Neuro gune

Basque Neuroscience Meeting

Vitoria-Gasteiz **2018**

Abstract Book

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CONTENTS

1. ORGANISATION

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2. PROGRAMME

Time	Activity	Duration (min)
9.00 - 9.30	Registration	*30*
9.15 - 9.30	Opening	15
9:30 - 10:20	*"Exploring encoding of emotional valence in circuits of the amygdala and insular cortex"* Opening Keynote by Anna Beyeler	50
10:20 - 11:00	Coffee Break (and Posters)	*40*
11:00 - 13:00	Oral Communications (6 talks x 20 minutes each)	120
	Track 1: Cellular and Molecular Neuroscience / Physiology	
	Track 2: Cellular and Molecular Neuroscience / Pathology	
	Track 3: Behaviour & Cognition, Imaging and Psychiatry	
13:00 - 15:00	Lunch (and Posters)	*120*
15.00 - 15:50	*"Prion-like dissemination of synuclein pathology using human brain-derived alpha-synuclein in monkeys"* Closing Keynote by Erwan Bezard	50
15:50 - 16:00	Closure	*10*

3. KEYNOTES

Opening Keynote
Anna Beyeler

"Exploring encoding of emotional valence in circuits of the amygdala and insular cortex"

Chair: **Olga Peñagarikano (UPV/EHU, Leioa)**

Closing Keynote
Erwan Bezard

"Prion-like dissemination of synuclein pathology using human brain-derived alpha-synuclein in monkeys"

Chair: **M. Cruz Rodríguez-Oroz (CIMA, Pamplona)**

4. SPONSORS

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TRACK 1: Cellular and Molecular Neuroscience / Physiology

ORAL PRESENTATIONS

Moderator: **Fernando García-Moreno (Achucarro, Leioa)**

(O1-1) ELECTROPHYSIOLOGICAL CHARACTERIZATION OF A1 ADRENOCEPTOR FUNCTION IN THE RAT LOCUS COERULEUS IN VITRO

Irati Rodilla, Aitziber Mendiguren, Joseba Pineda

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ABSTRACT

Introduction

α1 adrenoceptor (α1AR) is involved in the pathophysiology of different diseases in the CNS, and it could constitute a therapeutic target for neuropsychiatric disorders such as drug addiction or Alzheimer´s disease. This receptor has been found in the locus coeruleus (LC), the main noradrenergic nucleus in the CNS, by quantitative autoradiography and by RT-PCR techniques. Some authors have suggested that α1AR located in LC neurons decreases its activity during development to become absent in the adult rat. However, several indirect evidences suggest that α1AR remains functionally active in the adult rat LC. To date, the role of α1AR in the modulation of LC neurons activity has not been directly characterized.

Objective

The aim of this study was to characterize the role of α1AR in the regulation of the firing rate of LC neurons in adult rat brain slices.

Materials and Methods

We performed single-unit extracellular recordings in rat brain slices containing the LC. To characterize α1AR, we studied the effect of adrenergic receptor agonists in the presence and absence of adrenergic receptor antagonists. Furthermore, we studied the effect of modulators of different signalling pathways that may be coupled to α1AR.

Results

Perfusion with the α1AR/α2AR agonist noradrenaline (NA) (100 μM) and the α1AR agonist phenylephrine (PE) (100 μM) inhibited the firing rate of LC neurons. In the presence of α2AR antagonist RS 79948 (0.1 μM), NA and PE agonists increased the firing rate of LC neurons by 114 ± 23% and by 60 ± 12% respectively. The selective α1AR agonist cirazoline (10, 100 μM) also increased the firing rate of LC neurons by 60 ± 11% and by 68 ± 11% respectively. The increases in the firing rate induced by NA (100 μM), PE (100 μM) and cirazoline (10 μM) were blocked by the α1AR antagonist WB 4101 (0.5 μM), which indicates that these stimulatory effects were mediated by α1AR. NA (100 μM)-, but not cirazoline (10 μM)-induced stimulations were strongly reduced by the Gi/o protein inactivator pertussis toxin (500 ng·ml-1), but not by the G protein-activated inward rectifier potassium channel (GIRK) blocker BaCl2 (300 μM). The stimulatory effect of NA (100 μM) was reduced in the presence of the transient receptor potential channel (TRP) blocker 2-APB (10, 30 μM) by 18% and by 30% respectively. However, perfusion with the PKC inhibitor Go 6976 (1μM) or the cAMP dependent protein kinase inhibitor H-89 (10 μM) failed to modify the stimulatory effects induced by NA or by cirazoline.

Conclusion

α1AR activation increases the firing rate of LC neurons in adult rat brain in vitro through a signalling pathway that involves Gi/o proteins and TRP channels.

(O1-2) CB1 CONTROL OF HIPPOCAMPAL INHIBITORY CIRCUITS MODULATES RECOGNITION MEMORY

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ABSTRACT

The endocannabinoid system is a key regulator of memory processes. In particular, the cannabinoid receptor type-1 (CB1) is highly expressed in brain regions involved in learning and memory (e.g., hippocampus). In this work, we study the role of CB1 receptors in a subpopulation of hippocampal cells expressing the dopamine receptor type-1 (D1) and their involvement in object recognition memory (ORM). By using genetic, molecular, electrophysiological and behavioral approaches, we found that CB1 expression in hippocampal D1-positive cells is necessary and sufficient for long-term object recognition memory and training-induced synaptic plasticity. Furthermore, preliminary experiments show that this is due to changes in GABAergic transmission modulation. Overall, these results provide evidence of the fine-tuning cell-specific control of neurotransmission by the CB1 receptors in inhibitory microcircuits and its impact on cognitive processes.

(O1-3) ON-SITE GENERATION OF ADULT NEURAL STEM CELLS IN THE POSTNATAL DENTATE GYRUS

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ABSTRACT

Development of the dentate gyrus (DG) starts in the embryonic stages and continues in the postnatal period with a population of developmental neural stem cells (dNSC), which produce neurons, astrocytes and radial glia cells (RGCs). RGCs also give birth to neurons and astrocytes during this early postnatal period, and later in the young adult stage generate or differentiate into adult neural stem cells (NSCs) through a process yet unknown. Throughout life, NSCs give rise to neurons (granule cells, GC) through intermediate cells like amplifying neural progenitors (ANP) and neuroblasts (NB), and then differentiate into astrocytes, once they have ended their neurogenic divisions. NSCs are also able to divide symmetrically producing more copies of themselves (new NCSs). However, the small ratio of these symmetric divisions is not enough to overcome the differentiation of NSCs into astrocytes and their depletion. Therefore, the population of NSCs gets depleted with age. This phenomenon implies that the neurogenic output in the DG of the hippocampus is mainly determined by the initial pool of NSCs. However, how and when the NSCs get established in the subgranular zone (SGZ) of the dentate gyrus (DG) remains unknown. Using Nestin-GFP transgenic mice, in which NSCs can be readily visualized and analyzed, we observed that adult-like radial NSCs (rNSCs) can be identified, as nestin and GFAP-expressing cells with radial morphology and profuse arborization in the molecular layer, at postnatal day 7 (P7), but not earlier. From then on, the rNSC population increases in size reaching a maximum at P14-21. Moreover, when Nestin-GFP mice are injected with Bromodeoxyuridine (BrdU) once per day during different time windows of the first postnatal week (P2-P4 or P5-P7) the proportion of BrdU-labeled NSCs observed at P14 or P28 is significantly higher than when administered later during the second postnatal week (P8-P10 or P11-P13). These results support the notion that adult NSCs are generated in the early postnatal period. Furthermore, we analysed the postnatal Nestin-GFP transgenic mice crossed with the cyclin D2 knock-out mice (D2KO). In the D2 KO mice embryonic brain development is normal for the most part, but hippocampal cell

proliferation and neurogenesis are absent in the adult. We studied the effect of the lack of cyclin D2 in NSCs in five different postnatal time points (P0, P7, P10, P14, P28). According to our results, there are no differences in the total number of NSCs at P0, although already there are fewer cycling NSCs (defined as Nestin-GFP, GFAP cells in the SGZ and GCL). The DG is similar to the WT otherwise. Later at P7-P10, while in the WT NSCs increases drastically in total numbers compared to P0, the increase is much lower in the cD2 KO, resulting in significantly fewer NSCs in the cD2KO. This trend is maintained over time, with the difference in total number of NSCs between the WT mice and the cD2KO getting larger at P10 and P14. By P28 the population of NSCs has started to decrease in both, falling to a residual size in the cD2KO mice. We conclude that CD2-dependant mitosis is essential for the expansion of NSC pool from P7 to P14 and for the establishment of the adult NSC population. These results put together suggest that the adult rNSC population is established in the postnatal dentate gyrus within a discrete critical period and therefore adult hippocampal neurogenesis cannot be considered a mere continuation of hippocampal development.

(O1-4) PHAGOCYTIC MICROGLIA SECRETOME MODULATES ADULT HIPPOCAMPAL NEUROGENESIS

Valero J1,2, Díaz-Aparicio I1, Paris I1, Sánchez-Zafra V1, Alberdi E1,3,4, Matute C1,3,4, Sperlagh B5, Lemke G6, Sierra A1,2,3

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ABSTRACT

New neurons are constantly added to hippocampal memory circuits through adulthood. However, during adult neurogenesis the majority of the newborn cells undergo apoptosis and are quickly and efficiently phagocyted by resident microglia. We hypothesize that phagocytosis plays an active role in maintaining the homeostasis of the adult hippocampal neurogenic cascade by producing neurogenic regulators. First we compared the transcriptome of cultured naïve vs. phagocytic microglia using gene expression arrays. Gene ontology analysis revealed that phagocytosis triggered a neuromodulatory program in microglia. We found a significant upregulation of neurogenesis function and identified significant changes in 224 genes involved in functions related to different stages of the neurogenic cascade. Then, we analyzed the effects of the secretome of cultured phagocytic microglia in neurogenesis in vitro and in vivo. In vitro, conditioned media from phagocytic microglia induced the differentiation of cultured neuronal progenitor cells into a unique type of bipolar cell that we characterized as an astrocytic precursor cell. These cells presented proteins typically expressed by neuroprogenitor cells (nestin and GFAP) and astrocytes (GFAP, S100beta); showed a mixed intracellular calcium response to KCl and ATP, typical of precursor and astrocytic cells; and exclusively differentiated into astrocytic-like cells, lacking the capacity to produce neurons. In vivo, our data showed that intrahippocampal infusion of conditioned media from phagocytic microglia for 6 days decreased the long-term (28d after infusion) production of newborn neurons, indicating that factors released by phagocytic microglia modulate adult neurogenesis. Finally, we reasoned that phagocytosis impairment may also affect neurogenesis and utilized three different transgenic mouse models to block phagocytosis by knock-out of phagocytosis receptors (P2Y12, GPR34, MerTK/Axl). In vivo phagocytosis blockade was accompanied by a downregulation of the neurogenic cascade at different levels, from the proliferation of neuroprogenitor cells to the number of newborn

neurons, suggesting that microglial phagocytosis is required to maintain the neurogenic cascade. Altogether, our data indicate that phagocytic microglia may act as a cellular sensor of local cell death that modulates the adequate equilibrium between regional cell production (input) and expected cell survival/death (output) in the neurogenic niche, thus supporting the long term maintenance of adult hippocampal neurogenesis.
This work was supported by grants from the Spanish Ministry of Economy and Competitiveness with FEDER funds to A. S. (RYC-2013–12817), A. S. and J. V. (BFU2015–66689-R), from the Basque Government to A. S. (PI-2016-1-0011), and Ikerbasque start-up funds to J. V.

(O1-5) FUNCTIONAL CHARACTERIZATION OF EP2 RECEPTOR IN LOCUS COERULEUS NORADRENERGIC NEURONS IN RAT BRAIN SLICES

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ABSTRACT

Prostaglandin E2 (PGE2) is an inflammatory mediator that binds to specific EP receptors. In situ hybridization techniques have shown that mRNA for the Gs-coupled EP2 receptor is present in the brain. One of the brain areas expressing the EP2 receptor is the locus coeruleus (LC), the main noradrenergic nucleus in the central nervous system. However, the functional role for the EP2 receptor remains unknown. Therefore, our aim was to characterize pharmacologically the EP2 receptor in the LC by single-unit extracellular electrophysiological recordings in rat brain slices. Concentration-effect curves for the EP2 receptor agonist butaprost (0.01–10 μM) increased the firing rate of LC cells in a concentration-dependent manner (EC50=0.45 μM; Emax=74%). Likewise, the EP2 receptor agonist treprostinil (0.03–10 μM) stimulated the neuronal activity of LC cells (EC50=0.47 μM; Emax 76%). The concentration-effect curve for butaprost was shifted five-fold to the right by the selective EP2 receptor antagonist PF-04418948 (10 nM) but not by the EP3 receptor antagonist L-798,106 (10 nM) or the EP4 receptor antagonists L-161,982 (10 nM), suggesting that the effect of butaprost was mediated by activation of the EP2 receptor. Perfusion with the PKA activator 8-Br-cAMP (1 mM), the PKA inhibitor H-89 (10 μM), the HCN channel blocker ZD7288 (30 μM), or the Gαs blocker NF449 (10 μM) failed to significantly change butaprost (0.3–10 μM)-induced stimulation, which suggests that the effect of butaprost is not mediated by the Gαs/cAMP/PKA pathway. Furthermore, synaptic activity blockade with GABA and glutamate receptor antagonists or the antagonism of Kir6.2 channels with glibenclamide (3 μM) did not block butaprost-induced stimulation. However, the Gβγ-subunit blocker gallein (20 μM) or perfusion with low extracellular sodium prevented the butaprost-mediated stimulation, which indicates the involvement of Gβγ subunits and a sodium current. In conclusion, the EP2 receptor modulates LC noradrenergic cells by a Gβγ-mediated sodium current.

(O1-6) GLIA DISTINCTLY REGULATES LOCAL TRANSLATION IN AXONS UNDER PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

Andreia Batista, María Gamarra, Maite Blanco, Amaia Oulad and Jimena Baleriola

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ABSTRACT

Axons are able to respond to changes in their environment by recruiting mRNAs and producing proteins locally. Historically, intra-axonal protein synthesis was thought to be restricted to developing axons and for many years mature axons were thought to be translationally inactive. However, recent studies have shown that adult axons have more complex transcriptome and translatome tan previously expected, specially under pathological conditions, and local translation seems to be involved in axon regeneration after injury, it improves survival of motor

neurons in a mouse model of spinal muscular atrophy (SMA) and medietes neurodegeneration in models of amyloid pathology.
Although local translation is a phenomenon that is becoming widely accepted by the scientific community, many questions regarding its regulation remain anaswered. For instance, it is unknown whether intra-axonal protein synthesis is fully controlled by neurons or if sorrounding glial cells might contribute to the axonal translatome remotely. Here we show that both astroglia and microglia distinctly influence the ability of axons to produce proteins locally under basal and pathological conditions.

POSTERS

(T1-01) ISCHEMIA/STROKE RAPIDLY IMPAIRS MICROGLIAL PHAGOCYTOSIS IN VIVO

Sol Beccari, Takashi Umekawa, Ahmed Osman, Wei Han, Cecilia Dominguez, Ainhoa Plaza-Zabala, Virginia Sierra-Torre, Klas Blomgren, Amanda Sierra
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ABSTRACT

Microglial phagocytosis is an essential mechanism to maintain tissue homeostasis. In physiological conditions in the adult hippocampus, apoptotic cells are rapidly and efficiently phagocytosed by microglia. In response to phagocytic challenge induced by excitotoxicity or inflammation, microglia proportionally boost their phagocytic output to counteract the increased number of apoptotic cells, thus maintaining apoptosis and phagocytosis tightly coupled. Surprisingly, in a mouse model of cerebral hypoxia-ischemia we found the opposite. Using CX3CR1-GFP and CCR2-RFP mice, in which we can discriminate resident microglia from blood-derived monocytes, we have discovered that microglial phagocytosis is strongly uncoupled from apoptosis as early as 1d after ischemia in both postnatal day 9 (P9) and 3 month old (3m) adult brains. The phagocytic blockade was the result of reduced microglial surveillance and led to accumulation of non-phagocytosed apoptotic cells. Importantly, we have observed that this impairment occurred before blood-derived monocyte infiltration. Furthermore, while under physiological conditions microglia generally phagocytose by terminal branches (ball-and-chain mechanism), after ischemia the few phagocytic microglia detected engulfed dying cells by direct apposition to the soma. In addition, we also found some cases of phagoptosis, the engulfment of non-apoptotic cells, executed by microglia. The mechanisms underlying the microglial phagocytosis impairment are being investigated using primary and organotypic hippocampal slices under oxygen nutrient deprivation (OND). Accordingly, microglial phagocytic potential is a novel and yet unexplored therapy to promote clearance of apoptotic cells and anti-inflammatory response, in order to accelerate ischemic brain recovery.

(T1-02) 1-42 AB PEPTIDE INVOLVES RAC1 AND GLYCOGEN PHOSPHORYLASE IN HUMAN MICROGLIA MIGRATION AND PHAGOCYTOSIS

Alazne Arrazola Sastre, Miriam Luque, Francisco Llavero, José L Zugaza

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ABSTRACT

Small GTPases of the Rho family are implicated in important cellular processes such as cell migration and adhesion, protein secretion, and/or gene transcription. However, little is known about their role in Aβ-mediated responses in microglia. In this work we have reported that Rac1 is activated after stimulating human microglia with Aβ peptide.
Our results show that phagocytosis and migration are increased in Aβ-stimulated human microglia. Moreover, phagocytosis is reduced when adding a Rac1-binding domain peptide to Aβ-stimulated cells, which suggests a direct implication of this GTPase in microglial phagocytosis. Furthermore, our group has shown that the active form of the GTPase Rac1 interacts with glycogen phosphorylase (PYG) leading to its activation in IL-2-stimulated T cells. Here we have identified changes in the O-glycosylation of proteins when stimulating microglia with Aβ. This may be indicating that Aβ activates Rac1, which in turn activates PYG and the resulting glucose-1-

phosphate might be used to induce O-glycosylation in proteins. The identification of these proteins and their relation with microglial migration and phagocytosis still needs to be studied.

(T1-03) IMPLICATION OF AB1-42 PEPTIDE ON RAC1 AND GLYCOGEN PHOSPHORYLASE MOLECULAR PATHWAY IN HUMAN IMMORTALYZED ASTROCYTES.

Miriam Luque Montoro, Alazne Arrazole Sastre, Francisco Llavero, José L Zugaza

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ABSTRACT

Small GTPases of the Rho family, whose best characterized members include RhoA, Rac1 and Cdc42, are key players in important cellular processes such as cell migration and adhesion, protein secretion, and/or gene transcription. The deregulation of their activity can be involved in several processes such as cell transformation, astroglyosis and neurodegeneration, including Alzheimer's Disease (AD).

We have recently described an unexpected connection between Rac1 and the glycogen metabolism through glycogen phosphorylase (PYG) in T cells. On this basis, we have examined in human astrocytes whether Aβ1-42 peptide could activate a molecular pathway involving Rac1/glycogen phosphorylase molecular tandem. With this aim, we detected the presence of RhoA and Rac1 by immunoblotting, and also the presence of the three isoforms of PYG (brain, liver and muscle) by RT-PCR. Taking into account that Rac1 and PYG are expressed in human astrocytes, we stimulated these cells with Aβ1-42 peptide and we found a maximal PYG activation at 30 minutes. Furthermore, we have observed that Aβ1-42 peptide-induced Rac1 activation achieves at 15 minutes, being the maximum activation at 30 minutes. Our results provide a possible connection between Aβ1-42 peptide, Rac1 and glycogen metabolism in astrocytes.

(T1-04) AB1-42 PEPTIDE ACTIVATES TRANSIENTLY GLYCOGEN PHOSPHORYLASE VIA INTEGRIN-B 1 IN ASTROCYTES

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ABSTRACT

Although Alzheimer's disease (AD) is considered an illness that mainly affects neurons, recent studies have found molecular and functional abnormalities in astrocytes at brain from both animal models and brain of patients suffering from this pathology. In a recent study from our laboratory, we have described the effects of amyloid beta (Aβ1-42) peptide in the biology of astrocytes. However, the mechanism by which Aβ1-42 peptide activates the neurodegenerative program is still poorly understood. Here, we have examined the effects produced by Aβ peptide on the glycogen metabolism into the cultured primary astrocytes. We have observed that Aβ1-42 peptide produces a dramatic and transient reduction of intracellular glycogen stores, and this decrease is previously associated to a robust activation of the glycogen phosphorylase (PYG). Furthermore, pharmacological inhibition of integrin β1 prevents PYG activation and glycogen degradation induced by Aβ1-42 peptide. In addition, Aβ1-42 peptide-treated astrocytes reduce significantly the maximal respiratory rate and the respiratory capacity while pharmacological inhibition both integrin β1 and PYG rescue either the maximal respiratory rate or the respiratory capacity. Finally, we have deciphered where Aβ1-42 peptide binds to integrin β1 in the extracellular region. This binding site is located at the first 20 aas. at integrin β1 N-terminal. Our results provide insights into an unsuspected connection between Aβ1-42 peptide, Integrin β1 N terminal and glycogen metabolism in the same signal-transduction pathway and points out

integrin β1 and glycogen phosphorylase as potential targets to block the toxic effects of Aβ1-42 peptide in astrocytes.

(T1-05) MICROGLIAL ACTIVATION INDUCED BY OVEREXPRESSION OF HUMAN A-SYNUCLEIN PRECEDES DOPAMINERGIC DEATH IN A RODENT MODEL

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ABSTRACT

Neuroinflammation has been implicated in the physiopathology of Parkinson's disease (PD). Microglial and astrocyte activation is observed in the substantia nigra pars compacta (SNc) and striatum of PD patients and animal models of parkinsonism but their role in the beginning and progression of PD is still unknown. Our aim was to study the relationship between neuroinflammation and nigrostriatal degeneration in a rat model of progressive parkinsonism induced by overexpression of mutated A53T human α-synuclein (hα-syn) in the SNc. In vivo microglia activation studied by [18F]-DPA-714 positron emission tomography (PET) and post mortem molecular markers of neuroinflammation and dopaminergic lesion have been assessed at different time points of disease progression (24h, 72h, 1, 2, 3 and 16 weeks).
Within the AAV-hα-syn group, PET results showed an increase of microglial activity in the SNc from 2w until 16w post inoculation (p.i.; $p<0.05$ vs. 72h and 1w). By contrast, the striatum showed a significant increase in activated microglia only at 16w p.i. ($p<0.05$ vs. 2w). Moreover, significant TH+ cell loss was observed in the SNc since the 3w p.i. ($p<0.01$ vs. 24h; $p<0.05$ vs. 72h and 1w) that was maintained at 16w p.i. ($p<0.05$ vs. 24h). Significant reduction in striatal TH expression was observed from 72h p.i. ($p<0.01$ vs. 24h), up to 16w p.i. ($p<0.01$ vs. 24h) along with a higher expression of hα-syn. The Iba-1 microglial marker was increased in the SNc at 72h p.i. ($p<0.01$ vs. 24h; $p<0.01$ vs. 1w) and from 2w p.i. ($p<0.05$ vs. 24h and 72h) up to 16w p.i. ($p<0.05$ vs. 24h, 1w and 3w). In the striatum, there were no differences in microglial activation. In addition, GFAP+ astrocytes showed a transient increase in the SNc at 72h p.i. ($p<0.05$ vs. 24h) and at 16w p.i. ($p<0.05$ vs. 24h and 1w; $p<0.01$ vs. 2w). In the striatum, this increase occurred at 1w p.i. ($p<0.01$ vs. 24h, $p<0.001$ vs. 72h, 2w and 3w) and at 16w p.i. ($p<0.05$ vs. 24h, $p<0.001$ vs. 72h, 2w and 3w).
In conclusion, inflammation seems to play an important role in the development of dopaminergic degeneration. We observed that aggregation of hα-syn induced microglial activation only in the SNc, preceding neurodegeneration while astrocyte activation was present at later stages of neuronal degeneration, suggesting that glial cells have a different role in the neuroinflammatory process. (Proyectos colaborativos CIBERNED 2014/06).

