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PLENARY TALK

Brain mitochondria and metabolism on anxiety, behavioral drive and motivation

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We have recently reported individual differences in mitochondrial function in the nucleus accumbens on the link between stress, anxiety, and social competition. High anxious individuals are highly vulnerable to stress-induced depression. Importantly, high anxious rats display impaired mitochondrial function (respiration, membrane potential, ATP and ROS production) in the nucleus accumbens which is causally implicated in their disadvantage to achieve dominant status. Intra-accumbal infusion of nicotinamide, an amide form of vitamin B3 that boosts mitochondrial function, prevents the development of subordinate status in high anxious rats. Manipulations that modify anxiety levels transiently, such as acute stress or diazepam treatment, modulate social competitiveness and accumbal mitochondrial function. Notably, diazepam treatment when given either systemically or into the ventral tegmental area enhances accumbal mitochondrial respiration and ATP production along with increasing dominance behaviors. Furthermore, dominant mice show higher levels in accumbal energy-related metabolites than subordinate mice, as well as increased vulnerability to show social avoidance following social defeat. In subordinates, but not dominants, levels of these metabolites increase following chronic social defeat stress. Our findings have implications for the understanding of the mechanisms involved in individual differences in motivated behavior and vulnerability to stress.

ORAL PRESENTATIONS

O1.

Effects of the overexpression of IL-6 and IL-10 on the 'do-not-eat-me' signalling after the facial nerve axotomy

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The facial nerve axotomy (FNA) is an experimental useful model to study motor neuron death. After the injury, a slow death process of facial motor neurons is triggered and an innate immune response is concomitantly generated in the facial nucleus, including broad microglial activation. One of the mechanisms that regulate microglial activation is the 'do-not-eat-me' signalling, which consists on the interaction of microglial inhibitory receptors (such as CD200R, CX3CR1 and CD45) with their corresponding ligands in neurons and other glial cells (including CD200, CX3CL1 and CD22, respectively). Research developed in our laboratory showed that after FNA, animals overexpressing IL-6 (GFAP-IL6Tg) had an increase in motor neuron cell death, whereas animals overexpressing IL-10 (GFAP-IL10Tg) presented an increase in motor neuron survival. In both cases, facial motor neurons death was correlated with either a proinflammatory or an anti-inflammatory phenotype of microglia. In this study we aimed to analyse the regulation of 'do-not-eat-me' signalling after FNA and to explore if the overexpression of IL-6 and IL-10 exerted any effect on this signalling. With this purpose, adult GFAP-IL6Tg and GFAP-IL10Tg with the corresponding wild-type were lesioned and sacrificed at the following timepoints: 3, 7, 14, 21 and 28 dpi. Single immunohistochemical techniques were used to detect CD200, CD200R, CX3CL1 and CD45 in the facial nucleus of these animals. The results obtained were analysed under bright-field microscope and the area and intensity of staining were quantified with the AnalySIS program. Our results showed that the 'do-not-eat-me' ligands in neurons, CD200 and CX3CL1, are upregulated after FNA reaching a peak at 7 dpi, followed by a downregulation at 14, 21 and 28 dpi. Likewise, there is an increase of the 'do-not-eat-me' receptors CD200R and CD45 in microglia, macrophages and lymphocytes at early stages, reaching a peak at 7 dpi and decreasing at 14, 21 and 28 dpi. IL-10 overexpression exerts no effects on CD200, CD200R and CD45. CX3CL1, however, is upregulated in neurons at later stages compared to wild-type mice, suggesting a promotion of the neuronal survival. IL-6 overexpression exerts no effects on CD200 expression, but it downregulates the expressions of its receptor CD200R at later timepoints. Furthermore, IL-6 overexpression slows down the increase of CD45 expression in every single time-point after the injury. All these results indicate that after FNA, the 'do-not-eat-me' signals studied (CD200, CD200R, CX3CL1 and CD45) increase their expressions in an attempt to regulate microglial activation. However, at later stages, where the neuronal death peak takes place, there is a significant decrease of these molecules. IL-10 overexpression after FNA do not seem to exert beneficial effects on neuronal survival through CD200, CD200R and CD45, but CX3CL1 could be involved in the outcome. Finally, the increase in neuronal death observed in GFAP-IL6Tg under FNA is not mediated by CD200, but could be possibly related to a lesser increase in CD45, and CD200R downregulation observed at later stages, which could induce a more activated microglia. In summary, exploring 'do-not-eat-me' signals could help to establish the pathways involved in microglia inhibition and regulation, and constitute an interesting target for controlling neuroinflammation.

