused a significant cell death in both SH-SY5Y and SK-N-MC cells and altered adenosine and mGlu receptors expression. In addition, cell death and receptor alteration elicited by Aβ were prevented by t3ss. These results suggest [60]fullerenes as neuroprotective nanoparticles against Aβ toxicity involving modulation of GPCRs and open new future perspectives to be considered in the treatment of AD.

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

INTRACELLULAR AMYLOID REDUCTION AND PROTECTION FROM NEURONAL DEATH IN A MOUSE MODEL OF ALZHEIMER'S DISEASE AFTER A SINGLE INTRAPERITONEAL DOSE OF scFv-H3D6.

G. Esquerda-Canals [1], G. Rivera-Hernández [1], L. Giménez-Llort [2], J. Martí-Clúa [1], S. Villegas [1].
[1]. Facultat de Biociències. Universitat Autònoma de Barcelona, Barcelona.
[2]. Facultat de Medicina. Universitat Autònoma de Barcelona, Barcelona.

Alzheimer's disease is the most common form of dementia, with more than 44 million of patients worldwide. Because of the overwhelming impact of this neuropathology in both social and economic levels, treatments to prevent, stop or reverse it are desperately needful. In line with the promising tool of immunotherapy, our laboratory developed an anti-amyloid-β antibody single-chain variable fragment, scFv-h3D6. Previous *in vitro* studies demonstrated its ability to prevent amyloid-β-induced cytotoxicity in neuroblastoma cell-culture by withdrawing oligomers from the amyloid pathway. Furthermore, its efficacy was also supported by *in vivo* evidences at the behavioral and molecular levels in the 3xTg-AD mouse model of Alzheimer's disease.
The goal of this work was to examine neuronal vulnerability in those most affected regions of the brain such as the cerebral cortex, the hippocampus and the amygdala and, specially, to assess the ability of scFv-h3D6 to reduce the amyloid burden in these areas.
Five-months-old 3xTg-AD and non-transgenic females were sacrificed five days after an intraperitoneal injection of a single-dose of 100 µg of scFv-h3D6 or vehicle. The subsequent histological characterization allowed the double comparison between phenotypes and treatments. Results evidenced that cellular vulnerability is particular for each neuronal population. Specifically, transgenic females presented neurodegeneration of the pyramidal neurons from both the cerebral cortex and the Cornus Ammonis in the hippocampus, as well as of the neurons from the basal magnocellular amygdaloid nucleus. Excitingly, those transgenic females administered with scFv-h3D6 exhibited intracellular amyloid reduction and lower neuronal loss, showing that the neurodegenerative progression was slowed down by the treatment.
In conclusion, the present study demonstrates that scFv-h3D6 prevents from neuronal death and promotes intracellular amyloid clearance, evidencing its therapeutic potential for Alzheimer's disease.

1. Transtornos y reparación del sistema nervioso.
2. Neurociencia cognitiva y conductual.

COGNITIVE IMPAIRMENT INDUCED BY DELTA9-TETRAHYDROCANNABINOL OCCURS THROUGH HETEROMERS BETWEEN CANNABINOID CB1 AND SEROTONIN 2A RECEPTORS

X. Viñals[1]; E. Moreno[2]; L. Lanfumey[3]; A. Cordomí[4]; A. Pastor[5]; R. de La Torre[5]; P. Gasperini[6]; L.A. Howell[6]; L. Pardo[4]; C. Lluís[2]; E.I. Canela[2]; P.J. McCormick[6]; R. Maldonado[1]; P. Robledo[5]
[1]Neuropharmacology Laboratory, University Pompeu Fabra, Barcelona, Spain.
[2]Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Barcelona, Spain.
[3]CPN, INSERM UMR S894, Université Paris Descartes, UMR S894, Paris, France.
[4]Laboratori de Medicina Computacional, Unitat de Bioestadística, Facultat de Medicina, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain
[5]Human Pharmacology and Clinical Neurosciences Research Group, IMIM-Hospital del Mar Medical Research Institute, Barcelona, Spain
[6]School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ.

Activation of cannabinoid CB1 receptors (CB1R) by delta9-tetrahydrocannabinol (THC) produces a variety of negative effects with major consequences in cannabis users that constitute important drawbacks for the use of cannabinoids as therapeutic agents. For this reason there is a tremendous medical interest in harnessing the beneficial effects of THC. Behavioral studies carried out in mice lacking 5-HT2A receptors (5-HT2AR) revealed a remarkable 5-HT2AR-dependent dissociation in the beneficial antinociceptive effects of THC and its detrimental amnesic properties. We found that specific effects of THC, such as memory deficits, anxiolytic-like effects, and social interaction are under the control of 5-HT2AR, but not its acute hypolocomotor, hypothermic, anxiogenic and antinociceptive effects. In biochemical studies, we show that CB1R and 5-HT2AR form heteromers that are expressed and functionally active in specific brain regions involved in memory impairment. Remarkably, our functional data shows that co-stimulation of both receptors by agonists reduces cell signaling, antagonist binding to one receptor blocks signaling of the interacting receptor, and heteromer formation leads to a switch in G-protein coupling for 5-HT2AR from Gq to Gi proteins. Synthetic peptides with the sequence of transmembrane helices 5 and 6 of CB1R, fused to a cell-penetrating peptide, were able to disrupt receptor heteromerization in vivo leading to a selective abrogation of memory impairments caused by exposure to THC. These data reveal a novel molecular mechanism for the functional interaction between CB1R and 5-HT2AR mediating cognitive impairment. CB1R-5-HT2AR heteromers are thus good targets to dissociate the cognitive deficits induced by THC from its beneficial antinociceptive properties.

BEDDING INTAKE AS A NEW EXPERIMENTAL METHOD TO EVALUATE EMETOGENIC ANTINEOPLASTIC AND ANTIEMETIC DRUGS.

L. Núñez Navalón, G. Vera Pasamontes, N. A. Paniagua Lora, R. Abalo Delgado.
Área de Farmacología y Nutrición, Unidad Asociada al IQM y CIAL (CSIC) y Grupo de Excelencia de Investigación en Dolor URJC-CSIC-Banco de Santander (iDOL). Depto. CC. Básicas de la Salud, Fac. CC de la Salud, Universidad Rey Juan Carlos, Alcorcón (Madrid).

Introduction. Nausea and vomiting are amongst the most distressing adverse effects of chemotherapy, with a big impact on quality of life in cancerous patients. Although current antiemetic drugs are efficient for most patients, many of them do not respond or develop resistance to conventional antiemetics, such as 5-HT_3 antagonists. Thus, it is necessary to develop new antiemetic strategies. Rodents do not vomit and therefore indirect markers of nausea/emesis are used in order to evaluate emetogenic/antiemetic drugs on them. Bedding intake, a particular kind of pica (intake of non-nutritive substances) may be a pertinent indirect marker in preclinical studies.
Objectives. To validate bedding intake in the rat, as an experimental method to evaluate emetogenic and antiemetic drugs.
Material and methods. Male Wistar rats were isolated and observed for 4 hours after intraperitoneal administration of the 5-HT_3 antagonist granisetron (1 mg/kg) or saline, followed by the antineoplastic drug cisplatin (6 mg/kg) or saline. The number of times the rat ingested bedding from the cage were recorded in 15-min periods. After the 4-hour observational period, its change in body weight, and gastric weight and size were determined.
Results. Body weight did not change significantly throughout the experiment in any of the groups compared to control rats. Cisplatin significantly increased bedding intake and this was prevented by granisetron. Cisplatin also increased stomach weight and size, but granisetron only partially prevented the change in stomach weight induced by cisplatin. Granisetron alone did not induce any significant bedding intake or changes in gastric weight or size after the observational period.
Conclusions. Bedding intake is an indirect marker of nausea/vomiting in the rat that might be useful in the evaluation of new antiemetics for treatment/prevention of nausea/emesis associated to chemotherapy.

Acknowledgements. Supported by: SAF2012-40075-C02-01 (MINECO), S2010/BMD-2308, and PRIN13-CS20 (URJC). Thanks to Mª Carmen Merino for technical assistance.

1ª: Nuevos métodos y tecnologías (7)
2ª: Trastornos y reparación del sistema nervioso (5)

CANNABINOIDS RELIEVE PAIN IN A RAT MODEL OF 5-FLUOROURACIL-INDUCED NEUROPATHY

E. Pinto Rocha, G. Vera Pasamontes, R. Abalo Delgado.
Área de Farmacología y Nutrición, Unidad Asociada al IQM y CIAL (CSIC) y Grupo de Excelencia de Investigación en Dolor URJC-CSIC-Banco de Santander (iDOL). Depto. CC. Básicas de la Salud, Fac. CC de la Salud, Univ. Rey Juan Carlos, Alcorcón (Madrid).

Introduction: Clinical use of antineoplastic drugs is associated with the development of numerous adverse effects that many patients find intolerable, including peripheral neuropathy. Cannabinoids have relieved neuropathic pain in different animal models and are currently also being tested in patients for this. However, their therapeutic activities could be affected by their psychoactive properties.
Objective: The aim of this work was to determine the effect of cannabinoids in 5-fluorouracil (5-FU)-evoked neuropathy.
Methods: Adult male Wistar rats (250-400 g, n=8 rats/group) received an intraperitoneal injection of saline or 5-FU (150 mg/kg). Up to 15 days after administration, tests for mechanical allodynia and thermal hyperalgesia were performed. At different time-points, the non-selective agonist WIN 55,212-2 (WIN) or their vehicle were either systemically (intraperitoneal route, 1 mg/kg) or locally (intraplantar injection, 50 or 100 µg) applied. In order to test for central effects, the cannabinoid tetrad was performed.
Results: 5-FU-treated rats showed mechanical allodynia and thermal hyperalgesia 3 days after administration but mechanical allodynia was the only neuropathic sign persisting in the remaining experimental days. WIN alleviated mechanical allodynia both after local and systemic administration. No central effects were detected in the cannabinoid tetrad when WIN was systemically administered.
Conclusions: In a rat model of 5-FU-induced neuropathy, cannabinoids had an antinociceptive effect. This is a new experimental evidence of the usefulness and safety of cannabinoids for relieve of neuropathic pain induced by chemotherapy.
Acknowledgements. Supported by: SAF2012-40075-C02-01 (MINECO), S2010/BMD-2308, and PRIN13-CS20 (URJC).

1ª: Disorders and nervous system repair

NEW INSIGHTS FROM A MOUSE MODEL OF WILLIAMS-BEUREN SYNDROME

A. Navarro-Romero[1], L. Galera[1], R. Maldonado[1], V. Campuzano[2], A. Ozaita[1]
[1]Laboratorio de Neurofarmacología, Facultad de Ciencias de la Salud y de la Vida, Universidad Pompeu Fabra, Barcelona
[2]Laboratorio de Genética, Facultad de Ciencias de la Salud y de la Vida, Universidad Pompeu Fabra, Barcelona

Williams-Beuren syndrome (WBS) is a rare neurodevelopmental disorder (1 in 7,500 births) caused by a hemizygous deletion of 26-28 contiguous genes on chromosome band 7q11.23. At the neurological level, it is characterized by mild to moderate intellectual disability and a specific cognitive profile, including a characteristic hypersociable phenotype. The first mouse model for WBS featuring the same hemizygous complete deletion (WBS-CD) found in patients has been recently reported. The aim of this study was to characterize in WBS-CD mice the behavioral functioning and the brain relevant signaling pathways involved in cognitive performance in order to assess potential therapeutic approaches for this disorder. For this purpose, a battery of behavioral tests in young adult mice (2-3 months of age) was performed and then mice were sacrificed and hippocampal tissues were analyzed by immunoblot. WBS-CD mice poorly performed the novel object recognition, the novel place recognition and the inhibitory avoidance tasks. In addition, they showed deficits in the nesting assay and an alteration of the anxiety-like behavior in the marble burying test. At the biochemical level, immunoblot analysis from hippocampal samples revealed a hypoactivation of the Akt-mammalian target of rapamycin (mTOR) pathway, an alteration recently associated to intellectual disability disorders. In a first attempt to modulate these features in WBS-CD mice, we targeted the endocannabinoid system, a neuromodulatory system intimately associated to the Akt-mTOR signaling. Using the indirect cannabinoid agonist JZL184, a specific inhibitor of the monoacylglycerol lipase, we observed a partial recovery of the anxiety-like behavior in the marble burying test. Our data reveal the characteristic phenotype in a novel murine model of WBS and points to the endocannabinoid system as an appropriate target to treat specific behavioral features of the disorder.

Supported by BFU2012-33500 (MINECO, Spain) and Jérôme Lejeune Foundation (France). AN-R was supported by a FPU fellowship (MECD, Spain).

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

OPTOGENETIC CONTROL OF LOCOMOTOR ACTIVITY IN A RAT MODEL OF PARKINSONS DISEASE

Ivan Castela[1,3], Ledia F.Hernandez[1,2], Jose A. Obeso[1,2], Rosario Moratalla[2,3]
[1]. Centro Integral de Neurociencias AC (CINAC), Hospital HM Puerta del Sur, Madrid and CEU-San Pablo University, Madrid, Spain.
[2]. Center for Networked Biomedical Research on Neurodegenerative Diseases (CIBERNED), Madrid, Spain.
[3]. Cajal Institute_CSIC, Avda. Doctor Arce 37, 28002 Madrid

Parkinson´s disease (PD) is characterized by the loss of dopamine-containing neurons in the ventral tier of substantia nigra pars compacta (SNpc), which provides dopaminergic input to the caudal striatum. In PD patients such dopamine loss is most severe initially in the postero-lateral putamen, spreading with disease progression to other striatal regions. In this study we aim to manipulate through optogenetic techniques the motor behaviour in the 6-hidroxydopamine (6-OHDA) rat model. For this purpose, a local unilateral injection of 6-OHDA was delivered on the right dorsolateral striatum. The optogenetic control was achieved through viral expression of opsines (ChR2) carrying a CAMKII promoter to be expressed in the striatal projection neurons, the medium spiny neurons (MSNs). The virus and the optic fiber were placed in the dopamine depleted area. In order to establish the optimal parameters, we used different amount of viral particles and different intensity of laser power stimulation. Four weeks after the dopamine depletion and the viral injection, rats were stimulated with different protocols and motor activity was videorecorded twice a week for 9 mins during a 3 to 7 weeks period. After the behavioural experiments, rats were sacrificed for anatomical studies. We determined the degree of dopamine depletion, the spread of the viral infection and the neuronal activation by immunohistochemistry techniques.
Our preliminary results suggest that this technique may be used as a first step towards deciphering the role of the D1 and D2 striatal populations in the regulation of motor activity in the parkinsonian state and levodopa-induced dyskinesias. Funded by SAF2013-48532-R, PNDA and CIBERNED to RM, CIBERNED and SAF2012-40216-C02-01 to JO.

1ª: Trastornos y reparación del sistema nervioso
2ª: Nuevos métodos y tecnologías

RED LIGHT ATTENUATES RETINAL DAMAGE. IMPLICATIONS IN RETINAL NEURODEGENERATIVE DISSEASES.

Claudia Núñez Álvarez[1,2], Susana del Olmo Aguado[1,2], Jesús Merayo-Lloves[1,2] and Neville N. Osborne[1]
[1]Fundación de Investigación Oftalmológica, Oviedo , Asturias
[2]Universidad de Oviedo, Oviedo, Asturias

The aim of this study is to determine wether red light application of red light focused through dilated pupils of rat eyes given at the same time as raised intraocular pressure can attenuate neuronal damage to the retina and particularly their ganglion cells.
Male wistar (300g) rats were anesthetised and placed in a stereotaxic frame. The pupils from both eyes were dilated. The anterior chamber in one eye was cannulated with a 30G needle and the IOP was increased to 140mmHg to cause retinal ischemia. Raised IOP (60mins) was either delivered in darkness or under 3000lux of red light directed above the cornea. The contralateral eye was used as control. At different times after raised IOP (reperfusion) retinas were dissected and properly fixed or preserved for antigens localisation, qPCR or WB studies.
Raise IOP caused damage to the retina, indexed by changes in the localisation of a number of tissue antigens, following seven days of reperfusion. Moreover, at this stage an elevation of retinal GFAP, vimentin and HO-1 mRNAs were recorded. In addition a significant loss of retinal Ganglion cells and a decrease of the Ganglion cell markers Thy-1 and Brn3a was evident 15 days after reperfusion. These negative effects caused by raised IOP and reperfusion were significantly attenuated by red light treatment.
Ischemia to rat retina induced by elevated IOP results in a loss of retinal Ganglion cells and general damage to the retina. The negative effect of ischemia-reperfusion to the retina is significantly attenuated by delivery of red light in concrete conditions during the induction of the ischemia. These data supports the notion for the use of red light therapy in the treatment of glaucoma.