(T1-06) *DELICATE SUBCELLULAR IMMUNOLABELLING OF ASTROCYTES: GLAST VERSUS GFAP*

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ABSTRACT

Astrocytes are the most abundant glial cells in the central nervous system (CNS) of mammals. They are key active elements of the brain that participate to a variety of essential physiological processes in the healthy brain. Indeed, far from being merely passive cells providing structural support to neurons, astrocytes are now viewed as crucial active and dynamic elements of the brain circuitry: they participate in formation and maturation of synapses, receptor trafficking, control of the homeostasis of ions and energy metabolites and clearance of neurotransmitters. They also regulate the extracellular space volume and modulate synaptic plasticity at the "tripartite synapses". In astrocytes, Glial fibrillary acidic protein (GFAP) is the principal intermediate filament protein. GFAP antibodies are the most popular marker for astrocytes. However, due to its filament nature, GFAP immunolabeling may not show the complete surface of the astrocyte in microscopic preparations.

The goal of this study was to examine the best marker to identify astrocytes in the mouse CA1 hippocampus. For this purpose, we have used antibodies against GFAP (#G3893, Sigma-Aldrich, St. Louis, MO, USA, RRID: AB_477010) and the glutamate aspartate transporter (GLAST) (Anti-A522, rabbit, Ab#314; genereously supplied by Niels Christian Danbolt) in combination with a preembedding immunoperoxidase method for electron microscopy.

GFAP expression was very modest in CA1 astrocytes compared to GLAST, which was abundantly expressed. In fact, the GLAST antibody labeled four times more astrocytic processes than the GFAP antibody (GLAST: 0.955 (micrometer astrocytic membrane/micrometer2; GFAP: 0.24 micrometer astrocytic membrane/micrometer2). A plausible explanation could be based on the different molecular nature of GFAP and GLAST. As GFAP is a cytoskeletal protein assembled in intermediate filament packets, GFAP immunostaining is limited to the core in the main radial processes of the astrocyte. However, GLAST is expressed in the astrocytic plasmalemma further into the fine processes of astrocytes that normally lack GFAP.

These findings show that GLAST is an optimal astrocytic marker resulting in better detection of astrocytes than GFAP.

(T1-07) *MICROGLIAL ACTIVATION AND DE NOVO CANNABINOID TYPE-2 RECEPTOR EXPRESSION IN THE HIPPOCAMPUS OF A MOUSE MODEL OF TEMPORAL LOBE EPILEPSY*

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ABSTRACT

Medial temporal lobe epilepsy (MTLE) is the most common form of epilepsy with focal seizures, that present great resistance to treatment and severely impairs physical and mental health, affecting approximately 1% of human population. The causes of MTLE and its spontaneous, recurrent seizures are still unknown. The phenomenon of MTLE is always associated with the following changes: neuronal loss in CA1-CA3 and hilus, axonal and synaptic rearrangements of hippocampal circuits, granule cell dispersion (GCD) and increased neuronal excitability and inflammation.
Here, we have focused on microglial activation and CB2 receptor expression related to the inflammatory processes that could be involved in epilepsy. Pro-inflammatory M1 state microglia enters the anti-inflammatory M2 state by the CB2 receptor activation. Furthermore, this receptor has been shown to increase in brain injuries and in several types of neurodegenerative diseases. Here, we investigated the CB2 receptor expression in the hippocampus of a MTLE mouse model. For this purpose, we used two mutant mice: CB2-GFP and CB2-KO (generously supplied by Dr. Julián Romero).
The epileptic state induced by kainic acid promoted de novo expression of the CB2 receptor in the CB2-GFP mice hippocampus. CB2 receptors were mainly localized in the CA1 and CA2 pyramidal cell layer, and to a lesser extent in the CA3 and hilus. The CB2 receptor expression was accompanied by Iba-1 labeling microglia. These microglial changes were not observed in animals injected with saline. The Iba-1 immunofluorescence extended across the width of the CA1-CA2 stratum radiatum and oriens but was mainly concentrated in the pyramidal cell layer where colocalized with CB2. In the dentate gyrus, the hilus showed a discrete Iba-1 and CB2 labeling. CB2-KO mice also showed microglial activation in the same areas as in CB2-GFP mice. The identification of the microglia morphology in kainate-injected CB2-GFP and CB2-KO mice is under current investigation.

(T1-08) CONTRIBUTION OF ASTROGLIA TO LOCAL PROTEIN SYNTHESIS IN NEURITES

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ABSTRACT

Intra-axonal protein synthesis is an essential mechanism in neurons, so that axons can rapidly react to environmental stimuli. Recently, studies have shown that local protein synthesis has a key role not only in Nervous System development but also in cell death due to neurodegenerative signals. These studies showed β-amyloid1-42 (Aβ1-42) oligomers (related to Alzheimer's disease) increase intra-axonal translation in neurites of hippocampal neurons, leading to death. Our laboratory analysed contribution of astroglia to local protein synthesis in neurons. Our results showed that neurites and axons increase the number of translational hotspots in the presence of astrocytes in physiological conditions while in amyloid-stimulated conditions translation is blocked in the presence of glia. Moreover, these effects are relevant at neurites distant from the astrocyte, what suggests this regulation could be mediated by secreted factors. Because of that, we aim to determine if astrocytes modulate the local proteome in hippocampal neurons by releasing translation regulators within extracellular vesicles in both basal and amyloid-induced conditions. We have used conditioned medium from primary neurons cultures with or without astrocytes and treated or not with Aβ1-42 oligomers. We show that conditioned medium from neuron-astroglia cultures are able to increase local protein synthesis in naïve neurons, as the presence of astroglia does in co-cultures. These results suggest glial-secreted vesicles regulate local translation in neurites. On the other hand, we have analysed the role of astroglia in axonal RNA location in both physiological and pathological conditions. In neuron-astrocyte co-cultures, in amyloid-induced conditions, the number of RNA granules increases. These results suggest there is an uncoupling between RNA transport and translation.

An alternative explanation is that due to the blockade of translation in pathological conditions, detected RNA could be sent by the soma as a compensatory mechanism or that we are detecting an accumulation of untranslated mRNAs. Together, this work suggests an important role for astroglia in local protein synthesis, what could be relevant for therapeutic strategies for neurodegenerative diseases.

(T1-09) THE INFLUENCE OF THE MICROGLIA IN THE LOCALIZATION OF AXONAL RNA UNDER BASAL CONDITIONS AND IN PRESENCE OF THE AB OLIGOMERS

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ABSTRACT

Neurons are specialized cells whose function is to transmit information over long distances and there are multiple process that located far of the cellular body. So, it is not surprising that local translation plays an important role in these cells. Several degenerative disorders of the PNS (peripheral nervous system) have been related to deregulation of localization of axonal RNA and local translation, such as ALS (amyotrophic lateral sclerosis) and SMA (spinous muscular atrophy). But perhaps most surprising was the discovery of a translational response to b-amyloid in axons of the CNS (central nervous system), which implies that the synthesis of axonal proteins may also play a role in the pathogenesis of Alzheimer's disease (AD). The origin of these intra-axonal proteins was initially thought to be only the neuronal soma, but there is evidence that glia also contributes to the repertoire of RNAs and axonal proteins. Taking into account that previous work shows that the Aβ pathology is partially mediated by intra-axonal synthesis and that glia could affect the axonal translatome, our final objective is to determine the influence of the microglia in the localization of axonal RNA under basal conditions and in presence of the Aβ oligomers. Work from our lab has shown that microglia significantly changes the amount newly translated proteins in neurites under control conditions, but not in the presence of Aβ. Moreover, our results shows that microglia influences the quantity of RNA in neurites, which significantly increases in the absence of contact between neurons and microglia. Also, presence of Aβ has not influence in the quantity of RNA in neurites. In conclusion, our results suggest that microglia influences the recruitment of RNA in neurites in physiological but not pathological conditions, which correlates with increased local translation. The fact that increases of both RNA transport and translation is restricted to neurites that do not establish direct contact with microglia, suggests that these effects might be mediated by secreted factors. Finally, our results show no effect of microglia in the recruitment of RNA nor the local protein production. Thus, our immediate goal is to determine whether inflammation-mediated activation of microglia can eventually trigger changes in the local translatome and transcriptome in the context of amyloid-induced pathology.

(T1-10) ROLE OF MITOCHONDRIAL DIVISION INHIBITOR (MDIVI-1) IN MICROGLIAL ACTIVATION AND METABOLIC SWITCH

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ABSTRACT

Microglial cells are considered to be the resident macrophages of the brain. Their activation and polarization towards a pro-inflammatory phenotype is associated to the development of neurodegenerative disorders such as multiple sclerosis (MS). This process is commonly linked to a metabolic reprogramming of the cells, characterized by high rates of glycolytic function and supressed levels of oxidative phosphorylation. We reproduced the metabolic reprogramming in microglia in vitro by stimulating cells with lipopolysaccharide (LPS) plus Interferon-y. In an attempt to understand the mechanism regulating mitochondrial respiration abolishment, we characterized mitochondrial changes during the metabolic switch. We did not detect any change in mitochondria morphology. Moreover, we did not detect any change in mitochondria potential, thus indicating that the metabolic switch is not associated to mitochondrial dysfunction. Then, we studied the possible implication of mitochondrial dynamics in metabolic reprogramming using the mitochondrial division inhibitor-1 (Mdivi-1), which specifically blocks Drp1-dependent mitochondrial fission. In vitro treatment of microglia with Mdivi-1 induced a morphological change of microglia, showing a round-shaped morphology. Moreover, Mdivi-1 significantly reduced the expression of pro-inflammatory markers in LPS-treated cells, as analyzed by qPCR, and reduced the expression of the inducible oxide nitric synthase (iNOS). However, this inhibition does not match with any recovery effect of the mitochondrial phosphorylation ablation by LPS+IFNy. Since nitric oxide (NO) production by the iNOS has been linked to the microglial inability to repolarize towards an anti-inflammatory phenotype (Van des Bossche et al., 2016) we then checked whether Mdivi-1 could favour a repolarizing process. However, pretreatment with Mdivi-1 did not favour microglia polarization to an anti-inflammatory phenotype. These results suggest that Drp1, although potentially involved in microglia activation, does not play an essential role in metabolic reprogramming of microglia.

(T1-11) GLIAL CO-LOCALIZATION OF THE METABOTROPIC GLUTAMATE MGLU3 RECEPTOR AND THE CANNABINOID CB1 RECEPTOR IN THE MOUSE DENTATE GYRUS

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ABSTRACT

The hippocampal dentate gyrus receives the perforant path input from the entorhinal cortex. This pathway makes excitatory axo-spinous synapses in the outer 2/3 of the dentate molecular layer. Stimulation of its middle 1/3 induces mGlu3- and CB1- dependent-LTD. Seeking to elucidate the anatomical interaction of the glutamatergic and cannabinoid systems in the generation of the excitatory LTD in the dentate molecular layer, double immunocytochemical labelling was performed using specific antibodies against sequences of the CB1 and mGlu2/3 receptors. They were applied to hippocampi of mice genetically deprived of mGlu2 to determine the subcellular distribution of the CB1 and mGlu3 receptors in the middle 1/3 of the dentate molecular layer by high resolution electron microscopy.MGlu3 receptor labelling was restricted to astrocytes while CB1 receptor immunolocalization was foundin both astrocytes and neurons, as expected. Localization of mGlu3 receptor labelled processes lining CB1 receptor positive boutons and co-localization of both receptors in thin astroglial lamellae surrounding excitatory axo-spinous synapses were observed.

Coincidence of both receptors on astrocytic membranes provides a basis for intra-glia interactions between excitatory transmitter and lipid systems highlighting the functional role of glia at the tripartite synapse.

Keywords: astrocyte, glutamate receptors, endocannabinoid system, tripartite synapse, hippocampus
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(T1-12) INCREASED OLIGODENDROCYTE DENSITY AND HYPERMYELINATION IN ALZHEIMER´S DISEASE IS DRIVEN BY AMYLOID B

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ABSTRACT

Alzheimer´s disease (AD) is characterized by a progressive cognitive decline that correlates with the levels of amyloid β peptide (Aβ) oligomers. Strong evidences connect oligodendrocyte impairment with the onset of neurodegeneration in AD. However, knowledge about AD-associated alterations in oligodendrocyte and myelin is commonly regarded as secondary to the disease. Here, we reveal a dysfunction in oligodendrocyte population and myelin synthesis that could contribute to failures in axonal conduction in AD. First, we characterized the expression of myelin basic protein (MBP) in human postmortem samples of prefrontal cortex and hippocampus from healthy and AD subjects. Surprisingly, advanced stages of AD samples showed significant increased levels of MBP in both regions, specifically in CA3 and dentate gyrus. Consistent with these findings, upregulation in MBP expression was found in a triple-transgenic mouse model of AD, which correlated with Aβ oligomer burden. Furthermore, electron microscopy analysis of myelin sheath in corpus callosum samples showed that axons in triple transgenic mice are hypermyelinated and have myelin-related abnormalities. To determine the functional consequences of these white matter defects, we measured compound action potentials in corpus callosum of 18-month-old transgenic mice. Our results revealed that the conduction velocity is significantly lower in myelinated, but not in unmyelinated, axons of transgenic mice as compared to wild type mice. Concomitantly, adult transgenic mice showed a remarkable increase in the density of mature oligodendrocytes induced by changes in the differentiation rate along with a greater number and shorter nodes of Ranvier in the corpus callosum of 18-month-old transgenic mice. These changes in node size and density may underlie the reduction of conduction velocity we observed in AD transgenic mice. In addition, to characterize the molecular events underlying AD-related oligodendrocyte changes, purified rat oligodendrocytes were treated with oligomeric Aβ. In vitro analyses demonstrated that Aβ-mediated activity modulates oligodendrocyte differentiation and myelination, enhancing MBP expression by inducing local mRNA translation. Therefore, these results support the idea that oligodendrocyte and myelin dysfunction contributes to AD pathophysiology and point to Aβ as a primary driver of those alterations.

(T1-13) EVALUATION OF CB1 RECEPTORS IN OLIGODENDROCYTE POPULATIONS OF THE MOUSE BRAIN USING ELECTRON MICROSCOPY

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ABSTRACT

Myelin forming oligodendrocytes (OLs) develop from proliferating precursor cells (OPCs) in a process that contributes to the formation, maintenance and remodeling of the myelin sheath, as well as to repair processes in demyelinating diseases such as multiple sclerosis (MS). Cannabis-based medicines acting through cannabinoid CB1 receptors (CB1R) exhibit clinical utility in MS. Oligodendrocyte populations in culture express low levels of CB1R that promote lineage progression and mediate protection from excitotoxicity. Nevertheless, there is no conclusive evidence that CB1Rs are expressed and quantifiable in brain oligodendroglia. Here we applied electron microscopy (EM) techniques to investigate 1) the ultrastructural localization of CB1R in mature OLs and OPCs of the adult mouse brain, and 2) the role of CB1R in myelin formation/maintenance in vivo. Results from CB1R immunolabeling in brain sections from CB1-WT and CB1-KO mice show that 26% OLs in the corpus callosum, identified by ultrastructural features, are CB1R immunopositive at postnatal day 60 (P60). Using mice expressing EYFP under the control of the NG2 promoter (NG2-CB1-WT and NG2-CB1-KO), we measured around 7% of CB1R immunopositive OPCs profiles in CA1 stratum radiatum. Finally, quantification of g-ratios in the corpus callosum at the peak of myelination (P30) showed a tendency to an increased myelin thickness in small caliber axons from CB1-KO mice. These results corroborate the expression of CB1R in OLs and OPCs of the mouse brain and support the possibility that these receptor populations contribute to fine-tune the myelination process in vivo.

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Keywords: CB1 receptor, myelin, mature oligodendrocyte, oligodendrocyte precursor, NG2

(T1-14) CLEMASTINE MODULATES OLIGODENDROCYTES PROLIFERATION

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ABSTRACT

Multiple sclerosis is defined as a chronic inflammatory disease that leads to focal plaques of demyelination and oligodendocyte loss in white matter. High throughput screening identifies clemastine, a drug with anti-histaminic and anti-muscarinic properties, as a compound that promotes differentiation and myelination of oligodendroglia (Mei et al., 2014). Moreover, clemastine penetrates the blood-brain barrier efficiently and has a favorable safety profile with few adverse side effects, consequently it represents a promising candidate for multiple sclerosis therapy and human remyelination trials. We have analyzed the acute and chronic effects of clemastine in oligodendrocyte cultures. We observed that clemastine produces a significant increase in the number of oligodendrocyte progenitors (OPCs). However, we did not detect any change in the number of mature oligodendrocytes. These results suggest that clemastine could

modulate the proliferation of OPCs, more than its differentiation. In fact, we observed a significant increase in the expression of proliferation markers, Ki67+, in OPCs. Previous studies suggested that the action of clemastine is due to the interaction with the muscarinic cholinergic receptor Chrm1 (Met et al., 2016). So, we analyzed the function of this receptor in both populations, immature and mature oligodendrocytes. Muscarine induced an increase in cytosolic calcium in both populations, an effect that was significantly reduced in the presence of clemastine. However, the number of cells responding as well as the magnitude of the responses were three fold higher in OPCs versus mature oligodendrocytes. Altogether data demonstrated that OPCs are endowed with muscarinic receptors whose activation modulates their proliferation.

(T1-15) A PROTEOMICS APPROACH TO IDENTIFY CANDIDATE PROTEINS SECRETED BY MÜLLER GLIA THAT PROTECT GANGLION CELLS IN THE RETINA

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ABSTRACT

The retinal Müller glial cells, can enhance the survival and activity of neurons, especially of retinal ganglion cells (RGCs), which are the neurons affected in diseases such as glaucoma, diabetes, and retinal ischemia. It has been demonstrated that Müller glia release neurotrophic factors that support RGC survival, yet many of these factors remain to be elucidated. To define these neurotrophic factors, a quantitative proteomic approach was adopted aiming at identifying neuroprotective proteins. First, the conditioned medium from porcine Müller cells cultured in vitro under three different conditions were isolated and these conditioned media were tested for their capacity to promote survival of primary adult RGCs in culture. Mass spectrometry was used to identify and quantify proteins in the conditioned medium, and osteopontin (SPP1), clusterin (CLU), and basigin (BSG) were selected as candidate neuroprotective factors. SPP1 and BSG significantly enhance RGC survival in vitro, indicating that the survival-promoting activity of the Müller cell secretome is multifactorial, and that SPP1 and BSG contribute to this activity. Thus, the quantitative proteomics strategy identify proteins secreted by Müller glia that are potentially novel neuroprotectants, and it may also serve to identify other bioactive proteins or molecular markers

(T1-16) TRAUMATIC BRAIN INJURY-INDUCED ALTERATIONS IN THE ADULT DENTATE GYRUS

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ABSTRACT

Traumatic Brain Injury (TBI) is becoming a “silent epidemic” worldwide. Patients affected by TBI show serious neurological disorders such as decision-making and memory deficits, depression or aggressive behavior. Several of the important brain functions affected by TBI depend on the hippocampus, a brain structure very important for memory and learning that is highly vulnerable to this kind of injury. After an episode of TBI, the hippocampus suffers atrophy and alterations in synaptic transmissions. In addition, Adult Hippocampal Neurogenesis (AHN), a process involved

in memory, learning and control of anxiety, is impaired. We hypothesize that TBI induces long-term changes in both Neural Stem Cells (NSCs) and newborn neurons, subsequently impairing hippocampal and brain functioning. This project highlights the importance of considering NSCs and new neurons as novel targets in developing innovative strategic therapies against brain damage. We aim to understand what particular changes are induced in NSCs and newborn neurons by TBI, and what is the actual impact on brain functioning and behavior of these changes employing a mouse model. We have shown that NSCs modify their behavior readily in response to neuronal damage and changes in neuronal activity. Depending on the intensity of the stimulus they either boost transiently the generation of neurons (neurogenesis) and afterwards become depleted or they switch to a reactive phenotype (reactive NSCs, RNSCs) entering symmetric division and generating reactive astrocytes (RAs) (reactive astrogliogenesis) at the expense of their neurogenic potential. In both cases, the generation of ectopically newborn neurons with altered morphology and connectivity, ie., "aberrant neurogenesis" takes place, potentially affecting the processing of neuronal input into the hippocampus. We aim to investigate both the changes induced in NSCs and in newborn neurons in a multilevel and integrative manner. We propose that NSCs are central players together with astrocytes and microglia in the response to damage in the brain upon injury through three mechanisms. First, the accelerated depletion of the NSC population and subsequent long-term impairment of AHN; second, the NSC-dependent reactive gliosis, and finally the generation of "aberrant" neurogenesis. NSCs have been the focus of regenerative medicine because of their potential to replenish the neurons lost by injury or disease. We are here proposing and opposed function of NSCs that they are actually contributing to brain damage by the three proposed mechanisms (accelerated depletion, reactive gliosis and aberrant neurogenesis). Using a controlled cortical impact model of TBI in Nestin-GFP transgenic mice we have observed, 3 days after the impact, that the most affected area is the dorsal blade of the dentate gyrus, as shown by an anatomical alteration consisting of a thickening of the granule cell layer (GCL) and a migration or "bending" of this neuronal layer towards the cortex. These changes are accompanied by an increase in the expression of C-Fos, a marker of neuronal activation in the hilus. In addition, NSCs acquire a reactive-like morphology with thickening and increased branching of their processes, although their activation, as measured by incorporation of BrdU is not significantly altered. Furthermore, TBI triggers an expansion of neuroblasts (identified by doublecortin, DCX, expression) in the short term (3d). DCX+ neuroblasts also present cytoplasmatic hypertrophy and altered migration, being found with significantly higher frequency in the outer portions of the GCL. We are currently investigating concomitant changes in astrocytes and microglia, and in the electrophysiological properties of GCs and pyramidal cells of the hippocampus, as well as the changes underwent by the dentate gyrus in the long term.

(T1-17) COMBINATION THERAPY ENHANCES THE PARTIAL PROTECTION INDUCED BY COOLING AFTER NEONATAL BRAIN INJURY: A HISTOLOGICAL STUDY OF THE NEUROGENIC RESPONSE IN THE PIGLET SUBVENTRICULAR ZONE

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ABSTRACT

Background: Therapeutic hypothermia has become standard therapy for full-term neonates with moderate-to-severe neonatal encephalopathy. However, cooling does not fully protect the damaged brain and long-lasting neurological problems can occur. No established treatments exist aside from therapeutic hypothermia, so new therapies added during or after cooling could improve neuroprotection and/or enhance neurogenesis and repairing processes. Focusing on the neurogenic response in the subventricular zone of the newborn piglet, the main objectives of the present work were a) to evaluate the influence of a neonatal hypoxic-ischemic (HI) insult in the neural stem/progenitor cell pools, b) to study its possible modulation by hypothermia and c) to analyze any additive or synergistic response when administrating melatonin together with cooling.

Methods: 51 piglets were randomly assigned to six experimental groups: i) naïve animals (with neither HI-injury nor treatment, n=6); (ii) HI and normothermia (38.5ºC, n=7); (iii) HI and cooling at 35ºC (n=7); iv) HI and cooling at 33.5ºC (n=15); v) HI and cooling at 30ºC (n=7); and vi) HI and cooling at 35ºC plus i.v. Melatonin 30mg/kg at 10 min and repeated at 24h (n=9). Neuroblast chains around the subventricular zone were evaluated by hematoxylin-eosin routine staining and DCX immuno-histochemistry at 48 hours after HI. Cell proliferation was analyzed by quantifying the number of Ki67 positive cells whereas neural stem/progenitor cell proliferation was obtained after evaluating both Ki67 and Sox2 positive counts.

Results: Neonatal HI induced a significant reduction in neuroblast processes, cell proliferation and number of neural precursors. Cooling at 33.5ºC diminished the loss of neuroblast chains, an effect extended to the number of both Ki67 and Ki67/Sox2 positive cell counts. Cooling to 35ºC or 30ºC maintained cell proliferation but did not avoid the diminution of neuroblast number. Melatonin + cooling at 33.5ºC obtained better results than hypothermia alone, with similar values to those observed in naïve animals.