O2.

Effects of a caloric restrictive diet and a polyphenol in a rat model of induced obesity: impact on sweet preference, taste reactivity and adipose tissues

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Obesity is widely considered one of the major epidemic of modern times. However, much remains to be understood about both its development and consequences. It is believed that obese individuals could develop sugar resistance, and that it could be associated with a high preference for

sweet food and drink. In addition, the reversibility of diet-induced obesity and its consequences on sweet preference when adopting a restricted caloric diet is not known. The present study was aimed to evaluate whether a restricted caloric diet, administered alone or supplemented with a polyphenol, modified sweet taste preference and sweet taste reactivity in rats previously fed with the obesogenic cafeteria diet (CAF). We fed male Sprague-Dawley rats (n = 30) with the cafeteria diet (bacon, carrots, muffins, cookies, pâté, cheese and sugared milk) to induce obesity. A control group (STD; n = 10) was fed with standard chow for the duration of the study. After two months, the cafeteria group was subdivided in three subgroups fed as following: CAF (n = 10), remaining with cafeteria diet ad libitum; CAFr (n = 10), fed with the same cafeteria diet ingredients but placed under a caloric restriction of 30% vs the cafeteria diet; and CAFrO (n = 10), fed with the same restriction as the CAFr group plus an oral treatment of oleuropein (dose of 25 mg/kg/day), a polyphenolic compound found in green olives and olive leaves which we hypothesize should have an effect upon the parameters measured. After three months with the corresponding new diets, the animals conducted the two bottle preference and taste reactivity tests to evaluate respectively sweet preference and the hedonic and aversive behaviors evoked by the consumption of increasingly concentrated solutions of sucrose (0, 0.01, 0.03, 0.06, 0.1, 0.3, 0.6, 1 M). Both behavioral tests were first evaluated in a preliminary experiment to test for gender differences, showing that females reached their consumption peak at 0.1 M (117.3 ± 11.9 g intake/day), whereas males reached their peak at 0,3 M (112.1 ± 5.2 g intake/day). Differences were found in a higher concentration (0,6 M) in which males had a higher sucrose intake than females ($F_{(1, 14)}$ = 5.177; p = 0.039). In the cafeteria diet experiment performed with males, all groups reached their peak intake at 0.3 M, which is consistent with data from the preliminary experiment, with the STD group showing the highest intakes overall. More specifically, the STD intake was greater than CAF at 0.01 and 1 M, than CAFr at 0.01 M, and than CAFrO at 0,6 and 1 M, suggesting a differential effect of the oleuropein treatment with respect to the caloric restriction alone. Data of the taste reactivity test for the second experiment is being analyzed and expected to provide us with a deeper insight on the mechanisms underlying obesity and sugar consumption and resistance.

O3.

Severe cortical affectation after complex I subunit deletion in Cck-expressing cells