1ª: Trastornos y reparación del sistema nervioso
2ª: Nuevos métodos y tecnologías

COMPENSATORY SPROUTING TO METHAMPHETAMINE-INDUCED DOPAMINERGIC DEGENERATION

Noelia Granado[1,2], Sara Ares-Santos[1,2], Yousef Tizabi[3] and Rosario Moratalla[1,2,#]
[1]Instituto Cajal, Consejo Superior de Investigaciones Científicas, CSIC, Madrid, Spain
[2]CIBERNED, ISCIII, Madrid, Spain
[3]Department of Pharmacology, Howard University College of Medicine, Washington, DC, USA

Methamphetamine, a psychostimulant drug with high abuse potential, may more than double the risk of developing Parkinson's disease (PD). Studies in animals have shown that this drug produces persistent dopaminergic neurotoxicity in the nigrostriatal pathway. Since some compensatory changes to dopaminergic damage have been described after treatment with other dopaminergic neurotoxins like MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), 6-OHDA (6-hydoxydopamine) or MDMA (3,4-methylenedioxy-methamphetamine), we were curious to determine whether compensatory responses may also occur following METH treatment. Three established regimens of METH: a single high dose (1x30mg/kg), multiple lower doses (3x5 mg/kg) or (3x10 mg/kg) of METH with known neurotoxicity were applied. As expected, significant degeneration of striatal dopaminergic fibers were observed a day later in all cases. The damage was highest with the 3x10 mg/kg dose followed by 3x5 mg/kg, which was followed by 1x30 mg/kg. Moreover, regimen-dependent partial recovery was also noticed after 3 days. Interestingly, the recovery was also highest in 3x10 mg/kg, followed by 3x5 mg/kg, followed by 1x30 mg/kg. These partial recoveries were associated with a similar pattern of increase in tyrosine hydroxylase immunoreactivity and some fiber sprouting as evidenced by Gap-43 positive fibers. Additionally, METH treatment resulted in an increase in Iba-1 staining (reflective of microglia activation) after one day that was fully recovered by day 3. However, the increase in GFAP staining (reflective of astroglia activation) that was observed after one day was further increased by day 3. These results confirm that partial striatal recovery occurs following METH treatment and that astro- and micro-glia may have some role in this compensatory process. Nonetheless, further studies on long-term effects of METH and recovery mechanism(s) are warranted.

This work was supported by grants from the Spanish Ministry of Sanidad, Servicios Sociales e Igualdad, PNSD #2012/071, Spanish Ministry of Economía y Competitividad grant # BFU2010-20664, CIBERNED #CB06/05/0055 and Comunidad de Madrid ref S2010/BMD-2336 to RM. NG received a research contract NEUROSTEM-CM S2010/BMD-2336; SAS a JAE pre-doctoral fellowship

1. Trastornos y reparación del sistema nervioso

FRAGMENT C DOMAIN OF TETANUS TOXIN MITIGATES METHAMPHETAMINE NEUROTOXICITY IN MICE

Noelia Granado*[1,2] , Liliana Mendieta*[1,2], José Aguilera[2,3], Yousef Tizabi[4], Rosario Moratalla[1,2]
1 Instituto Cajal, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain
2 Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain.
3 Institut de Neurociències and Departament de Bioquímica i de Biologia Molecular, Facultat de Medicina, Universitat Autònoma de Barcelona (UAB), Cerdanyola del Vallès, Spain.
4 Departament of Pharmacology, Howard University College of Medicine, Washington, DC, USA

Background: The C-terminal domain of the heavy chain of tetanus toxin (Hc-TeTx) is a non-toxic peptide with demonstrated in vitro and in vivo neuroprotective effects against striatal dopaminergic damage induced by MPP+ (1-methyl-4-phenylpyridinium) and 6-hydoxydopamine, suggesting its possible therapeutic potential in Parkinson's disease. Methamphetamine (METH), a widely abused psychostimulant, has selective dopaminergic neurotoxicity in rodents, monkeys and humans. This study was undertaken to determine whether Hc-TeTx might also protect against METH-induced dopaminergic neurotoxicity and the consequent motor impairment. Methods: For this purpose, we treated mice with a toxic regimen of METH (4 mg/kg, 3 consecutive i.p. injections, 3h apart) followed by 3 injections of 40 ug/kg of Hc-TeTx into grastrocnemius muscle at 1h, 24h and 48h post METH treatment.
Results: We found that Hc TeTx significantly reduced the loss of dopaminergic markers tyrosine hydroxylase (TH) and dopamine transporter (DAT) and the accompanying increases in silver staining (a well established degeneration marker) induced by METH in the striatum at 3 and 7 days after METH treatment. Moreover, Hc-TeTx prevented the increase of neuronal nitric oxide synthase (nNOS), but did not affect microglia activation induced by METH. Stereological neuronal count in the substantia nigra indicated loss of TH-positive neurons after METH that was not significantly affected by Hc-TeTx. Importantly, impairment in motor behaviors on days 1 and 3 post METH treatment, were significantly reduced by Hc-TeTx.
Conclusions: Here we demonstrate that Hc TeTx can provide significant protection against METH-induced neurotoxicity and motor impairment. Thus, Hc-TeTx fragment may represent a potential therapeutic drug for METH-abusers. This work was supported by grants from the Spanish Ministry of Sanidad, Servicios Sociales e Igualdad, PNSD #2012/071, Spanish Ministry of Economía y Competitividad grant # BFU2010-20664, CIBERNED #CB06/05/0055 and Comunidad de Madrid ref S2010/BMD-2336 to RM. NG received a research contract NEUROSTEM-CM S2010/BMD-2336; SAS a JAE pre-doctoral fellowship

1ª: Trastornos y reparación del sistema nervioso

EFFECT OF D3 RECEPTOR BLOCKADE ON L-DOPA-INDUCED DYSKINESIA IN AN ANIMAL MODEL OF PARKINSON DISEASE.

Oscar Solís [1,2], Jose Ruben Garcia-Montes [1,2], Rosario Moratalla [1,2]
[1]. Instituto Cajal, Consejo Superior de Investigaciones Científicas, CSIC, 28002 Madrid, Spain
[2]. CIBERNED, Instituto de Salud Carlos III, Madrid, Spain

Background: Although the dopamine D3 receptor (D3R) is principally located in the ventral striatum, overexpression of D3R in the dorsal striatum has been reported following L-DOPA treatment in dopamine-denervated animals. This has drawn attention to the potential importance of the D3R in L-DOPA-induced dyskinesia (LID).
Methods: To study the role of D3R in LID, we assessed the effects of PG01037 (D3R-preferring antagonist) on LID in the 6-OHDA mouse model of Parkinson's disease (PD). Mice were treated with L-DOPA (10 mg/kg) followed by PG01037 (10 mg/kg) after 15 min (to evaluate the effect in the expression and development of LID). Axial, limb and orolingual dyskinetic symptoms were evaluated as abnormal involuntary movements and the rotarod test used as a measure of the antiparkinsonian effect of L-DOPA. Alterations in FosB and histone3 (pAcH3) activation were examined by immunohistochemistry 1 h after the last L-DOPA injection.
Results: PG01037 treatment to hemiparkinsonian mice decreased dyskinesia development upon chronic exposure to L-DOPA, attenuating dyskinesia once established, without affecting the antiparkinsonian effect of L-DOPA. The expression of FosB and pAcH3 was associated with the development and expression of dyskinesia. Moreover, PG01037 significantly reduced FosB and pAcH3 when co-administered with L-DOPA. We demonstrate that targeting D3R can modify both the behavioral and molecular consequences of L-DOPA treatment.
Conclusions: Our results demonstrate that D3R modulates the development of dyskinesia by targeting D1R-mediated intracellular signaling and suggest that decreasing D3R activity may help to ameliorate LID.

This work was supported by grants from the Spanish Ministerios de Economía y Competitividad (SAF2013-48532-R) and PNSD #2012/071, and ISCIII, CIBERNED (CB06/05/0055) and Comunidad de Madrid ref. S2011/BMD-2336 to RM.

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

DEPRESSIVE-LIKE SYMPTOMS IN A RESERPINE-INDUCED MODEL OF FIBROMYALGIA IN RATS

EM González-Soler, A. Blasco Serra, F. Escrihuela Vidal, F. Martínez Expósito, A. Cervera Ferri, S. Martínez Bellver, A. Luque García, M.C. Blasco Ausina, J. Martínez Ricós, V. Teruel Martí, A.A. Valverde Navarro.
Dept. Of Human Anatomy and Embryology. Faculty of Medicine and Dentistry. Universitat de València, Valencia.

Objective: The aim of this work is to test whether rats undergoing the reserpine-induced fibromyalgia model show depressive-like symptoms. This model is based on central monoamine depletion following repeated administration of reserpine.
Methods: Male Sprague-Dawley rats were used. Habituation and handling procedures were performed for 5 days in order to reduce the animal's stress and discomfort. Experimental rats were injected with reserpine 1 mg/kg for three consecutive days; control rats were injected with vehicle 1 mg/kg. Animals were exposed to a Novelty-Suppressed Feeding Test adaptation or to a modified Forced Swimming Test in order to detect signs of depression.
Results: Animals administered with reserpine showed a higher latency to feed from the centre ($t=3.085$; $p<0.007$), and approached ($t=-4.303$; $p<0.001$) or smelled ($t=-2.980$; $p<0.009$) the central food a fewer number of times than control rats in the Novelty-Suppressed Feeding Test. Control of in-cage food consumption after the test confirms that increased latency to feed was not caused by differences in appetite between control and experimental groups. Reserpine-treated animals showed higher immobility ($t=3.581$; $p<0.006$) and lower swimming ($t=-2.509$; $p<0.026$) times in the Forced Swimming Test than vehicle-treated. $p<0.05$ was considered statistically significant.
Conclusion: These results suggest the presence of depressive-like disorder in the reserpine-induced fibromyalgia animal model, and thus provides with further evidence for the monoamine dysfunction theory in the pathogenesis of this disease. This is another step in the development of an appropriate animal model of fibromyalgia, critical for further basic research in its pathogenesis and treatment options.

1. Systems neuroscience
2. Disorders and repairment of nervous system

PURINERGIC RECEPTORS ARE IMPLICATED IN THE MODULATION OF THE UBIQUITIN-PROTEASOME SYSTEM (UPS) AFTER ADMINISTRATION OF LIPOPOLYSACCHARIDE (LPS).

L. De Diego-García[1], A. Sebastián-Serrano[1], C. Martínez-Frailes[1], M. Ramírez-Escudero, M. Díaz-Hernández[1].
[1].Veterinary School, Complutense University of Madrid, Madrid

ATP and other nucleotides act as neurotransmitters that activate specific membrane receptors denominated purinergic receptors. The abundance and variety of these receptors present in the Central Nervous System (CNS), presumes that they play an important role in its physiology. On this way, it has been reported that these receptors play an important role in the neural physiology, being involved in both the axonal growth and establishing of synaptic contact. On the other hand, its dysfunction has been related with neurodegenerative and neuroinflammatory processes. Interestingly, others groups have described that the ubiquitin-proteasome system (UPS), an intracellular system involved on degradation of most of intracellular proteins in a specific and selective manner, plays also a relevant role on these same processes. Taking into account that in a well-characterized model of neuroinflammation, the intraperitoneal administration of lipopolysaccharide (LPS), it has been described that occurs both a failure of the UPS activity and changes in purinergic signaling, we wonder if both phenomena are linked. To address this question we have studied whether the purinergic receptors can modulate the UPS activity and/or if the UPS can influence in the purinergic signaling.

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

ANALYSIS OF AUTOPHAGY IN NEURONS AND ITS ROLE IN AD MICE MODELS

I. Benito Cuesta [1], F. Wandosell Jurado [1,2]
[1]. Centro de Biología Molecular Severo Ochoa (CBMSO), CSIC-UAM, Madrid.
[2]. Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid.

Autophagy is an important constitutive process in neurons which, as postmitotic cells, cannot dilute harmful organelles or excess/toxic proteins through cell division. They may require a delicate balance between the formation and clearance of autophagic vesicles (AVs), which remain at low levels, to maintain the proteostasis. This process appears to be damper in some neurodegenerative diseases like Alzheimer's Disease (AD). In AD some data revealed that AVs accumulate within dystrophic neurites, suggesting a progressive dysfunction of macroautophagy and a failed maturation to lysosomes. However, there is some controversy about the role of autophagy in AD as it has been described as a degradative route for Ab peptide but also as responsible for its production.

The interest of our group is to analyse more deeply the autophagy process in normal neuronal function and in a mouse model of AD as a possible therapeutic target. We have employed as neuronal systems the SH-SY5Y cell line and cerebellar granule neuron cultures from the AD mouse model *B6.Cg-Tg (APPSwe,PSEN1dE9)/J*; denoted as APP/PS1) and from their wild-type littermates. Our data suggests that some degradation systems may be saturated in brain extracts obtained from AD patients and from the APP/PS1 mouse model. We have tested some previously described autophagy inductors like the well-stablished in cell lines rapamycin, though there is some controversy about its effect in neurons. We have observed a slight increase in autophagy flux and degradation induced by rapamycin, as well as a decrease of the A□ levels secreted by cultured neurons from APP/PS1 mice. However, based on our *in vivo* data, we couldn't appreciate a significant reduction in A□ levels (1 -40 or 1-42) in APP/PS1 mice when treated with rapamycin for two months. Thus, putative use of rapamycin as a therapeutic approach in AD should be questionable.

1ª: Trastornos y reparación del sistema nervioso
2ª: Sistemas homeostáticos y neuroendocrino

THE ROLE OF P2X7 RECEPTOR EXPRESSION IN MICROGLIAL CELLS DURING THE COURSE OF FAMILIAR ALZHEIMER DISEASE

C. Martínez-Frailes, L. De Diego-García, Á. Sebastián-Serrano, M. Díaz-Hernández
Departamento de Bioquímica y Biología Molecular IV, Facultad de Veterinaria, Universidad Complutense, Madrid.

Purinergic P2x7 receptor is an ionotropic receptor that opens a cation-permeable ligand-gated ion channel in response to binding of extracellular ATP. This receptor is widely distributed in the central nervous system, being expressed by the majority of cell linages, such as neurons, oligodendrocytes and microglia.
Previous works of our group have demonstrated, using a transgenic mouse model of Familiar Alzheimer Disease (FAD), that *in vivo* pharmacological blockage of P2x7, reduces senile plates number and size by increasing the non-amyloidogenic processing of amyloid precursor protein (APP) in neurons.
Indeed, it has been previously reported by other groups, that P2x7-expressing microglial cells tend to appear preferentially in contact with amyloid plates. This initial finding led to describe that P2x7 expression in microglial cells plays an important role in several physiological processes related with the Alzheimer disease progression, such as, microglial activation, reactive oxygen species production, neuronal death, cytokines release, and amyloid-beta peptide phagocytosis.
In this work, we focus in evaluating how the temporal expression of P2x7 by microglial cells along the course of the disease, impacts in the assembling dynamic of amyloid plates. To affront this challenge, we have generated a novel transgenic mouse that expresses both, mutated human APP and GFP reporter protein under the control of P2x7 promoter.
Data obtained shows that only a small percentage of microglial cells started expressing P2x7 once they were in contact with the senile plates. This percentage decreased in the last stages of the disease. According to these results, using a well-characterized neuroinflammatory model, intraperitoneal injection of LPS, we confirmed that P2x7 expression on microglial cells decreased in an aging-related way.

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

FUNCTIONAL RECOVERY AND WHITE MATTER REPAIR AFTER EXOSOMES ADMINISTRATION IN DIFFERENT EXPERIMENTAL ANIMAL MODELS OF STROKE.

M. Gutiérrez Fernández, L. Otero Ortega, B. Rodríguez Frutos, J. Ramos Cejudo, B. Fuentes, E. Medina, E. Díez Tejedor.
Hospital Universitario La Paz, IdiPAZ, UAM, Madrid, España.

Objectives: To investigate white matter repair after exosomes administration in two experimental models of subcortical stroke: cerebral infarct and hemorrhage.
Material and Methods: Subcortical ischemic stroke was induce by Endothelin-1 in striatum. Collagenase IV was used to induce subcortical hemorrhagic stroke into striatum. Intravenous exosomes or saline only were administrated as treatment into both different animal models of stroke. Exosomes were isolated from culture of adipose mesenchymal stem cell and they were characterized by Nanoshight, Electronic microscope, Western blot and Inmunoflorescece. Proteins contained into exosomes were analyzed by Orbitrab. We analyzed functional recovery by Rotarod, beam walking and Rogers tests. Lesion volume and tract connectivity were studied by magnetic resonance image. Anterograde and retrograde tracers were used to analyze axonal sprouting. Finally, myelin formation was analyzed by cryomielin.
Results: Proteomics analysis of exosomes identified more that 2000 proteins, many of them involved in intercellular communication. DiI labeled-Exosomes were detected in peripheral organs (liver, lung and spleen). After 28 days, treated groups showed smaller functional deficit compared to control groups in both hemorrhagic and ischemic animal models. Moreover, treated group showed an increase in tract connectivity at 7 and 28 days compared to control groups. Also, animals which received exosomes showed an increase axonal sprouting and myelin formation at 28 days after stroke in both hemorrhagic and ischemic stroke. The treated groups also showed higher levels of white matter-associated markers in the injured area than the control groups.
Conclusion: White matter integrity in different subcortical strokes is in part restored by exosomes treatment, probably mediated by repair molecular factors implicated in axonal sprouting, remyelination and oligodendrogenesis. These findings are associated with improved functional recovery in both kinds of strokes.