Conclusions: Melatonin augments hypothermic protection of hypoxic-ischemic-induced reduction in neurogenesis in the subventricular zone of the newborn piglet.

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(T1-18) ATP LINKS THE INDUCTION OF REACTIVE NEURAL STEM CELLS BY NEURONAL HYPEREXCITATION

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ABSTRACT

Besides generating neurons, the NSCs dwelling in the adult hippocampus generate astrocytes, more copies of themselves and even olygodendrocytes after genetic manipulation. The balance between excitatory and inhibitory input regulates the behavior and fate of hippocampal NSCs. Thus, in normal conditions NSCs generate mostly and astrocytes and symmetric self-renewing division cannot compensate for the natural decline of the population. Making NSCs reluctant to GABAergic tonic input results in higher activation (more of them are recruited to enter the cell cycle) and increasing the frequency of symmetric NSC-generating divisions. Triggering administration of the glutamamte agonist kainic acid (KA) also recruits NSCs in higher number, but without altering the asymmetric versus symmetric division, thus resulting in accelerated depletion of NSCs and long-term impairment of neurogenesis. Finally, when neuronal hyperexcitation is high enough to trigger seizures, NSCs become reactive (React-NSCs), becoming multibranched, hypertrophic, losing their broccoli-like apical arborization and entering massively

in mitosis. React-NSCs divide symmetrically generating more copies of themselves that will ultimately differentiate into reactive astrocytes (RAs). Therefore NSCs are able to contribute to the neuroinflammatory response at the expense of abandoning their neurogenic potential. We are now investigating whether the induction of React-NSCs is a direct effect of neuronal hyperexcitation or a consequence of the inflammatory response of astroglia and microglia after seizures. This is an intriguing point as the astroglia and microglia-driven inflammatory response tend to decrease NSC activation, reducing their proliferation and neurogenesis. We are mimicking bacterial and viral infection in the hippocampus, plus testing the direct effect of cytokines on the NSC population, and comparing the results with models of neuronal hyperexcitation (KA injection and traumatic brain injury, TBI). These models also induce neuroinflammation, but in contrast the inflammatory models that we are using induce little or none neuronal hyperactivity, thus allowing us to dissect their separate effects. Our current hypothesis is that NSCs becoming React-NSCs could happen together with the astroglial and microglial reactive response, but is actually independent of it. Our work suggests that there is direct link between hyperactive/excitotoxic neurons and NSCs that is responsible for their conversion into React-NSCs. Furthermore we have identified ATP, acting on P2X receptors (P2XR) present in NSCs (both in vivo and in vitro) as a possible key mediator between hyperactive neurons and NSCs. Intrahippocampal injection of ATP mimics the effect of the high dose of KA, strongly inducing React-NSCs with very similar characteristics to the ones explained above (massive activation, hypertrophy and symmetric division). We will now use inducible Nestin-CreERT2-Rosa26YFP mice for lineage tracing studies after hippocampal injection of ATP. Further, we can recapitulate in vitro the results in vivo. Nestin-GFP-expressing NSC and progenitor cells express P2XR in culture. When ATP is added to neurosphere culture and increase in cell proliferation as well as a cytoplasmic hypertrophy and increment of the gliogenic differentiation is provoked. Thus we have a valuable tool to dissect the molecular signaling pathways triggered by the binding of ATP to P2XR in NSCs.

(T1-19) CHRONOLOGICAL CONSTRUCTION OF HIGH ORDER SENSORY PROCESSING CIRCUITS IN EMBRYONIC BIRDS

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ABSTRACT

The early generation program of the pallium reflects the evolutionary origin of the neocortex. The developmental novelties that triggered neocortical evolutionary formation can be now identified as events happening in the mammalian dorsal pallium exclusively. Here we present preliminary results of our group in the search of those divergent events by comparing the early developing pallium of mouse and chick. We focus on the neurogenic timing of pallial neurons. Homologous developmental events must maintain an equivalent timing. Therefore, the chronological order in which the different neurons of the cortical circuit are born should be preserved in any cortical homologue of non-mammalian species. To test the homology the dorsal ventricular ridge, a suggested neocortical homologue in birds, we injected chick embryos with the thymidine analogue EdU at selected developmental timepoints. We describe the chronological generation of neurons at each of the nuclei of the visual and somatosensory pallial circuits. We researched the birthdate of both GABAergic interneurons and glutamatergic projecting neurons at the specific nuclei of the tri-synaptic pallial circuit: entopallium, dorsal nidopallium and arcopallium. Interestingly, interneurons and projecting neurons of each nuclei are generated

synchronously, a feature common to mammals. However, we found that the neurons participating in the sensory circuit of the avian dorsal ventricular ridge were not generated in an equivalent order to that of the mammalian cortical canonical circuit. Essential divergences are 1) the birthdate of thalamic-recipient sensory neurons, which are the first ones generated in the avian circuit, and 2) the lack of contribution to the sensory circuit of the latest generated neurons. These crucial developmental differences suggest that both circuits are not derived from a common ancestor circuit, and their functional correlation could be a product of evolutionary convergence.

(T1-20) PROTECTIVE EFFECTS OF DIETARY OMEGA-3 FATTY ACIDS ON ADULT NEUROGENESIS AGAINST AN INFLAMMATORY CHALLENGE

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ABSTRACT

Formation of new neurons (i.e. neurogenesis) during adulthood only occurs in two brain regions, which are the subventricular zone of the lateral ventricles and the hippocampus. In the hippocampus, newborn neurons incorporate into existent circuitries and are involved in memory formation and consolidation. Adult hippocampal neurogenesis can be regulated by different lifestyle factors, and among them stand out inflammation and diet, such as omega-3 polyunsaturated fatty acids (n-3 PUFA) intake. The former is associated with a decreased number of newborn neurons, whereas the latter is anti-inflammatory and promotes the formation of new neurons. The aim of this work was to investigate whether a n-3 rich diet is able to prevent the detrimental long-term effects of an acute systemic inflammation on adult hippocampal neurogenesis. To test the hypothesis, 1.5 months old mice were fed with n-3 rich or n-3 deficient diets. One month later, mice were exposed to an inflammatory event induced by a single LPS injection, which was previously demonstrated to decrease neurogenesis. At the age of 4 months, mice were sacrificed to analyze adult neurogenesis, microglia, and astrocytes. Our results reveal that the area covered by microglial cells and the production of new neurons are concomitantly decreased in mice fed with an unbalanced diet that received a LPS injection compared with the other experimental groups. Nevertheless, further analyses are ongoing in order to verify changes in microglial morphology and production of new neurons.
Our data suggest that a n-3 rich diet protects the hippocampus from the detrimental effects of systemic inflammation in the morphology of microglial cells and adult neurogenesis.
This work has been supported by the Spanish Ministry of Economy and Competitiveness with FEDER funds to A.S. (BFU2015-66689-R and RYC-2013-12817) and Ikerbasque startup funds to J.V.

(T1-21) ELECTROPHYSIOLOGICAL ACTIVITY OF DENTATE GYRUS NEURONS AFTER TRAUMATIC BRAIN INJURY

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ABSTRACT

Traumatic brain injury (TBI) affects millions of people representing a major public health concern however, treatment options are limited. Even after mild TBI many individuals suffer from long term neuropsychological impairments such as memory loss and learning deficits. The

hippocampus is essential for learning, memory consolidation and mood control and is highly vulnerable to TBI. Some of the observed impairments could be related to the alterations in adult hippocampal neurogenesis (AHN). Therefore, we propose that the population of neural stem cells (NSCs) and the process of AHN is significantly altered, which might account for some of the symptoms associated with TBI. As a result the electrophysiological properties of newborn and preexistent neurons in the Dentate Gyrus (DG) circuitry are changed, altering brain functioning. Ongoing research is aimed at examining on how TBI affects activity of post – TBI born immature and mature granule cells in the DG. At several timepoints after TBI or SHAM surgery, horizontal brain slices containing DG were prepared and electrophysiological activity of granule cells was evaluated with whole-cell patch-clamp recordings. Preliminary results suggest that neuronal intrinsic membrane properties, such as resting membrane potential (RMP) and input resistance (Ri) remain unchanged. However a trend for increased excitability of immature granule neurons 3 days after TBI, when compared to SHAM controls was observed. We hypothesize that early alterations of neuronal properties in the DG could represent the basis for long-lasting changes in the hippocampal circuitry.

(T1-22) ***BIRTH-SEQ, A NEW METHOD TO ISOLATE DIVIDING-DATED CELLS***

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ABSTRACT

Understanding the appearance of neocortex in evolution requires the study of the formation of sensory processing circuits during embryonic development. These circuits appear in sensory structures of the telencephalon of different amniotic species, such as the mammalian neocortex and the dorsal ventricular ridge of birds. However, we still do not know the evolutionary history of these circuits. If they were homologous they would have evolved from a common ancestral circuit that was already present more than 300 million years ago and that has not substantially changed since then. To study this conservation we aim to compare the development of the circuit in selected amniote species. First, we shall investigate homology of neural stem cells (NSCs), the cells from which circuits originate. Secondly, we shall determine the evolutionary relationship of neural circuits by analysing the embryonic history, brain position and neuronal diversity of the neurons that construct them.

Thus, a fundamental step to be able to investigate the evolutionary history of neural circuits is the isolation of progenitors knowing the region of the brain and the neurogenic moment in which they have been generated. For this, we have developed Birth-Seq, a method for the isolation of neural progenitors that is valid for all amniotic species. The method combines traditional birthdating methods (with thymine analogues) and FACS sorting. We marked the dividing progenitor cells by intravenous injection of ethynyl deoxyuridine (EdU) in chicken embryos at selected stages of development. Two hours post-injection, the embryonic brains were disaggregated. By means of a modified non-toxic developing of the EdU labelling, we isolated the dividing progenitors, using fluorescence activated cell sorting (FACS). Finally, in order to validate the identity of the cells and their viability, we conducted a study of their gene expression using qPCR. Birth-seq will allow us to compare birth-dated cells between brain regions and species, ultimately bringing light into the evolutionary history of vertebrate brains.

(T1-23) ***LPA1 LABELS REACTIVE NEURAL STEM CELLS AND REGULATES THEIR ACTIVATION***

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ABSTRACT

Neural stem cells (NSCs) persist in the hippocampus of most mammals and are able to generate neurons through adulthood, a process known as adult neurogenesis. We discovered that epileptic seizures originated in the hippocampus shift the essentially neurogenic cascade towards reactive astrogliogenesis. In addition to this abolition of the neurogenic program, NSC transformation into reactive astrocytes (RAs) contributes to hippocampal sclerosis and presumably inflammation. Thus, we are interested in characterizing NSC-derived RAs and comparing them to those RAs differentiated from parenchymal astrocytes. For this purpose, we identified and validated the LPA1-GFP transgenic mouse line in which lysophosphatidic acid receptor 1 drives the expression of GFP.
In LPA1-GFP mice, GFP labels specifically hippocampal NSCs. This feature let us analyze their population and activation properties in our epilepsy model. We performed a time course following intrahippocampal kainic acid (KA) injection and monitored the generation of GFP-positive RAs that progressively lose the transgene expression and the concomitant reduction of the GFP-positive NSC population. The prolonged GFP expression during seizure-induced NSC transformation into RAs defines a time window of weeks in which these cells are different from other RAs. Functionally, in a mouse line null for LPA1 we have observed that the increased NSC activation induced by seizures declines when compared to their wild type counterparts. These results suggest that LPA1 is involved in the cell cycle entry of adult NSCs and could be used to manipulate adult neurogenesis.

(T1-24) EXPOSURE TO NEUROTROPHINS BDNF AND NT3 REPROGRAMS HUMAN ECTOMESENCHYMAL DENTAL PULP STEM CELLS TO NEURAL CREST PROGENITORS WITH INCREASED NEUROGENIC AND GLIOGENIC DIFFERENTIATION POTENTIAL

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ABSTRACT

Human Dental Pulp Stem Cells (hDPSC) derived from the Neural Crest (NC) during embryo development have been traditionally described as ectomesenchymal stem cells with a particularly great capacity to give rise to connective tissue-related cell lineages such as osteoblasts, chondroblasts, and adipocytes. However, recent evidence suggests that DPSCs may also retain characteristics of true NC progenitor cells which are also competent to differentiate to neural cells. Given their multi-lineage differentiation potential, high expansion rates in culture, and easy accessibility by a simple tooth extraction, these cells are regarded as ideal candidates to regenerate both connective and nervous tissues by cell therapy.
However, the widespread practice of using Fetal Bovine Serum (FBS)for in vitro hDPSCit's maintenance and expansion especially in neural differentiation protocols, can provoke serious safety concerns even in the case of autologous cell therapies.
For this reason, we set out to compare the profile, fate and changes of gene expression of hDPSCs cells cultured either using a serum-free specific mesenchymal stem cell medium StemPro MSC TM (STP) or the standard culture mediuma DMEM+ 10% FBS. Our initial results showeds that STP alloweds hDPSCs to grow as non-adherent floating DPSC-derived dentospheres with a low rate of proliferation , contrary to , DMEM+ 10% FBS culture medium, which induceds a monolayer

cell attachment with much faster proliferation rates. Next, we determined that the expression of mesenchymal/connective tissue markers expression, pluripotency core factors and neurotransmitter receptors were unaltered between both media conditions.However and unexpectedly, we found that neurotrophic factor receptors NTRK2(TrkB) and NTRK3(TrkC) were overexpressed in hDPSC grown in serum-free STP medium. Interestingly, stimulation of those receptors by addition of their natural ligands, BDNF and NT3 to the STP medium, induced a large increase on the expression of NC markers (HNK1 and , P75) and a pluripotency core factors (SOX2, OCT4 and, NANOG) in hDPSC cultures. These changes were consistent with a reprogramming process were hDPSCs acquired characteristics of early NC progenitors, not only competent to differentiate to mesenchymal lineages but also to neural cells. To test this hypothesis, we grew in parallel hDPSCs in (i) DMEM + FBS, (ii) STP, and (iii) neurotrophin-supplemented STP media for 7 days, and then we plated the cells in another Neurocult ™ neural differentiation serum-free medium. When we assessed the neural differentiation potential of hDPSCs in each condition, we found out a very large and significant increase of double positive cells for the neuronal markers Ddoublecortin (DCX) and nuclear neuronal marker NeuN, as well as for the glial Schwann cell markers s100beta and p75, specifically in hDPSC cultures that had been preconditioned with neurotrophin-STP. These results showed an enhancement of the neurogenic and gliogenic differentiation potential of hDPSCs, induced by a short-term pretreatment with BDNF and NT3 neurotrophins in serum-free STP medium.
In conclusion, we present a new protocol to enrich hDPSC cultures with cells with NC progenitor characteristics and increased neurogenic and gliogenic differentiation potential. This work has been financed by “Ramón y Cajal” programs RYC-2013-13450 and RYC-2012-11137 and MINECO SAF2015-70866-R, University of the Basque Country (GIU16/66, UFI 11/44), and Basque Government (GV/EJ; IT831-13) and University of Basque country (upvPIF//13/268).

(T1-25) DIFFERENT DISTRIBUTION OF RBPMS IN THE NEONATAL AND ADULT PIG RETINA

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ABSTRACT

RNA binding protein with multiple splicing (RBPMS) is expressed exclusively in retinal ganglion cells (RGCs) in the Central Nervous System and can label all RGCs in normal retinas of mouse, rat, guinea pig, rabbit, cat and monkey, but its function in these cells is not known. Due to limited knowledge regarding RBPMS, we have analyzed the expression of RBPMS in the retina of young and adult pigs in vivo, in vitro and ex vivo and compared the results to human and rat. RBPMS was also studied in organotypic retinal cultures from pig and rat. In neonatal pig retina, RBPMS was localized in the RGCs somas, whereas in adult it was localized in the inner plexiform layer, the same location as adult human retina. In contrast, in neonatal and adult rat retinas, RBPMS is located always in the soma. We have further shown RBPMS localization in the RGCs axons in the retina during the RGCs degeneration in pig organotypic retinal cultures. Further experiments should be done to elucidate the role of RBPMS in regeneration of RGC axons as well as its role in dendritic plasticity in the pig and human retina.

(T1-26) EVALUATION OF LONG-TERM DESIPRAMINE ADMINISTRATION ON ALPHA-2 ADRENOCEPTORS REGULATING NORADRENALINE AND SEROTONIN RELEASE IN RAT FRONTAL CORTEX

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ABSTRACT

Antidepressant drugs take time to develop their effect, typically 2-3 weeks. It has been shown that desensitization of α2-adrenoceptors is a common response to the chronic treatment with antidepressant drugs that increase synaptic noradrenaline (NA) concentration. The present study was undertaken to elucidate the effect of repeated treatment with the tricyclic antidepressant desipramine (DMI) (twice daily, 14 days. 72 hours of washout period) on the frontal cortex (FC) noradrenergic and serotonergic transmission by rat brain microdialysis, focusing on the effect of α2-adrenoceptors sensitivity. Acute administration of the α2-adrenoreceptor agonist clonidine (0.3 mg/kg i.p.) decreased NA in FC (Emax=-45±7%; $p<0.0001$). Administration of the α2-adrenoreceptor antagonist RX821002 (1 mg/kg i.p.) enhanced NA concentration (Emax=268±48%; $p<0.0001$). For α2-adrenoreceptor-mediated serotonin (5-HT) release inhibition (Emax=-30±9%; $p<0.0001$), a higher dose of clonidine was needed (0.9 mg/kg i.p.). RX821002 administration (1 mg/kg i.p.) did not modulate 5-HT concentration in the FC. After long-term desipramine treatment, a high dose clonidine injection (0.9 mg/kg i.p.) decreased NA concentration in the FC in control (Emax =-42±12%; $p<0.0001$) and treated groups (Emax=-40±12%; $p<0.0001$). In the same way, when 5-HT concentration was measured after clonidine administration (0.9 mg/kg i.p.) a similar decrease of 5-HT was observed in control (Emax=-60±10%; $p<0.0001$) and treated groups (Emax=-59±6%; $p<0.0001$). These findings indicate that long-term administration of DMI is not able to desensitize the α2-adrenoreceptor subpopulation that exert an inhibitory control on 5-HT release by serotonergic terminals in the FC.

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Keywords: α2-adrenoreceptor, prefrontal cortex, desipramine, noradrenaline, serotonin, clonidine

(T1-27) A2A- AND A2C-ADRENOCEPTOR SUBTYPES EXPRESSION IN POSTMORTEM PREFRONTAL CORTEX OF SUBJECTS WITH SCHIZOPHRENIA

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ABSTRACT

α2A- and α2C-adrenoceptor subtypes are present in the human prefrontal cortex (PFC) and could play a role in schizophrenia. Moreover, α2-adrenoceptors are targets for different antipsychotic drugs. In this context, the density of both α2-adrenoceptor subtypes and their pre/postsynaptic expression has not been deeply evaluated in the PFC of schizophrenic subjects.
α2A- and α2C-adrenoceptor protein expression was determined by Western Blot in postmortem PFC of 24 subjects with an antemortem diagnosis of schizophrenia, and 24 controls. Both groups were matched by age, gender, and postmortem delay. Twelve of the schizophrenic subjects were taking antipsychotics at death (based on positive blood toxicological analysis), while the other 12 where antipsychotic-free (negative toxicology). α2A- and α2C-adrenoceptor expression was measured both in a preparation of synaptosomes and in postsynaptic membrane fractions, and was normalized for actin immunoreactivity as loading control.
α2A-adrenoceptor protein expression in synaptosomes showed a non-significant trend to increase (+37%, p=0.114) in schizophrenia subjects compared with controls. When subjects were divided regarding antipsychotic treatment, there was a significant increase in α2A-adrenoceptor expression in antipsychotic-treated (+78%, p=0.025) but not in antipsychotic-free subjects compared with controls. α2A-adrenoceptor expression in postsynaptic fraction was significantly increased in schizophrenia subjects vs controls (+71%, p=0.026). Again, the increase was significant in antipsychotic-treated subjects (+131%, p=0.014) but not in antipsychotic-free subjects. α2C-adrenoceptor protein expression was not significantly different between schizophrenia subjects and controls in synaptosomes and postsynaptic fraction, neither in antipsychotic-treated or antipsychotic-free subjects.
In conclusion, α2A-adrenoceptor protein expression was increased in PFC of schizophrenia subjects receiving antipsychotic treatment. This increase was stronger in the postsynaptic fraction compared to synaptosomes (which include both pre- and postsynaptic membranes). These results might be a consequence of the α2A-adrenoceptor antagonistic properties of some antipsychotic drugs.
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(T1-28) ACUTE THC EXPOSURE ALTERS CB1 RECEPTOR LOCALIZATION AND INDUCES ULTRASTRUCTURAL CHANGES IN THE HIPPOCAMPUS OF YOUNG-ADULT MICE

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ABSTRACT

Cannabis is the most widespread illicit drug in the world and its main psychotropic ingredient Δ9-tetrahydrocannabinol (THC) exerts psychoactive effects through the activation of the cannabinoid receptor type 1 (CB1). This receptor is expressed in different neuronal subpopulations and glial

cells in the central nervous system and plays an important role in astrocyte function and modulation of neuronal synaptic transmission and plasticity. Marijuana use often begins during adolescence, a highly susceptible period for environmental stimuli to alter functional and structural organization of the developing brain. However, no much information is available on the fine anatomical changes taken place in neurons after THC consumption during adolescence. In addition, the impact of adolescent THC consumption on the localization of CB1 receptor in the brain remains unknown.
To investigate this, young adult C57BL6J mice were subcutaneously injected with 5 mg/kg of Δ9-THC (once) or vehicle. After half an hour, they were deeply anesthetized andperfusion-fixed through the heart. Then, a preembedding immunogold method for high resolution electron microscopy was used to study the CA1 hippocampus.
49.57% ± 1.39% of the total area analyzed in sham and 67.80% ± 1.13% in THC ($p < 0.001$***, Mann Whitney test) corresponded to dendritic profiles. Furthermore, a huge increase in the number of dendritic mitochondria was observed in THC exposed mice relative to sham ($p < 0.001$***, Chi-square).
Furthermore, 78.40% ± 0.81% of the inhibitory terminals and 21.91% ± 2.06% of the excitatory terminals were CB1 immunopositive in sham. In THC-treated mice, the proportion of CB1 immunopositive inhibitory terminals decreased significantly (47.74% ± 13.27%; $p < 0.001$***, Mann Whitney test), and not significant differences were detected in theCB1 immunopositive excitatory terminals (17.55% ± 2.39%; $p > 0.05$, Kruskal-Wallis test). Furthermore, there was a significant reduction in the CB1 receptor immunopositive astrocytic processes in THC with respect to sham (23.05% ± 3.99% vs 35.13% ± 4.29%, respectively) ($p < 0.01$**, Mann Whitney test), as well as an increase in CB1 immunoparticle density (0.40 ± 0.06 vs 0.20 ± 0.02 particles/μm) ($p < 0.01$**, Mann Whitney test). Finally, both the proportion of neuronal CB1 immunopositive mitochondria (18.66% ± 1.06%) and the percentage of astroglial CB1 immunopositive mitochondria (13.49% ± 2.28%) were reduced in acuteTHC (11.32% ± 0.68%; $p < 0.001$***, Mann Whitney test and 6.08% ± 1.39%; $p = 0.049$*, Mann Whitney test), respectively). Altogether, these data indicate the existence of fast brain adaptations that support the behavioral alterations caused by cannabis intoxication.

(T1-29) ULTRASTRUCTURAL CHANGES IN THE CEREBELLAR PARALLEL FIBER TERMINALS OF MICE LACKING CB1 RECEPTORS

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ABSTRACT

The endocannabinoid system (ECS) plays two clearly different roles depending on the developmental stage of the brain. Thus, besides its main well known function as a modulator of the neurotransmitter release, in vivo and in vitro studies have demonstrated that the cannabinoid CB1 receptor plays a critical role in axonal growth and guidance during brain development.
The ECS is highly expressed in the cerebellum from early embryonic stages, but also during the postnatal development and in the adulthood. While several studies have demonstrated a practically normal phenotype of the CB1-knockout (CB1-KO) mice, others highlighted the deficits they have in motor coordination and its mechanisms compared to the CB1-wild-type (CB1-WT) mice. Therefore, we hypothesized that the functional and behavioral deficits observed in the CB1-KO should have an anatomical correlate in the cerebellum, in particular, in the CB1 receptor-expressing parallel fiber terminals (PFT).