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Leigh syndrome, the most frequent infantile mitochondrial disease, causes failure to thrive, ataxia and cognitive decline. A wide range of genetic mutations, in the nuclear or mitochondrial genome, can trigger Leigh syndrome and results in most cases in early death. We generated an animal model for Leigh syndrome lacking a mitochondrial complex I subunit (Ndufs4KO mouse). It recapitulates the majority of the clinical signs present in Leigh syndrome patients, such as: ataxia, growth retardation and early lethality. In these animals, specific brain areas presented progressive neuronal deterioration and gliosis: cerebellum, vestibular nuclei and olfactory bulb. While motor alterations are ubiquitous in Leigh syndrome patients, cortical affectation and cognitive decline are also widely reported. However, the severe phenotype and short lifespan of the Ndufs4KO mice hinders the study of these aspects. Therefore, we set to develop a cortical-centered Ndufs4KO. Given that Cck (cholecystokinin) gene is extensively expressed in the brain cortex, we created an animal lacking Ndufs4 gene in Cck expressing cells (Ndufs4cKO^{Cck} mouse). As expected from a more restricted Cre expression, Ndufs4cKO^{Cck} mice, in contrast to Ndufs4KO, did not present a reduction in lifespan or body weight. Although, they manifested motor coordination problems. Furthermore, gliosis and neuronal death was detected in different cortical areas: motor cortex (M1 and M2), somatosensory cortex (S1 and S2), cingulate cortex (Cg1, Cg2, PrL and IL) and visual cortex (V2ML). Several other brain areas manifested inflammatory response in the absence of overt lesions, like: hippocampus, striatum and thalamus. Detailed behavior phenotyping of these animals is underway to establish the contribution of each brain area to the disease symptoms, providing novel insight on the cellular and molecular mechanisms leading to cognitive decline in mitochondrial disease.

O4.

Kinetic microglia/macrophages travel through hypoxic palisades in glioblastoma multiforme to eventually phagocytose tumoral debris

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Glioma, the most common primary brain tumor remains incurable while its tissular architecture is yet poorly known. However, understanding of the tumors' microanatomy could help elucidate mechanisms implicated in tumor progression and suggest new effective therapies, especially for glioblastoma multiforme, the most aggressive form of glioma. Pseudo-palisades have been classically described as a high density area of glioblastoma multiforme cells escaping from harsh necrotic conditions in their core. Importantly, reports suggest this migration could contribute to the invasion of new brain areas. By analysing human biopsies we spot for the first time a high immune component in palisades. More precisely, we observe tumor-associated microglia/macrophages (TAMs) structurally conforming these palisades. When modelling *in vitro* the hypoxic and hypoglycemic conditions of the palisades with mouse cell lines, we see that hypoxia provokes morphological changes in microglia, adopting an elongated shape and increasing their motility, in contrast with tumor cells. Moreover, by means of confocal microscopy we detect the orientation of TAMs in these structures and we suggest motility mainly towards the necrosis. Reconstructions of these areas indicate that TAMs, probably guided by the tumoral fibers, arrive to the necrotic focus to phagocytose tumoral debris, including GFAP fragments and pyknotic nuclei. Hence, we demonstrate immune cells are able to navigate through pseudo-palisades and become phagocytic when the environment is favorable, therefore shedding light to the understanding of glioblastoma multiforme microenvironments and the network between microglial and tumor cells, approaching the possibility of manipulating TAMs to eradicate the pernicious tumor cells. *This work was supported through grants from the Spanish Ministry of Economy and Competitiveness (RYC2010-06729, SAF-2010-21274, SAF2013-45178-P and SAF-2015-64123-P).*

O5.

Isolation rearing effects and neuroanatomical characterization of the prefrontal cortex of the Roman rats

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The aim of this study has been to further characterize a proposed new animal model for the study of schizophrenia-related symptoms. We have tested the effects of a chronic environmental manipulation such as the isolation rearing of the animals at a behavioural and neuroanatomical level. We subjected the Roman rats to a 12-weeks isolation rearing, which is known to induce and potentiate schizophrenic-like symptoms, and we have observed that, compared to the RLA strain, RHAs display isolation-induced deficits in prepulse inhibition, increased anxiety-related responses, hyperactivity and long-term reference memory deficits. At the neuroanatomical level, we also observed a significant strain effect in volume in certain brain areas such as prefrontal cortex, hippocampus and dorsal striatum. It has been widely acknowledged in the literature that schizophrenia characterizes by a reduction in volume in certain brain areas, including the prefrontal cortex, and while the behavioural profile has followed our initial expectations of more profound deficits induced by social isolation in the RHA-I rats, we have obtained paradoxical results on the volume estimation analysis. However, given the results obtained in which we have seen a difference in volume between the two strains in prefrontal cortex (RHA < RLA), we wanted to address the rationale behind it. In this regard, the quantification of dendritic spine density in pyramidal neurons and parvalbumin neurons, which are thought to be responsible for the differences in volume and are related to the cognitive symptoms of schizophrenia, constitute an important aspect to be taken into account in the characterization of the Roman rats. From the stereological analysis of parvalbumin-expressing neurons we found no differences in number between the two strains of Roman rats, which is in line with the literature. The differences in dendritic spine density are being currently analysed. *Supported by grants PSI2013-41872-P, PSI2017-82257-P, 2017-SGR-1586, 2014SGR-1587, and FI (I.O.), FPI (A.S.G.) and FPU (C.T.E.) fellowships.*