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

MITOCHONDRIAL DIVISION INHIBITOR 1 (MDIVI-1) PROTECTS NEURONS AGAINST EXCITOTOXICITY THROUGH THE MODULATION OF INTRACELLULAR Ca^{2+} SIGNALING AND MITOCHONDRIAL FUNCTION.

A. Ruiz, E. Alberdi, C. Matute
Departamento de Neurociencias, Achucarro Basque Center for Neuroscience, Universidad del País Vasco (UPV/EHU) y Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED), Leioa, Bizkaia.

Overactivation of the dynamin related protein 1 (Drp1) triggers an imbalance in mitochondrial dynamics towards fission and has been implicated in brain ischemia and neurodegeneration. However, how mitochondrial fission contributes to intracellular Ca^{2+} homeostasis disruption and neuronal death during excitotoxicity is not fully understood. In this work, we have analyzed the effects of Drp1 inhibition by mdivi-1 on NMDA-induced excitotoxicity in primary cortical neurons. NMDA triggered Drp1 dephosphorylation at Ser637 and mitochondrial fission that was inhibited by mdivi-1. Drp1 inhibitor strongly attenuated NMDA-induced calpain activation and neuronal death, suggesting a modulation of cytosolic Ca^{2+} ($[Ca^{2+}]_i$) signals by mdivi-1. Indeed, live cell imaging experiments showed that mdivi-1 depleted ER Ca^{2+} stores and reduced both NMDA-induced $[Ca^{2+}]_i$ and mitochondrial ($[Ca^{2+}]_m$) overloads. In addition, after mdivi-1 incubation neuronal mitochondria were depolarized, basal mitochondrial respiration reduced, and eventually NMDA-produced spare respiratory capacity drop attenuated. However, mdivi-1 turned out to protect neurons against kainate and thapsigargin, which did not trigger mitochondrial fission, whereas knock-down of Drp1 did not prevent NMDA-induced either mitochondrial fragmentation nor cell death. In summary, our results provide evidence that mdivi-1 protects neurons from excitotoxic Ca^{2+} homeostasis disruption by regulating intracellular Ca^{2+} signaling and mitochondrial function through a Drp1-independent mechanism.

Supported by MINECO (SAF2013-45084-R), Gobierno Vasco and CIBERNED

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

THE THERAPEUTIC POTENTIAL OF INTRAVENTRICULARLY INJECTED MESENCHYMAL STEM CELLS IN CHRONIC DEMYELINATING DISEASES

P. Cruz-Martinez [1], J. Jones [1], S. Martinez [1,2]
[1]. Instituto de Neurociencias, Univ. Miguel Hernández (UMH-CSIC), San Juan, Alicante, SPAIN.
[2]. IMIB-Hospital Universitario Virgen de la Arrixaca, Universidad de Murcia, Murcia, SPAIN.

During the past decade, it has been demonstrated the therapeutic potential of mesenchymal stem cells (MSCs) in various neurodegenerative disorders, including demyelinating diseases. However, this effect was generally observed only locally, in the surrounding area where the MSCs were transplanted. Moreover, current treatments modifying the pathological mechanisms are capable of ameliorating the disease symptoms, but are frequently insufficient to repress the progressive loss of myelin and promote functional recovery. Thus, in order to achieve general remyelination in various brain structures simultaneously, bone marrow-derived MSCs were transplanted into the lateral ventricles of chronic demyelinated mice. In this manner, the cells may secrete soluble trophic factors into the cerebrospinal fluid (CSF) and boost the endogenous oligodendrogenic potential of the subventricular zone (SVZ). The results indicated an enhanced recruitment of oligodendrocyte progenitor cells (OPCs) within the corpus callosum (CC) over time, which was correlated with an increase in myelin content. Electrophysiological studies, together with electron microscopy analysis corroborated that the newly formed myelin was functional. Whereas the number of astrocytes seemed to be unaffected, an enhancement in the proliferation of neural stem progenitor cells (NSPCs) was detected in the SVZ, possibly due to their contact with the tropic factors released in the CSF. Hence, the findings of this study revealed that MSCs intraventricular-injection is a feasible method to elicit a paracrine effect in the oligodendrogenic niche of the SVZ, which is prone to respond to the secreted factors and therefore promoting oligodendrogenesis and functional remyelination.

1ª: Trastornos y reparación del sistema nervioso
2ª: Neurobiología del desarrollo

GRAY MATTER CHANGES IN PARKINSON'S DISEASE WITH FREEZING OF GATE

M. Delgado-Alvarado[1], L. García-Penton[2], H. Jimenez-Urbieta[1], B. Gago[1], J. Ruiz-Martinez1[1,5], A. Bergareche[1,5], J.F. Marti-Masso[1,4,5], C. Caballero[2], M. Carreiras[2,3,4] and M.C. Rodriguez-Oroz[1,3,5]
[1]Instituto de Investigación Sanitaria Biodonostia, San Sebastián, Guipuzcoa.
[2]BCBL, Basque Center on Cognition, Brain and Language, San Sebastián, Guipuzcoa.
[3]Ikerbasque, Basque Foundation for Science, Bilbao, Vizcaya.
[4]UPV/EHU, Universidad del País Vasco, Bilbao, Vizcaya.
[5]Unidad de Trastornos del Movimiento del Hospital Universitario Donostia, San Sebastián, Guipuzcoa.

Objectives: To investigate differences in white (WM) and gray matter (GM) integrity between patients with Parkinson's disease (PD) with freezing of gate (PD-FOG) and without FOG (PD-nonFOG) with a similar cognitive profile.
Matherial and methods: 27 PD-FOG patients (age 71.67 ± 8.05), 21 PD-nonFOG (age 70 ± 5.81) and 21 healthy controls (age 66.24 ± 7.16) were studied. FOG was defined as a score ≥1 in item 3 of the FOG questionnaire (Giladi et al. 2000). Participants were clinical and cognitively evaluated (z scores for attention and working memory, executive function, memory, visuospatial function, and language). A MRI study (3-T Magnetom Trio Tim scanner, Siemens AG, Erlangen, Germany) was also obtained, acquiring T1-MRI and DW-MRI images. Results were considered statistically significant at a conservative threshold of $p<0.01$ corrected.
Results: There were no differences between PD-FOG and PD-nonFOG in age ($p=0.4$), years of education ($p=0.5$), gender ($p=0.1$), depression scale ($p=0.26$) and cognition i.e., mean composite (all $p>0.5$). Compared with controls, PD-FOG group had reduction of GM volume in the in right inferior temporal, fusiform, Heschl's, inferior frontal and superior parietal gyri, in the right temporal pole, superior temporal sulcus and right/left amygdala. No difference between PD-FOG and PD-nonFOG and between PD-nonFOG and controls were observed. There was a negative correlation between GM volume and FOG questionnaire score in right fusiform and inferior temporal giry ($p=0.012$; $r= -0.493$). There were no differences in WM among groups.
Conclusions: To our knowledge, this is the first study comparing groups of PD-FOG and PD-nonFOG patients with similar cognitive profile, showing that there are no differences in GM or WM. In contrast, the presence of FOG is associated with GM atrophy mainly in the right hemisphere, in areas involved in cognitive processing such as the temporal areas as well as in the amygdala.

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

SYSTEMICALLY ADMINISTERED EXOSOMES MEDIATE RECOVERY IN THEILER'S MOURINE ENCEPHALOMYELITIS VIRUS ANIMAL MODEL OF MULTIPLE SCLEROSIS.

J. Ramos-Cejudo[1], M. Fernández-Fournier[1], M. Gutiérrez-Fernández[1], B. Rodríguez-Frutos[1], L. Otero-Ortega[1], A. Feliú[2], F. Carrillo Salinas[2], M. Mecha[2], C. Guaza[2], T. Navarro-Hernanz[3], S. Cerdán[3], E. Díez-Tejedor[1]

[1] Department of Neurology and Stroke Center, Neuroscience and Cerebrovascular Research Laboratory, La Paz University Hospital, Neuroscience Area of IdiPAZ Health Research Institute, Autónoma University of Madrid, Madrid, Spain

[2] Neuroimmunology Group, Functional and Systems Neurobiology Department, Cajal Institute, CSIC, Madrid, Spain

[3] Laboratory for Imaging and Spectroscopy by Magnetic Resonance (LISMAR), Institute of Biomedical Research Alberto Sols, CSIC-UAM, Madrid, Spain

Introduction: Exosomes (EXO) are small vesicles of 40 to 150nm released by different cell types, mediating intercellular communication. An animal model that has recently gained relevance in the study of Multiple Sclerosis is Theiler's Mourine Encephalomyelitis Virus (TMEV) model. We aimed to study if the administration of exosomes derived from mesenchymal stem cells could mediate recovery in the TMEV model.

Methods: Exosomes purified from human adipose tissue-derived mesenchymal stem cells were intravenously administered (25ug) to SJL/J mice undergoing demyelinating disease induced by TMEV injection (2x10^6 viral units) on day 60 post infection. Animals (n=27) were randomly divided into 3 groups: Sham, neither viral infection nor treatment (n=7); TMEV-VH, viral infection and saline administration (n=10); TMEV-EXO, viral infection and exosomes administration (n=10). Mice were evaluated clinically and by 7-tesla Magnetic Resonance Imaging (MRI) on day 15 after treatment.

Results: Motor activity was improved in TMEV-EXO animals compared with TMEV-VH treated mice ($P<0.05$). MRI analysis showed partial resolution ventricular atrophy in TMEV-EXO treated animals ($P<0.05$) as well as an improvement in brain connectivity in TMEV-EXO treated mice compared with TMEV-VH animals. Proteomic content of exosomes in cell supernants was analyzed by Orbitrap identifying over 1,300 proteins, including a number of trophic factors and signaling molecules.

Conclusions: Our results suggest that exosomes derived from mesenchymal stem cells derived from the adipose tissue have the potential to mediate recovery after central nervous system demyelination and might provide a novel approach to treat autoimmune diseases such as Multiple Sclerosis.

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

BDNF DELIVERY TO THE ISCHEMIC RAT BRAIN BY ULTRASOUND-TARGETED MICROBUBBLE DESTRUCTION IN SUBCORTICAL STROKE

B.Rodríguez-Frutos[1], J.Ramos-Cejudo[1], M.Gutiérrez-Fernández[1], L.Otero-Ortega[1], P.Martínez-Sánchez[1], I.Sanz-Barahona[1], T.Navarro-Hernanz[2], S.Cerdán[2], E. Díez-Tejedor[1]

[1] Department of Neurology and Stroke Center, Neuroscience and Cerebrovascular Research Laboratory, La Paz University Hospital, Neuroscience Area of IdiPAZ Health Research Institute, Autónoma University of Madrid, Madrid, Spain

[2] Laboratory for Imaging and Spectroscopy by Magnetic Resonance (LISMAR), Institute of Biomedical Research Alberto Sols, CSIC-UAM, Madrid, Spain

Objectives- Ultrasound- targeted microbubble destruction (UTMD) is a non-invasive imaging technique used in stroke patients that can be used for drug delivery. In a previous study from our group, brain-derived neurotrophic factor (BDNF) administration demonstrated efficacy on white matter repair in subcortical stroke in rats. The purpose of this study was to analyze whether BDNF encapsulated in microbubbles with focused ultrasound could be more effective than BDNF alone in an experimental animal model of subcortical ischemic stroke.

Material and Methods-Ischemia was induced by injection of endothelin-1. Sprague-Dawley rats were randomly assigned in three study groups: 1-BDNF; 2-BDNF+UTMD; 3-Control (saline). Treatments were i.v. administered at 24h after stroke. We analyzed: BDNF biodistribution, functional evaluation (Beam Walking, Rotarod and Rogers' tests), lesion size and Apparent Diffusion Coefficient (ADC) as well as fiber tract integrity on Magnetic Resonance Imaging (MRI) and white matter repair markers [A2B5, CNPase, MOG and Olig-2] at 7d and 28d.

Results-BDNF-treated animals showed less functional deficit than control animals ($p<0.05$). Compared with BDNF group, BDNF+UTMD animals showed significantly less deficit in Rogers' test at 28d ($p<0.05$). Although T2-MRI did not show differences in lesion size between groups, ADC maps showed higher diffusion coefficient in BDNF and BDNF+UTMD groups compared with controls at 28d ($p<0.05$). Diffusion Tensor Imaging (DTI) tractography analysis revealed augmented fiber tract connectivity at 28d in BDNF+UTMD animals in comparison with both BDNF and control groups ($p<0.05$). The levels of white matter repair-associated markers (A2B5, CNPase, MOG and Olig-2) were higher in BDNF+UTMD animals compared with BDNF and control groups ($p<0.05$).

Conclusions- BDNF administration with focused UTMD increased BDNF brain levels, improved functional outcome and white matter repair processes (oligodendrogenesis, remyelination and fiber connectivity) compared to BDNF alone after subcortical ischemic stroke in rats.

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

INTRAMUSCULAR GRAFTING OF HEALTHY BONE MARROW CELLS PRESERVES THE MUSCULAR FUNCTION IN AN ANIMAL MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

D. Pastor [1], C. Pastore [2], S, Martínez [1, 3], E. Geijo-Barrientos [1]
[1] Instituto de Neurociencias de Alicante. UMH-CSIC. San Juan, 03550 Alicante. España
[2] Hospital Universitario de San Juan. San Juan, 03550 Alicante. España.
[3] Instituto Murciano de Investigación Biosanitaria, Murcia, España and CIBERSAM

Amyotrophic lateral sclerosis (ALS) is a motorneuron degenerative disease, characterized by degeneration of upper and lower motor neurons that leads to progressive muscle paralysis. We have studied, in an animal model of ALS (the SOD1/G93A$^{+/-}$ mutant mouse) the potential protective effect on spinal motoneurons of bone marrow cells (BMC) grafted directly into Tibialis Anterioris (TA) muscle.
BMC were obtained from femurs of healthy GFP^{+} or SOD1/G93A mice and injected in TA muscle of experimental animals at 75 days of age. We used two control groups: healthy animals of the same strain not transplanted (B6SJL; n=5) and SOD1 affected animals injected with saline (SHAM; n=6) and two experimental groups: SOD1 affected animals injected with BMC from affected SOD1 animals (ISO, n=5) and SOD1 affected animals injected with BMC from healthy littermates (BMC, n=7). At 115 days of age, animals were anesthetized with Ketamine and Xilacine and we recorded the amplitude of the compound muscular action potential (CMAP) of the TA muscle evoked by supramaximal stimulation of the sciatic nerve.
The comparison of the CMAP amplitudes obtained in the four groups with a one way ANOVA revealed significant differences ($p<0.05$) between the SHAM group (11.85 ± 6.12 mV) and the B6SJL (23.08 ± 0.55 mV) and BMC (11.98 ± 5.81 mV) groups. However, there were no differences between the SHAM and the ISO (7.74 ± 1.57 mV) groups. Data given as mean±s.d.
These results suggest that intramuscular transplantation of BMC obtained from healthy animals is able to preserve, at least partially, the muscular function deteriorated in ALS,

Funding: MICINN FONDOS FEDER REF. BFU2011-27326, EUCOMMTOOLS, PROMETEO/2009/028, PROMETEOII/2014/014, Allen Institut for Brain Science, TERCEL RD06/0010/0023/RD12/0019/0024.

1ª: Trastornos y reparación del sistema nervioso

DYRK1A OVEREXPRESSION IMPACT ON SIGNALING NETWORKS: A SYSTEMS BIOLOGY APPROACH

M. Ortega[1,3], E. Sabidó[2,3], M. Dierssen[1,3]
[1]Systems Biology Program, Centre for Genomic Regulation (CRG), PRBB, Barcelona, Spain;
[2]CRG/UPF Proteomics Unit, PRBB, Barcelona, Spain
[3]Universitat Pompeu Fabra, PRBB, Barcelona, Spain

Trisomy for human chromosome 21 results in Down syndrome (DS), the most complex and common genetic perturbation leading to intellectual disability. Dual-specificity tyrosine (Y)-phosphorylation kinase 1A (Dyrk1A) regulates fundamental cellular functions such as cell proliferation and survival and is a plausible candidate gene to explain DS phenotypic abnormalities. We demonstrated that the inhibition of DYRK1A kinase activity with epigallocatechin-3-gallate (EGCG), a flavonol compound found mainly in green tea, rescues the cognitive phenotype in TgDyrk1A mice (only overexpressing the kinase) but also in trisomic mice (Ts65Dn) and in DS humans, suggesting that, normalization of Dyrk1A activity is sufficient to rescue the cognitive and the neuronal phenotype. However, the molecular mechanism through which EGCG rescues the DS phenotypes remains elusive.
In this project, we aim to investigate the molecular effects at the proteome and phosphoproteome level induced by in vivo overexpression of Dyrk1A in the hippocampus of wild type and transgenic mice (TgDyrk1A) and the impact of treatment with EGCG using mass spectrometry-based proteomics to assess significant changes in the abundance of specific proteins and phosphosites amongst the different states. Preliminary data from network analysis revealed that in vivo overexpression of Dyrk1A have a huge impact in altering important cell functions such as mitochondrial regulation and cytoskeleton proteins. Those could be good candidates for the dysfunctions already described in DS patients. We also observed that EGCG can partially rescue DS-related proteins abundance deficits suggesting that they are regulated by DYRK1A kinase activity.
The laboratory of M.D. is supported by DIUE de la Generalitat de Catalunya (Grups consolidats SGR 2014/1125). Supported by MINECO (SAF2013-49129-C2-1-R) and EU (Era Net Neuron PCIN-2013-060). The CRG is a Center of Excellence Severo Ochoa SEV-2012-0208. The CIBER of Rare Diseases is an initiative of the ISCIII.