In this study, the ultrastructural features of the adult CB1-KO mice parallel fiber terminals were assessed by electron microscopy in lobes 5 and 10 of the cerebellum.
CB1-KO mice had fewer parallel fiber-Purkinje cells dendritic spines (PCDS) synaptic contacts in lobe 5 than CB1-WT (CB1-KO: 2.476±0.106; CB1-WT: 3.109±0.137; p=0.0004***) and longer synapses (CB1-KO: 343.8±6.622; CB1-WT: 303.6±5.372; p<0.0001***). No differences were found in lobe 10. Moreover, the CB1-KO PFT were 25% larger in lobe 10 than in lobe 5 (lobe 10: 0.56±0.015; lobe 5: 0.42±0.011; p<0.0001***) and both lobes had less vesicles close to the active zone in CB1-KO (lobe 5: 53.71±1.029; lobe 10: 46.47±1.625) versus CB1-WT (lobe 5: 66.94±1.798; lobe10: 53.23±1.873). Furthermore, synaptic vesicles were more dispersed in PFT of CB1-KO lobe 5 than lobe 10.
Taken together, our findings indicate that the lack of CB1 receptors alters some ultrastructural features of the PFT. Expanded synaptic terminals with decreased vesicular density near the active zone suggest the existence of compensatory mechanisms particularly in lobe 5. These morphological changes observed in the parallel fiber terminals highlight the adaptive capacity of the cerebellar circuits in order to keep functionality as efficient as possible.

(T1-30) THE SUBSYNAPTIC DISTRIBUTION OF THE ENDOCANNABINOID SYSTEM IN MOUSE CORTICAL BRAIN

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ABSTRACT

The CB1 receptor is mainly localized at presynaptic plasma membrane, where it takes part in the retrograde signaling regulating the neurotransmitter release at different synapses (1). However, the CB1 receptor can also be found in other subcelullar compartments, for instance in somatodendritic membranes or intracellularly in mitochondrial membranes and it can also be located in other type of cells as astroglia or microglia (2). A very useful method for studying in detail the synaptic CB1 receptor and reducing the contamination and the impact of other compartments is to purify synaptic particles called synaptosomes (3, 4).
Therefore, in order to isolate a mouse cortical synaptosome fraction, one of the purposes of the present study was to validate a subfractionation procedure based on differential and density gradient centrifugation in sucrose. For this aim, immunofluorescence technique was used for the quantification of the particles depending on their size and their neuronal or glial origin. Secondly, synaptosomes were fractionated collecting the presynaptic active zone, postsynaptic density and nonsynaptic fractions (extrasynaptic) using “Phillips” protocol, which is based on the differential sensitivity to pH and detergents of the different fractions (5). The quality of the procedure was validated by Western blot technique using specific antibodies against proteins of different fractions.
The biochemical distribution of the cannabinoid system elements was studied in the subsynaptic fractions. The CB1 receptor was found in the extrasynaptic fraction, together with the different subtypes of the Gi/o proteins. The cannabinoid interacting protein 1a (CRIP1a) was also located in the extrasynaptic fraction. Regarding 2-arachidonoilglycerol (2-AG) production system, the phospholipase C-β1 (PLCβ1) and Gq protein were found in the extrasynaptic fraction and diacylglycerol lipase (DAGL) was observe mainly in the postsynaptic density, although in was also located in the extrasynaptic fraction. The monoacylglycerol lipase (MAGL), the principal 2-AG degradation enzyme, was found in the extrasynaptic fraction.

The biochemical distribution of the cannabinoid system elements at the synaptic level is in concordance with the retrograde signaling function assigned to the cannabinoid system.

(T1-31) GABA SIGNALING IN OLIGODENDROCYTE PROGENITORS PROMOTES DIFFERENTIATION, MYELINATION AND REMYELINATION

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ABSTRACT

Demyelination is the main pathological hallmark in multiple sclerosis. Oligodendrocyte precursor cells (OPCs) are the primary source of remyelination after a demyelinating insult since these cells are able to proliferate, migrate and differentiate into myelinating oligodendrocytes (OLs). This process may be mediated by neuron-glia interactions that involve several molecules such us growth factors and neurotransmitters like GABA. We previously reported that the expression and function of GABAA receptors in cultured OLs are regulated by axon-to glia interactions (Arellano et al., 2016). In the current study, we have investigated the role of the GABAergic system in OPC differentiation, myelination and remyelination in two in vitro models. We found that cultured OLs express the two GABA-synthesizing enzymes GAD65/67 and MAOB as well as both GABAA and GABAB receptors. Surprisingly, they release GABA to the culture medium which may regulate oligodendrocyte maturation in an autocrine and/or paracrine manner. Indeed, selective activation of GABAB receptors with Baclofen in purified rat OLs and cerebellar organotypic slice cultures promotes the production of myelin associated glycoprotein (MAG) and myelin basic protein (MBP), an effect that is attenuated in the presence of its antagonist CGP55485. In addition, modulation of GABA signaling attenuates lysolecithin-induced demyelination in cerebellar organotypic cultures and may contribute to the subsequent remyelination. These results indicate that signaling through GABA receptors regulates OPC maturation and point at these receptors as a possible therapeutic target to enhance remyelination in demyelinating diseases.

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Arellano RO, Sánchez-Gómez MV, Alberdi E, Canedo-Antelo M, Chara JC, Palomino A, Pérez-Samartín A and Matute C. (2016). Axon-to-glia interaction regulates GABAA receptor expression in oligodendrocytes. Mol Pharmacol, 89:63-74.

(T1-32) MECHANISM OF PROSTAGLANDIN E2 AND OPIOID INTERACTION IN THE INSPIRATION-GENERATING PREBÖTZINGER COMPLEX

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ABSTRACT

Prostaglandin E2 (PGE2) is an inflammatory mediator that also depresses vital breathing movements seemingly from the pre-Bötzinger Complex (preBötC), the brainstem central pattern generator. This inhibitory effect on breathing is mediated by Gi/o-coupled EP3 receptors and may lead to serious clinical complications, particularly in neonates, such as apneas, sudden unexpected postnatal collapse or even sudden infant death syndrome. In addition, preBötC inspiratory neurons express μ-opioid receptor that mediates opioid-induced, potentially fatal, respiratory depression. However, the possible interaction between PGE2 and opioids to induce

major respiratory disruption, e.g. during infections and surgery, remains to be elucidated. Therefore, we performed live time-lapse calcium imaging on organotypic brainstem slices of both wild-type mice (WT) and mice lacking the EP3 receptor (Ptger3-/-). PGE2 (10 and 100 nM) or the μ-opioid receptor agonist DAMGO (0.5 and 5 μM), concentration-dependently reduced the Ca2+ signaling frequency in WT. Furthermore, cellular connectivity and synchronicity decreased and the network rewired to gain small-world features (i.e. local clusters connected via a few hubs). In addition, the μ-opioid receptor antagonist naloxone (5 μM) or the inwardly rectifying potassium channel blocker SCH-23390 (15 μM) administration prevented DAMGO's inhibitory effect. Notably, in Ptger3-/-, PGE2 did not affect respiration-related frequency and the reduction in frequency induced by DAMGO (5 μM) was delayed on time, suggesting an interaction. Furthermore, when DAMGO (5 μM) was applied after PGE2 (100 nM) it did not induce any further respiratory depression. Thus, PGE2 and μ-opioids may converge in a common signaling pathway. Indeed, phosphodiesterase 4 blocker rolipram (5 μM) hindered DAMGO and PGE2-induced reduction in Ca2+ signaling frequency, suggesting mutual dependency on the cAMP pathway. This could identify new therapeutic targets for alleviating opioid-induced respiratory depression and improve analgesic treatment regimens.

(T1-33) ENDOCANNABINOID SIGNALLING AT NEURONAL NUCLEI. STUDY OF DIACYLGLYCEROL LIPASE (DAGL) ENZYMATIC ACTIVITY AND 2-ARACHIDONOYLGLICEROL (2-AG) HYDROLYSIS IN NUCLEAR MATRIX

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ABSTRACT

Beyond the canonical location of phosplipase C-β1 (PLCβ1) / Diacylglycerol lipase-α (DAGLα) signalling cascade near the postsynaptic density of excitatory synapses, regulating the synaptic function (1), we have described the presence of PLC /DAGL dependent mechanism for 2-AG production in neuronal nuclei (2). Recently, we have also described in neuronal nuclei the expression of 2-AG metabolizing enzymes, suggesting that their distribution profile in different nuclear compartments, and their different colocalization degrees with DAGL, could condition their role in regulating 2-AG levels at this location (3).
In this context, due to the high expression of phosplipase C-β1 (PLCβ1) and Diacylglycerol lipase-α (DAGLα) at nuclear matrix, the aim of the present work was to evaluate the DAGL activity and the potential enzymatic activities involved in 2-AG degradation at this nuclear compartment.
To this aim, nuclear matrix fractions were isolated from intact nuclei, and 2-AG production was determined by liquid chromatography and mass spectrometry (LC-MS). The study of DAGL activity was performed by using 1-estearoil-2-arachidonoyl-gylcerol (SAG) as exogenous substrate, and the enzyme kinetic parameters (KM and Vmax) were obtained. The role of 2-AG metabolizing enzymes either by hydrolytic pathway, monoacylglycerol lipase (MAGL) and monoacylglycerol lipase containing Alpha/beta-hydrolase domain 12 (ABHD12), or by the oxidative pathway, cyclooxygenase-2 (COX-2), was studied by determining the impact of these enzymes inhibition on 2-AG accumulation. Additionally, serine-hydrolase activity in nuclear matrix was determined by measuring the arachidonic acid (AA) production after including 2-AG and/or 1-AG as exogenous substrates.
Previous to evaluate the enzymatic activity of DAGL, we performed some different experiments in order to set the amount of protein, the incubation times, and the amount of ditiotreitol (DTT) to

be included in the assays. Under our experimental conditions, the inhibition of 2-AG metabolizing enzymes (MAGL, ABHD6, COX-2) did not modify 2-AG accumulation, suggesting that 2-AG accumulation measurement can be used as a good approach for determining DAGL activity kinetic parameters. The 2-AG accumulation curves obtained in the presence of increasing concentrations of SAG were analysed using the classical Michaelis-Menten equation for enzyme kinetics, obtaining the Michaelis-Menten constant (KM) and the maximum reaction rate (Vmax) for DAGL activity (KM=179,8 ± 15,8 μM and Vmax=1,3 ± 0,22 pmol/μg/min). These parameters are consistent with those described for DAGLα isoform. Although 2-AG accumulation did not change by inhibiting serine- hydrolase activity, this could be explained by the low levels of 2-AG produced, not enough to induce that metabolizing enzymatic activity. In fact, when 2-AG or 1-AG were added at high concentrations (10μM) a remarkable serine–hydrolase activity was revealed. Double-immunofluorescence and Western Blot analysis revealed that only ABHD12 hydrolase was expressed at nuclear matrix, suggesting that this enzyme could be responsible of the enzymatic activity observed in this nuclear compartment. Due to the lack of selective inhibitors for ABHD12, we determined the role of this enzyme by measuring the inhibition of serine–hydrolase activity by methyl arachidonyl fluorophosphonate (MAFP) in nuclear matrix fractions. Although MAFP is able to inhibit ABDH12, ABHD6 and FAAH enzyme activities, it inhibited AA accumulation with a potency (IC50 = 1 nM) close to the affinity described for its interaction with ABHD12.

Our results show that 2-AG production at neuronal nuclei occurs mainly at nuclear matrix fraction. Also, at this nuclear compartment the analysis of enzyme kinetics suggests that DAGL activity is mediated by DAGLα isoform, and the main enzyme regulating 2-AG levels appears to be ABDH12 serine hydrolase. Future studies will have to be aimed at understanding the physiological function of 2-AG in the neuronal nucleus.

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(T1-34) CELLULAR MECHANISMS UNDERLYING THE BENEFICIAL EFFECTS OF A LONG-TERM ENVIRONMENTAL ENRICHMENT IN MICE CHRONICALLY EXPOSED TO ALCOHOL DURING ADOLESCENCE

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ABSTRACT

Abusive alcohol consumption during the adolescence, known as binge drinking (BD), has become one of the main problems that concern our society. Studies in the last decade indicate that the endocannabinoid system (ECS) function is affected in ethanol dependence. An enriched

environment (EE) significantly facilitates recovery from brain injury through the interaction with functional changes occurring along brain development. Therefore, EE may have beneficial effects on the alcohol-induced brain damage. In this investigation, the cognitive and neurobehavioral effects of the EE after chronic ethanol intake during adolescence were evaluated, as well as the mechanisms by which EE counteracts the effect on the CB1 receptor-dependent excitatory LTD impairment observed in the adult mouse hippocampus induced by after adolescent BD practice. On the other hand we have studied the mechanisms by which enrichment exerts a beneficial effect in the normal brain.
Male C57BL6 mice were exposed to a 4 day drinking-in-the-dark procedure during adolescence. The animals were given free access to alcohol or water for 2-h sessions during three consecutive days, and a 4-h session on the 4th day. Then the animals during the withdrawal period (15 days) were reared under two different conditions: standard laboratory condition (SC) and EE. Cognitive and neurobehavioral tests were performed in the last 4 days of the withdrawal period (p70-p73). Then, from p74 to p78 mice were sacrificed and electrophysiological techniques were applied.
A significant lower recognition ($p<0.001$***), spatial ($p<0.5$*) and associative ($p<0.001$***) memory was observed in the alcohol treated standard group (EtOH) compared to the untreated shams. Besides, the EtOH showed worse motor coordination skills ($p<0.5$*) and balance ($p<0.5$*) associated with adolescent chronic ethanol consumption. However, enriched EE-OH group showed a significant recovery of the three memories studied ($p<0.001$***), as well as motor coordination ($p<0.01$**) and balance ($p<0.001$***). No significant long-term depressive changes were found between the different groups ($p>0.5$). However, an anxiolytic effect in both EE-OH and EE-H20 enriched groups was observed ($p<0.001$***).
Synaptic stimulation of the medial perforant path (MPP) (10min@10Hz) failed to elicit a CB1-dependent excitatory long term depression (LTD), as previously shown (Peñasco et al., 2015). However, LTD was rescued in EE-OH treated mice that was CB1, mGluR5, mGluR1 and 2-AG dependent, as the antagonists AM251 (4μm), MPEP (10μm), CPCCOet (7μm) and the DAGL-alpha inhibitor THL (10μm) , respectively, abolished the LTD. LTD was unaffected by URB 597 (2 μm), a relatively selective FAAH inhibitor, indicating that anandamide (AEA) was not involved. MPP synaptic stimulation (10min@10Hz) in EE-H20 group triggered a TRPV1-dependent excitatory long term potentiation (LTP), as this LTP was blocked by AMG 9810 (3 μm) and, interestingly, by URB 597. However, the CB1, mGluR5 and mGluR1 antagonists and THL did not affect LTP.
These results suggest that EE may have potential benefits in brain recovery after adolescent binge drinking through cannabinoid-dependent synaptic plasticity mechanisms.

(T1-35) RESTRAINT STRESS IMPAIRS ENDOCANNABINOID-DEPENDENT PLASTICITY AT DENTATE GLUTAMATERGIC SYNAPSES IN YOUNG ADULT MICE

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ABSTRACT

The endocannabinoid (eCB) system plays a central role in the control of stress responses and, reciprocally, the effects of stress on the eCB system are complex, regionally specific, and time-dependent relative to exposure to stress. However, how the restraint stress impacts upon the hippocampal eCB system and the eCB-dependent plasticity at the medial perforant path (MPP) synapses in the hippocampal dentate molecular layer is poorly reported.
A decrease in DAGL-α and PLCβ1was noticed in the hippocampi of acute and chronic restraint stress versus control mice ($p<0.01$, $n=6$). However, the cannabinoid type 1 receptor (CB1R) expression was reduced in acute stress ($p<0.05$, $n=6$), but not in chronic stress mice ($p>0.05$, $n=6$).

In the light microscope, a down regulation of the CB1R was observed in the dentate molecular layer in both groups (acute stress: p<0.001; chronic stress: p<0.01 vs. control). Synaptic stimulation of the MPP (10 min, 10 Hz) triggered a long term depression (LTD) of the excitatory synaptic transmission (p<0.01, n=16) in control mice that was completely abolished by selective CB1 receptor (AM251: p<0.01, n=8.), TRPV1 channel (AMG9810: p<0.01, n=5.) and GABAb receptor (CGP55845: p<0.01 n=7.) antagonists. Furthermore, this novel form of eCB-dependent plasticity required the metabotropic glutamate receptor 5 (mGluR5), L-type Ca2+ channels, and 2-AG synthesis. However, the same MPP stimulation protocol (10 min, 10 Hz)in acute restraint stress triggered a long term potentiation (LTP) which was blocked by administration of both TRPV1 (AMG9810: p<0.001, n=13) and NMDA receptor (D-APV: p<0.001, n=7) antagonists. This LTP also involved L-type Ca2+ channels and anandamide (AEA) synthesis. Finally, chronic restraint stress impaired the LTD observed in control mice (p<0.01, n=10). Increasing endogenous 2-AG and AEA by the MAGL inhibitor (JZL184) and FAAH inhibitor (URB597) respectively, did not recover LTD but elicited a potentiation of the excitatory synaptic transmission (p<0.001, n=7). However, increasing endogenous 2-AG and antagonizing both, CB1 receptors and TRPV1 by simultaneous application of AM251 and AMG9810, recovered the LTD observed in control mice (p>0.05, n=9). Also increasing endogenous AEA and antagonizing only TRPV1 recovered the LTD observed in control mice (p>0.05, n=7).
In summary, chronic and acute stress conditions differentially alter the endocannabinoid-mediated plasticity at glutamatergic synapses in the dentate molecular layer.
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Key Words: endocannabinoid-LTD, perforant path, Ex vivo electrophysiology

(T1-36) EVOLUTION OF BDNF FULL-LENGTH/TRUNCATED RECEPTOR RATIO AND COGNITIVE/GENERAL FUNCTIONING AFTER A FIRST EPISODE OF PSYCHOSIS

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ABSTRACT

Inflammatory and neuroplasticity hypotheses have demonstrated their role in the pathophysiology of schizophrenia. Adequate functioning of both signaling pathways is crucial to avoid cognitive deterioration in such patients. The results about the relationship between peripheral levels of BDNF and cognitive performance are contradictory. In this study, we hypothesized that the expression of different types of BDNF receptors and its evolution during 1 year would influence general and cognitive function in patients with a first episode of psychosis (FEP).
BDNF full length (TrKB-FL) and truncated receptors' peripheral levels were assessed in a sample of 97 FEP patients and 97 matched healthy controls, and the ratio TrKB-FL/TrKB-T (from now on FL/T) was calculated for each one. Cognitive and general functioning was measured at inclusion and 2 years later.
A high baseline ratio FL/T was related to a better cognitive functioning (general, verbal memory, working memory and premorbid IQ) with a significant interaction with educational level, both with baseline and 2 years cognitive performance.
The increase of the FL/T ratio through the 2 years follow-up period positively influenced in general but not in cognitive functioning.
A higher level of functional BDNF receptor versus the truncated one was associated with better cognitive performance of patients both at the beginning of the disease and after 2 years.
The increase in the FL / T ratio meant that patients had better overall long-term functioning.

(T1-37) ENRICHED ENVIRONMENT IMPROVES COGNITIVE AND NEURAL MECHANISMS MEDIATING SYNAPTIC PLASTICITY IN AN EXPERIMENTAL MODEL OF SCHIZOPHRENIA.

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ABSTRACT

Increasing evidences point to prefrontal cortex dysfunction in schizophrenia underlying cognitive disorders. Remarkable efforts are being done to understand and overcome those cognitive impairments. N-methyl D-aspartate receptor (NMDAR) hypofunction hypothesis has been notably successful to explain the pathophysiological findings and symptomatology of schizophrenia. Thereby, NMDAR blockade induced by MK-801 administration in rodents represents a useful tool to identify new therapeutic approaches. In this regard, enriched environment (EE) could play an essential role. Using a multilevel approach of behaviour, electrophysiology and protein analysis, we showed that a short-term exposure to EE in adulthood ameliorated object-place associative memory impairment observed in postnatally MK-801-treated Long Evans rats. Moreover, EE in adult life restored long-term potentiation (LTP) in hippocampal-medial prefrontal pathway abolished by MK-801 treatment, and this was associated to several modifications in the expression of proteins related to glutamatergic neurotransmission in mPFC. Taken together, these findings suggest that EE may be a useful approach to manage enduring perturbations linked to schizophrenia and shed new light on the neurobiological effects of EE to reverse the actions of MK-801.

(T1-39) *ENRICHED ENVIRONMENT IN ADULTHOOD RESCUES BEHAVIORAL IMPAIRMENT AFTER HEAVY ALCOHOL CONSUMPTION DURING ADOLESCENCE*

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ABSTRACT

Alcohol is the most consumed psychoactive substance and is becoming one of the major concerns of our society. The heavy episodic drinking or binge drinking is becoming more and more common in adolescence. Brain maturation does not occur until early adulthood and maturation of brain areas involved in higher functions occur in later adulthood. Thus, neurotoxic drugs could lead to developmental problems in the maturing brain. Specifically, excesive alcohol consumption results in hippocampal, prefrontal cortical and cerebellar damage. Enriched environment (EE) is increasingly gaining relevance as it exerts beneficial changes in the brain. The aim of this study was to measure the long-term effects of alcohol consumption in the brain and to evaluate whether EE would recover alcohol-related brain impairments. For this goal, we treated C57BL6 mice with alcohol during the 4 weeks of adolescence, followed by an abstinence period during the early adulthood; then the mice were exposed to EE. Thereafter, behavioral tests were conducted to measure the effects of alcohol and EE: thigmotaxis for anxiety-like behaviors; novel object recognition test (NORT) for object recognition memory; beam walking balance test (BWBT) and the novel location recognition test (NLRT) for location memory. There was not any significant difference in anxiety-like behavior. The exploration time of mice in EE and standard conditions was similar. However, a significant lower object and spatial recognition memory was observed in the EtOH group, meaning they were not able to discriminate. Similar results were observed in the balance test. Interestingly, the EE-OH group recovered discrimination and balance capacity showing similar values to the control group. Therefore, alcohol impairs recognition and spatial memory as well as balance, but EE is able to recover them.

TRACK 2: Cellular and Molecular Neuroscience / Pathology

ORAL PRESENTATIONS

Moderator: **Joaquín Castilla (CIC bioGUNE, Derio)**

(O2-1) MICRORNA-TRANSCRIPTOME INTERACTION ANALYSIS AND MICRORNA MODULATION IN A MOUSE MODEL OF RETINITIS PIGMENTOSA.

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ABSTRACT

Purpose: To identify differentially expressed MicroRNAs (miRs) that might play important roles in the etiology of retinal degeneration in a genetic mouse model of retinitis pigmentosa (rd10 mice) at initial stages of the disease, and to analyze the therapeutic potential of the modulation of differentially expressed miRs.
Methods: microRNAs-mRNA interaction networks were generated for analysis of biological pathways involved in retinal degeneration. miR modulation was achieved via subretinal injections of AAV containing sequence-specific inhibitors. Contralateral eyes were used as controls, which received the same AAV vector solution expressing scramble miRs sequences. Evaluation of the effect of miR modulation was performed by electroretinography and by histological analysis.
Results: Out of more than 1900 microRNAs analyzed, we selected 19 microRNAs on the basis of: 1) a significant differential expression in rd10 retinas compared to control samples; and 2) an inverse expression relationship with predicted mRNA targets involved in biological pathways relevant to retinal biology and/or degeneration. By inhibiting one microRNA, we were able to achieve a significant preservation of the visual function and the number of photoreceptors.
Conclusions: This study contributes to our understanding of the etiology and progression of retinal degeneration and might help to identify new potential miR-based therapeutic approaches.