O6.

Implications of local IL-10 overexpression in the neuron-microglia communication and hippocampal neurogenesis during aging

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Hippocampus is one of the brain regions more vulnerable to age-related neuronal deterioration and several studies have shown that neurogenesis in the subgranular layer of the dentate gyrus decreases dramatically with age. The production of new granular neurons plays an important role in hippocampus-dependent memory and learning, being both processes affected in the aging. The process of neurogenesis is intimately affected by the microenvironment, thus, inflammation and microglial activation reduce the promotion of new neural cells. However, depending on the specific microglial activation phenotype, microglial cells can exert detrimental or beneficial functions to the neurogenesis process. This microglial phenotype depends not only from the cytokine environment but also from the communication that they established with neurons and other glial cells. During aging, the brain presents a pro-inflammatory microenvironment with high levels of oxidative molecules and high glial activation. In that way, we expect that in advanced ages this inflammatory microenvironment could produce alterations in the microglia-neuron communication interfering negatively in the neurogenesis process. Then, the objective of this study is to analyse if the overproduction of the anti-inflammatory interleukin-10 (IL-10) in the central nervous system has an impact in the hippocampal neuronal population during physiological aging. For this purpose, we used adult (4-6 months) and aged (18-22 months) mice wild-type and with astrocyte-targeted production of IL-10 (GFAP-IL10Tg). By immunohistochemical analysis we observed a high microglial activation phenotype and microglial cell density in aged GFAP-IL10Tg transgenic mice. Moreover, by flow cytometry we detected a higher number of lymphocytes and CD11b+CD45high microglia/macrophages in the aged transgenic animals respect to aged wild-type animals. Although the total number of hippocampal neurons was not affected neither by the age or the IL-10 overexpression, a reduction of neuroblasts in the dentate gyrus of aged GFAP-IL10Tg mice was observed. This result correlates with a decrease in the spatial learning capacity and memory observed in the aged transgenic mice after a Morris water maze test. Finally, we analysed different microglial receptors involved in the microglia-neuron communication, such as CD200R, CX3CR1 and SIRPα. The disruption of this dialog leads to inflammation and as consequence to a decrease of the neurogenesis. In GFAP-IL10Tg from both ages, the microglial/macrophage population showed an increase of CD200R and a decrease of CX3CR1 expression respect to adult wild-type mice. This pattern of expression was the same in aged wild-type animals. No differences were observed in the SIRPα expression. We also analysed the CD200 and CD47 ligands expression in the hippocampal neurons. Our results showed that approximately half of the total number of neurons expressed CD47 and/or CD200 markers. A decrease in CD200 expression and a tendency to decrease in CD47 expression was observed in both aged animals, although, no effects were found by the overexpression of IL-10. These results indicate that the alterations observed in microglial cells by IL-10 overexpression are associated to inflammatory changes and have an impact in the hippocampal process of neurogenesis during the physiological aging. *This work was supported by Ministerio de Economía y Competitividad (BFU2014-55459 y BFU2017-87843-R).*

O7.