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

MONITORING PROTOCOL FOR ADDRESSING AND EARLY DIAGNOSIS IN ENU-INDUCED GLIOMAS

A. García-Blanco, S. Bulnes, H. Cepeda, H. Bengoetxea, J.V. Lafuente.
Faculty of Medicine and Odontology, Basque Country University. Leioa. Vizcaya

Homecage systems have been used in psychiatric disease and for neuropharmacological assays in animal models but not as a diagnostic tool. CNS tumours in animal models are in vivo detected by imaging methods but there is not any standardized protocol for the early clinical suspicion. The aim of this work is to propose a monitoring protocol including a homecage system in order to complement visual observation in the early diagnosis of ENU-gliomas.
35 Sprague-Dawley rats developed glioma after prenatal exposition to N-ethyl-N-nitrosourea. The welfare and some clinical parameters (food and water intake, % of time moving, number of rearings, the total of walked distance and % of exploration) were monthly recorded from 4th month up to death age using Morton&Griffith scale and the homecage system (Phecom) respectively.
Results divided animals into three groups: Group A (good welfare and no clinical changes); Group B (good welfare but increase in any of recorded parameters) and Group C (bad welfare and a decrease in any of recorded parameters). A temporary evolution from Group A (5th month of age) to Group C (7th of 8th months) was observed. Rats belonging to Group B seem to be asymptomatic, but they showed mild weight loss and increased activity. Morphologically they correspond to the early state of ENU-glioma. To take in consideration these data could be a useful approach for the early diagnosis of experimental tumours. Rats from group C frequently showed severe physical handicaps being in both groups the distance the most representative parameter modified.
Homecage systems are a useful way to detect changes in the clinical state of experimental animals suggesting the presence of base pathology before detection of clinical symptoms.

1ª Cognitive and Behavioral Neuroscience
2ª Disorders and nervous system repair

IMPAIRMENT OF THE GABAERGIC SEPTOHIPPOCAMPAL CONNECTION IN A MOUSE MODEL OF TAUOPATHY

H. Soler[1,2], M. González[1,2], D. E. Parruca da Cruz[1,2], S. E. Rubio[1,2], J. Avila[2,3], E. Soriano[1,2] and M. Pascual[1,2]

[1.] Department of Cell Biology, University of Barcelona, Barcelona, Spain

[2] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED, ISCIII), Spain

[3] Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain

Alzheimer's disease (AD) modifies the functioning of cortical networks, including altered patterns of synchronous activity and deficit in cholinergic septohippocampal (SH) innervation. The GABAergic component of the SH pathway (SHP) regulates synchronous hippocampal rhythms by controlling the activity of interneurons. Recently, we have reported a dramatic decrease in the GABAergic SHP in a mouse model with a considerable accumulation of amyloid-β deposits (Rubio et al., 2012). Another characteristic feature of AD is the hyperphosphorylation and aggregation of the microtubule-associated protein Tau. To evaluate the role of tauopathy in the GABAergic SHP, we analyzed VLW transgenic mice overexpressing human mutated Tau. Our characterization data show that pyramidal neurons and some Parvalbumin(PV)-positive hippocampal interneurons accumulate phosphorylated forms of Tau (P-Tau) in 2- and 8-months-old (mo) VLW mice. In addition, no P-Tau accumulation was present in GABAergic SH neurons.

Furthermore, using well-characterized tracing experiments, we demonstrate an early onset of GABAergic SHP deterioration in PV-positive interneurons in 2-mo VLW mice. In 8-mo VLW littermates, this alteration was more severe. No major loss of GABAergic SH neurons or PV-positive hippocampal interneurons was observed, thereby indicating that this decline is not caused by neuronal loss, but by the reduced number and complexity of GABAergic SH axon terminals. We thus conclude that GABAergic SH innervation is specifically reduced on PV-positive interneurons accumulating P-Tau in 2- and 8-mo VLW mice. These data, together with our previous results, indicate that the GABAergic SHP is impaired in response to both amyloid-β and P-Tau accumulation, thereby suggesting that cognitive deficits and altered patterns of synchronous activity in AD patients could be caused by the loss of GABAergic SH axons, which modulate hippocampal rhythmic activities. Our findings identify a new target for therapeutic intervention in AD.

1ª: Disorders and nervous system repair
2ª: Systems Neuroscience

NEUROPROTECTIVE EFFECT OF TAURO-URSO-DEOXYCHOLIC ACID ON AXOTOMIZED RETINAL GANGLION CELL.

A. Gracia-Llorente[1], V. Gómez-Vicente[2*], F. Germain[1], N. Cuenca[3], P. de la Villa[1],
[1]Facultad de Medicia, Universidad de Alcalá, Alcalá de Henares, Spain.
[2]Facultad de Optica, Universidad de Alicante, Alicante, Spain.
[3]Facultad de Biología, Universidad de Alicante, Alicante, Spain.

Retinal ganglion cell (RGC) degeneration underlies the pathophysiology of diseases affecting the retina and optic nerve. Several studies have previously evidenced the anti-apoptotic properties of the bile constituent, tauroursodeoxycholic acid, in diverse models of photoreceptor degeneration. The aim of this study was to investigate the effects of systemic administration of tauroursodeoxycholic acid on axotomy-induced damage in the mouse retina using a functional and morphological approach. Tauroursodeoxycholic acid was administered intraperitoneally before and after optic nerve section. One week after insult, patter electroretinograms showed reductions in amplitude, thus indicating the death of RGC. Quantitative morphological evaluation of whole-mount retinas demonstrated a reduction in the density of retinal ganglion cells. Systemic administration of tauroursodeoxycholic acid attenuated the functional impairment induced by optic nerve section, which correlated with a higher retinal ganglion cell density. Our findings sustain the efficacy of tauroursodeoxycholic acid administration *in vivo*, suggesting it would be a good candidate for the pharmacological treatment of degenerative diseases coursing with retinal ganglion cell loss.

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

Neuro-specific FAIM-L stabilizes RIP1 and is essential for TNFR1-induced NF-κB activation

K.M.O. Galenkamp[1], M. Turch-Anguera[1], P. Carriba[1], J.X. Comella[1]
[1]. Cell signaling and Apoptosis. Vall d'Hebron Research Institute, Barcelona

Objectives: FAIM-L is the long and neuro-specific isoform of the Fas Apoptosis Inhibitory Protein (FAIM), primarily described as a protein involved in the inhibition of death receptor (Fas and TNFR1) induced apoptosis in neurons. Recently, we identified FAIM-L to be a critical protein in Alzheimer's disease, since we observed that it is crucial for TNFα-induced rescue from Amyloid-β-induced cell death in cortical neurons. In addition, we observed downregulation of FAIM-L in human brain samples with advanced stages of Alzheimer disease. In the present study, we assessed the molecular mechanism by which FAIM-L mediates TNFα-induced protection against Amyloid-β-induced cell death. For this purpose, we characterized the role of FAIM-L in NF-κB signaling

Material and Methods: For the purpose, we used Western Blot, immunocytochemistry, and qRT-PCR to study the regulation of TNFα-induced activation of NF-κB. In addition, we assessed FAIM-L binding partners and ubiquitination of proteins of interest by (co-)immunoprecipitation and subsequent Western Blot.

Results: We observed that downregulation of FAIM-L inhibits TNFα-induced p65 translocation to the nucleus, which concurred with a lack of IκBα degradation. Moreover we show that overexpression of FAIM-L does not affect the kinetics of TNFα-induced IκBα degradation, indicating that the sole expression of FAIM-L is essential for TNFα-induced activation of NF-κB. Next, we characterized the effects of FAIM-L on the ubiquitination state of RIP1. We were able to show that FAIM-L affects RIP1 ubiquitination and that downregulation of FAIM-L decreases the stability of RIP 1.

Conclusion: These data contribute to a better understanding of the function of FAIM-L in the nervous system and provide directions for the targeting of FAIM-L in Alzheimer disease.

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

POTENTIATION OF PERIPHERAL IMMUNE-MEDIATED OPIOID ANALGESIA BY SIGMA-1 RECEPTOR ANTAGONISM: STUDIES ON INFLAMMATORY PAIN

M.A. Tejada[1,2], A. Montilla-García[1,2], S.J. Cronin[3], J.M. Penninger[3], J.M. Baeyens[1,2], E.J. Cobos[1,2]
[1]. Faculty of Medicine and Neuroscience Institute, University of Granada, Granada, Spain
[2]. Institute of Molecular Biotechnology, Viena, Austria

Objectives: Immune cells at the inflamed site release pronociceptive substances contributing to inflammatory pain. However, these cells also produce endogenous opioid peptides (EOP) whose analgesic potential is barely explored. It is known that σ1 receptor antagonism enhances opioid drug-induced analgesia. Our aim was to study whether σ1 receptors are able to modulate peripheral immune-mediated opioid analgesia during inflammation.
Materials & Methods: Inflammatory hyperalgesia to mechanical (paw pressure) and thermal (plantar test) stimuli was assessed 3 h after the intraplantar administration of carrageenan to CD-1 mice. The drugs used were the selective σ1 antagonists BD-1063 and S1RA, the σ1 agonist PRE-084 and the peripherally acting opioid antagonist naloxone methiodide. The content of β-endorphin in the inflamed paw was determined by ELISA. To block the actions of this EOP in the inflamed paw, we administered an antibody against β-endorphin. The immune cells at the inflamed site and their changes by the in vivo administration of an antibody targeting Ly6G (a molecule involved in neutrophil infiltration) were determined by FACS.
Results: Administration of σ1 antagonists abolished inflammatory hyperalgesia. This effect was reversed by either PRE-084 or naloxone methiodide, indicating that it requires from the activation of peripheral opioid receptors. We found a marked increase in β-endorphin levels in the inflamed paw, and the capture of this EOP by a selective antibody completely abolished the antihyperalgesic effects of σ1 antagonism. Administration of an anti-Ly6G antibody decreased neutrophil infiltration, β-endorphin levels and the antihyperalgesic effects induced by the σ1 antagonists. Therefore, EOPs-containing neutrophils in the inflamed site are necessary for the effects of σ1 antagonism.
Conclusions: σ1 receptor inhibition produces opioid analgesia at the inflamed site by enhancing the effects of EOPs from immune cells. This mechanism maximizes the analgesic potential of immune cells that will naturally accumulate at the painful site, the inflamed area.

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

Topic 7.

Homeostatic and neuroendocrine systems

ESTROGEN RECEPTOR β EXPRESSION FLUCTUATES DURING THE ESTROUS CYCLE CONTRIBUTING TO THE BALANCE OF THE PITUITARY CELL PROLIFERATION

PA. Pérez, JP. Petiti, IA. Wagner, E Sabatino, AL. De Paul, AI. Torres, S. Gutiérrez.
Centro de Microscopia Electrónica, INICSA-CONICET, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

17β-estradiol (E2) levels fluctuate during the estrous cycle regulating pituitary cell proliferation through its specific estrogen receptors (ER) α and β. Although an important number of studies have demonstrated the function of ERα in pituitary cell proliferation, the role of ERβ in the growth of pituitary cells is not well known. This background leads us to study the expression of ERβ during the estrous cycle and analyze if this subtype of ER modulates the expression of cell cycle regulators.
Pituitary glands from female Wistar rats at different stages of the estrous cycle (proestrus, estrus and diestrus) and GH3 cells transfected to over-expression of ERβ (GH3β+) were used. E2 and the agonists of ERα (PPT) and ERβ (DPN) were employed. ERα and β expression was quantified by flow cytometry, cyclin D1 and CDK4 expression was detected by Western Blot and the GH3 proliferation was analyzed by BrdU technique. The statistical analysis used was ANOVA-Tukey.
34.12 ± 7.7 % of pituitary cells obtained from rats in diestrus expressed ERβ, 35.4 ± 2.5 % were ERβ+ in the proestrus, with a decrease in the ERβ expression in estrus, with an 18.79 ± 1.1% of ERβ+. However, ERα levels were similar during the estrous cycle. In pituitary cells from rats in proestrus and estrus the cyclin D1 levels were increased in relation to those found in diestrus. Over-expression of ERβ in GH3 cells decreased the pituitary cell proliferation and the cyclin D1 level expression.
These results indicate that ERβ levels in the pituitary gland fluctuate during the estrous cycle and suggest that its expression is modulated by E2. The ERβ exerts an inhibitory role on the anterior pituitary cell proliferation with participation of the cyclin D1, adjusting the cell population and its functionality to the body's requirements.

NEONATAL LEPTIN ADMINISTRATION NORMALIZES SOME BUT NOT ALL THE LONG TERM NEUROENDOCRINE CHANGES INDUCED BY MATERNAL DEPRIVATION IN RATS

V. Mela[1], F. Díaz[2,3], Jesús Argente[2,3,4], J.A. Chowen[2,3] Maria-Paz Viveros[1]
[1]. Department of Physiology (Anim Physiol II), Faculty of Biology. Complutense University Madrid, Spain
[2]. Department of Endocrinology, Hospital Infantil Universitario Niño Jesús, Instituto de Investigación la Princesa, Madrid, Spain
[3]. CIBEROBN Instituto Carlos III Madrid, Spain
[4]. Department of Pediatrics, Universidad Autónoma de Madrid, Madrid, Spain.

Introduction and Objectives: Maternal deprivation for 24 hrs on postnatal day (PND) 9 (MD) markedly reduces leptin levels coinciding with the peak of the physiological neonatal leptin surge (PND 9-10) and has diverse long-term neuroendocrine effects. We hypothesized that replacement of leptin would normalize these alterations.

Methods: MD was carried-out in Wistar rats for 24 hrs on PND 9. Female and male MD and control rats were treated from PND 9 to13 with rat leptin (3 mg/kg/day sc) or vehicle. Sexual behavior was measured in males at PND84 and rats were sacrificed at PND90. Serum estradiol and testosterone levels were measured by ELISA and RIA and genes related to reproduction and metabolism were measured by RT-PCR in the hypothalamus (HT).

Results: MD females showed a decrease in estradiol levels and leptin treatment reversed this effect. In males, testosterone levels were increased by leptin treatment. The following changes were observed in the hypothalamus: 1) MD increased NPY and POMC mRNA levels in males, with leptin treatment normalizing only NPY; 2) In controls, leptin increased POMC and CART in males and orexin and leptin receptor mRNA levels in both sexes; 3) In MD males leptin normalized the rise in NPY, but not POMC, in males and did not stimulate CART or leptin receptor mRNA levels in either sex. With respect to sexual behavior in males: 1) Leptin treatment decreased ejaculation latency in the control group, but not in MD rats; 2) Leptin treatment tended to increase ejaculation frequency in the control group and reduce it in MD rats, 3) MD tended to decrease the mount/intromission ratio and leptin treatment normalized it.

Conclusion: Neonatal leptin treatment and MD induce diverse long-term neuroendocrine effects that are sex-dependent. Leptin replacement reversed/attenuated some, but not all the long-term neuroendocrine alterations induced by MD.

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

RELAXIN3 RECEPTOR ACTIVATION INDUCES ERK PHOSPHORYLATION IN SPECIFIC CHAT SEPTAL NEURONS

H. Albert-Gascó[1], S.Moustafa[1], A. García Avilés[1], I. Arnal-Vicente[1], N. Gomes[1], M. Larrosa-Flor[1], FE. Olucha-Bordonau [1]AM Sánchez-Pérez
Departamento de Medicina, Universidad de Jaume I, Castellón de la Plana, (Castellón).

The septal area has traditionally been seen as a relay station which connects telencephalic areas with regions of the brain stem. This concept rose from the observation of a complementary set of neural connections from nucleus incertus to the medial septum and lateral septum that regulate the hippocampal theta rhythm. Upon stimulation of the NI in urethane-anesthetised rats increase hippocampal theta rhythm. In contrast; NI electrolytic lesions cause an abolishment of hippocampal theta rhythm produced by reticularis pontis oralis (RPO) stimulation. RPO stands as a main theta rhythm generator. The nucleus incertus is the main area where Relaxin3 peptide is synthesized. In vitro studies have demonstrated that relaxin3 treatment is able to induce Erk1-2. The aim of this work is to elucidate whether or not relaxin3 receptor agonist are able to induce Erk phosphorylation in the septum in *in vivo*. Three experimental groups were considered; **naïve** animals, which did not, received any treatment. **R3/I5** animals, treated with relaxin3 peptide agonist (5ug/ul); and **sham** animals, rats which underwent surgery but only received vehicle. Each of the three groups were sacrificed at; 20 and 90 minutes after either R3/I5 agonist or vehicle injection. Brain was isolated in cold, areas dissected out and, homogenized. Erk phosphorylation was measured by Immunoblot and Immunohistochemistry. Immunoblot results were normalized to the levels of total Erk. Cerebellar Erk phosphorylation was not altered by R3/5 infusion. However, in the septum and the amygdala, Erk phosphorylation significantly increased up to 50% in R3/I5 injected cases compared to naïve and sham animals. Our results suggest that Erk phosphorylation is both time dependent and neuron specific at the septal areas. Mainly, we found co-localization between phosphorylated Erk and cholinergic neurons. These data provides *in vivo* evidence for the role of relaxin3 inducing Erk phosphorylation in the amygdala and septum and furthers confirms the role of nucleus incertus in regulating septal cholinergic activity via relaxin3 ascending projections.