(O2-2) CANNABINOID MODULATION OF CORTICAL-NIGRAL TRANSMISSION THROUGH THE MEDIAL PREFRONTAL CIRCUITS OF THE BASAL GANGLIA IS ALTERED IN HEMIPARKINSONIAN RATS

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ABSTRACT

Evidences regarding the potential therapeutic role of cannabinoid compounds in Parkinson's disease (PD) are abundant. As a matter of fact, medial prefrontal (mPF) basal ganglia circuits are extensively regulated by cannabinoids. Moreover, this circuit shows relevance in the appearance of apathy in some PD patients. The aim of this study was to determine the modulation of cortical information transmission through the mPF circuits by the CB1 receptor agonist WIN 55,212-2 in hemiparkinsonian rats. To do so, electrical stimulation of the mPF cortex, and simultaneous single-unit extracellular recordings of substantia nigra pars reticulata (SNr) were carried out, in anaesthetized SHAM and 6-hydroxydopamine (6-OHDA)-lesioned rats.
Both spontaneous and cortically evoked SNr neuron activities were altered after dopaminergic denervation. Neurons from 6-OHDA-lesioned rats had lower firing rates and a more irregular firing pattern, along with modified responses to cortical stimulation. Administration of WIN

55,212-2 (125 µg/Kg, i.v.) in SHAM and 6-OHDA-lesioned rats had no effect on the firing rate, but caused a reduction in its burst activity. Regarding the cortically evoked activity of SNr neurons, WIN 55,212-2 (125 µg/Kg, i.v.) administration totally abolished information transmission through the mPF circuits in SHAM rats. However, in 6-OHDA-lesioned rats, WIN 55,212-2 (125 µg/Kg, i.v.) administration decreased the information transmission through the hyperdirect pathway, but such transmission remained intact in both direct and indirect trans-striatal pathways of the mPF circuits of the basal ganglia.
These results may help to grasp how the cannabinoid system functionality is altered after dopamine denervation, and can contribute to the understanding of what possible role may cannabinoids have as therapeutics in Parkinson's disease.
Supported by Government of the Basque Country IT 747-13 and Spanish Government SAF2016-77758-R(AEI/FEDER,UE). M.A. is supported by MECD fellowship.

(O2-3) E46K ALPHA-SYNUCLEIN PATHOLOGICAL MUTATION CAUSES CELL AUTONOMOUS TOXICITY WITHOUT ALTERING PROTEIN TURNOVER OR AGGREGATION

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ABSTRACT

Alpha-synuclein (aSyn) is the main driver of neurodegenerative diseases known as synucleinopathies, yet the mechanisms underlying this toxicity remain poorly understood. To investigate aSyn toxic mechanisms, we have developed a primary neuronal model in which a longitudinal survival analysis can be performed by following the overexpression of fluorescently tagged wild-type or pathologically mutant aSyn constructs. Most aSyn mutations linked to neurodegenerative disease hindered neuronal survival in this model, of which the E46K mutation proved to be the most toxic. While E46K induced robust PLK2-dependent aSyn phosphorylation at serine 129, inhibiting this phosphorylation did not alleviate aSyn toxicity, strongly suggesting that this pathological hallmark of synucleinopathies is an epiphenomenon. Optical pulse-chase experiments with Dendra2-tagged aSyn versions indicated that the E46K mutation does not alter aSyn protein turnover. Moreover, since the mutation did not promote overt aSyn aggregation, we conclude that E46K toxicity was driven by soluble species. Finally, we developed an assay to assess whether neurons expressing E46K aSyn affect the survival of neighbouring control neurons. Although we identified a minor cell non-autonomous component spatially restricted to proximal neurons, most E46K aSyn toxicity was cell autonomous. Thus, we have been able to recapitulate the toxicity of soluble aSyn species at a stage preceding aggregation, detecting cell non-autonomous toxicity and evaluating how some of the main aSyn hallmarks are related to neuronal survival.

(O2-4) ZINC MEDIATED ACTIVATION OF EGFR PATHWAY PLAYS A ROLE IN THE INDUCTION OF HYPERPROLIFERATION AND REACTIVATION OF NEURAL STEM CELLS AFTER SEIZURES.

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ABSTRACT

Epidermal growth factor receptor (EGFR) is widely expressed by activated neural stem cells and progenitors and its stimulation induces cell proliferation of neurogenic niches. EGFR inhibition impairs astrocytic differentiation as well as the induction of mitosis. Kainic acid (KA) infusion into hippocampus has been used as a model of Mesial Temporal Lobe Epilepsy and it has been recently described as a hyperexcitatory stimulus responsible for hyperproliferation and exhaustion of the hippocampal neurogenic niche. Interestingly, after KA, a strong release of both HB-EGF and Zn+2 occurs activating the EGFR signalization pathway. In the present work, we demonstrate the presence of EGFR receptor in both in-vitro and in vivo in hippocampal neural stem cells and progenitors. EGFR signalization pathway activates during the firsts days of KA infusion. Moreover, Zn+2 infusion recapitulates hiperproliferation and niche astroglial reactivity. EGFR signalization of in vitro cultured hippocampal NSC and progenitors, either in presence of EGF, Zn+2 or HB-EGF plus Zn+2 can be blocked efficiently by the chemotherapeutic Gefitinib. This drug is a reversible inhibitor of the EGFR in clinical phase IV. Moreover, when administered at 10mg/Kg twice a day for 3 days is able to reduce aberrant cell hyperproliferation stimulated by Zn+2 or KA neuronal hyperexcitation and ameliorates glial reactivity, thus protecting from the progressive reduction of neurogenesis increasing the numbers of doublecortin immature neurons.
This work has been financed by “Ramón y Cajal” programs RYC-2013-13450 & RYC-2012-11137 and MINECO SAF2015-70866-R.

(O2-5) MIR-219A-5P-LOADED EXOSOMES INDUCE OPC DIFFERENTIATION AND EAE IMPROVEMENT MORE EFFICIENTLY THAN LIPOSOMES AND NANOPARTICLES.

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ABSTRACT

Multiple sclerosis (MS) is a central nervous system (CNS) disease in which myelin is damaged by an autoimmune attack. Although there are several treatments to modulate the immune system, remyelination promoters are not still available. The use of microRNAs and more concretely hsa-miR-219a-5p, has been proposed as oligodendrocyte precursor cell differentiation mediator and

therefore as a remyelination inductor. Nevertheless, the administration of microRNA to the CNS is a tricky question and delivery systems, such as the use of exosomes, nanoparticles and liposomes are under study.
The goal of this work is to encapsulate miR-219a-5p in several delivery systems such as Nanoparticles (NPs), Liposomes (LPs) and Exosomes (EXs) and to address the ability of these vehicles to promote OPC differentiation in vitro. Then, the most promising delivery system will be selected to address its ability to induce remyelination in an animal model of MS, the experimental autoimmune encephalomyelitis (EAE).
PLGA Nanoparticles and DSPC Liposomes were loaded with miRIDIAN microRNA mmu-miR-219a-5p. Exosomes were obtained from HEK-293T cells infected with pLKO-mmu-miR219a-5p plasmid. All the respective controls were done. Samples were characterized by Nanotrack Particle Analysis and Cryo-TEM. Droplet Digital PCR was used to quantify miR-219a-5p in the exosomes. Up-take and differentiation studies were performed in OPC primary cultures obtained from P2 C57BL/6 mice and analysed by confocal microscopy and qPCR respectively. MOG35-55 induced EAE model was carried out and clinical evolution monitored daily. Treatments were intranasally administered and T-11 Nuclear Magnetic Resonance was performed to characterize the EAE lesions. Animal procedures were approved by the pertinent ethical committee.
Preliminary results showed higher levels of miR-219a-5p and up-take for LPs and NPs in comparison with EXs. However, EXs were able to induce OPC differentiation whereas LPs and NPs did not. Therefore, exosomes were selected for the in vivo study. EXs were intranasally administered to the animal model producing a decrease in the clinical score of mice.
To conclude, miR-219a-5p was able to be encapsulated in LP, NP and EXs. However only EXs were able to induce OPC differentiation in vitro and to decrease EAE score after intranasal administration. All things considered, exosomes have shown to be a proper microRNA delivery system for CNS diseases. These results open a promising and feasible remyelination therapy for MS and other neurodegenerative diseases.
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(O2-6) DOPAMINERGIC DEGENERATION ALTERS NOCICEPTIVE INTEGRATION IN THE LOCUS COERULEUS

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disorder classically defined by motor symptoms. However, the disease is also characterized by non-motor problems such as pain and anxiety, which are often undertreated and limit the quality of life of these patients. Previous studies have demonstrated that PD is associated with dysfunctional neuronal activity of the noradrenergic nucleus, locus coeruleus (LC), which is involved in pain modulation and anxiety. The aim of the study was to examine if dopaminergic degeneration alters neuron activity and TH and pERK1/2

expression in the LC and to evaluate behavioural response to painful stimuli and anxious situations. To do that, we carried out first electrophysiological single-unit extracellular recordings in the LC and later nociceptive and anxious behaviour, in sham and 6-hydroxydopamine (6-OHDA) lesioned rats. In LC neurons, dopaminergic denervation reduced basal spontaneous activity and increased the duration of the suppression period evoked by the application of a noxious stimulus. This latter effect was enlarged with alpha-2-adrenoceptor agonists, which produced a significantly longer inhibition in the lesioned animals. Relative to TH and pERK1/2 expression, no differences were observed between groups. In the behavioural evaluation, parkinsonian rats supported less weight on the paw contralateral to the lesion, although mechanical and thermal sensitivity and anxiety were similar to sham animals. These results confirm that dopaminergic degeneration induces modifications in basal neuron activity and signal transmission of painful stimuli throughout the LC without altering the TH and pERK1/2 expression. However, dopaminergic depletion has minor impact in the nociceptive and anxious behavioural performed tests.
Supported by SAF2016-77758-R (AEI/FEDER, UE) and IT 747-13. EPR has a predoctoral fellowship from the Basque Government.
KEY WORDS: locus coeruleus, 6-OHDA lesion, Parkinson’s disease, pain, anxiety

POSTERS

(T2-01) MICROGLIAL PHAGOCYTOSIS OF APOPTOTIC CELLS IS IMPAIRED BY GENETIC CYSTATIN B DEFICIENCY, A MOUSE MODEL OF PROGRESSIVE MYOCLONUS EPILEPSY (UNVERRICHT-LUNDBORG DISEASE)

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ABSTRACT

Microglial phagocytosis of apoptotic cells is an essential component of the brain regenerative response in neurodegenerative diseases. The molecular crosstalk between apoptosis and phagocytosis ensures the coupling of the two processes, resulting in a rapid removal of the neuronal corpses and preventing the spillover of intracellular contents. In addition, phagocytosis triggers an anti-inflammatory response in microglia that further contributes to maintain the brain parenchyma homeostasis. We have recently described that microglial phagocytosis of apoptotic cells is very efficient in adult neurogenic niches in physiological conditions, as well as during apoptotic challenge induced by excitotoxicity or inflammation. Unexpectedly, phagocytosis is impaired in mouse and human mesial temporal lobe epilepsy (MTLE) due to a complex mechanism that involves reduced process motility, reduced expression of phagocytic receptors, and disrupted apoptotic "find-me" molecule ATP gradients due to the widespread release of ATP during seizures. Here we extend our studies to a genetic model of epilepsy induced by deficiency of cystatin B (CSTB), progressive myoclonus epilepsy. CSTB is an inhibitor of cysteine proteases such as cathepsins B, L, and S, which are lysosomal proteins involved in proteolysis. We first demonstrate that the number of apoptotic cells increases while microglial phagocytosis is impaired as early as P14 in CSTB deficient mice, before seizure onset. To test if this blockage is a cell-autonomous or an envirnonment-driven effect due to the hyperexcitation of the circuitry, we first show that both CSTB and downstream cathepsins are actually expressed by acutely purified microglia from the adult hippocampus. Next, we analyze the effect of reduced microglial CSTB expression in an in vitro model of phagocytosis of apoptotic cells. Our in vivo data show that microglial phagocytosis impairment is restricted to the granullar cell layer and more particularly to the apoptotic cells in close contact with the cFos positive neurons, hinting towards an environment driven phenomenon. All these data suggest that microglial phagocytic impairment is an early feature of hippocampal damage in epilepsy and opens novel therapeutical approaches for epileptic patients based on harnessing microglial phagocytosis.

(T2-02) METABOLIC CROSS-TALK BETWEEN AUTOPHAGY AND PHAGOCYTOSIS IN MICROGLIA DURING ISCHEMIC BRAIN INJURY

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ABSTRACT

Autophagy and phagocytosis are the two major degradation pathways in charge of recognizing, delivering, and digesting intracellular and extracellular cargo, respectively. Although autophagy can be virtually found in all mammalian cells, phagocytosis is mainly executed by immune cells, including microglial cells. Microglia are the resident macrophages of the brain, where they efficiently engulf and digest brain derived cargo such as apoptotic cells by phagocytosis, preventing the spillover of toxic material to the parenchyma and ensuring appropriate brain health and function. Although autophagy in microglia has been scarcely studied, data in peripheral macrophages points to a role for many core autophagy proteins during the phagocytosis of apoptotic cells. Therefore, we hypothesize that autophagy and phagocytosis may be mutually-limiting processes in microglia since they may partially compete for the same cellular resources. This relationship may be relevant during ischemia/stroke, where unpublished data of the laboratory indicate that phagocytosis of apoptotic cells by microglia is deficient. To test our hypothesis, we have used an in vitro oxygen and nutrient deprivation (OND) model in BV2 microglial cell line cultures, primary microglia, and organotypic slices. Our results indicate that OND time-dependently enhances autophagy flux in microglia. Moreover, preliminary data suggests a down-regulation of the microglial phagocytic response after autophagy flux induction. Next, we will evaluate if autophagy induction impairs microglial phagocytosis at the recognition and/or degradation level by the pharmacological and/or genetic inhibition of autophagy. Finally, we will test the efficacy of autophagy inhibitors as a potential strategy to recover the phagocytic activity of microglia during ischemic brain injury.

(T2-03) CRISPR/CAS GENE EDITING FOR IN DUCHENNE MUSCULAR DYSTROPHY CULTURES TO TEST NEW TREATMENTS FOR THE DISEASE.

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ABSTRACT

Background:
Gene editing techniques have the possibility of becoming a permanent cure to many genetic disorders, but the delivery hurdle of these techniques will delay their practical application, particularly in neuromuscular disorders where muscle represents an extremely large and widespread target. However, these techniques have a more immediate application in the generation of better disease models in a field where not many good human myogenic cultures are available.
Methods:
The aim of our studies is to use gene editing to generate a specific DMD deletion in healthy control myoblasts cultures and then correct this deletion also by gene editing, the first objective will provide us with a model to test many possible mutation-specific treatments, such as antisense oligonucleotides, while the second is a proof of concept of a possible therapeutic gene editing option.
Results:
For our first objective, we have designed specific guide RNAs to edit the DMD gen removing exon 52 in order to reproduce a common deletion in Duchenne patients. We have confirmed the efficacy of our designs and selected the best candidates in HEK293 cells. Once selected, the best candidate gRNAs have been transfected in a control myoblast culture to create this new "DMD-like" culture. To complete our second objective, we have designed gRNAs targeting exon 51 and followed the same methods, this time on a DMD culture missing exon 52, to restore dystrophin expression.

Conclusion:
Issues such as safe and efficient delivery to target regions stop gene editing from being an immediate therapeutic option, but, as shown in this poster, gene editing is a useful tool to create better disease models to help in the development of other treatments and could be a feasible option to ex vivo treatment of cultures before autologous transplant.

(T2-04) MYTOBLOTS FOR THE EVALUATION OF NEW TREATMENTS IN NEUROMUSCULAR DISORDERS.

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ABSTRACT

Background
New therapies for neuromuscular disorders often require to be studied in patient's cell cultures. Many potential therapies for Duchenne Muscular Dystrophy (DMD) aim to alter the expression of key proteins, such as dystrophin or utrophin, but, as muscle cell cultures from DMD patients are scarce and do not grow or differentiate well, only a limited number of candidate drugs are tested. Moreover, dystrophin and utrophin quantification by western blotting requires a large number of cultured cells; so fewer compounds are evaluated as thoroughly as it is desirable.
Methods
We have developed a quantitative assessment tool using fewer cells to contribute in the screening of better drug candidates: an “in-cell-western”, also known as cytoblot, is a quantitative immunofluorescence assay performed in microplates that makes possible the quantification of proteins directly in cell culture, combining the specificity of western blotting with the reproducibility and throughput of ELISA.
Results
Our group has recently optimized the assay (‘myoblot’) to quantify several muscle proteins in differentiated myoblast cultures and we are using this method to assess dystrophin restoration treatments, drugs that aim to increase the expression of utrophin and others that alter the differentiation of cultures.
Conclusion
We expect that the use of myoblots will accelerate the development and validation of new therapies.

(T2-05) FUNCTIONAL, METABOLIC, AND MORPHOLOGICAL CHANGES IN MICROGLIAL MITOCHONDRIA AFTER PHAGOCYTOSIS

Mar Márquez Ropero, Victor Sánchez Zafra, Irune Díaz-Aparicio, y Amanda Sierra Saavedra

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ABSTRACT

Functional, metabolic, and morphological changes in microglial mitochondria after phagocytosis
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Phagocytosis of cellular debris is an essential process for tissue homeostasis because it prevents the spillover of cellular contents and actively suppresses the initiation of the inflammatory response. In the brain, phagocytosis is performed by microglia, the resident immune cells. In macrophages, phagocytosis leads to a metabolic rewiring which supports long-term changes in

their function in a process known as trained immunity. With the aim to test if microglia undergo a similar metabolic switch through phagocytosis we performed a gene array comparing naïve and phagocytic primary microglia. Data obtained clearly pointed towards to an upregulation of the glycolytic pathway genes and a downregulation in those related to the oxidative phosphorylation pathway. To test whether these changes in gene expression altered microglial metabolism, we used a microglial cell line (BV2). We first analyzed the mRNA expression for key enzymes of both glycolysis and oxidative phoshorylation by RTqPCR in BV2 cells and found that several genes implied in mitochondrial pathway were downregulated but there were no evidences of upregulation in any of the analyzed genes for the glycolytic pathway. Afterwards, we directly assessed the metabolic behavior of microglia using a Seahorse extracellular flux analyzer. We found that as a consequence of phagocytic process, microglia suffered an evident impairment in both glycolysis and oxidative phosphorylation. To further confirm the alteration of mitochondrial pathways we performed a more exhaustive analysis of mitochondrial morphology and dynamics transfecting BV2 cells with GFP-mito7 plasmid comparing naïve and phagocytic cells. The results obtained from these experiments will help us to understand the mechanisms through which mitochondria regulate phagocytosis efficiency, which is essential in the correct elimination of cellular corpses both in neurodegenerative diseases and in healthy conditions.

(T2-06) UBIQUITINATION OF RNGO/DDI1 AND SEVERAL PROTEASOMAL SUBUNITS BY UBE3A, THE E3 LIGASE INVOLVED IN ANGELMAN SYNDROME

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ABSTRACT

Angelman syndrome is a complex neurodevelopmental disorder caused by the loss of maternal expression of a single gene, UBE3A. The E3 ligase coded by this gene is known to build up K48-linked ubiquitin chains, a modification historically considered to target substrates for degradation by the proteasome. However, a change in protein abundance is not proof that a candidate UBE3A substrate is indeed ubiquitinated by UBE3A.We have here used an unbiased ubiquitin proteomics approach, the bioUb strategy, to identify 79 proteins that appear more ubiquitinated in the Drosophila photoreceptor cells when Ube3a is overexpressed. We found a significantly high number of those proteins to be proteasomal subunits or proteasome-interacting proteins, suggesting a wide proteasomal perturbation in the brain of Angelman patients. We focused on validating the ubiquitination by Ube3a of Rngo, a proteasomal component conserved from yeast (Ddi1) to humans (DDI1 and DDI2), but yet scarcely characterized. Ube3a-mediated Rngo ubiquitination in fly neurons was confirmed by immunoblotting. Using human neuroblastoma SH-SY5Y cells in culture, we also observed that human DDI1 is ubiquitinated by UBE3A, without being targeted for degradation. The novel observation that DDI1 is expressed in the developing mice brain, with a significant peak at E16.5, strongly suggests that DDI1 has biological functions not yet described that could be of relevance for Angelman syndrome clinical research.

(T2-07) THE EFFECT OF BUSPIRONE ON NEURONAL ACTIVITY AND AMINO ACID RELEASE FROM SUBSTANTIA NIGRA PARS RETICULATA OF A RAT MODEL OF PARKINSON´S DISEASE

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ABSTRACT

The pathophysiology of Parkinson's disease (PD) and L-DOPA-induced dyskinesia (LID) is associated with dysfunctional neuronal activity and changes in the oscillatory activity and synchronization of the basal ganglia (BG) nuclei and motor cortex circuits. Serotonin-based therapies have shown promising results in the treatment of PD and LID. Interestingly, buspirone, a partial agonist of 5-HT1A receptors, has demonstrated antidyskinetic properties in preclinical and clinical trials but the mechanisms involved in this therapeutic effect are not fully understood. The aim of the present study was to investigate the antidyskinetic effect of buspirone on the main BG output nuclei; the substantia nigra pars reticulata (SNr). To this aim, in vivo microdialysis and single-unit extracellular recordings were carried under urethane anaesthesia in control, 6-hydroxydopamine (6-OHDA) hemilesioned or dyskinetic rats. Glutamate and GABA release in SNr was measured, and neuronal activity parameters, oscillatory activity and synchronization between the SNr and the motor cortex were analysed. In microdialysis experiments, local administration of buspirone (50-500 nM) in SNr increased glutamate and GABA levels in control rats but not in 6-OHDA lesioned and dyskinetic animals. In electrophysiological experiments, local administration of buspirone (0.25-2 nmol) in SNr, but not systemic administration (0.6125-5 mg/kg), inhibited neuron activity in all groups of animals. Furthermore, low-frequency oscillatory activity and synchronization between SNr and motor cortex was unaltered after drug application although it is increased in 6-OHDA hemilesioned and in dyskinetic rats. The present findings help shed light on the mechanism of action of buspirone in the treatment of dyskinesia.
Supported by SAF2016-77758-R (AEI/FEDER, UE) and IT747-13. SV has a predoctoral fellowship from UPV/EHU.

(T2-08) INTERACTIONS BETWEEN TRPV1 CHANNELS AND CANNABINOID CB1 RECEPTORS IN MOUSE DENTATE GYRUS AND AFTER KAINATE-INDUCED EPILEPTIC SEIZURES

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ABSTRACT

The control of glutamate release by cannabinoid type 1 (CB1) receptors localized at excitatory synapses plays a protective role in the modulation of neuronal circuits affected by epileptic seizures. The transient potential vallinoid receptor type 1 (TRPV1) also regulates excitatory and inhibitory synaptic transmission and plasticity, and the up-regulation of this channel after epileptic seizures has been reported to contribute to the altered epileptic brain activity. As TRPV1 and CB1 receptors localize to postsynaptic and presynaptic loci, respectively, of excitatory and inhibitory synapses in the dentate molecular layer, it is plausible to hypothesize that both receptors get reciprocally involved in pathological states, such epilepsy. Here we investigate the

effect of the TRPV1 absence on the expression and function of CB1 receptors in the mouse dentate molecular layer, under normal conditions and in a model of medial temporal lobe epilepsy (MTLE).

In normal conditions, the expression of MAGL-α, DAGL-α, FAAH and CB1 receptor is increased in whole hippocampi of TRPV1-KO versus WT mice. Subcellularly, CB1 receptor immunopositive excitatory terminals increased significantly throughout the dentate molecular layer. Furthermore, 37.25 ± 3.28% of the excitatory synaptic terminals in WT and 47.59 ± 4.1% in TRPV1-KO were CB1 receptor immunopositive in the inner 1/3 of the dentate molecular layer. In the outer 2/3 targeted by the perforant path synapses, 28 ± 1.33 % of the excitatory synapses in WT and 31 ± 1.44% in TRPV1-KO were CB1 receptor immunolabeled. Functionally, the CB1 receptor-dependent inhibition of the excitatory perforant path synaptic transmission was significantly enhanced in TRPV1-KO versus WT.