Dissecting a vestibular circuit controlling food intake

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The vestibular system has classically been associated with balance and motion sickness. It is widely accepted that MS develops with the occurrence of neural mismatches between the integrated input of sensory information under motion and the memory of previous experience. Motion sickness is characterized by an unpleasant feeling resulting from an alteration in autonomic functions, such as a reduction in ambulatory activity, food intake and body temperature. Accordingly, vestibular stimulations also induce these autonomic changes, recapitulating motion sickness. However, a genetically-defined, specific vestibular nuclei circuitry involved in the regulation of autonomic functions has not been established. Here, given that glutamatergic neurons in the vestibular nuclei constitute the main cell-type in the region, we have used an optogenetic approach to dissect the vestibular connectome regulating physiological responses, modulating *in vivo* the activity of glutamatergic vestibular somas and select projections in freely-moving mice. Our results demonstrate that stimulation of glutamatergic vestibular neurons is sufficient to reduce motor activity, body temperature and intake of low-palatability food (regular chow). Interestingly, no food intake decrease was observed using highly palatable diet, therefore revealing a motivational component and ruling out physical impairment. Additionally, we define the importance of a glutamatergic vestibulothalamic circuit in appetite suppression. Ongoing analyses will provide the glutamatergic vestibular connectomic definition and address the specific contribution of its projections in regulating motion sickness-related physiological responses. Overall, this data underscores a potential role of the

glutamatergic vestibular connectome in the development of motion sickness.

O8.

Driving outside of the lane. Issues with promoters driving Cre recombinase expression in the central nervous system

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The Cre-lox system is a widely used strategy for generating conditional knock-out mice, and the neuroscience field is no exception. The interest for cell-specific deletion of a gene of interest has led to the use of lines such as glial fibrillary acidic protein (GFAP)-Cre and synapsin 1 (Syn1)-Cre. However, close examination of these lines unveils several issues related to Cre-recombination. Firstly, GFAP-Cre drives recombination in astrocytes, but also in many neurons in the brain. Secondly, both promoters can lead to peripheral tissue recombination of the target gene. Here we present a multifaceted approach to the characterization of these issues to demonstrate that the use of just one of them can lead to incorrect assumptions about the model: conventional polymerase chain reaction to detect the recombined allele for the gene of interest in tail and brain homogenates; flow cytometry to detect Cre protein in different cell populations (astrocytes, microglia or neurons), previously purified using magnetic cell-separation; crossing with the RiboTag reporter mouse in order to identify previous recombination in neurons, astrocytes or microglia.

O9.

Adaptive immune system in Parkinson's disease: the latent foe

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Neurodegenerative diseases have a high and increasing prevalence in our ageing society. Ostensibly, this leads to an important socio-economic burden. General cell-autonomous mechanisms such as oxidative stress, protein degradation machinery impairment and lysosomal dysfunction are implicated in neuronal dysfunction and, at the end, neuronal death. However, little is known and few efforts have been invested in understanding the non-cell-autonomous mechanisms. During the last few years, emerging evidences have suggested the important role of the immune system in this group of disorders. Focusing on Parkinson's disease (PD), a neurodegenerative disorder based on the specific loss of the dopaminergic neurons located in the midbrain region, neuroinflammation has been demonstrated as a keystone for the neurodegenerative progression. However, there is controversy in the role of the adaptive immune system in PD. Although it was initially reported that there was a 10-fold increase of T cell brain infiltration in the substantia nigra *pars compacta* of PD people compared with age-matched healthy controls, it remains controversial due to a novel study in which no differences were observed. Thus, the main goal of this project was to analyze the T cell brain infiltration in human post-mortem tissue of both PD people and healthy controls in order to break through this problematical and hazy question. Moreover, we wanted to reproduce these results in different PD animal models, using them to answer rising questions about their specific function and to describe novel therapeutic targets. In the post-mortem human tissue of PD people we found a T cell brain infiltration in the substantia nigra *pars compacta* as it was previously described. However, these lymphocytes were mainly cytotoxic T cells. This adaptive immune response linearly correlated with dopaminergic cell loss. Then, we lead to study the T cell brain infiltration in two different PD animal models: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated mice and the overexpression of human wild-type α-synuclein in both rats and mice. We found a striking immune response in the overexpression of human wild-type α-synuclein in rats in contrast with the other animal models. In that model, T lymphocyte invasion also correlated with dopaminergic cell loss. From these obtained results, we can conclude that there is an adaptive immune response in PD and, therefore, a therapeutic treatment targeting it could be quite relevant to delay the progression of the disorder.