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

1. Sistemas homeostáticos y neuroendocrino
2. Neurociencia cognitiva y conductual

REGULATION OF FEEDING BEHAVIOR AND GLUCOSE HOMEOSTASIS IN A DOWN SYNDROME MOUSE MODEL

M. Fructuoso [1,2,3], E. Philippe [4], J. Castel[4], R. Denis [4], S. Luquet[4], H. Le Stunff [4], C. Magnan [4], N. Janel, JM Delabar [4], M. Dierssen [1,2,3]

[1]Center for Genomic Regulation (CRG), E-08003, Barcelona, Spain.
[2]University Pompeu Fabra (CEXS-UPF), E-08003, Barcelona, Spain.
[3]CIBER of Rare Diseases (CIBERER), E-08003, Barcelona, Spain.
[4] University Paris Diderot, UMR8251, Paris, France.

Down syndrome (DS) results from a trisomy of chromosome 21 leading to anomalies of both nervous and endocrine systems. Differences in feeding practices and reduced self-control may contribute to the prevalent overweight in DS. However, energy balance disturbances could also explain the phenotype.

To address this issue, we have studied feeding behavior, glycemic control and energy expenditure in a trisomic mouse (TS65Dn) and its normosomic littermates (2N). Ts65Dn mice fed with standard chow diet (SC) showed lower eating rate and less number of SC meals than 2N mice. However, the average SC intake and the duration of the meals were higher. We studied the glycemic and insulinaemic profiles after administration of a glucose load by the oral glucose tolerance test. Interestingly, Ts65Dn mice showed a more rapid recovery of basal glycemic profiles after administration of a glucose load, suggesting a better glycemic control, previously reported in DS patients.

Exposition to a high-fat diet (HFD) induces obesity and feeding behavior changes in Ts65Dn mice. HFD is often associated with the installation of insulin resistance state and as a compensatory mechanism through the up-regulation of pancreatic β cell mass and insulin secretion. We have also studied glycemic control, energy expenditure and the endocrine pancreatic function in Ts65Dn after HFD regime and compared it with SC regime and its 2N littermates.

Taken together, the results will help to better understand the complexity of the obesity phenotype in Down syndrome and ultimately improve future therapeutic solutions.

Acknowledgements: Foundation Jerôme Lejeune and Spanish Ministry of Economy and Innovation SAF2013-49129-C2-1-R funded this work.

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

THE METABOLIC-DISRUPTOR ROLE OF CHLORPYRIFOS IN APOE3 TRANSGENIC AND C57BL/6N MALE MICE: FROM FEEDING BEHAVIOR TO HORMONAL IMBALANCE

F. Peris-Sampedro [1,2,3], M. Cabré [1,4], P. Basaure [1,2,3], T. Neri-Gómez [5], I. Reverte [1,2], M.T. Colomina [1,2]
[1]. Research in Neurobehaviour and Health (*NEUROLAB*), Universitat Rovira i Virgili, Tarragona
[2]. Department of Psychology and Research Center for Behavioural Assessment (*CRAMC*), Universitat Rovira i Virgili, Tarragona
[3]. Laboratory of Toxicology and Environmental Health, School of Medicine, *IISPV,* Universitat Rovira i Virgili, Reus, Tarragona
[4]. Department of Biochemistry and Biotechnology, Universitat Rovira i Virgili, Tarragona
[5]. Department of Biology, Universidad Autónoma de México, México DF

Nowadays, obesity and type 2 diabetes have become pandemic. In the light of this trend, research has been extended to new or, so far unsuspected risk factors. Although epidemiological studies linked pesticide exposure to both diseases outcomes, underlying mechanisms remain unclear. Multiple signals from gut and fat tissue convey in the brain to control feeding and energy balance, where hypothalamus and serotonergic system are key mediators. Our results highlighted gene x environment interactions: apolipoprotein E3 (apoE3) mice were more prone to become overweighed than apoE2 and apoE4 upon exposure to chlorpyrifos (CPF), a common pesticide used worldwide. Moreover, it has been shown that CPF increases impulsivity and alters monoamine systems. We aimed to characterize neuroendocrine and metabolic effects of a subchronic CPF ingestion in both apoE3 and C57BL/6N adult male mice. Animals were fed a standard or a CPF-supplemented (2 mg/kg body weight/day) chow for 8 consecutive weeks. We examined body weight, food intake, lipid and glucose homeostasis, metabolic biomarkers, insulin levels and resistance, as well as leptin and ghrelin profiles. Moreover, we studied the expression of the serotonin transporter (SERT) and the 5-HT2A receptor in hypothalamic areas. CPF increased food intake, glucose and total cholesterol concentrations, and tended to elevate acyl ghrelin levels. Nonetheless, excess weight gain and increased leptin levels were inherent to apoE3 mice. Moreover, the propensity towards a diabetic profile was notably higher in these animals, as they showed a higher homeostatic model assessment index for insulin resistance and higher insulin levels. The expressions of SERT and 5-HT2A receptor were generally increased in exposed animals, suggesting lower levels of serotonin in the hypothalamus. These data support the metabolic-disruptor role of CPF, and point to a markedly susceptibility of the *APOE3* genotype. It is worth asking whether both factors could contribute to the incidence of such diseases.

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

Fiona Peris-Sampedro
fiona.peris@urv.cat

DYSBIOTIC MICROBIOME AND ITS IMPACT ON THEILER'S VIRUS DEMYELINATING DISEASE OUTCOME

FJ Carrillo-Salinas[1], A Feliú[1], M MechaM[1], L Mestre[1], R del Campo[2], C Espejo[3], X Montalbán[3], JC Álvarez-Cermeño[2], LM Villar[2], C Guaza[1]
[1] Instituto Cajal, CSIC; Madrid, Spain
[2]Hospital Universitario Ramón y Cajal, Madrid, Spain
[3]Hospital Vall d'Hebron; Barcelona, Spain

Alterations in the balance of the gut microbioma have been associated with detrimental or protective effects in experimental autoimmune diseases. It is unknown the capacity of commensal gut bacterial to modify Theiler's virus induce demyelinating disease (TMEV-IDD) a model of primary progressive MS. In this study we investigated whether the oral treatment with a broad spectrum of antibiotics (ABX) influence immune responses to brain TMEV infection and the later disease outcome. Female SJL/J mice were infected intracerebrally with $2x10^6$ PFU of TMEV. Mice were provided autoclaved drinking water supplemented with ampicillin, neomycin sulfate, metronidazole and vancomycin and were sacrificed at 15 days pi or at 80 days pi to assess the influence of ABX in the outcome and severity of the disease. Pyrosequencing studies of fecal samples showed that ABX treatment completely deplete bacterial microflora. Brain TMEV infection modified the intestinal flora compared with control mice. One of the most significant findings include those related to microglia since TMEV-infected mice showed microglial activation restricted only to the striatum whereas ABX-TMEV mice displayed persistent microglial activation in cerebral cortex and expanded inflammatory response to most caudal brain structures such as brain stem. ABX-TMEV mice present less CNS $CD4^+$T, $CD8^+$T and Tregs cells than TMEV-infected mice. A similar profile was observed in cervical lymph nodes but in mesenteric lymph nodes not only T cells were reduced also macrophages resulted significantly decreased by ABX treatment. ABX-TMEV treated mice maintained for long-term disease showed increased mortality, but the surviving mice displayed similar motor activity than TMEV mice. Significant changes in the expression profile of cytokines and chemokines were also observed in the cerebral cortex of TMEV and ABX-TMEV mice. Our data demonstrate that antibiotic modification of gut commensal bacterial influence brain and peripheral immune responses to TMEV infection and the course of TMEV-IDD.

This work was supported by the MINECO grant SAF2013-4274R and Red Española de Esclerosis Múltiple RD12/0032/0008.

Francisco Javier Carrillo-Salinas; francisco.carrillo@cajal.csic.es

1. Homeostatic and neuroendocrine systems.
2. Disorders and nervous system repair.

NEO-EPITOPES EMERGING IN THE DEGENERATIVE HIPPOCAMPAL GRANULES OF AGED MICE ARE TARGET OF NATURAL ANTIBODIES OF IGM TYPE

G. Manich[1], I. Cabezón[1], E. Augé, M. Pallàs[2], A. Camins[2], C. Pelegrí[1], J. Vilaplana[1]
[1]. Departament de Fisiologia, Facultat de Farmàcia, Universitat de Barcelona, Barcelona
[2]. Unitat de Farmacologia i Farmacognòsia, Facultat de Farmàcia, Universitat de Barcelona, Barcelona

Pathological granular structures, described as Periodic Acid Schiff granules due to their reactivity with this staining, appear progressively with age in the hippocampus of most mice strains, and especially in the senescence-accelerated mouse prone-8 strain. These structures are formed by degenerative mechanisms that occurs mainly in astrocytes' processes but that can also affect structures of the surrounding neuropil. We recently reported that granules contain a neo-epitope recognized by IgM antibodies that are present as contaminants in many commercial antibodies obtained from mouse or rabbit ascites or serum. In the present work we hypothesised that these IgMs directed against the neo-epitope are, in fact, natural antibodies. Natural antibodies are generated spontaneously from foetal stages without previous contact with external antigens. Their reactivity pattern is determined evolutionarily and they are remarkably stable within species and even between species. Some of these natural antibodies are able to recognize neo-epitopes that are formed in cell remnants or in apoptotic or altered cells, and they can thus intervene in their controlled elimination. To test if the IgMs anti-neo-epitope are natural antibodies, we evaluate the presence of IgM anti-neo-epitope in mouse sera from SAMP8, ICR-CD1 and BALB/C strains at 3 and 6 months of age. Moreover, we tested the presence of these antibodies in sera from ICR-CD1 and BALB/C mice born and maintained under specific opportunistic pathogen-free conditions until their sacrifice. In all animals from all strains and in any of the tested ages and conditions, we observed that the sera contained IgMs anti-neo-epitope, thus indicating that the IgMs anti-neo-epitope are natural antibodies. The existence of these natural antibodies not only would explain why they are very frequently found as contaminants in commercial antibodies but also would open a new approach in the therapy and diagnosis of pathological brain processes, based on natural IgMs and neo-epitopes.

1ª: Sistemas homeostáticos y neuroendocrino
2ª: Trastornos y reparación del sistema nervioso

TUNING THE BRAIN FOR MOTHERHOOD: PROLACTIN-LIKE CENTRAL SIGNALLING IN VIRGIN, PREGNANT AND LACTATING FEMALE MICE

H. Salais-López[1], E. Lanuza[2], C. Agustin-Pavón[1], F. Martinez-García[1]
[1]. Facultat de Ciències de la Salut, Universitat Jaume I, Castelló de la Plana, Spain
[2]. Facultat de Ciències Biològiques, Universitat de València, València, Spain

Prolactin is essential for the correct expression of maternal behaviour in rodents. Administration of prolactin on top of a proper gonadal steroid background accelerates the onset of maternal behaviour in neophobic virgin female rats. In mice, by contrast, the specific role and period of action of prolactin remains unclear. In this work, we aim to characterize the actions of this hormone in the brain throughout the reproductive cycle of the female mouse (virgin, late-pregnant and lactating mice). Therefore, we employ the immunohistochemical detection of the phosphorylated (active) form of STAT5 (pSTAT5), a pivotal element in the signalling cascade of the prolactin receptor. We also intend to discriminate the contribution of alternative lactogens during pregnancy by suppressing the hypophyseal release of prolactin in an additional group of late-pregnant females.

The brain of virgin female mice shows a variable yet overall moderate pattern of pSTAT5 immunoreactivity. Conversely, late-pregnant and lactating females display a widespread, stable pattern of pSTAT5 immunostaining comprising areas of the sociosexual brain involved in the expression of maternal behaviour, e.g. the septal region, medial and central extended amygdala, preoptic, anterior and tuberomammillary hypothalamus and some thalamic, midbrain and brainstem nuclei (periaqueductal grey, raphe and parabrachial nuclei, among others). The pattern of pSTAT5 immunoreactivity is not affected by administration of bromocriptine, suggesting it to be elicited by non-hypophyseal lactogenic agents, likely placental lactogens.

In sum, our results reflect how the shaping of the maternal brain in mice is taking place prior to parturition and suggest that lactogenic agents are important candidates in the development of maternal behaviours already during pregnancy.

Funded by MINECO/FEDER (BFU2013-47688-P) and Government of Castilla-La Mancha/FEDER (PEIC11-0045-4490).

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

CORTISTATIN-14 INHIBITS INSULIN SECRETION IN PANCREATIC BETA CELLS

Manuel Castellano-Muñoz[1], Alex Rafacho[2], Paloma Alonso-Magadalena[1], Esperanza Irles[1], Melisa Bello[1], Beatriz Merino[1], Sergi Soriano[3], Pau Iborra[1], Antonia Ruiz[1], Raúl M. Luque[4], Ángel Nadal[1], Iván Quesada[1].

1,Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM) y Universidad Miguel Hernández de Elche, Elche 03202, Alicante, Spain.

2,Department of Physiological Sciences Center of Biological Sciences, Federal University of Santa Catarina, 88040-900, Florianópolis, SC, Brazil.

3,Departamento de Fisiología, Genética y Microbiología. Universidad de Alicante, 03080, Alicante, Spain.

4,Departamento de Biología Celular, Fisiología e Inmunología de la Universidad de Córdoba, Spain.

Cortistatin (CORT-14) is a neuropeptide commonly expressed in inhibitory neurons of the cerebral cortex, having a strong structural, pharmacological and functional homology to somatostatin (SST). In addition to having roles in the central nervous system, both peptides also regulate endocrine secretion. However, the cellular mechanisms supporting this role are poorly characterized. We studied the potential role of CORT-14 and SST in the insulin-secreting pancreatic β cells of the Islets of Langerhans. Using insulin secretion assays, we observed that both CORT-14 and SST reduced insulin release. The reduction in insulin secretion was paralleled by a decrease in glucose-induced calcium levels observed by fura-2 calcium imaging. Additionally, the effect of CORT-14 in β cell calcium load was blocked by specific SST-R5 receptor antagonists, suggesting a higher affinity of CORT-14 for this receptor. Using perforated patch clamp experiments we demonstrated that application of CORT-14 hyperpolarized pancreatic pancreatic β cell thus decreasing action potential firing. Additionally, CORT-14 reduced calcium currents in whole cell patch clamp experiments. Our results suggest that the binding of CORT-14 to SST-R5 receptors leads to pancreatic β-cells hyperpolarization and triggers the inhibition of calcium channels, thus reducing calcium load and consequently insulin secretion.

1. Excitabilidad neuronal, sinapsis y glía: mecanismos celulares
2. Sistemas homeostáticos y neuroendocrino

OREXINS/HYPOCRETINS REGULATE THC-INDUCED HYPOTHERMIA, ANTINOCICEPTION AND ANXIOLYTIC-LIKE EFFECTS

Á. Flores de los Heros, M. Julià-Hernández, R. Maldonado López, F. Berrendero Díaz.
Laboratory of Neuropharmacology, Department of Experimental and Health Sciences, Pompeu Fabra University, Barcelona

Emerging evidence suggest the existence of a cross-talk between orexinergic and endocannabinoid signalling. Thus, orexin signalling has been reported to stimulate the synthesis of 2-arachidonoyl glycerol leading to retrograde inhibition, suggesting that endocannabinoids might contribute to several orexin effects. Moreover, orexins modulate the rewarding effects of cannabinoids through orexin receptor-1 (OXR1) signalling. In the present work we evaluated the role of orexin transmission in the acute pharmacological effects induced by delta-9-tetrahydrocannabinol (THC), the main psychoactive compound of the *Cannabis sativa* plant. We employed orexin-deficient mice as well as C57BL6/J mice preteated with the OXR1 antagonist SB334867 (5 mg/kg) or the orexin receptor-2 (OXR2) antagonist TCSOX229 (10 mg/kg). Locomotion, hypothermia and analgesia were assessed following acute administration of THC at 5 and 10 mg/kg. Additionally, anxiety-like behaviour was evaluated using anxiolytic (0.3 mg/kg) and anxiogenic (5 mg/kg) doses of THC in the elevated plus maze. THC-induced amnesic-like effect was also assessed in the object recognition task. Orexin-deficient mice presented decreased THC-induced hypothermia and antinociception in the hot plate test, as well as reduced anxiolytic-like effect. Conversely, no differences between genotypes were observed in hypolocomotion and analgesia in the tail immersion paradigm, as well as in anxiogenic- and amnesic-like effects induced by THC. Pretreatment with TCSOX229, but not with SB334867, was able to mimic the findings observed in orexin knockout mice. Immunoblot analysis revealed that orexin-deficient mice show normal CB1 levels in all brain regions analysed. Immunofluorescence studies showed reduced THC-induced c-Fos expression in the central amygdala and the preoptic area of orexin knockout mice. Therefore, our results indicate that orexins modulate some cannabinoid-induced effects such as hypothermia, supraspinal antinociception and anxiolysis, probably through OXR2 signalling and independently from OXR1.