Kainic acid-induced status epilepticus in mice was followed during 4 hours after kainic acid application. The behavioral score showed that seizures were milder in TRPV1-KO than in WT. Furthermore, dispersion of granule cells accompanied by a decrease in CB1 receptor immunoreactivity was observed two weeks after the kainic acid administration in both WT and TRPV1-KO.
Current experiments are digging into the CB1 receptor expression in cell populations of the TRPV1-KO mouse model of MTLE.

(T2-09) N-TERMINAL ACETYLATION STABILIZES ALPHA-SYNUCLEIN PROTEIN

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ABSTRACT
Increased alpha-synuclein (aSyn) protein levels causes neurodegenerative diseases like Parkinson´s disease or dementia with Lewy Bodies, grouped as synucleinopathies. Still, the mechanisms by which altered aSyn protein levels causes neuronal death are unknown. In vivo, all aSyn molecules are cotranslationally modified by an acetyl group attached to the alpha-amino group of the N-terminal amino acid by the enzyme NatB. 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 in neurons has not been addressed. Recently, our laboratory has developed a neuronal model based on automated microscopy that enables longitudinal tracking of individual primary living neurons expressing aSyn and survival analysis. Using Cox proportional regression models the specific contribution of different factors (e.g. mutations and protein expression levels) can be quantified and dissected. To address the role of aSyn N-terminal acetylation in neurons we generated three N-terminal aSyn mutants; D2A and D2P that alter and block aSyn N-terminal acetylation respectively, and D2E, a conservative mutant. The toxicity of these mutants was evaluated by longitudinal survival analysis. Mutant D2P exhibited lower protein levels when expressed in primary cortical neurons and consequently it decreased the risk of neuronal death compared to normal aSyn forms. Using an optical pulse-chase methodology that allows measuring protein half-life in situ in primary neurons we found that D2P mutant decreased aSyn stability compared to wild-type aSyn. Our results strongly suggest that N-terminal acetylation stabilizes aSyn protein modulating its toxicity. We are currently evaluating N-terminal acetylation-dependent molecular mechanisms that lead to aSyn destabilization.

(T2-10) A FUNCTIONAL DICHOTOMOUS INPUT ORGANIZATION IN THE EXTERNAL GLOBUS PALLIDUS

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ABSTRACT

The basal-ganglia (BG) circuits form a complex loop with the cortex and the thalamus that is involved in action selection and movement control. In the last two decades, the role played by the external globus pallidus (GP) in these subcortical circuits has changed drastically. Conventional ideas view the GP as a simple relay nucleus composed of only one population of cells part of the indirect pathway but this vision has been challenged by our recent findings describing two types of GP neurons. Indeed, the majorities of GP cells (≈75%) are called prototypic neurons and represent classic GP neurons because they always send axons to the subthalamic nucleus, whereas arkypallidal neurons formed a novel subpopulation (≈25%) of neurons that only send their axons to the striatum. One classic assumption in BG models is that all GP neurons receive inhibitory GABAergic inputs from striatopallidal neurons and excitatory glutamatergic inputs from subthalamic neurons. However, it is currently not known whether this classic scheme of synaptic connectivity applies to both populations of GP cells. To dissect the functional inputs organization of prototypic and arkypallidal neurons we carried out in vivo extracellular recordings and juxtacellular labeling coupled to selective optogenetic inputs manipulation of striatopallidal and subthalamic pathways using transgenic mice. Our results highlight a functional division of inputs organization between prototypic and arkypallidal neurons which support the view that arkypallidal neurons may not belong to the traditional indirect pathway but represent instead a novel inputs node to access classic BG circuits through non-conventional inputs.

(T2-11) BENEFICIAL EFFECTS OF POLYUNSATURATED FATTY ACIDS ADMINISTRATION IN A PARTIAL LESION MODEL OF PARKINSON'S DISEASE

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ABSTRACT

Parkinson's disease (PD) is characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta and the subsequent striatal dopamine deficiency that lead to the classical motor symptomatology. There is substantial evidence supporting that nutraceuticals such as, polyunsaturated fatty acids (PUFAs), could constitute novel promising neuroprotective compounds for PD treatment acting on mitochondrial dysfunction, oxidative stress, excitotoxicity and neuroinflammation. In the present study, we assessed the neuroprotective effect of docosahexaenoic acid (DHA) and in its hydroxyl-derivative, hydroxydocosahexaenoic acid, (OHDHA), in 6-hydroxidopamine (6-OHDA) partially lesioned rats by using behavioural and immunohistochemical tests.

The animals were daily treated with DHA (50 mg/kg), OHDHA (50 mg/kg), vehicle or saline, by intragastric administration, 1 month before and 4 months after the striatal lesion with 6-OHDA. OHDHA-treated rats exhibited the best behavioural improvement in the amphetamine-induce rotational test. In line with this result, these animals showed the highest TH-immunopositive striatal fibers. In addition, the OHDHA treatment decreased the percentage of activated microglia cells (Iba-1+ cells) in the striatum with similar values to that observed in the contralateral side, suggesting a modulatory role on the neuroinflammatory component of the disease.
Taking all the above mentioned results, it may be concluded that nutraceutical compounds, particularly OHDHA, may contribute to a reduction on the progression of PD.
Acknowledgments: This project was partially supported by the Spanish Ministry of Economy and Competitiveness (RTC-2015-3542-1), the Basque Government (Consolidated Groups, IT 747-13 and IT 907-16). The authors also wish to thank the intellectual and technical assistance from the ICTS NANBIOSIS, more specifically by the Drug Formulation Unit (U10) of the CIBER-BBN at the UPV/EHU. S. Hernando thanks the Basque Government for the fellowship grant.

(T2-12) SYSTEMATIC REVIEWS AND META-ANALYSIS IN ANIMAL MODELS FOR TRANSLATIONAL RESEARCH IN PSYCHIATRIC PATHOLOGIES

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ABSTRACT

The aim of this work is to assess the clinical efficacy of anti-inflammatories in animal models of depression as an approach to translational research and their possible application as therapeutic interventions in depression. We will conduct a systematic review and meta-analysis of all available evidence to evaluate the hypothesis that suggests a crucial role for inflammation in the development of depression as a consequence of chronic exposure to social stressors. Depression is one of the most common diseases in the world, being the fourth leading cause of disability worldwide. Current therapeutic approaches achieve at most a 30-35% remission of depressive symptoms in the adult population. As a result, the probability of recurrence of depression after 10 years is about 67%, and about 85% after 15 years. Systematic reviews of the effectiveness of anti-inflammatory interventions in animal models of depression will serve to strength translational research and provide relevant information on methodological issues related to systematic measures of intervention in animal models of disease. In summary, the aim of this study is to evaluate by means of systematic reviews and meta-analyses the efficacy of anti-inflamatory drugs in animal models of depression. We would like to highlight that if feasible, this study will be followed by others to try to gain a clear picture of the translationality of animal models of affective diseases to humans.

(T2-13) DETECTION OF AMYLOID FIBRILS USING PLASMONIC CHIRALITY

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ABSTRACT

The detection of amyloid fibrils at low concentration is pivotal in the early diagnosis of several neurodegenerative disorders and development of therapeutic strategies. Herein, we report a novel methodology for the specific identification of amyloid fibrils using chiroptical effects in plasmonic nanoparticles. The formation of amyloid fibrils based on α-synuclein was probed using gold nanorods, which showed no apparent interaction with monomeric proteins but effective adsorption onto fibril structures via noncovalent interactions. The amyloid structure drives a helical nanorod arrangement, resulting in intense optical activity at the surface plasmon resonance wavelengths. This sensing technique was successfully applied to brain homogenates of patients affected by Parkinson's disease, wherein disease-related fibrils were detected through chiral signals from gold nanorods in the visible and near IR, whereas healthy brain samples did not exhibit any meaningful optical activity. The technique was extended to the specific detection of infectious recombinant prions, confirming the wide potential of the technique. The intense chiral response driven by strong dipolar coupling in helical gold nanorod arrangements allowed us to detect amyloid fibrils down to nanomolar concentrations.

(T2-14) MICROGLIA REDUCES EXTRACELLULAR AMYLOID AND REDUCES SYNAPTIC DYSFUNCTION INDUCED BY B-AMYLOID PEPTIDE IN ALZHEIMER´S DISEASE

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder and the most common cause of progressive cognitive decline in the aged population. Accumulation of β-amyloid (Aβ) peptide and the synaptic dysfunction are the main hallmarks of AD neuropathology. Some studies have reported that neurons cultured in presence of oligomeric Aβ have selective alterations in synaptic compartments compared to control neurons. Loss of synapses, as seen by loss of synaptophysin immunoreactive pre-synaptic terminals, occurs early in AD and is considered the best pathological correlate of cognitive decline.

Microglia are innate immune cells of the brain that mediate responses to pathogens and injury. Some discoveries pointing to the key role of microglia on synapses opens new research routes in neurodegeration research. A recent publication shows that microglia mediates early synapse loss in AD models. In contrast, activation of microglia by immunotherapy or the proinflammatory cytokine macrophage colony stimulating factor (MCSF) results in a more efficient Aβ degradation. Thus, the possible dual role of microglia in AD is becoming more and more relevant and further study is needed.

To study the role of microglia in the control of synapse number we performed immunofluorescence, western blot techniques to measure the levels of pre- and post-synaptic markers in neurons cultured alone or together with microglia in the presence or absence of Aβ oligomers. We performed immunoprecipitation for Aβ detection in the culture media.

We observed a significant reduction of synaptic marker synaptophysin labelling in primary neuron cultures in presence of Aβ compared with controls and this synaptic loss was partially reverted when neurons were co-cultured with microglia. Moreover, we found that microglia reduced extracellular amyloid in microglia-neuron co-cultures in presence of extracellular Aβ oligomers. Additionally, microglial activation by MCSF further reduces extracellular amyloid load. Overall, these results showed the Aβ capacity of damaging synapses in neuronal primary culture. Microglia is able to reduce extracellular Aβ and also reduces synaptic damage in vitro. These

results strongly suggest that Aβ oligomers are deleterious to synaptic function either by interfering directly or indirectly with neurons, and microglial activity and its modulation could constitute an important therapeutic target for preventing the synapse loss.
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(T2-15) CONTRIBUTION OF NEURON AND GLIAL CELLS TO ABETA-INDUCED COMPLEMENT ACTIVATION IN ALZHEIMER´S DISEASE

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ABSTRACT

Synaptic dysfunction is increasingly viewed as a key hallmark of Alzheimer's disease (AD) at early stages, given than it is the best pathological correlate with cognitive decline. But the cellular mechanism by which β-amyloid peptide (Aβ) may affect synapses remains unclear. Recent discoveries point towards the crucial role of glial cells via complement cascade on synapse loss in AD models. However, the involvement of neurons and glial cells in the activation of the complement cascade in the presence of Aβ needs further clarification.
To study the contribution of neuron, microglia and astrocytes on the activation of complement cascade in AD, we performed immunofluorescence techniques to measure the levels of complement proteins (C1q and C3) in neurons, microglia and astrocytes cultures alone or co-cultures neuron-microglia and neuron-astrocytes in the presence or absence of Aβ oligomers. Preliminary results show that C1q is mainly expressed in microglia compared with neurons and astrocytes and that C3 is expressed in all three cell types. Also, Aβ treatment increases C1q and C3 expression in microglia and astrocytes respectively. Moreover, the presence of microglia in neuron-microglia cocultures alters the expression of complement components C1q and C3 in neurons, in that Aβ peptide increases the expression of both C1q and C3 in neurons in the coculture.
Overall, these results indicate that both neurons and glial cells express proteins of the complement and that Aβ promotes the activation of the complement cascade with the contribution of both neurons and glia. Further clarification of the contribution of each cell type in the Aβ induced complement cascade activation is necessary for understanding the role of this pathway in the synapse pathology in AD.
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(T2-16) *SOLUBLE OLIGOMERIC AMYLOID-BREGULATES NMDA RECEPTOR ACTIVITY AND PROMOTES DENDRITIC AND SPINE COMPLEXITY THROUGH A MECHANISM INVOLVING INTEGRIN B1 AND PKC ACTIVATION*

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ABSTRACT

Accumulation of soluble oligomeric forms of amyloid-β (Aβ) in the brain is a relevant early event in Alzheimer's disease (AD) etiopathogenesis. Here, we have investigated the molecular mechanisms of early synaptic changes induced by subtoxic levels of Aβ oligomers in neurons. Using a combination of pharmacological, immunochemical and calcium imaging approaches in primary neuron cultures, we found that Aβ oligomers differentially increased NR2B-containing NMDA receptors (NMDAR), postsynaptic density protein 95 (PSD95) in neuronal surface as well as in synaptosome fractions. This resulted in enhanced synaptic NMDAR-dependent calcium influx by mechanisms involving NR2B subunit phosphorylation by activation of integrin β1 and PKC signalling. Next, we examined synaptic changes in mouse hippocampal neurons following short exposure to Aβ by high resolution imaging of dendritic arborization and spines in a long-term ex vivo model from organotypic cultures of mouse hippocampus. An algorithm-based analysis revealed an Aβ-induced increase in total dendritic spine density, specifically in the number of stubby spines, which was reverted with β1 integrin and PKC inhibitors. Additionally, analysis of dendritic complexity based on a 3D reconstruction of the whole neuron morphology indicated an increase in the apical, but not in the basal, dendrite length and branching in CA1 neurons of Aβ-treated organotypic hippocampal slices. In conclusion, these results revealed that short exposure to Aβ oligomers can alter NMDAR distribution and function in neurons via integrin β1 and PKC activities, and promote an increase in spine density and dendritic complexity of hippocampal neurons. The relevance of these synaptic changes to AD initiation warrants further investigation.
Supported by Mineco, CIBERNED, Ikerbasque, UPV/EHU and Basque Government

(T2-17) *TRX80 INDUCES INTRACELLULAR BETA AMYLOID METABOLIZATION THROUGH AUTOPHAGY-LYSOSOMAL PATHWAY REGULATION.*

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ABSTRACT

Aggregation and accumulation of amyloid-beta (Aβ) is believed to be of great importance in the pathogenesis of Alzheimer disease (AD). In this study, we investigated the effect of Trx80 in in vivo and in vitro models of Aβ pathology. We developed transgenic models of Drosophila Melanogaster that overexpresses human Trx80, human Aβ42 or both; exclusively in the central nervous system. We found that Trx80 prevents Aβ42 accumulation in the brain and rescues the reduction in lifespan and locomotor impairment seen in expressing flies. We showed that Trx80 induces autophagolysosome formation and reverse the inhibition of Atg4B-Atg8a/b pathway

caused by Aβ42. These effects were confirmed in human neuroblastoma cells with an effect of Trx80 on reducing Aβ42 levels and activating the autophagic machinery.

(T2-18) DEFECTIVE FUNCTION OF AMYOTROPHIC LATERAL SCLEROSIS-LINKED GENES IMPACTS AUTOPHAGY AND MYOGENIC CAPACITY OF MYOBLASTS

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder characterized by death of motor neurons and muscle atrophy. For year, the muscle wasting of ALS patients has been assumed to stem exclusively from MN denervation, but recent lines of evidence suggesting an impaired regenerative capacity of muscle in ALS have challenged this view. We have collected consistent data from the repression of TDP-43, SOD1 and FUS in human myoblasts to support that genes linked to the inherited forms of ALS affect the myogenic capacity. Because autophagy plays an essential role in many aspects of cell physiology and participates in the pathogenic mechanisms of ALS, we have studied whether altered autophagy may mediate these myogenic defects. Lentiviral-induced silencing of TDP-43, FUS or SOD1 in the human myoblast line 8220 caused different changes of autophagic markers, suggesting that each of the silenced genes impact autophagy flux in different ways. While knockdown of TDP-43 impaired autophagolysosome degradation and led to increased autophagic biogenesis, knockdown of FUS halted the biogenesis of autophagy. However, both of them resulted in reduced cellular recycling, which was supported by the abnormal accumulation of autophagic vesicles (as indicated by electron microscopy) and aggregation of p62. In contrast, knockdown of SOD1 caused a dramatic upregulation of autophagic flux, although incapable to clear p62 aggregates. Treatment of control myoblasts with chloroquine, an inhibitor of autophagosome-lysosome fusion, induced similar defects on myogenesis than gene silencing. Collectively, this data supports autophagy as an important intrinsic factor for maintaining the regenerative capacity of muscle stem cells, and perturbations on this pathway may underlie myogenic defects associated to genetic causes of ALS.

(T2-19) OPTIMIZATION OF NON-INVASIVE STIMULATION PROCEDURES FOR THE RECOVERY IN SPINAL CORD INJURED PATIENTS

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ABSTRACT

Introduction: Spinal Cord Injury (SCI) produces the disruption of the top-down neural pathways, causing a permanent loss of motor and sensory function caudal to the level of injury. It has been demonstrated that magnetic stimulation at spinal cord level can induce recovery in SCI patients. Aims: This study analyses current stimulating strategies using Transcranial Magnetic Stimulation (TMS) coils for the stimulation of the spinal cord. The aim is to study the electromagnetic field distribution generated with the TMS coil using 3D computational models of the spinal cord to understand the main neural structures that are involved when the stimulation is done. In that

way, and optimization of selectivity/precision and stimulation depth with the objective of maximizing neural activation and synaptic plasticity can be done.
Methods: Different numerical methods together with realistic human body models are used for the analysis of the electromagnetic field distribution along the spinal cord. Different parameters such as, intensity, frequency, coil type or location have been studied. This methodology can be applied for macroscopic simulation (tissue level) and also for microscopic level, where different analytical models of single fibers and neurons are used to model the neural mechanisms.
Results: The obtained results show the effect of the intensity, frequency, coil type and location on the field distribution at the spinal cord level using different high-resolution anatomical models. Different parameters should be used depending on the target tissue and depth desired for each application.
Conclusions: The potential of using computational simulation platforms with very realistic human models has been tested to optimize and improve current stimulation strategies using TMS for SCI patients.

(T2-20) ROLE OF IRF5-P2X4R AXIS IN THE PATHOGENESIS OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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ABSTRACT

Microglia are the resident macrophages of the central nervous system (CNS), survey the brain microenvironment for signals of injury or infection, and are essential for the initiation and resolution of pathogen- or tissue damage–induced inflammation. Understanding the mechanism of microglia-specific responses during pathology is hence vital to promote regenerative responses. We have previously observed that P2X4 receptor modulates microglia activation and that the potentiation of P2X4R signaling in microglia ameliorated experimental autoimmune encephalomyelitis (EAE) symptoms, an animal model of multiple sclerosis. Microglia conversion into the P2X4R+ reactive state is driven by interferon regulatory factor 5 (IRF5), which is involved in innate immune activation. Indeed, we observed that microglia treated with lipopolysaccharide showed an increase in IRF5 nuclear translocation. In the EAE model, we showed that IRF5 was upregulated at the peak and recovery phase and that its expression matched P2X4R expression and correlates to the neurological score. In order to check its role in EAE pathogenesis we immunized with MOG IRF5-/- mice. Compared to wild type mice, IRF5-/- mice showed a significant delay in the appearance of neurological symptoms but, on the contrary, an absence of improvement in the recovery phase. We did not detect any significant alteration in the number of infiltrating T cells and B cells in spinal cord lesions or parenchyma, nor in the CD4 T cell response. However, IRF5-/- mice had an increase in myelin damage, in axonal damage and in the number of Iba1+ positive cells in the lesions. Moreover, confocal imaging analysis revealed that there was higher accumulation of myelin debris in parenchyma and inside microglia/macrophages. Further experiments were needed to determine whether this increase represents increase phagocytosis or inefficient degradation of myelin. Importantly, our results pinpoint a microglial pathway involved in EAE recovery and remyelination and suggest that potentiating this axis could have therapeutic potential to stop MS progression.

(T2-21) GENES RELATED TO MENDELIAN FORMS OF ALS ARE INVOLVED IN MYOGENESIS: DEEP IMPLICATIONS OVER MUSCLE ATROPHY IN AMYOTROPHIC LATERAL SCLEROSIS

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder characterized by the death of motor neurons (MN) and muscle atrophy. Classically, muscle atrophy has been assumed to arise from MN denervation, but recent research has proposed that this process may be muscle-autonomous. To address the hypothesis that alterations of adult muscle regeneration may be primary events in ALS pathophysiology we have silenced genes related to familial inheritance of disease, such as TDP-43, SOD1, FUS, in myoblasts and studied whether the myogenic program is affected. The results show that these genes play a crucial role in the differentiation process of myoblasts to mature myotubes, which is particularly evident in the case of SOD1 knockdown. We infer that, at least in some forms of ALS, myogenic defects may contribute to the general a progressive wasting of muscles, and raise the question of whether they are early events that occur prior to MN degeneration.

(T2-22) GLYCOLYSIS IMPAIRMENT IS A CONSISTENT METABOLIC FEATURE IN CELLULAR MODELS OF ALS

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ABSTRACT

There is a growing body of evidence supporting that amyotrophic lateral sclerosis (ALS), a neurodegenerative disease that preferentially affects motor neurons, presents with systemic metabolic disturbances. The facts that insulin resistance and type I diabetes are risk factors of ALS, and that fast glycolytic fibres are the first affected, devise a range of evidences to postulate that altered energy production from glucose may be an early pathogenic event in ALS. To verify this hypothesis we have silenced three genes involved in the Mendelian forms of ALS (TDP-43, SOD1 and FUS) and have performed a comprehensive study of the glycolytic pathway, in human muscle cells. The results indicate that the loss of function of each of the three genes produce a consistent decrease in the glycolytic capacity through different ways. These findings suggest that peripheral glycolysis may have fundamental implications in the etiopathogenesis of ALS.

(T2-23) *SILENCING ALS-RELATED GENES IN MUSCLE INDUCES A DECREASE IN MITOCHONDRIAL ATP PRODUCTION*

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a degenerative disease of motoneurons characterized by a progressive weakness of voluntary muscle until complete paralysis and patient death due to respiratory failure. A bulk of clinical and experimental evidences imply the existence of a basal and systemic hypercatabolic state in ALS patients and animal models, and hence has allowed to recognize metabolic alterations in non-neuronal tissues as primary pathogenic events. Skeletal muscle atrophy of ALS is assumed to stem from motoneuron denervation, but others and we have begun to postulate that a disease-specific imbalance of energy metabolism in muscle fibres might be the cause of the neurodegenerative process. In this work we have used an in vitro muscle cell model in which various genes involved in the Mendelian form of ALS (TDP-43, SOD1 and FUS) have been silenced. The data show that the silencing of each of the three genes induces a significant and consistent decrease in the production of mitochondrial ATP, which is more pronounced in TDP-43 knockdown cells. Paradoxically, TDP-43 deficiency produces a profound increase of the maximal respiratory capacity, which may provide a compelling explanation for the high mitochondrial membrane potential and superoxide levels found in these cells. These results provide further evidence to support that metabolic events in muscle are primary to disease and offer new insights on the pathophysiology of ALS.

(T2-24) *INVOLVEMENT OF THE MURF-ATROGIN AXIS IN THE MUSCLE ATROPHY OF ALS*

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ABSTRACT

Muscular atrophy is the loss of muscle fibre size produced by the imbalance between protein degradation and synthesis. Muscular atrophy can be a consequence of multiple physiological and pathological conditions, such as aging (sarcopenia), muscle disuse, infections, drug toxicity or primary muscle diseases of autoimmune or genetic origin as dystrophies. Perturbations of cellular energy metabolism are among the key factors that account for the shift towards increased protein degradation leading to muscle wasting. There is a relationship between energy metabolism and the signalling pathway that activates the muscle atrophy pathway Atrogin-1 /

Foxo3 / MuRF-1 through protein degradation. To analyse if this pathway is involved in the muscle atrophy that is specific of ALS, we have silenced three genes involved in the Mendelian forms of ALS (TDP-43, FUS and SOD1) in a human muscle cell model. The results indicate that reductions in the production of ATP induced by each of the three silenced genes lead to increased protein catabolism through the activation of Atrogin-1 / FOXO3 / MuRF-1 pathway. This finding suggests that muscular atrophy of ALS could stem from the activation of specific a pro-atrophic pathway, which in turn is secondary to alterations of energy metabolism in muscle.