O10.

Presenilin regulates tau phosphorylation and inflammation during neurodegeneration

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Alzheimer's disease (AD), the most common cause of dementia, is characterized by gradual loss of cognitive abilities, especially memory. AD main pathological features include amyloid-β deposition and hyperphosphorylated tau protein accumulated in neurofibrillary tangles in addition to neuronal loss, neuroinflammation and autophagic dysfunction. Dominantly inherited mutations in the Presenilin (PS) genes, the catalytic subunit of γ-secretase complex responsible for amyloid-β generation, are the major cause of familial AD. Moreover, PS mutations are linked with frontotemporal dementia, which is characterized by accumulation of aggregated phosphorylated tau. At present, the molecular mechanisms by which PS mutations lead to tau phosphorylation are largely unclear. Interestingly, loss of PS function in brain-specific PS1/PS2 (PS) conditional double knockout (cDKO) mice results in increased cerebral tau phosphorylation, neurodegeneration and inflammation. In this work, we studied tau pathology and its relationship with astrocytic and microglial activation, autophagic function and memory in control and PS cDKO mice during aging. Our results show an age-dependent tau phosphorylation in the cortex and hippocampus of PS cDKO mice associated with increased p25/Cdk5 levels, not only in neurons, but also in astrocytes, microglia and oligodendrocytes. This phenotype is associated with increased autophagic, microglial and astrocytic markers. Interestingly, inactivation of PS in human tau transgenic mice results in altered tau pathology and exacerbated memory impairments. Taken together, these results provide evidence that loss of PS function in neurons leads to tau hyperphosphorylation, autophagic dysfunction, brain inflammation and memory loss during neurodegeneration. *This study was funded by grants from Ministerio de Economía, Industria y Competitividad (MINECO) SAF-2016-80027-R and Instituto Carlos III (CIBERNED) CB/06/05/0042.*

POSTERS

Cognition and mental disorders

P1.

Neurobiology of sensorimotor gating deficits in the Roman rat strains: studies of c-Fos expression and COMT activity

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Schizophrenia is characterized by positive, negative and cognitive symptoms, which are associated with anomalies in brain structure and neuronal activity. However, the precise relationship between brain changes and specific clusters of symptoms remains unclear. Prepulse inhibition (PPI) of the startle response is a measure of sensorimotor gating related to cognitive symptoms that is impaired in schizophrenia and animal models of the disease. In this study, we aimed at unravelling the neural circuits involved in the differential PPI levels that characterize the inbred Roman high- (RHA) and inbred low-avoidance (RLA) rat strains. The RHA and RLA rat strains were bidirectionally bred for their good versus non-acquisition of two-way active avoidance, respectively. As a consequence of this selective breeding, both strains present a range of phenotypical differences. In particular, compared with RLA rats, the inbred RHA strain is characterized by several schizophrenia-like phenotypes, such as PPI impairments, cognitive deficits, augmented central dopaminergic tone, increased volume of the lateral ventricles and reductions of hippocampal and medial prefrontal cortex (mPFC) volumes. In the present study, RHA and RLA rats underwent a single PPI session and they were euthanized 2h later to obtain brain samples for the study of c-Fos expression, which is an indirect marker of neuronal activity. Control groups consisted of rats from both strains that were submitted to either background noise ('no-pulse' control group) or startle pulses alone ('startle' control group). Thus, there were three conditions (no-pulse, startle and PPI groups) for each strain. As expected, RHA rats displayed lower PPI than the RLAs. Regarding the c-Fos expression in the mPFC, factorial ANOVA showed 'strain × condition' and 'strain' effects, indicating that RLA rats showed overall higher c-Fos expression. This was especially due to the fact that the different conditions markedly affected the RLA c-Fos activation (i.e. RLA rats in the PPI condition displayed much higher c-Fos activation than the 'no-pulse' and 'startle' control groups: $p < 0.05$ Duncan's test), while experimental conditions did not significantly influence c-Fos expression in the RHA rats (i.e. there were no statistical differences among RHA rats in the PPI condition and the 'no-pulse' and 'startle' control conditions). No between-strain differences in c-Fos activation were observed in the cingulate cortex, nucleus accumbens, striatum, amygdala and hippocampus. To study more specifically the underlying mechanisms of PPI, assays of catechol-O-methyl transferase (COMT) enzyme activity, an enzyme that has been linked to cognitive symptoms of schizophrenia, were conducted. No statistical differences in COMT activity of the mPFC and the hippocampus were found between RHA and RLA rats. The present results of c-Fos expression suggest that a reduction of mPFC activation may be involved in sensorimotor gating deficits. This finding is in line with those reported in schizophrenia patients and lends further support to the construct validity of RHA rats as a model of schizophrenia-related features. *Work supported by PSI2017-82257-P, 2017-SGR-1586 grants, and FPU (C.T.E.), FPI (A.S.G.), and FI (I.O.) fellowships.*