1. Homeostatic and neuroendocrine systems
2. Disorders and reparation of the nervous sytem

CHANGES IN THE MORPHOLOGICAL AND NEUROCHEMICAL PROPERTIES OF CORNEAL COLD SENSORY NEURONS AND THEIR PERIPHERAL AXONS AND BASAL TEARING RATE IN AGED MICE

A. Íñigo-Portugués[1], I. Alcalde[1], O. González-González[1], J. Gallar[2], J. Merayo-Lloves[1], C. Belmonte[1, 2].
[1]. Instituto Universitario Fernández-Vega. Universidad de Oviedo. Asturias
[2]. Instituto de Neurociencias. Universidad Miguel Hernández-CSIC. San Juan de Alicante. Alicante.

Purpose: To determine in aging mice the proportion, morphology and neurochemical characteristics of trigeminal ganglion cold primary sensory neurons and of their cold peripheral axons innervating the cornea and the relationship with basal tearing rate.
Materials and Methods: Corneas and trigeminal ganglions (TG) of TRPM8-EYFP mice of different ages ranging from 90 to 720 postnatal days were studied. Basal tearing was measured in anesthetized animals, using phenol red threads. Corneal nerves expressing EYFP protein and neuronal class III beta-tubulin were identified in whole mount corneas using immunocytochemical techniques. TG corneal neurons were labeled with fast blue onto the cornea in anesthetized mice. Then, TG were processed for immunofluorescence techniques against Peripherin, NF200, TrkA, CGRP and GFRα3 and counted.
Results: In 3-months mice, $TRPM8^+$ sub-basal nerve fibers represent 22,4 % of the total number of sub-basal nerve branches; most of them were beaded axons finally ramifying in the uppermost epithelium as a cluster of beaded nerve terminals. Less abundant, longer and narrower fibers lacking beads and ending as a single or double bulbous terminal branch were also found. The total number of $TRPM8^+$ sub-basal nerve filaments and epithelial terminals decreased non-linearly with age. In the TG two populations of TRPM8 corneal neurons with different neurochemical signature were identified and classified as intense (IF-EYFP) and weak (WF-EYFP) immunofluorescent neurons and their neurochemical properties changed with age. In parallel, basal tearing rate was altered in aged mice.
Conclusions: Alteration of basal tearing rate was observed in aged mice and which could be related with the disturbances in morphological and neurochemical properties of corneal cold sensory neurons.

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

LACK OF NEURONAL IL-6 IMPAIRS GROWTH AND HINDERS HIGH-FAT DIET-INDUCED OBESITY IN MICE

O. Fernández Gayol[1], M Giralt[1], G Comes[1], A Molinero[1], J Hidalgo[1]
[1] Institut de Neurociències. Facultat de Biociències. Universitat Autònoma de Barcelona, Bellaterra (Barcelona)

Interleukin-6 (IL-6) is a pleitropic cytokine involved in the control of body weight as evidenced by results with IL-6-deficient mice, which develop mature onset obesity. Moreover, transgenic mice with astrocyte-targeted production of IL-6 are resistant to high-fat diet-induced obesity, highlighting the role of centrally produced IL-6 in regulating weight. Results from our group show that astrocyte-specific IL-6 KO mice are bigger and more prone to high-fat diet-induced obesity.
Objectives: to characterize the role of neuronal IL-6 in the regulation of body weight.
Material and methods: conditional knock-out mice for IL-6 in neurons (neu-IL6KO) were generated using the Cre-lox technology. Male and female mice were weighed until age 11 weeks and measured on weeks 10 (nose-to-anus distance) and 13 (tibia). Activity was assessed at age 10 weeks with the open field test. Male and female neu-IL6KO and floxed controls were fed a high-fat diet (58% kcal from fat) for 12 weeks and their body weight and food intake compared with those of mice fed chow (18% kcal from fat). Hypothalamic neuropeptide mRNAs were quantified using qPCR.
Results: Neu-IL6KO mice weigh less, a difference seemingly explained by a shorter length and higher activity, rather than decreased fat depots. When fed a high-fat diet, both male and female neu-IL6KO mice had a lower final weight, partially explained by lower liver weight and reduced white adipose tissue depots. However, only female neu-IL6KO mice had impaired weight gain, suggesting an additional mechanism by which neuronal IL-6 hinders body weight gain. No differences in food intake were observed between genotypes, although neu-IL6KO males tended to eat less. Moreover, there were no alterations in the expression of hypothalamic neuropeptides regulating food intake and energy expenditure.
Conclusions: Lack of neuronal IL-6 impairs linear growth and response to a high-fat diet in an opposite way to astrocytic IL-6.

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

RELEASE OF CALCITONIN GENE-RELATED PEPTIDE IN NORMOTENSIVE YOUNG INDIVIDUALS DURING A MAXIMAL EXERCISE TEST.

A. Aracil [1,2], D. Pastor [1,2], J. M. Sarabia [2], S. Guillén, [2,3], R. López-Grueso, [2], M. Moya [2]
[1] Instituto de Neurociencias, UMH-CSIC (Alicante)
[2] Centro de Investigación del Deporte, UMH (Alicante)
[3] Hospital IMED, Elche (Alicante)

Objective: Calcitonin gene-related peptide (CGRP) is an inotropic, chronotropic and vasodilator agent that decreases arterial stiffness, thus lowering blood pressure. CGRP increases in plasma after bouts of exercise. Given the fact that during exercise systolic blood pressure increases, we examined if the exercise-induced release of CGRP was related with the magnitude of the blood pressure elevation. **Methods:** Twenty-one young and normotensive students (24.4 ± 0.7 years, BMI: 23.2 ± 0.5 $Kg \cdot m^{-2}$; 14 male, 7 female) participated after signing an informed consent. Participants performed a maximal ergoespirometry on a treadmill, and an automated sphygmomanometer was placed on the left arm. Venous blood samples were taken at rest, maximal effort and recovery trough an intravenous catheter placed on the right antecubital vein. Plasma CGRP concentration ([CGRP]) was measured by enzyme-immunoassay. **Results:** At maximal effort, [CGRP] increased 3.0 ± 0.72 fold in respect to baseline (p=0,002, paired t test). Systolic blood pressure also increased at maximal exercise (131.6 ± 3.3 vs 177.0 ± 4.3, baseline vs maximal effort, respectively, p<0,001, t test). A positive significant correlation was found among the systolic blood pressure and [CGRP] (r=0,261; p=0,04). **Conclusion:** Acute bouts of maximal exercise elicit increases of circulating CGRP. CGRP concentration seems to correlate with systolic blood pressure, thus opening the question of which physiological mechanisms can be involved in the exercise-induced CGRP release.

1.- Sistemas homeostáticos y neuroendocrino.

Topic 8

New methods and technologies

BIOFUNCTIONALIZED MICROFIBERS FOR ULTRASENSITIVE NEURAL RECORDINGS

H. Vara Rivera, J.E. Collazos Castro.
Hospital Nacional de Parapléjicos, Toledo

Long-term viability and biocompatibility are main concerns regarding currently available microelectrodes for neural interfaces and implants. Carbon microfibers (CMFs) coated with conducting polymers may provide a solution for long-term recording of activity from small groups of neurons. Attaching cell adhesion molecules to the electro-sensitive surface might further improve electrode-neuron contact, thus enhancing neural signal fidelity, simultaneously enhancing biocompatibility. We fabricated biofunctionalized microelectrodes consisting of 7-µm diameter CMFs coated with poly(3,4-ethylenedioxythiophene) doped with poly[(4-styrenesulfonic acid)-*co*-(maleic acid)] (PEDOT:PSS-*co*-MA), and subsequently linked the adhesion molecule N-Cadherin to the polymer surface. 20-, 50- and 250-µm-long microelectrodes were fabricated. Electrode properties were characterized by cyclic voltammetry and by chronoamperometric techniques, revealing that PEDOT:PSS-*co*-MA coating increases charge storage capacity (which predicts a longer electrode life and lesser electrode failure). Electrode ability to detect neural activity was evaluated by recording spontaneous multi-unit activity and sharp wave-ripple complexes, as well as evoked field excitatory postsynaptic potentials (fEPSPS) in rat hippocampal slices *in vitro*. The effects of electrode length and functionalization were compared. PEDOT:PSS-*co*-MA coating reduced the electrical background noise of electrophysiological recordings, although it had no significant effect on the amplitude of recorded biopotentials. On the other hand, surface biofunctionalization with N-Cadherin increased the amplitude of the recorded multi-unit activity, leading to a double signal-to-noise ratio with respect to bare CMF electrodes. Further reduction in the noise was observed in the longest, 250-µm biofunctionalized electrodes. Analysis of evoked fEPSPs showed no changes in input/output curves nor in paired-pulse facilitation due to polymer coating or biofunctionalization, ruling out eventual acute deleterious effects of these electrodes at the synaptic level. In light of these results, electrode biofunctionalization procedure described here opens a new ground for the development of advantageous neural recording systems.

Áreas Temáticas:

1ª (8) Nuevos métodos y tecnologías
2ª (6) Trastornos y reparación del sistema nervioso

EFFECT OF INTRANASAL ADMINISTRATION OF ENCAPSULATED GDNF IN AN ANIMAL MODEL OF PARKINSON´S DISEASE

O. Gartziandia[1,2], E. Herrán[1,2], J.A. Ruiz-Ortega[3,4], C. Miguelez[3,4], M. Igartua[1,2], J.V. Lafuente[5], J.L. Pedraz[1,2], L. Ugedo[3], R.M. Hernández[1,2]

[1]NanoBioCel Group, Laboratory of Pharmaceutics, University of the Basque Country (UPV/EHU), School of Pharmacy, 01006, Vitoria, Spain;

[2]Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Vitoria, Spain.

[3]Dpt. Pharmacology, School of Pharmacy, University of the Basque Country (UPV/EHU), 01006, Vitoria, Spain.

[4]Dpt. Pharmacology, Faculty of Medicine and Dentistry, University of the Basque Country (UPV/EHU), 48940, Leioa, Spain,

[5]LaNCE, Dept Neurosciences, University of the Basque Country (UPV/EHU), Leioa, Spain

BACKGROUND: Neurotrophic factors, such as the glial cell- derived neurotrophic factor (GDNF), are promising alternatives to treat Parkinson's Disease (PD). However, their short half-life and rapid degradation after *in vivo* administration has limited the clinical use. Recently, the intranasal delivery has appeared as an alternative to release GDNF directly to the brain.

OBJECTIVES: To evaluate the *in vivo* neuroprotective effect of intranasally administered GDNF, encapsulated in Chitosan-nanostructured lipid carriers (CS-NLCs), in the 6-hydroxydopamine (6-OHDA) rat model.

MATERIAL AND METHODS: Rats were rendered parkinsonian by the administration of 6-OHDA into the medial forebrain bundle and immediately after treated for two weeks with intranasal GDNF (Groups: Sham, CS-NLCs-without GDNF, CS-NLCs loaded with GDNF, 2.5 µg GDNF/day, and GDNF in PBS solution ,2.5 µg GDNF/day). During the following seven weeks, motor impairment was evaluated using the cylinder test and amphetamine-induced rotational behaviour. At the end of the study, the degree of dopamine denervation was analyzed using the tyrosine hydroxylase immunostaining.

RESULTS: At the end of the treatment, the group of rats treated with CS-NLC-GDNF showed significantly less amphetamine-induced rotations as well as a reduction of forelimb asymmetry compared to the other rat groups. The behavioural improvement was significantly correlated with the number of remaining cells in the substantia nigra compacta and TH-positive striatal density.

CONCLUSIONS: These results demonstrate that the administration of CS-NLCs loaded with GDNF by intranasal route, induces an improvement in the degree of injury in the striatum and SNc, and suggest that it could be a promising therapy for the treatment of PD.

Support by Saiotek S-PE13UN048, Ministerio de Economía y Competitividad SAF2013-42347-R, Ministerio de Sanidad FIS PI12/00613 and UPV/EHU UFI 11/32.
O. G. has a fellowship from the UPV/EHU.

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

IN VIVO CALCIUM IMAGING TO IDENTIFY DOPAMINERGIC NEURONS IN THE VENTRO-LATERAL SUBSTANTIA NIGRA PARS COMPACTA

L. F. Hernandez [1,2], Marcelo Mendonça[3], Joaquim Alves da Silva[3], Jose A. Obeso [1,2] , Rui M. Costa[3]
[1].Centro Integral de Neurociencias AC (CINAC), Hospital HM Puerta del Sur, Madrid and CEU-San Pablo University, Madrid, Spain.
[2] -Center for Networked Biomedical Research on Neurodegenerative Diseases (CIBERNED), Madrid, Spain.
[3] -Champalimaud Neuroscience Programme, Champalimaud Center for the Unknown Lisbon, Portugal.

Understanding why cell loss occurs predominantly in the ventro-lateral Substantia Nigra *pars compacta* (SNpc) in early stages of disease evolution and devising therapies to halt such progression is a major current goal for Parkinson's Disease therapeutics. Indeed, unravelling the mechanisms and factors underlying such selective vulnerability may provide ultimate insight into the etiopathogenesis of the neurodegenerative process and its extension to other regions of the nervous system.
With the help of a novel tool, the miniaturized epifluorescence microscope, we are aiming to record the activity profiles of the medial and lateral SNpc neurons. This miniaturized (1.9 g) integrated epifluorescence microscope enables high-speed cellular imaging across ~0.2 mm2 areas in awake behaving animals.
Using a cre-recombinase strategy we were able to express a genetically encoded calcium indicator (gcamp 6f) specifically in SNpc dopaminergic neurons.
We implanted those devices in TH-cre mice and imaged calcium transients in SNpc dopaminergic neurons before and after intrastriatal 6-OHDA injection. Here, we shall present initial original data showing that is possible to identify individual SNpc neurons, their specific topography and activation pattern during a motor task (running wheel and open field behavior). Finally, we want to assess the possibility of defining a pattern of SNpc neuronal loss after 6-OHDA striatal injection.

GRAPHENE DERIVATIVES AS SCAFFOLDS FOR EX VIVO SURVIVAL AND MATURATION OF DOPAMINERGIC SN4741 CELLS

N. Rodríguez-Losada [1], R. Wendelbo[2], E. Arenas[3], JA Aguirre[1]
[1]. Facultad de Medicina. Universidad de Málaga, Málaga
[2]. Abalonyx AS, Oslo, Norway
[3]. Department of Medical Biochemistry and Biophysics. Karolinska Institutet, Stockholm, Sweden

Carbon nanomaterial graphene (G) can form a three-dimensional porous structure with efficient bioconjugation and cell differentiation properties, providing a promising scaffold for neural regeneration. **Aims:** To study this putative new application of G, we cultured a clonal substantia nigra dopaminergic neuronal progenitor cell line (SN4741) in presence of G as scaffold. **Methods:** Cells were cultured in DMEM/10% FCS to about 80% confluence and incubated with different concentrations (0.001 to 1 mg/ml) of three chemically different G derivatives (G oxide (GO); partially reduced GO (PRGO) and fully reduced GO (FRGO)) and two different presentation matrixes as powder and films. Cell viability was measured by the MTT assay. To study cellular characterization, morphology and assessment of cell engraftment into G films, we analyzed the immunostaining of the neuronal marker NeuN, the anti-rat Beta-3-tubulin antibody, and the anti-rabbit DCX as immature neuronal marker. Lactate dehydrogenase was measured in the culture supernatant. **Results:** We found similar increase of survival and metabolism (30-40%) at low concentrations of PRGO and FRGO (0.05-0.01 mg/ml) compared with the higher concentration (1 mg/ml), no changes were seen in the GO group. PRGO or FRGO films showed an increased in the effective anchorage capacity to nest into the G matrix and in the maturation of the dopaminergic SN4741 cells. **Conclusions:** G scaffolds could offer a powerful platform for neural stem cells, direct cell conversion techniques and neural tissue engineering.

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

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

INTERACTIVE EXPLORATORY DATA ANALYSIS TOOL IN ALZHEIMER'S DISEASE

D. Furcila[1, 2], J. Morales[3], A. Rodríguez[4], J. DeFelipe[1, 2, 5], L. Alonso-Nanclares[1, 2, 5]
[1]Laboratorio Cajal de Circuitos Corticales. Centro de Tecnología Biomédica,
Universidad Politécnica de Madrid, Campus Montegancedo s/n, Pozuelo de Alarcón 28223, Madrid (Spain)
[2]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), C/Valderrebollo 5, 28031 Madrid, Spain
[3]Cajal Blue Brain Project, CeSViMa, Universidad Politécnica de Madrid (UPM), Campus Montegancedo S/N, Pozuelo de Alarcón 28223 Madrid, Spain
[4]Departamento de Arquitectura y Tecnología de Sistemas Informáticos, ETSIINF, UPM, Campus de Montegancedo s/n, 28660 Boadilla del Monte, Madrid, Spain
[5]Instituto Cajal (CSIC), Avda. Doctor Arce, 37, Madrid 28002 (Spain)

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive cognitive impairment, and a variety of neuropathological changes, which are not homogenous between different brain regions within a given patient or between patients. The availability of techniques to explore the brain provides neuroscientists a wealth of data that is difficult to analyze, as a result of both its volume and its complexity.
In order to overcome this problem, we propose to apply a new interactive exploratory data analysis tool, MorExAn (Morphology Exploratory Analyzer), which has been specifically designed to facilitate the study of complex neuroscientific data.
In the present study, we used brain tissue from 10 patients with AD (age range 76 - 90 years old). We included quantitative stereological data of several histological features (e.g., neuron and plaques density) obtained from different hippocampal regions, as well as neuropsychological data and common clinical variables.
Simultaneous analysis of data using MorExAn allows researchers to immediately detect possible differences between regions and relationships between different types of data.
Thus, MorExAn provide us the possibility to relate histopathological data with neuropsychological and clinical variables. The aid of this interactive visualization tool brings us the possibility to find unexpected conclusions beyond the insight provided by simple statistics analysis, as well as to improve neuroscientists' productivity.