(T2-25) GENE SILENCING OF TDP43, FUS AND SOD1 REVEALS CHANGES IN CELL PATHWAYS AND PROCESSES LINKED TO ALS

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ABSTRACT

A major challenge to understand motor neuron degeneration manifesting as amyotrophic lateral sclerosis (ALS) is to link biologically the many different genetic causes of the disease. ALS has been associated with alterations of the endoplasmic reticulum (ER) and the mitochondria, together with stress granules and protein aggregates that often contain TDP43. Here we show that gene silencing of 3 ALS-linked genes TDP43, FUS or SOD1 disturbs pathways and processes that are altered in ALS, including the expression of a suite of factors related to the ER, mitochondrial energy production and zinc homeostasis, the last of which is critical for stress granule formation. The findings suggest that several ALS associated pathways and processes are interdependent. Hence, this can explain why mutations in many different genes can cause ALS; with the corollary that interventions that target any of these interdependent processes could mitigate the underlying defects and help to slow or halt the progression of the disease.

(T2-26) COMBINING ELECTROPHYSIOLOGICAL RECORDING AND MICRODIALYSIS TO STUDY NEUROPHYSIOLOGICAL DEFICITS IN A RAT MODEL OF SCHIZOPHRENIA

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ABSTRACT

The brain's ability to integrate different behavioral and cognitive processes relies on its capability to generate neural oscillations in a cooperative and coordinated manner. Drugs and diseases alter this "oscillatory homeostasis" that results in abnormal behavior and/or cognitive deficit. By using the ketamine model of schizophrenia our lab has recently shown that subanesthetic doses of ketamine alters the spatio-temporal patterns of the oscillatory activity in the cortico-basal ganglia network of healthy, freely moving rats. Subanesthetic doses of ketamine induce aberrant hyper-synchronization of the whole cortico-basal circuit where the tandem theta-HFO seems to act as the main actor in the hyperlocomotion shown by the animals. Although ketamine is considered an uncompetitive antagonist (channel pore blocker) of the NMDA receptor, it has effects in the activation of AMPA, μ-, κ-, and δ-opioid receptors, acts as sigma σ1 and σ2 receptor agonist, partial agonist of the dopamine D2 receptor, ligand of the serotonin 5-HT2A receptor, potentiator of 5-HT3 receptor and others, including the muscarinic acetylcholine receptor antagonists. To better understand the relationship between behavior, electrophysiology and pharmacology of this model of acute schizophrenia here we aim at combining electrophysiological recordings with microdialysis in chronically implanted rats. By analyzing the

interaction between neurophysiological signatures, behavior and in situ neuropharmacological measurements, we are able to establish a link between neurotransmitter release and alterations in oscillatory activity that opens a door to further investigations devoted to elucidating to what extent these interactions also reflect the prominent neurophysiological/neuropharmacological deficits observed in schizophrenic patients.

(T2-27) ULTRAESTRUCTURAL AND FUNCTIONAL CHANGES OF DOPAMINERGIC SYNAPSIS IN A RAT MODEL OF PROGRESSIVE PARKINSONISM

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ABSTRACT

Aggregation of α-synuclein (α-syn) in intracytoplasmic Lewy bodies and the loss of dopaminergic neurons are key pathological feature of Parkinson´s disease (PD). Recent studies provide evidence that synaptic and axonal abnormalities occur before the degenerative loss of neuronal cell bodies and these can be attributed to synaptic accumulation of α-syn. In addition, failure of synaptic mitochondria to generate adequate ATP has been implicated as a causative event preceding loss of synaptic networks in neurodegenerative disease. However, it is not entirely clear the impact of impaired mitochondrial dynamics induced by α-syn on neurodegeneration. Thus, our aim was to study the temporal sequence of ultrastructural and functional changes in dopaminergic striatal terminals associated with α-syn overexpression. For that purpose, ultrastructural changes by electron microscopy in TH+ striatal terminals and mitochondrial function using Seahorse XF96 in isolated striatal synaptosomes, have been assessed after bilateral inoculation in the SNc of recombinant adeno-associated viral vectors (rAAV) encoding for A53T mutated human α-syn (hα-syn) at different time points (24 h, 72 h, 1, 2 and 4 weeks post-inoculation, p.i.). Robust and persistent expression of hα-syn was observed in the nigrostriatal pathway starting at 72 h p.i. in the SNc and at 1 week p.i. in striatum. We observed reduced TH+ density at the striatal level at 2 weeks p.i. ($p<0.05$) and 4 weeks p.i. ($p<0.01$) and in the SNc at 4 weeks p.i. ($p<0.05$). Ultrastructural examination of striatal tissue also revealed signs of neurodegeneration such as dystrophic and swollen morphology of dopaminergic axons as shown by an increase in their area ($p<0.05$) and tortuosity ($p<0.05$) at 4 weeks. We also observed higher number of autophagic vesicles within the dopaminergic terminals at 2 weeks p.i. ($p<0.05$), being more pronounced at 4 weeks p.i. ($p<0.01$) along with an increase in their size. Regarding synaptic functionality, significant reduction of mitochondrial respiration has been observed in striatal synaptosomes at 1 week p.i. (basal respiration, $p<0.01$; proton leak, $p<0.05$; ATP production, $p<0.01$). Our results indicate that pathological α-syn in dopaminergic striatal terminals seems to induce functional synaptic changes that precede axonal pathology, autophagic disruption and the onset of dopaminergic degeneration. (PI14/00763)

(T2-28) THE BORRELIA BURGDORFERI BACTERIUM: A NOVEL CONTACT-DEPENDENT INDUCER OF PERIPHERAL NERVE DEMYELINATION

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ABSTRACT

Schwann cells form myelin sheaths around large diameter axons in the peripheral nervous system, and this is essential for rapid saltatory conduction of nerve impulses. Myelin breakdown, demyelination, is a universal outcome of a remarkably wide range of conditions that involve disturbance to Schwann cells or the nerve environment, whether due to genetic or acquired disease, toxicity or microbial infections. Strikingly, demyelination is often seen in Lyme neuroborreliosis (LNB), which is considered one of the most dangerous of all manifestations of Lyme disease, the most common arthropod-borne infectious disease in temperate regions of the northern hemisphere. It is caused by infection with the spirochete Borrelia Burgdorferi (Bb). The early local reaction to the deposition of the bacteria in the skin is followed by the hematogenous dissemination of the spirochete, which results in the colonization of different tissues and organs such as the skin, nerves and brain. In this study, using in vitro analyses, we show that Borrelia Burgdorferi is a potent contact-dependent inducer of peripheral nerve demyelination, by activating several receptor and intracellular signalling pathways to strongly repress myelin gene and protein expression, throwing light on one of the most intriguing pathological features of Lyme neuroborreliosis.

(T2-29) THE RNA-BINDING PROTEIN HUR/ELAVL1 CONTROLS A CORE GENE REGULATORY CIRCUITRY ESSENTIAL FOR MPNST GROWTH AND METASTASIS

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ABSTRACT

Neurofibromatosis type 1 (NF1) is one of the most common genetic disorders among humans, affecting 1:3500 individuals worldwide. The hallmark of NF1 is the development of multiple benign peripheral nerve sheath tumours known as dermal and plexiform neurofibromas. Approximately 10% of plexiform neurofibromas undergo malignant transformation into malignant peripheral nerve sheath tumors (MPNSTs), aggressive and highly metastatic soft tissue sarcomas that are essentially incurable. Schwann cells are the crucial pathogenic cell type in NF1. The RNA-binding protein (RBP) HuR is aberrantly expressed in several types of cancers, in which it regulates the expression of several cancer-related proteins. We observed that HuR expression

was significantly increased in NF and MPNST samples compared to normal nerves by IHC, qPCR and WB analysis, and found a strong correlation between HuR expression and degree of malignancy. RIP-CHIP analysis showed that the number of mRNAs bound to HuR increased as malignancy progresses. Amongst them, several ones with well-defined roles in oncogenesis were identified. Lentiviral-mediated HuR silencing in vitro and in vivo blocked both MPNST growth and metastasis. This work points to a key master regulatory role of HuR in controlling gene expression patterns in MPNST, and identify HuR as a possible therapeutic target for these tumours.

(T2-30) ASTROCYTES RESTORE CONNECTIVITY AND SYNCHRONIZATION IN DYSFUNCTIONAL CEREBELLAR NETWORKS OF ATM-KO MODEL OF ATAXIA TELANGIECTASIA

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ABSTRACT

Evidence suggests that astrocytes play key roles in structural and functional organization of neuronal circuits. To understand how astrocytes influence the physiopathology of cerebellar circuits, we cultured cells from cerebella of mice that lack the ATM gene. Mutations in ATM are causative of the human cerebellar degenerative disease Ataxia-Telangiectasia. Cerebellar cultures grown from Atm−/− mice had disrupted network synchronization, atrophied astrocytic arborizations, and higher numbers of synapses per neuron than wild-type cultures. Chimeric circuitries composed of wild-type astrocytes and Atm−/− neurons were indistinguishable from wild-type cultures. Adult cerebellar characterizations confirmed disrupted astrocyte morphology, increased GABAergic synaptic markers, and reduced autophagy in Atm−/− compared to wild-type mice. These results indicate that astrocytes can impact neuronal circuits at levels ranging from synaptic expression to global dynamics.

TRACK 3: Neurology, Behaviour & Cognition, Imaging and Psychiatry

ORAL PRESENTATIONS

Moderator: **Ana M. González Pinto (BioAraba, HU Araba, UPV/EHU; Vitoria-Gasteiz)**

(O3-1) GENE THERAPY FOR DRAVET SYNDROME

Neurology > Clinical Neurology

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ABSTRACT

Dravet Syndrome (DS) is a severe epileptic encephalopathy with infantile onset, characterized by refractory seizures, increased risk of sudden death, as well as mental, behavioural and motor comorbidities. In most cases, the genetic basis is a haploinsufficiency caused by mutations in SCN1A, which encodes the alpha subunit of a voltage-dependent Na^+ channel (Nav1.1). Due to the complex physiopathology of DS, etiological approaches such as gene therapy have unique chances to obtain a global improvement in the life of these patients. We aim to deliver a functional copy of the SCN1A gene to the brain using High-Capacity adenoviral vectors (HC-Ad). To provide proof of concept about the feasibility of this approach we generated a preliminary vector prototype carrying a codon-optimized SCN1A cDNA under the control of a ubiquitous promoter sequence (CAG). The expression cassette inserted into de vector genome was stable in E. Coli and gave rise to viable HC-Ad particles following a standard rescue and amplification protocol. The resulting HCA-CAG-SCN1A vector was able to infect neurons and increase the amount of Nav1.1 in a dose-dependent manner. Biodistribution analysis using HC-Ad vectors encoding GFP demonstrated efficient transduction of neurons upon intracerebral administration. Finally, in vitro luciferase reporter assays were performed to select a regulatory sequence with preferential activity in GABAergic/paravalbumin-expressing inhibitory neurons. In summary, the results obtained so far indicate that gene therapy base on HC-Ad vectors is a viable option for the treatment of DS.

(O3-2) IDENTIFICATION OF FACTORS PROMOTING RESILIENCE TO COGNITIVE IMPAIRMENT IN TG2576 MICE

Behavioural and Cognitive Neuroscience

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ABSTRACT

Amyloid plaques and neurofibrillary tangles are the primary pathophysiological lesions in Alzheimer´s disease (AD). However, recent neuroimaging and cerebrospinal fluid biomarker studies have shown that fibrillar β-amyloid (Aβ) accumulation occurs in approximately one-quarter to one-half of cognitively normal older adults. Interestingly, in our lab we have identified that, like in humans, some "rare" transgenic AD-mice (Tg2576 14-16 month-old), maintain intact cognition in the Morris water maze (MWM) test despite presenting severe amyloid pathology. Thus, in this project we aimed to study, the molecular mechanisms and/or pathways that confer resilience to dementia in healthy individuals in the Tg2576 line. A group of aged-Tg2576 mice with intact cognition (similar performance to wild type mice in the MWM test) was selected as "good learners" (GL) and were compared with a group of Tg2576 mice with poor performance, that were identified as "poor learners" (PL). Two hours after a block of trials of hidden platform training, animals were sacrificed for their neuropathological characterization. The brains of Tg2576 GL with an intact cognition in the MWM test showed amyloid and tau pathology similar to that observed in Tg2576 PL. Interestingly, a significant increase in the key memory related protein pCREB, was observed in the prefrontal cortex of Tg2576 GL mice compared to Tg2576 PL mice. Now, by using microarrays, transcriptome analysis of dorsal hippocampus of Tg2576 GL and PL mice have been achieved to analyze differential gene expression in order to indentify protective-factors that might confer the "resilience" to cognitive impaiment observed in GL mice. The identification of these disease-protective factors may point towards new biomarkers of disease progression and/or new potential drug targets for preventing or even treating AD.

(O3-3) SOCIOECONOMIC STATUS CORRELATES OF LINGUISTIC SKILLS AND WORKING MEMORY IN A LOW-RISK PRETERM SAMPLE DURING ADOLESCENCE AND YOUNG ADULTHOOD.

Behavioural and Cognitive Neuroscience

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ABSTRACT

Background: Socioeconomic status (SES) has a crucial impact on cognitive functioning during childhood; with pervasive consequences in early adulthood. However, the SES-cognition relationship in prematurity has only been studied throughout the infant stage. Methods: The sample consisted of 32 participants, 16 preterms and 16 controls, of both sexes and aged between 11 and 36 years. Familiar, monoparental and self SES, as well as, linguistic skills and working memory were evaluated. Results: As expected, statistically significant differences were found between the groups for the clinical variables; such as gestational age, birth weight, birth height and cranial perimeter. Likewise, statistically significant differences were observed in digits forward (□prem=9.00±2.03, □cont=10.50±2.19 U=180.0 p=0.05) and increasing digits (□prem=8.81±2.81, □cont=10.63±1.67 U=185.5 p=0.03), in which lower scores were obtained by the group of preterm individuals. Statistically significant correlations were discerned in the group

of preterms between the self SES and all the domains of language (rhopeabody=.67 p□0.001, rhopeabody_errors=-.59 p=0.002, rhophonetic_fluency=.74 p=0.001, rhosemantic_fluency=.53 p=0.008), as well as in the computation of working memory (rhodigits_forward=.76 p=0.03, rhodigits_backward=.63 p=0.01, rhoincreasing_digits=.55 p=0.01, rhototal_digits=.79 p=0.001). Conclusions: Low-risk preterms during adolescence and early adulthood showed a lower cognitive performance in some tests related to working memory. Moreover, in the group of low-risk preterm individuals a relationship was found between the personal SES and all the cognitive domains. Therefore, the present study extols the crucial value of personal SES in those who were born prematurely since it seems to be linked to cognitive performance in adolescence and early adulthood.

(O3-4) EEG OSCILLATIONS: VISUAL-MOTOR CONNECTIVITY DURING STIMULUS-PACED REPETITIVE MOVEMENTS AND THEIR INFLUENCE ON MOTOR STRATEGY AND AND TEMPORAL PERCEPTION

Neurology > Clinical Neurology

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ABSTRACT

Portable technological advances in combination with advanced data analysis techniques is opening a new world for tracking and managing chronic diseases. In various neurodegenerative and neuropsychiatric diseases, temporal and rhythmic abnormalities exist. In Parkinson's disease (PD) for example, patients have an impaired ability to discriminate between temporal deviations. The mechanism of using ones internal clock to follow a particular rhythm is altered in Schizofrenia. This investigation explores the use of various tests on a tablet application to determine natural and optimal temporal frequency, analyze predictive and reactive movement strategies, and quantify internal clock activity in healthy controls. In order to further explore the oscillatory activity related to rhythmic and temporal tasks, our team recorded EEG activity for each test. In further studies, these parameters potentially could be used to classify, track and characterize symptoms and progression of neurodegenerative and neuropsychiatric patients.

(O3-5) ASSESSMENT OF EFFECTIVE CONNECTIVITY IN RAT CORTICO-BASAL GANGLIA NETWORK DURING BEHAVIORAL AND PHARMACOLOGICAL CHANGES

Behavioural and Cognitive Neuroscience

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ABSTRACT

The brain's ability to integrate different behavioral and cognitive processes relies on its capacity to generate neural oscillations in a cooperative and coordinated manner. Local neuronal structures work as a single system, but are involved in a wide interconnected network. The interectation in and between them have become a cutting-edge topic in neuroscience and computer science.

Cross-frequency coupling (CFC) has recently been proposed as one of the mechanisms involved in organizing brain activity. Recently, our lab has shown how dopaminergic drugs affect the spatio-temporal organization by analyzing CFC patterns of the oscillatory activity in the cortico-basal ganglia network of healthy, freely moving rats.
In this communication we aim at understanding how interactions across different structures and oscillatory brain rhythms serve to orchestrate the brain processes and shapes the information transmission across different cortico-basal ganglia structures. We will assess the effective connectivity, meaning the direction of interaction in and between these structures. To do that, we will adapt and develop new signal analysis techniques that will be used to evaluate the changes induced by different behavioral states (rest or moving) or by pharmacological modulation.

POSTERS

(T3-01) THE CONTRIBUTION OF COGNITIVE REHABILITATION TO QUALITY OF LIFE IN THE ELDERLY: LONGITUDINAL CLINICAL TRIALS

Behavioural and Cognitive Neuroscience

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ABSTRACT

Introduction

The quality of life in the elderly is reduced by factors such as cognitive decline, social support and healthy problems. Therefore, cognitive and functional rehabilitation programs are necessary to improve quality of life. The aim of the current study was to investigate the effectiveness of RehacoG cognitive rehabilitation program to improve quality of life after intervention (3 months) and longitudinal follow-up (12 months).

Methods

One hundred and twenty-four participants with a mean age of 79 years (8.85) were randomized to the rehabilitation (RehacoG) or control group. RehacoG group (n=62) received cognitive rehabilitation for attention, processing speed, learning and memory, language and executive functions for 3 months (3 sessions/week of 60 minutes). The control group (n=62) performed occupational tasks with the same frequency and duration. Both interventions were administered in group format. Quality of life was assessed through the Satisfaction With Life Scale (SWLS) at baseline (T0), after treatment (T1) and 12 months follow-up (T2).

Results

No significant differences were found between groups in sociodemographic or quality of life scores at baseline. Repeated measures ANOVA (time x group) showed significant differences in quality of life between RehacoG group and control group after the intervention (np2=0.11, p=0.001) showing the RehacoG group higher quality of life scores. Moreover, the RehacoG group showed significant differences in quality of life (n2p=0.11, p=0.001) compared to the control group after 12 months follow-up showing higher scores.

Conclusions

The RehacoG group showed significant improvement in quality of life after treatment and 12-month follow-up. The results of this study support the idea that intervention programs as RehacoG improve overall quality of life in the elderly, making it susceptible to be used as part of prevention programs and promoting elderly population health.

The work has not been published

(T3-02) BEHAVIOURAL EVALUATION OF A TRANSLATIONAL ANIMAL MODEL OF SCHIZOPHRENIA

Behavioural and Cognitive Neuroscience

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ABSTRACT

Schizophrenia (SZ) is a chronic and disabling psychiatric disorder affecting about 1% of the population worldwide. Schizophrenia comprises positive and negative symptoms as well as

cognitive deficits. Epidemiological and experimental studies indicate that infections during the gestational period represent a risk factor to develop SZ along lifetime, which in combination with stressful events in adolescence may lead to the SZ onset. The aim of the present study was to create a traslational “double-hit” animal model of SZ in male and female mice, based in maternal immune activation (MIA, hit-1)—injection of poly(I:C) to pregnant dams, 7.5 mg/kg i.p.—and social isolation (SI, hit-2) in the peri-pubertal period (3-11 weeks). In the four experimental groups (hit-1, hit-2, double-hit and control) locomotion and anxiety were assessed using the Open Field Test (OFT), and the cognitive status (declarative/episodic memory) was evaluated by means of the Novel Object Recognition Test (NORT). No differences were observed in the spontaneous locomotor activity between any of the groups, neither in females nor in males. However, an increase in the percentage of time spent in the centre of the OFT was significantly associated to the hit-1 (MIA) only in female mice ($F[1,53]=4.252$; $p=0.044$, $n=57$). Moreover, a significant decrease in the discrimination index in the NORT was also associated to the hit-1 (MIA) in the subgroup of female mice ($F[1,55]=7.266$; $p =0.0093$, $n=59$). These preliminary results indicate that MIA produces a greater impact in female mice inducing an anxiolytic-like phenotype and cognitive impairments.
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(T3-03) ALTERATIONS IN A MICE MODEL OF ALZHEIMER´S DISEASE: CHARACTERIZATION OF TG2576 STRAIN.

Behavioural and Cognitive Neuroscience

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ABSTRACT

Background or Introduction
Tg2576 mice expressing the Swedish mutation of the amyloid precursor protein (APPs) constitute an opportunity for exploring the pathophysiology and neurobiology of Alzheimer's disease (AD). Tg2576 transgenic mice develop amyloid plaques in addition to progressive cognitive decline. Although cognitive function in Tg2576 has been extensively characterized by biochemical and behavioral techniques, the electrophysiological basis of mnemonic function is poorly understood. Our objective is the electrophysiological characterization of this model to relate previous knowledge with a new approach to study the pathophysiology of AD.
Material and methods
Cognitive impairment in each animal was assessed by means of fear conditioning and Morris water maze tests. Histopathological markers were also evaluated (amyloid and tau).
Chronic electrophysiological recordings from prefrontal cortex screws and six electrodes located in the hippocampus during freely moving and sleep were obtained during freely moving activity. Behavioral states were divided into awake rest, awake movement, slow sleep and REM sleep conditions.
Power spectrum, cross-frequency coupling and imaginary coherence estimates were obtained and compared across behavioral states and genotypes. Age effects were investigated by covariance analyses.
Results
Our results show electrophysiological alterations both, during wakefulness and sleep. The frequency of theta oscillation during movement and REM sleep were affected by age and genotype. Transgenic mice showed higher levels of beta-gamma power compared with wild type animals. In addition, intercritical spikes were observed in ~75% of the transgenic mice while no epileptic activities were detected in WT animals. The topography of this activities suggest that they are originated in the dentate gyrus and are more likely to occur during sleeping periods.
Conclusions

Electrophysiological activity had been proposed to play a role in memory consolidation processes. Here we show that the Tg2576 mice model of AD shows severe alterations in the electrophysiology of hippocampus and prefrontal cortex that could explain some of the cognitive deficits of the model.

(T3-04) INCREASED IMPULSIVITY FOLLOWING NIGRAL DEGENERATION AND CHRONIC PRAMIPEXOLE TREATMENT IN A RAT MODEL OF PARKINSON'S DISEASE

Behavioural and Cognitive Neuroscience

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ABSTRACT

Impulse control disorders (ICD) is a common side effect of dopaminergic treatment in patients with Parkinson's disease (PD), frequently associated with dopamine agonists (DA) such as the D2/D3 DA pramipexole (PPX). The pathophysiology of ICD is poorly understood and there is a need for reliable animal models. We have assessed the behavior of parkinsonian rats by inoculation of adeno-associated viral vectors (AAV) encoding for A53T mutated hα-syn in the substantia nigra pars compacta (SNc) and control (AAV-GFP) rats under chronic PPX treatment. Eighteen weeks post-injection, animals were treated with 0.25mg/kg PPX daily during four weeks and the impulsive behavior was analyzed with the 5-Choice Serial Reaction Time Task paradigm in ON and OFF medication. Dopaminergic lesion induced significant progressive motor deficit after the AVV-hα-syn injection ($p<0.001$). Before DA treatment, dopaminergic lesion increased the premature responses rate (waiting impulsivity) that was significantly higher during the 4 weeks of PPX treatment under ON state ($p<0.05$). Perseverative responses (compulsive behavior) did not change over the experiment in either lesion or control group. By contrast, variables related to attention showed similar pattern (decrease in accuracy, increase in omissions). The post mortem histological analyses shown a clear dopaminergic lesion in AAV-hα-syn rats with a decrease in the number of TH+ neurons (30%, $p<0.05$) in SNc and dopamine transporter immunostaining in the striatum (64%, $p<0.05$), as well as the appearance of hα-syn inclusions in both brain nuclei. In conclusion, these results indicate that the dopaminergic lesion and the chronic PPX treatment increased the impulsive behavior as well as changed the molecular markers of the dopaminergic pathway in the parkinsonian animal model. All these findings make this model suitable as a valid tool to investigate the pathophysiology of ICD in PD (DFG11/019, PI11/02109).

(T3-05) MOTOR LEARNING MEDIATED BY MOTOR TRAINING WITH A MYOELECTRIC INTERFACE FOR REHABILITATION AFTER STROKE

Behavioural and Cognitive Neuroscience

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ABSTRACT

Local brain damage due to stroke frequently affects the initiation of motor commands and/or their descending flow to the spinal cord. This leads to a pathological muscle coordination in the limbs opposite to the injured hemisphere. There is evidence that the brain and the lower sensorimotor circuitry can change or reorganize itself in response to sensory input, experience and learning. Several studies have confirmed that functional recovery mediated by motor training entails a learning process in patients with motor impairment. Myoelectric interfaces, which allow controlling a robotic exoskeleton based on electromyographic activity, have been proposed and used in motor training paradigms for rehabilitation. However, the existing systems allow the control of only up to 2 degrees of freedom (DoFs) of a wearable exoskeleton, which have hindered the training of functional movements involving proximal and distal segments of the arm and hence, the potential to elicit motor recovery. In this study, we investigated the viability of using a novel myoelectric interface to control a 7-DoF upper limb exoskeleton during functional tasks. Moreover, we evaluated if the proposed system can be used to elicit motor learning in a 5-session intervention in 10 healthy participants. Additionally, the perception of the participants about different features of the system was studied for their validation and future optimization.

(T3-06) FUNCTIONAL MAPPING OF NEURONAL CIRCUITS RESPONSIBLE OF SOCIAL DEFICITS IN AN ANIMAL MODEL OF AUTISM

Behavioural and Cognitive Neuroscience

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ABSTRACT

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social behavior and communication, together with the presence of limited interests and restrictive/ repetitive behaviors. The latest prevalence of ASD is approximately 1 out of 59 children, being one of the primary health issues worldwide. The septal area and the striatum have been proposed to be involved in the processing and modulation of motivation, mood and affection, which are core domains of the social behavior. Lacks of these social features are widely observed in ASD patients. Genetic, advanced imaging and clinical studies have linked many brain abnormalities and neural pathologies with autism, including irregularities in brain structures involved in social cognition. In this work, we investigated cell activation after distinct stimulus in both lateral septum (LS) and nucleus accumbens (NAcc), main structures responsible for motivation, using a genetic mouse model of autism (Cntnap2-knockout). Eight week animals (wild-type and mutant, male and female) were exposed to social stimulus (WT mice of same sex) and object stimulus for 90 seconds. Interestingly, we found that Cntnap2-knockout mice doubted significantly more times with the object compared to its wild-type counterparts. This notable

attention that mutant mice showed with the object was accompanied by increased neuronal activation in the nucleus accumbens as measured by expression of the immediate early gene cFos. However, we did not observe significant changes between knockout and wild-type mice neither in NAcc nor LS in regard to neuronal activation as we expected. Future experiments will allow us to determine any potential abnormalities in the encoding of the motivational valence underlying deficits in social interactions in this model.

(T3-07) CHARACTERIZATION OF VOCAL COMMUNICATION SYSTEM IN A MOUSE MODEL OF AUTISM

Behavioural and Cognitive Neuroscience

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ABSTRACT

Autism spectrum disorder describes a group of heterogeneous neurodevelopmental disorders, characterized by deficits in social communication and social interaction, and the presence of repetitive behaviors and restricted interests. Multiple lines of evidence have lined the CNTNAP2 gene with autism and, specifically, with the 'language development' endophenotype. In addition, CNTNAP2 genetic variants have been associated with language disorders such as specific language impairment, dyslexia, selective mutism and stuttering, as well as with language development in the general population. Animal models provide a good insight into the neurogenetic basis for speech and communication disorders. It has been previously demonstrated that a mouse knockout (KO) for the Cntnap2 gene, a validated model of autism, displays abnormal vocal communication, as shown by reduced number and irregular type of ultrasonic vocalizations (UsV) emitted by pups upon maternal separation at postnatal day 7. It is generally accepted that the type of ultrasonic vocalization (characterized by their sound frequency and length) confers important information for vocal communication in mice, but it is a field underexplored. In the present work, we aimed at characterizing the developmental trajectory in the pattern of emission of ultrasonic vocalizations in Cntnap2-KO mice compared to wild-type. We found slight differences in terms of number, frequency and duration between genotypes. To investigate if these abnormalities were associated to potential neuroarchiteture defects in cortico-striatal circuits in this model, known to modulate vocal communication and where Cntnap2 is highly expressed, we performed an anatomical characterization of the striatum. Our preliminary data indicate that no obvious differences, when analyzing the strisoma/matrix percentage in this structure, exist between both genotypes. Additional studies will provide insight into the molecular contribution of Cntnap2 to vocal communication.

(T3-08) COGNITIVE CHARACTERIZATION OF MDX MICE MODEL OF DUCHENNE MUSCULAR DYSTROPHY

Behavioural and Cognitive Neuroscience

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ABSTRACT

Duchenne muscular dystrophy (DMD) is a genetic neuromuscular disorder that affects principally to skeletal and cardiac muscle causing progressive degeneration and weakness. In the 30% of the patients it can accompanied by central nervous system abnormalities, such as cognitive impairments, learning disabilities and depressive disorders. There is no cure known and the average life expectancy is around 20-30 years old.
In this work we have characterized the behavioral phenotype of 4 months old mdx mice, the most used animal model of the disease, by marble test, freezing test and holeboard test. We have also realized MRI analysis of the dissected brains. In addtion, we have done neuronal cultures from control and mdx mice fetus to characterize intracellular calcium levels and different protein expression by immunocytochemistry.
Since most of the research in Duchenne muscular dystrophy is focused to the skeletal and cardiac muscle, this study can be useful to approach other features of the disease.

(T3-09) PET IMAGING OF CROSSED CEREBELLAR DIASCHISIS AFTER LONG-TERM CEREBRAL ISCHEMIA IN RATS.

Imaging

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ABSTRACT

Introduction: Crossed cerebellar diaschisis (CCD) is a decrease of regional blood flow and metabolism in the cerebellar hemisphere contralateral to the injured brain hemisphere as a common consequence of stroke1,2. Despite CCD has been detected in patients with stroke using neuroimaging modalities, the evaluation of this phenomenon in rodent models of cerebral ischemia have been scarcely evaluated so far. Moreover, the preclinical evaluation of CCD might contribute to the understanding of the role of CCD on stroke pathophysiology. For this reason, the main purpose of this study is to evaluate the CCD using positron emission tomography (PET) imaging with 2-deoxy-2-[18F]fluoro-D-glucose ([18F]FDG) after long-term cerebral ischemia in rats for the first time so far.
Methods: In vivo PET imaging studies with [18F]FDG were performed to explore the alterations in glucose metabolism before (day 0) and at 1, 3, 7, 14, 21 and 28 days following transient focal cerebral ischemia in rats. Likewise, magnetic resonance imaging (MRI-T2W) studies were carried out to assess the extent of brain damage before rats were subjected to nuclear studies. Finally, neurological evaluation was applied to evaluate rat functional recovery following long-term ischemia.
Results: In the ischemic territory, PET with [18F]FDG showed a significant decrease of the glucose metabolism (cortex and striatum) followed by a progressive recovery during the first week and a secondary decrease later on. Conversely, the cerebellum showed a significant contralateral hypometabolism at days 7 and 14 after reperfusion evidencing the presence of CCD. Finally, neurological behavior showed major impaired outcome at day 1 after ischemia followed by a significant recovery of the sensorimotor function from days 7 to 28 after experimental stroke.
Conclusions: Taken together, these results provide valuable knowledge regarding the role of CCD after experimental stroke and suggest that the degree of cerebellar diaschisis after cerebral ischemia might be predictive of neurological recovery in rats.

(T3-10) PROMOIJ: A NEW TOOL FOR SEMI-AUTOMATIC ANALYSIS OF CELL PROCESSES MOTILITY

Imaging

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ABSTRACT

Microglial cells, the immune cells of the central nervous system, continuously survey the brain parenchyma to detect alterations and maintain tissue homeostasis. The gold standard technique to study motility involves the use of two-photon microscopy to obtain images from living animals. This technique generates large amounts of 4D images (XYZT) which are manually analysed using tedious and time consuming protocols. In addition, motility analysis is frequently performed using Z-projections of image stacks with the loss of 3D information and accuracy. To overcome these limitations we developed ProMoIJ, an ImageJ tool to perform automatic motility analysis of cell processes in 3D. It includes several ImageJ macros that allow batch processing for registration, background subtraction, and bleaching correction of the images. The main core of the tool is formed by two ImageJ macros, to manually select the process to be analyzed and to automatically reconstruct its 3D skeleton. Several motility data are extracted from each skeleton: process length at each time, length variation per minute, retraction, protraction, tip position, and tip motility. We have validated the data obtained with ProMoIJ by comparing them with manually obtained data by three different researchers using an assisted reconstruction protocol. Our results show that the use of ProMoIJ presents several advantages compared to manual analysis: 1) it reduces the time required for the analysis, 2) it is less sensitive to experimenter bias, and 3) it produces more consistent data. To the best of our knowledge, ProMoIJ is the first freely available tool for automated analysis of microglial motility that facilitates the analysis of 3D motility of cell processes by reducing the time required to obtain results, and increasing the accuracy and reproducibility of the data.

(T3-11) INTEGRATION AND FATE OF HUMAN DENTAL PULP STEM CELLS GROWN IN NEUROGENIC MEDIA AFTER INTRACEREBRAL GRAFT INTO ATHYMIC NUDE MICE.

Neurology > Clinical Neurology

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ABSTRACT

Adult human molars contain cell populations able to grow and produce neuronal-like phenotypes. Cells from the periodontal ligament have been shown to acquire neuronal-like lineage previous expansion with alpha-MEM plus 10% calf serum and posterior neuronal induction with DMEM/F12. On the other side, Dental Pulp Stem Cells (DPSCs) possess strong osteogenic potential and are actively studied for their possible therapeutic use in bone tissue repair.

In the present work, we want to address the phenotypic fate of adult DPSCs cultured from the very beginning with neurogenic media (NeuroCult) usually used for the expansion of adult Neural Stem Cells (NSC). Our result shows that DPSC cells are able to grow and survive without a need of serum addition using this type of culture media as NSC do. When DPSCs were grafted intracranially into athymic nude animals, DPSCs localized close to vasculature, which were detected as positive cells for human-nestin, showing no tumorigenic and/or osteogenic potential. Our results show that NeuroCult NSC culture media is compatible for the growth of human dental pulp stem cells for graft purposes. This work has been financed by “Ramón y Cajal”

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(T3-12) *ALTERATIONS OF LIPID METABOLISM DEFINE POTENTIAL CIRCULATING BIOMARKERS OF AMYOTROPHIC LATERAL SCLEROSIS*

Neurology > Clinical Neurology

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is the most common neurodegenerative motor neuron disease. Currently there are no biomarkers of disease or treatment. A growing bulk of evidences suggests that part of the disease process lies on alterations on the energy metabolism, which is observed as a clinic state of systemic hypermetabolism. This metabolic state may give rise to consistent changes on circulating metabolites that relate to disease severity, and hence become biomarkers. To address this question, we have performed a comprehensive analysis of the metabolomic profile in serum from 71 fasting subjects (39 ALS patients and 32 healthy controls) using different chromatography techniques. In a first untargeted metabolomic approach, a total of 446 metabolites were detected, 30 of which were altered in ALS patients (without statistical significance in multivariate analysis). There was a general increase in triglycerides with long/very long chain fatty acids (FA) and a particular increase in nervonic ω-9 FA (NA, C24: 1). In addition, a significant reduction of the ratio between FAs C16: 1 and C16: 0, indicative of deficient delta9-desaturase activity, were found in patients. A second targeted lipidomic study confirmed alterations in these FAs and revealed further changes in several ω-6 and ω-3 FAs. The reduction of delta9-desaturase activity was associated with shorter times to non-invasive mechanical ventilation (NIMV) (HR=5.032, p <0.01), gastrostomy (HR=23.7, p <0-001) and death (HR=4.68 p <0.012) in a 3 years follow-up. Altogether, there is no metabolic signature that discriminates ALS patients from controls in the studied cohort, but certain changes in individual lipid metabolites (in particular delta9 desaturation index) are postulated as potential biomarkers with prognostic value for ALS.

(T3-13) *HOW PERSONALITY CAN MODIFY QUALITY OF LIFE IN THIRD-AGE UNIVERSITY STUDENTS.*

Psychiatry > Clinical Psychiatry

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ABSTRACT

Introduction: We live long lives, but quality of life has been set aside sometimes. There are several elements that affect the quality of life. The most important are: physical health, psychological health, social relationships and the environment. These domains vary depending on the activities that the person do in his daily routine.

General purpose: Our aim is to test if university in third-age students lead to a good quality of life. We also want to analyze if personality can modify how quality of life is perceived.
Methodology: These four domains have been measured with the WHOQOL-BREF in 54 third-age students at the "Aulas de la experiencia" at the University of the Basque Country. Personality has also been studied with the Eysenck test to see if it could modify the quality of life.
Results: Our results showed that third-age students have a good quality of life in general. We did not find relation between sex gender and neuroticism. Also, neuroticism was related to a worst quality of life. There was no relation between quality of life and extraversion o psicoticism.
Conclusions: This study demonstrated that the university in the third-age is an experience that improves physical and psychological health, social relationships and the environment, that is, the quality of life. It also demonstrated that a high level of neuroticism is a risk factor to develop a worse quality of life.
Keywords: "Quality of life", "University of Third Age", "Eysenck" and "Neuroticism".

(T3-14) ALPHA-7 NICOTINIC AGONISTS FOR COGNITIVE DEFICITS AND NEGATIVE SYMPTOMS IN SCHIZOPHRENIA: A META-ANALYSIS OF RANDOMIZED DOUBLE-BLIND CONTROLLED TRIALS

Psychiatry > Clinical Psychiatry

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ABSTRACT

Background: Most individuals with schizophrenia suffer some cognitive dysfunction and persistent negative symptoms, predicting long-term functioning. Current antipsychotic treatments have little or no effect on these domains. Novel pharmacological agents have emerged treating to solve this problem. The role of nicotinic cholinergic system has been proposed to have a key role in the symptomatology of schizophrenic patients. Aim: Assess the efficacy of α-7 nicotinic agonists (α-7 nAChR) in the treatment of cognitive and negative symptoms in schizophrenia. Methods: An extensive literature search was conducted to identify randomized double-blind placebo-controlled trials (RCTs) evaluating the effects of α-7 nAChR in cognitive and negative symptoms in schizophrenia, published up to 18 January 2018. References were retrieved through searching the following databases: PubMed, Embase and Cochrane. The effects of α-7 nAChR were evaluated for individual cognitive domains affected in schizophrenia as proposed by the MATRICS initiative, and negative symptoms using psychiatric clinical scales. Results: 13 studies were identified. Only 5 of them provided raw scores for cognitive domains, and 7 for negative symptoms. We found no statistical differences in none of the cognitive domains as well as in negative symptoms. The risk of bias was low and evidence based on outcomes from the meta-analysis was rated as "moderated" as per the GRADE guidelines. Conclusions: α-7 nAChR may not be effective in the treatment of cognitive deficits and negative symptoms in patients with schizophrenia. Future work should evaluate and control the effect of confounding variables and consider the inclusion of First Episode Psychosis.

(T3-15) THE COMPLEX ASSOCIATION BETWEEN THE ANTIOXIDANT DEFENSE SYSTEM AND CLINICAL STATUS IN EARLY PSYCHOSIS

Psychiatry > Molecular Psychiatry

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ABSTRACT

Background: Oxidative stress is a pathophysiological mechanism potentially involved in schizophrenia. There is evidence that patients who have experienced just a single episode of psychosis have increased levels of lipid peroxidation and a decreased total antioxidant capacity. The total antioxidant activity of extracellular fluid can be calculated by adding endogenous and food-derived antioxidants. Total antioxidant status (TAS) is considered to have great potential in the search for biomarkers of functional damage in psychiatric disorders, given its association with the pathophysiology of schizophrenia spectrum disorders.
Objective: The objective of our study was to assess the relationship between total antioxidant status (TAS) and the functional status of patients with a first episode of psychosis (FEP), at the early course of the disease. We hypothesized that antioxidant status would be associated with both, the short and long-term functioning and clinical outcome in these patients.
Methods: a sample of 70 patients aged between 9 and 17 years with a FEP, were followed up for a period of two years. Blood samples were drawn to measure TAS levels at three time points: at baseline, at one year, and at two years. Clinical symptoms and functioning were also assessed at the same time points using various scales. Linear regression analysis and was performed to investigate the relationship between TAS and clinical status at each assessment, adjusting for potential confounding factors. In addition, we used longitudinal linear models to analyse the relationship between the evolution of TAS and changes in clinical scale scores. Data are presented in terms of beta coefficients with p values and the corresponding 95% confidence intervals.The distribution of clinical variables was grouped in different percentiles to assess dose-response in the relation between clinical variables and TAS.
Results: At baseline, patient´s score on Children's Global Assessment Scale (CGAS) was directly and significantly associated with TAS (B=0.005; $p<0.05$) and surprising this association was reversed after one and two years of follow-up (B= -0.011, $p<0.01$ and B= -0.009, $p<0.05$ respectively) and longitudinal linear models confirmed this relationship between TAS and CGAS (β= -0.006, p= 0.004, 95% CI: (-0.010, -0.002)). When CGAS were distributed in percentiles to analyse dose-response in this relation, a monotonic increase of the TAS was observed, with higher CGAS at baseline. In contrast, at one and two year follow-up, this monotonic effect was also significant, with a negative relationship between TAS values and CGAS.
Conclusions: Altogether our results indicates that, in the early stages of the illness, FEP patients with a poorer clinical status have a lower antioxidant capacity but in the long term, this correlation is reversed and their antioxidant defence capacity seems to improve as a compensatory response mechanism of the body. This finding should be seriously considered, as it suggests that the antioxidant treatments currently under study should be only applied during the early stages of the illness or, at least, their long-term use is more questionable.

(T3-16) OSCILLATORY REPRESENTATIONS OF PRE-STIMULUS VISUAL PREDICTIONS IN HIERARCHICAL PREDICTIVE CODING FRAMEWORK

Behavioural and Cognitive Neuroscience

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ABSTRACT

The neural correlates of hierarchical predictive coding or predictive processing (Clark 2013) in visual domain have been largely investigated (Howhy 2013, Clark 2016), however, the effect of time uncertainty in this framework is still unclear. The current state-of-the-art literature investigated the post-stimulus neural correlates which represent prediction errors in the predictive processing framework. In the present study, we evaluate the biomagnetic correlates of visual prediction (focusing on predictions rather than prediction errors) in pre-stimulus brain activity.

Participants were presented with four visual Gabor patches in a serial order (i.e. entrainers) followed by a fifth one (i.e. target) after a certain time lag. The orientation of the Gabor could either change in a gradual (predictable orientation) or a random (unpredictable orientation) manner across entrainers up to the target. In addition, the timing of the entrainers can be either fixed (predictable timing) or variable (unpredictable timing). We evaluated the differential MEG correlates between predictable and unpredictable target orientation when the timing was either fixed or variable.

Twenty-five healthy participants took part in the study. Data were acquired using 306-channel MEG (Elekta, Neuromag) system. Data analyses were performed employing Fieldtrip after Maxwell filtering. The time-frequency estimation was performed using a Hanning taper within a range of 1 to 90 Hz (with a resolution of 1 Hz).

We observed significant positive clusters (unpredictable > predictable when timing is predictable) within beta oscillations (25 - 29 Hz) during the pre-stimulus window (i.e. –400 - 0 ms). This cluster reflects the reduction in power in predictable trials compared to unpredictable trials. We also observed significant positive clusters (unpredictable > predictable when timing is unpredictable) within gamma oscillations (45 - 48 Hz) during the pre-stimulus window (i.e. –400 - 0 ms). This cluster is more anteriorly distributed reflecting the increased utilization of top-down resources. The results reflect significant contribution of beta and gamma oscillations to the visual predictions in predictive processing framework.

(T3-17) HISTONE ACETYLATION AND METHYLATION AT GRM2 PROMOTER IN POSTMORTEM PREFRONTAL CORTEX OF SUBJECTS WITH SCHIZOPHRENIA

Psychiatry > Molecular Psychiatry

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ABSTRACT

Schizophrenia (SZ) is a chronic and incapacitating mental illness with positive (e.g. hallucinations), negative (e.g. apathy) and cognitive symptomatology. Therapeutically used drugs, typical and atypical antipsychotics, are effective against positive symptoms but do not improve the cognitive ones. Actually, atypical antipsychotics have been reported to deteriorate cognition [1,2]. In line with this, metabotropic glutamate receptor 2 (mGlu2R) agonism has been reported to have a

positive effect on cognition [3,4].In brains of SZ subjects, a decrease in mGlu2R protein and in its codifying gene GRM2 expression have been described. This reduction seems to be higher in those SZ subjects that received antipsychotic treatment [5,6]. The mechanism by which chronic atypical antipsychotic treatment induces a decrease in GRM2 expression has been recently described. Briefly, chronic atypical antipsychotics, through 5-HT2A receptor antagonism, increase HDAC2 transcription leading to decreased histone 3 acetylation at GRM2 promoter in PFC of SZ subjects [7,8].
Thus, the aim of this study was to quantify post-translational histone modifications known to correlate with transcriptional activation and repression at GRM2 promoter in PFC of SZ and control subjects.
Twenty two subjects were included in the study: eleven had an antemortem diagnosis of SZ and were matched by age, gender and postmortem-delay to eleven subjects without any history of psychiatric disease. Five of the SZ subjects were under antipsychotic treatment at death and six were antipsychotic-free (positive and negative toxicology in blood, respectively). Epigenetic modifications at GRM2 promoter were determined by immunoprecipitation of native chromatin (ChIP) in postmortem PFC. Briefly, human PFC chromatin was immunoprecipitated with antibodies against permissive H3K4me3, AcH3, AcH3K9, AcH3K27, AcH4K5 and AcH4K16 and repressive H3K27me3 modifications. The immunoprecipitated DNA was then submitted to quantitative real-time PCR (qPCR) for amplification of promoter regions at GRM2. Experiments in SZ subjects and matched controls were always performed in parallel. Statistical analysis to compare epigenetic modifications was performed by one-way ANOVA.
By this research we have shown that the studied epigenetic modifications H3K4me3, H3K27me3, AcH3, AcH3K9, AcH3K27, AcH4K5 and AcH4K16 are all associated to GRM2 promoter in human PFC. Preliminary results do not observe any significant alteration of the studied epigenetic modifications at GRM2 promoter in SZ subjects, possibly due to the low number of samples. However, although non-significantly, we observed a decrease in permissive marks associated to GRM2 promoter in those SZ subjects that did not receive antipsychotic treatment (Δ =24-79%). On the other hand, in antipsychotic-treated SZ subjects acetylation of histones associated to GRM2 promoter was not significantly increased (Δ=20-323%).
Although, non-significantly, our results in PFC of antipsychotic-free SZ subjects point towards to a lower histone acetylation and H3K4me3 at GRM2 promoter gene. This reduction in permissive epigenetic marks may lead to a reduced transcription of GRM2 gene. However, our results in PFC of antipsychotic-treated SZ subjects do not support previously described downregulation of GRM2 gene and protein expression. The divergence between present and published results might be due to the variety of antipsychotic drug treatment found in our samples opposite to the exclusively clozapine-treated SZ subjects used in previous studies. Further studies in order to increase the number of samples belonging to each group, will provide a more real representation of epigenetic regulation at GRM2 promoter in SZ.
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