Areas:
1st: New methods and technologies.
2nd: Cognitive and behavioral neuroscience.

SIMULTANEOUS MONITORING OF MONOAMINES, NUCLEOTIDES AND NEUROPEPTIDES IN BOVINE ADRENAL CHROMAFFIN CELLS WITH HIGH-RESOLUTION MASS SPECTROMETRY DETECTION

A. Wojnicz[1,2,3], R. de Pascual[1,2], J. Avendaño-Ortiz[1,2], L. Ruiz-Pascual[1,2], B. Fiz del Cerro[1,2], A. G. García[1,2,3] and A. Ruiz-Nuño[1,3]

[1]Instituto Teófilo Hernando and [2]Departamento de Farmacología y Terapéutica, Facultad de Medicina, Universidad Autónoma de Madrid, Spain. [3]Servicio de Farmacología Clínica, Instituto de Investigación Sanitaria, Hospital Universitario de la Princesa, Universidad Autónoma de Madrid, Madrid, Spain

Introduction and Objetives: The primary function of adrenal medullary chromaffin cells is the synthesis and storage of the catecholamines noradrenaline (NA) and adrenaline (AD) into their chromaffin vesicles, and their exocytotic release into the blood stream by Ca^{2+}-dependent exocytosis. Other monoamines, nucleotides, and opiates such as leucine-enkephalin (LENK) and methionine-enkephalin (MENK) are also co-stored and co-released with the catecholamines. Here we present a novel high-resolution method of liquid chromatography in tandem with mass spectrometry (LC-MS/MS) to monitor simultaneously in the same batch of bovine chromaffin cells (BCCs) 13 compounds.

Material and methods: We homogenized BCC maintained in primary cultures during 24 h, with ice cold 3.01% perchloric acid. After centrifugation and addition of internal standards (IS): isoprenaline and D-Ala2 LENK, the chromatographic separation was performed during 12 min under gradient conditions through a mobile phase consisting of 0.2% formic acid and acetonitrile with flow rate of 0.6 mL/min. The analytes were detected using the mode dynamic multiple reaction monitoring.

Results: We validated the analytical method according to the recommendations of EMA and FDA through tests of precision, accuracy, stability, sensitivity, and specificity. In three BCC batches from different cultures, the method permitted the simultaneous quantitative determination of the catecholamines dopamine, NA, and AD as well as the monoamines serotonine, L-glutamic acid, gamma-aminobutyric acid, histamine, and metanephrine. We could also monitor the cell levels of ADP, AMP, cyclic AMP, LENK and MENK.

Conclusions: We have developed a high-resolution LC-MS/MS method to determine in the same BCC batch the levels of 13 compounds. The method will allow the study of the selective effects of neurotrophic factors and/or secretagogues on the components of this rich messenger cocktail in primary cultures of BCCs that have been amply used to inquire into the fine tuning of the regulatory mechanisms of Ca^{2+} signaling, exocytosis, and endocytosis.

Key words: Bovine chromaffin cells; catecholamines; neuropeptides; LC-MS/MS

Supported by SAF 2013-44108-T from MINECO, Spain; FPU 12/02220 to AW; FIS No CA 12/00122 to ARN

EFFECT OF N-TERMINAL ACETYLATION ON ALPHA-SYNUCLEIN TOXICITY

I. Iñigo[1], L. Larrea[1], L., Samayoa[2], B. Carte[1], L. Neri[1], M. Martínez-Goikoetxea[2], I. Zuriguel[2], R. Aldabe[1,3,*] and M. Arrasate[1,3,*].
[1] Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Navarra.
[2] School of Sciences, University of Navarra, Pamplona, Navarra.
[3] IDISNA, Pamplona, Navarra.
[*] Authors contributed equally to this work

Alpha-synuclein (aSyn) causes neuronal death in Parkinson´s disease (PD). Therefore, decreasing aSyn levels constitutes a possible therapeutic strategy to stop or cure PD. *In vivo*, all aSyn molecules are posttranslationally modified by an acetyl group attached to the alpha-amino group of the N-terminal amino acid by the enzyme NatB. Some *in vitro* evidences suggest that this modification stabilizes the helical structure of the aSyn N-terminal domain and affects the lipid binding and aggregation capacity of the protein. However, the biological significance of aSyn N-terminal acetylation is not fully understood. To elucidate the role of this modification in neurons, we generated mutants that affect aSyn N-terminal acetylation. Using a new optical pulse-chase methodology that allows us to measure protein half-life *in situ* in primary neurons, we found that a mutant that impedes N-terminal acetylation decreases aSyn stability. Accordingly, this mutant exhibits lower protein levels and a lower risk of neuronal death (as measured by longitudinal survival analysis) than normal aSyn. Our results suggest that aSyn levels and toxicity could be modulated by N-terminal acetylation. We are currently evaluating whether inhibiting NatB enzyme modulates aSyn toxicity. (Supported by: FP7-Marie Curie IRG (Project ID:2248499), Ramón y Cajal Program MICINN (RYC2008-0325), Spanish Ministry of Economy and Competivity MINECO (BFU2013-48703-P), Fundación Tatiana Pérez de Guzmán El Bueno and CIMA project)

1ª: Nuevos métodos y tecnologías
2ª: Trastornos y reparación del sistema nervioso

ULTRASTRUCTURAL STUDY OF THE TRANSCYTOSIS OF GOLD NANOPARTICLES COATED WITH 8D3 ANTIBODY ACROSS BLOOD-BRAIN BARRIER IN MICE

I. Cabezón[1], G. Manich[1], M. Pallàs[2], A. Camins[2], C. Pelegrí[1], J. Vilaplana[1]
[1]. Departament Fisiologia, Facultat de Farmàcia, Universitat de Barcelona, Barcelona.
[2]. Unitat Farmacologia i Farmacognòsia, Facultat de Farmàcia, Universitat de Barcelona, Barcelona.

The 8D3 antibody is directed against the mouse transferrin-receptor and has been proposed as molecular Trojan horse to carry pharmacological substances across the blood-brain barrier. In the present work we studied the dynamics that 8D3 antibody follows in the endothelial cells of mouse brain capillaries after in vivo administration by using transmission electron microscope (TEM) techniques. We first covalently attached the 8D3 antibody to gold nanoparticles (AuNPs) and the resulting conjugate was then intravenously administered to male ICR-CD1 mice. Animals were sacrificed after different times of recirculation (10min, 30min, 2,5h and 24h) and brain samples were then processed to localize the conjugate using TEM. The observations indicated that, after 10 minutes of recirculation, most of the AuNPs were individually localized in endocytic vesicles inside the brain capillary endothelial cells, and few of them inside endosomes containing from 2 to 4 AuNPs. Moreover, some AuNPs were still attached to the luminal surface of the apical membrane of these cells, initiating in some cases the process of endocytosis by a clathrin coated pit. At 30 minutes of recirculation, the percentage of vesicles containing more than one AuNP increased. After 2,5h and 24 hours of recirculation, all AuNPs were already internalized and the number of AuNPs per vesicle notably increased, observing sometimes more than 20 AuNP per endosome. Only in sporadic cases, individually AuNPs could be observed in the basal membrane of the endothelium. We conclude that, when attached to 8D3, AuNPs are individually internalized to the endothelial cell by a clathrin coated pit endocytosis and thereafter the resulting vesicles become derived to bigger vesicles in which AuNPs finally accumulate in high numbers. Although a few AuNPs can be observed in the basal membrane, AuNPs mostly tend to accumulate in the endothelial cells but not to cross the blood-brain barrier.

1ª: Nuevos métodos y tecnologías
2ª: Sistemas homeostáticos y neuroendocrino

STUDY OF THE POSSIBLE TRANSCITOSIS THROUGH THE BLOOD-BRAIN BARRIER USING A PROTEIN CARGO LINKED TO AN ANTIBODY AGAINST THE MOUSE TRANSFERRIN RECEPTOR BY A THIOETHER BOND

I. Cabezón[1], G. Manich[1], M. Pallàs[2], A. Camins[2], J. Vilaplana[1], C. Pelegrí[1]
[1]. Departament de Fisiologia, Facultat de Farmàcia, Universitat de Barcelona, Barcelona
[2]. Unitat de Farmacologia i Farmacognòsia, Facultat de Farmàcia, Universitat de Barcelona, Barcelona

The possible transcytosis of a protein cargo linked by a thioether bond to the 8D3 antibody, which is directed against mouse transferrin-receptor (TfR) present in brain capillary endothelial cells (BCECs), is studied here. The protein cargo used in this work is a rabbit anti-GFAP antibody, which is easily detectable by immunohistochemistry. Initially, an immunohistochemical study with the 8D3 and the anti-GFAP antibodies was performed on mouse brain slices to determine the absence of cross-reactivity. As this was not observed, the 8D3 antibody was linked to the anti-GFAP antibody by a thioether bond using a protein-protein cross-linking kit. The double positive staining observed in blood vessels, where no GFAP protein exists, confirmed the formation of conjugates. Thus, conjugates were administered to male ICR-CD1 mice intravenously and they were sacrificed after 2,5 h of recirculation. As a control, the same procedure was performed using rat IgG instead of 8D3. The conjugate components were then localized by double immunofluorescence on brain sections. Samples from mice administered with the 8D3-anti-GFAP conjugate showed the presence of both antibodies on BCECs, but not in brain parenchyma. Colocalization of both stainings indicated that the conjugate components remain linked between them. Moreover, the granular appearance suggested the internalization of the conjugate in endocytic vesicles. To more precisely locate the conjugate in brain capillaries, a triple immunostaining using an anti-laminin antibody was performed. In this case, the granular staining of both conjugate components appeared externally delimited by the laminin staining, thus corroborating that conjugates were retained in the BCECs. Regarding control samples, no staining was observed. In conclusion, when administered in vivo, the 8D3-anti-GFAP conjugate formed by linking both antibodies by thioether bonds allows guiding the anti-GFAP antibody to the BCECs and probably producing their endocytosis. However, 2,5 h after administration, the cargo did not cross the blood-brain barrier.

1ª: Nuevos métodos y tecnologías
2ª: Sistemas homeostáticos y neuroendocrino

DESIGN AND IMPLEMENTATION OF COST EFFECTIVE IMPLANTS FOR CHRONIC RECORDING IN SMALL RODENTS

M.J. Nicolás, E. Lopetegui, S. Arrieta, I. Cordón, M. Alegre, J. Artieda, M. Valencia
Laboratorio de Neurofisiología Clínica, Centro de Investigación Médica Aplicada (CIMA), Universidad de Navarra, IdiSNA, Navarra Institute for Health Research, Pamplona, Spain,

This communication presents the design and implementation of chronic implants for neural recording in small rodents. Nowadays, many experimental neurophysiology set ups aim to record the oscillatory activity in awake and free moving animals. Recording several brain areas simultaneously in small animals and the need of moving the electrodes with high precision in order to record unitary activity make commercial solutions too expensive.
With the aim to obtain customizable and cost effective solutions, here we propose the use of 3D print technology combined with some steel components. Tested on models of Parkinson's and Alzheimer disease, they have shown to perform as well as commercial devices. Main advantages are the cost effectiveness, low weight and capability to proceed with multi-structure recordings in freely moving animals. We also present a complete description of the methodology to build the recording electrodes including an equispaced multi-channel probe to record neural activity across the different layers of the cortex and hippocampus. We consider that the current approach could serve to generate versatile and custom recording systems using elements and components that we can be afforded easily and very cost effective.

1. Nuevos métodos y tecnologías
2. Neurociencia de sistemas

DO FMRI AND HR-DOT DETECT SIMILAR FUNCTIONAL CHANGES? A COMPARATIVE STUDY IN HUMAN PREFRONTAL CORTEX.

E. Hernández Martín[1], O. Casanova González[1], F. Marcano Serrano[1] C. Modroño Pascual[1] J. Plata Bello[1,2], J.L González Mora[1].
[1]. Facultad de Medicina. Universidad de La Laguna, Santa Cruz de Tenerife.
[2]. Servicio de Neurocirugía. Hospital Universitario de Canarias, Santa Cruz de Tenerife.

Functional near infrared spectroscopy (fNIRS) is an optical tool to measure changes in the absorption of near infrared light in the cerebral cortex. fNIRS allows an estimation of changes in cerebral oxygenated (HbO_2) and deoxygenated hemoglobin (HbR) concentration due to local brain activation. These hemodynamic changes can also be measured with functional magnetic resonance imaging (fMRI) through the blood oxygenated level dependence (BOLD) signal.
Due to the complexity of activations detectable during cognitive tasks and low relationship between noise and signal, the correlation between both techniques has been studied in motor areas.
Our goal is to study whether both techniques detect activations in functional areas of the prefrontal cortex associated with the execution of a given cognitive paradigm.
The same paradigm was repeated four times with a participant using an MRI scanner and fNIRS equipment. The fMRI data were acquired with a 3T GE scanner using a spiral sequence to correct the field distortions produced by frontal sinus. A Nirx Medical System using diffuse optical tomography (DOT) was used to acquire the fNIRS data. The latter were pre-processed using a band-pass filter (0.004-0.04 Hz). Images collected from both techniques were co-registered to the structural images, normalized to the standard template and then, analyzed using SPM8. We generated a contrast image comparing blocks of tasks vs rest for each probe (p-value < 0.001). The results were spatially related using a conjunction analysis between fMRI and DOT-fNIRS (p-value < 0.05).
The conjunction analysis revealed a large number of voxels in the common areas between HbR-BOLD and HbO_2Sat-BOLD images, while there were a smaller number of voxels in the common areas between HbO_2-BOLD.
The results show that HbR data from fNIRS have a larger number of common voxels with BOLD in the prefrontal cortex during the cognitive process than the other fNIRS data.

1ª: Nuevos métodos y tecnologías.
2ª: Neurociencia cognitiva y conductual.

QUANTITATIVE STUDY OF THE BRAIN AND TEMPORAL LOBE STRUCTURES IN CONTROL AND ALZHEIMER DISEASE CASES USING MAGNETIC RESONANCE IMAGING. A PRELIMINARY STUDY.

E. Artacho-Pérula[1], JC. Delgado-García[1], F. Mansilla-Legorburo[2], J. Florensa-Vila[3], A. Rábano-Gutiérrez[4], M.V. Zelaya-Huerta[5], A. Insausti[6], MM. Ubero-Martínez[1], G. Artacho-Trillo-Figueroa[1], A. Mohedano-Moriano[1], R. Insausti[1]
[1]Medical School, University of Castilla-La Mancha, Albacete, Spain.
[2]Radiology Service, MR Unit, Complejo Hospitalario Universitario de Albacete (CHUA), Albacete, Spain.
[3]Radiodiagnostic Service, Hospital Nacional de Parapléjicos (HNP), Toledo, Spain.
[4]BT-CIEN, FRS, Instituto de Salud Carlos III, Madrid, Spain.
[5]NavarraBiomed, Fundación Miguel Servet, Biobank Research Unit, Pamplona, Spain.
[6]Department of Health, Physical Therapy School, Public University of Navarra, Tudela, Spain.

The quantitative evaluation of brain morphology and, especially, the temporal lobe, are widely used to obtain data helpful in the differentiation of cortical changes in Alzheimer disease (AD) versus controls. The value of the magnetic resonance imaging (MRI) to identify early changes in the brain is a valuable tool to establish a reliable diagnosis and an early treatment We have used two control and two AD cases (kindly provided by the BT-CIEN Fundación Reina Sofía –Madrid- and NavarraBiomed –Pamplona-). Brains were fixed in paraformaldehyde, and analyzed by MRI in both 1.5T and 3T scanners (CHUA –Albacete– and HNP –Toledo–, respectively) using modified sequences for ex-vivo images. Once the MRI study was performed, the temporal lobe serially sectioned at 50-µm thick consecutive sections. Anatomical references, cytoarchitecturally described, were applied to MRI images to delimitate regions of interest. Both brain were blocked coronally, *ex-vivo* MRI at 1.5T and 3T were obtained, and histological sections were analyzed to get size and shape estimators as well as surface area and volume of different structures. In spite of the low number of cases analyzed, the association among different measurements was high; in addition, we found a more significant volume decrease in the temporal lobe relative to total brain size in AD cases; moreover, surface area was reduced in AD cases as well as an increase in size of the lateral ventricle of the temporal horn. MRI studies combined with simple and efficient morphometric and stereological methods for quantitative analysis might improve a quick assessment of brain changes in the patient's diagnosis and treatment from the start of the disease. *Support from the Education, Culture and Sports of Castilla-La Mancha Autonomic Government Project PPII-2014-013-A.*

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

CELL MEMBRANE MICROARRAYS FOR THE STUDY OF MITOCHONDRIAL ENZYMES ACTIVITY AND LIPIDOMIC PROFILE IN PARKINSONIAN MONKEYS.

G. Barreda-Gómez[1], A. Pérez-Valle[1], M.D. García-Fernández[1,2], J. Garate[3], R. Fernández[3], T. Tolentino-Cortez[1], J.A. Fernández[2], E. Astigarraga[1]
[1]. IMG Pharma Biotech S.L., Kabi 612, 48160-Derio (Spain)
[2]. UPV/EHU, Faculty of Medicine and Dentistry, Dept of Pharmacology, 48940-Leioa (Spain)
[3]. UPV/EHU, Faculty of Science and Technology, Dept of Physical-Chemistry, 48940-Leioa (Spain)

Several evidences suggest that mitochondrial dysfunction is involved in the pathogenesis of Parkinson's disease (PD) through the selective cell death of dopaminergic neurons. The first evidence supporting this hypothesis appeared when it was found that long exposure to the inhibitor of complex I of mitochondrial electron transport chain, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), produced parkinsonism in humans and monkeys. However, long term effects in mitochondrial electron transport chain functionality and in the lipid composition of cellular membrane derived from chronic exposure to MPTP are not yet thoroughly determined.
To face these questions, we have developed cell membrane microarrays from different brain areas and tissues of control and MPTP-treated non-human primate (*Macaca fascicularis)*. These microarrays revealed themselves as a useful tool for the evaluation of the NADH:ubiquinone oxidoreductase (complex I), succinate dehydrogenase (complex II) and cytochrome c oxidase (complex IV) activities. The versatility of cell membrane microarrays enable the use of MALDI Imaging Mass Spectrometry (MALDI-IMS) in order to determine the lipidomic profile, assisted by Artificial Neuron Networks for the analysis of the huge amount of data obtained.
MPTP treatment induced an increase of the succinate dehydrogenase activity in olfactory bulb, putamen, caudate and substantia nigra, where it was observed a decrease in cytochrome c oxidase activity. It is worth mentioning that lipidomic profile was also altered in these areas as an outcome of the treatment, being especially relevant the reduction in the phosphatidylserine (38:1) content. Otherwise, in the cerebellum, where a decrease of both enzymatic activities was recorded, this phosphatidylserine (38:1) was increased. Therefore, the MPTP treatment not only modulates the mitochondrial electron transport chain enzymes, but also seems to alter other mitochondrial enzymes that regulate the lipid metabolism, such as the phosphatidylserine decarboxylase.

1º New methods and technologies
2º Theoretical and Computational Neuroscience

This work was supported by Zabalduz (MD. García-Fernández) and UPV/EHU (J .Garate) pre-doc Fellowships

PERGOLA: BOOSTING VISUALIZATION AND ANALYSIS OF BEHAVIORAL LONGITUDINAL DATA BY UNLOCKING GENOMIC ANALYSIS TOOLS

J. Espinosa-Carrasco[12], M. Fructuoso[12], I. Erb[12], C. Notredame[12], M. Dierssen[12]
[1]Centre for Genomic Regulation (CRG), Dr. Aiguader 88, 08003 Barcelona, Spain.
[2] Universitat Pompeu Fabra (UPF), Barcelona, Spain

The analysis of complex temporal sequences of behavior is an important tool for understanding the nervous system. Thanks to the rapid developments of technologies in the field of behavioral neuroscience it has become possible to collect large amounts of longitudinal data or big behavioral data (BBD). In order to understand these data it is key to have good tools for its visualization and analysis. Dealing with such a huge amount of sequential data is not a new problem in biology. Genomics, for instance has provided us with a widely-used tools for genome visualization such as the genome browser as well as very efficient tools for sequence analysis such as BEDtools. So why not to apply these tools for the understanding of longitudinal behavioral data? Here we present the Python bEhavioRal GenOmebrowser LibrAry (Pergola), a python library that makes longitudinal behavioral data compatible with popular desktop genome browsers and genomic analysis tools. Pergola accomplishes data format conversion using a simple correspondence between behavioral and genomic data. Behavioral longitudinal data can be then displayed in genome browsers, simplifying both data aggregation and the integration with other sources of data such as environmental data or downstream analyses. Data conversion also allows the user to analyze the data using genomic tools. We show some applied examples of behavioral data visualization in the framework of feeding behavior analysis. Furthermore we demonstrate simple operations on the data to characterize differential behavioral patterns between normal and pathological conditions.

1ª: Nuevos métodos y tecnologías
2ª: Sistemas homeostáticos y neuroendocrino

SPATIO-TEMPORAL MAPS TO STUDY COLONIC FAECAL PROPULSION *IN VITRO*. APPLICATION TO DIABETIC EXPERIMENTAL MODELS.

E. Alonso Ordóñez, G. Vera Pasamontes, R. Abalo Delgado.
Área de Farmacología y Nutrición, Unidad Asociada al IQM y CIAL (CSIC) y Grupo de Excelencia de Investigación en Dolor URJC-CSIC-Banco de Santander (iDOL). Depto. CC. Básicas de la Salud, Fac. CC de la Salud, Universidad Rey Juan Carlos. Alcorcón (Madrid).

Introduction. Diabetes mellitus (DM) is amongst the most frequent chronic non-transmittable diseases. Numerous complications, due to sustained hyperglycaemia, limit expectancy and quality of life. Amongst these complications, peripheral neuropathy and diabetic gastroparesia are well known. Colonic motility alterations (constipation, diarrhoea), which may also be extremely bothersome to patients, are not so well characterised. This is necessary in order to optimize treatment.
Objectives. To characterise colonic faecal propulsion in diabetic rats, using a new *in vitro* method and spatio-temporal maps.
Methods. Male Wistar rats received streptozotocin (STZ, 60 mg/kg, i.p.) or its vehicle and changes in body weight, food and water intake, and mechanical sensitivity were determined 4 weeks after. At this time point, rats were sacrificed and the colon dissected away, emptied of faecal pellets, and placed inside a horizontal organ bath filled with warmed Krebs-Henseleit solution and bubbled with carbogen. The colon was fixed at both ends and, after equilibration, a dried faecal pellet covered with resin was inserted through the oral end. Colonic movements and faecal pellet propulsion were recorded for 1 hour using a video camera located above the organ bath. Each minute of the video, the location of the faecal pellet throughout the colon was determined to build spatio-temporal maps.
Results. In STZ-treated rats, the typical symptoms and complications of diabetes (polyphagia, polydipsia, reduced weight gain, mechanical allodynia) were reproduced. Spatio-temporal maps showed the existence of 3 different patterns of colonic faecal propulsion, in both control and STZ-treated animals. Colonic propulsion was slightly reduced in STZ-treated compared to control rats.
Conclusions. Spatio-temporal maps might be useful to characterise *in vitro* the alterations of colonic motility associated to different diseases and drug treatments, and to better determine the contribution of the central and enteric nervous system in them.
Acknowledgements. Supported by: SAF2012-40075-C02-01 (MINECO), S2010/BMD-2308, and PRIN13-CS20 (URJC).

1ª: Nuevos métodos y tecnologías (7)
2ª: Trastornos y reparación del sistema nervioso (5)

NANOTECHNOLOGY: A NOVEL CELL-DIRECTED GENE THERAPY TO REDUCE INFLAMMATION

C. Ulecia Morón[1,2], A. Romero-Sandoval[1]
[1]. Department of Pharmaceutical and Administrative Sciences, Presbyterian College School of Pharmacy, Clinton, SC, USA
[2]. Department of Physiology, Anatomy and Cell Biology, Pablo de Olavide University, Seville, Spain

Introduction: Up to 50% of the patients that undergo major surgeries develop chronic postoperative pain, while 10% of patients with minor surgeries develop this type of chronic pain. This indicates that the extent and duration of postoperative inflammation and wound healing process are involved in the development of chronic postsurgical pain.
Objectives and Methods: The purpose of this study is to develop a cell-directed gene therapy using nanotechnology, mPEI nanoparticles specifically, to promote a more rapid and efficient wound healing by targeting macrophages. Macrophages play an essential role in the activation, maintenance and deactivation of inflammation and tissue repair process by a phenotypic shift from an M1 phenotype (pro-inflammatory) to an M2 phenotype (anti-inflammatory). We hypothesize that the over-expression of the scavenger receptor gene CD163 in human macrophages will promote an M2 cellular phenotype that will result in the resolution of inflammation and a more efficient wound healing process. We further postulate that this approach would promote a more efficient wound healing process following major surgeries, which would reduce the risk of developing chronic postoperative pain.
Results: We have previously shown that the induction of CD163 in macrophages successfully promotes an anti-inflammatory phenotype. We confirmed the over-expression of CD163 in M1 macrophages. Using an *in vitro* scratch assay with primary human keratinocytes and fibroblasts, we also observed that M2 macrophages promote a more rapid and efficient wound healing process through a unique interaction with fibroblasts.
Conclusions: CD163 seems to play a critical role in inflammation, as well as in the wound healing process by promoting an anti-inflammatory phenotype in human macrophages. Our studies suggest that this approach could result in the prevention of the development of chronic postsurgical pain following major surgeries.

Keywords: chronic postsurgical pain, macrophages, CD163, mPEI nanoparticles, gene therapy, cytokines, inflammation, wound healing.

1ª: New methods and technologies
2ª: Disorders and nervous system repair

NEURO SONIFICATION OF EEG SIGNALS AS A TOOL FOR THE DIAGNOSIS AND REHABILITATION OF PATIENTS WITH MOTOR AND/OR COGNITIVE DISABILITIES

J. Sala [1], M. Quevedo [1,5], D. Ibañez [6], E. Foglia [4], A. Soria-Frisch [6], S. Dunne [6], M. Dierssen [1,2,3]
[1] Centre for Genomic Regulation (CRG)
[2] Universitat Pompeu Fabra
[3] CIBERER
[4].Universitat de Barcelona (UB)
[5].Institut Hospital del Mar d'Investigacions Mèdiques
[6].Starlab Barcelona S.L.

Our hypothesis is that it is possible to identify patterns of electroencephalographic (EEG) signals of specific emotions and to develop a sonification method with the aim of creating an Alternative Communication Protocol (ACP) for communication and rehabilitation of patients with cerebral palsy through a Brain Computer Interface (BCI). This requires: 1) Induce specific emotional states and identify the EEG signal containing valuable information related to the different emotional states, 2) Extract the specific features corresponding to specific emotional states from the sub-signals obtained by multiwavelet decomposition of EEG signals; 3) Design a brain-computer interface (BCI) for the communication with completely paralyzed patients. The device should allow auditory feedback of multiple EEG characteristics in real time. The BCI used for EEG recording is Enobio® (Neuroelectrics) that allows, in real time, accurate, adequate and low cost wireless interface with the brain activity. The audiovisual databases of stimulus used for the validation of the approach are the International Affective Picture System (IAPS) and the International Affective Digital Sounds (IADS). We developed an experimental protocol for validation, and a pipeline for the synchronization of stimulus presentation and EEG recording, In a pilot study we have tested the validity of this system in healthy volunteers.

We have registered about 15 EEG sessions of emotion induction with the IAPS and IADS, to validate the emotional response associated with the stimuli. We ensured that the recognition and evocation of emotions and their respective correlation in EEG can be used for the development of a parametric sonification in real time, especially in the case of patients with motor and/or cognitive disabilities.

Funded by a CRG Award

1. Nuevos métodos y tecnologías
2. Neurociencia cognitiva y conductual

Topic 9

History, Teaching, Release and Ethics

MEDICAL SCHOOL STUDENTS IN NEUROSCIENCE DIVULGATION: BRAIN AWARENESS WEEK 2015

I Ballesteros-Yanez[1]; FJ Sancho Bielsa[1]; JC Albertos Marco[1]; C De La Rosa Prieto[1]; J De Las Heras Salord[1]; S Díaz Sánchez[2]; E Fairén Jiménez[1]; AM Flores-Cuadrado[1]; J Frontiñán Rubio[1]; J Mayordomo Cava[1]; D Saiz Sánchez[1]; MD Vidal[1]

1 Universidad de Castilla-La Mancha. Facultad de Medicina. Ciudad Real.

2 Universidad de Castilla-La Mancha. Facultad de Ciencias y Tecnologías Químicas, Ciudad Real.

The Faculty of Medicine of Ciudad Real celebrated last March the Brain Awareness Week (BAW). The BAW is an annual global campaign to increase public awareness of the progress and benefits of brain research. On this occasion, we have organized a series of educational activities for people of all ages, including children, adolescents and adults. In order to benefit our students with a non-formal learning about neuroscience, some of the organized activities in brain were prepared by 1st and 2nd year students. It was a volunteer activity and the students who wanted to participate organized content, prepared a guidance of activities and selected bibliographical and audiovisual resources to be used. Seventy-six students prepared activities for a total of 416 children and adolescent visitors. The activities included: exhibitions about comparative anatomy, physiology of the brain, neuronal cultures and biochemical function, a poster session, “Draw the brain lobules” workshop and “building a neuron” workshop. Once tested and carried out the scheduled activities, we proceeded to step a satisfaction survey. The assessment by students participants was very favorable, 100% of respondents "agree / strongly agree / totally agree" with the educational benefits of this activity and being fully prepared to participate in future editions. Brain Awareness Week at the Faculty of Medicine of Ciudad Real, has established itself as a highly educational and fun activity that students enjoy learning and teaching. This activity was financially support by *Vic. Economía y Planificación, Vic. Estudiantes, Vic. Cultura y Extensión Universitaria, Vic. Investigación, Dpt. Ciencias Médicas* from UCLM, SENC and FENS.

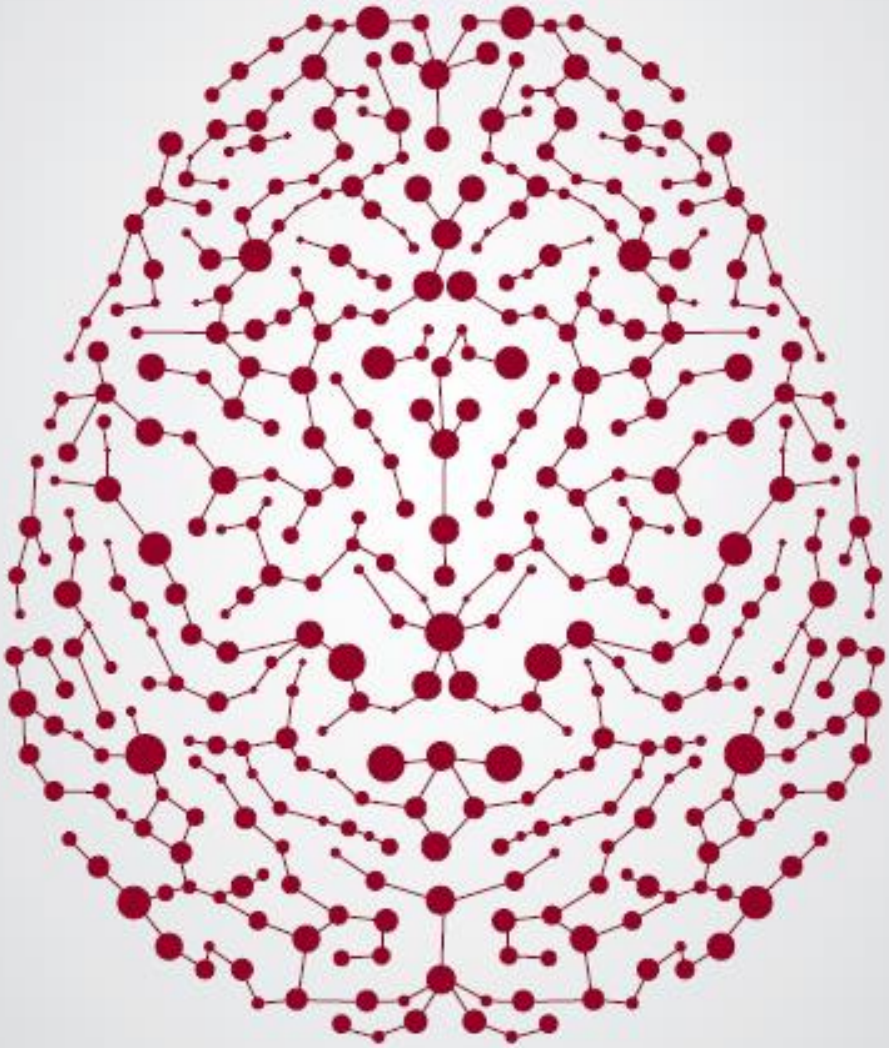
1. Historia, Docencia, Divulgación y Ética

SENC

sociedad española de neurociencia

16 National Congress of the Spanish Society of Neuroscience

GRANADA | SPAIN 23 - 25 September 2015



senc2015@kenes.com

www.senc2015.com