P2.

Schizophrenia-relevant symptoms and antipsychotic/pro-psychotic drug effects in the Roman rat strains

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Up until now, basic research devoted to the study of the psychobiological anomalies of schizophrenia, as well as of its treatments, has used animal models in which some psychotic-like symptoms are induced by psychostimulant drugs (amphetamine, apomorphine). However, there is a need of animal models that present better construct validity, e.g. animal lines spontaneously showing phenotypes associated to the psychotic spectrum (enhanced sensitivity to psychostimulants, attentional and/or cognitive anomalies, increased exploratory activity) and negative symptomatology (anhedonia, social interaction deficits). Several lines of evidence suggest that the RHA-I (inbred Roman high-avoidance) rat strain, which have been psychogenetically selected for their good vs extremely poor acquisition in the two-way active avoidance task in the shuttle box, presents a neurobehavioral profile which is consistent with such goals compared with the RLA-I (inbred Roman low-avoidance) strain. Our results show that the Roman strains differ in sensorimotor gating –i.e. impaired pre-pulse inhibition of the acoustic startle response (prepulse inhibition) levels in RHA-I rats–, as well as deficits in social interaction and spatial working memory (Morris Water Maze) in the RHA rat strain. In order to further characterize the RHA-I strain, pharmacological studies are being conducted. Previous results from our lab show a significant improvement of prepulse inhibition performance with haloperidol (typical antipsychotic) in the RHA but not in the RLA strain. A general deficit of prepulse inhibition has also been observed with administration of MK801/dizocilpine (non-competitive NMDA antagonist), and such a deficit is more clearly attenuated by clozapine (atypical antipsychotic) in RLA-I than RHA-I rats. Moreover, the MK801-induced impairment of social interaction is only present in the RHA strain. Current studies are being carried out to study whether clozapine shows differential between-strain antagonism of the above mentioned MK801 effects. *Work supported by PSI2013-41872-P, PSI2017-82257-P, 2017SGR-1586, 2014SGR-1587 grants, and FI (I.O.) and FPU (C.T.E.) and FPI (A.S.G.) fellowships.*

P3.

Interaction between orexin/hypocretin system and insulin-like growth factor type 1: role in modulation of mood and cognition

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Hypothalamic orexinergic neurons have emerged as an important system in neuroendocrine homeostasis through a broad innervation throughout the brain. Orexins are mainly involved in the sleep/wake cycle, but also in the regulation of mood and cognition, showing a role in different neurodegenerative and neuropsychiatric diseases. On the other hand, our laboratory has demonstrated the protective properties of insulin-like growth factor 1 (IGF-1) through its pleiotropic receptor (IGF-1R), specially promoted by exercise, that has important beneficial effects in mood and cognition along aging. Although, all mechanisms involved in exercise are not completely understood. Orexinergic system depends on an intact GH axis (where IGF-1 is implicated), and regulates it at the same time. Here we show a functional link between IGF-1 and orexin activity in the effects of exercise on cognition, mood and sleep/wake status, along life in mice. Indeed, mice lacking IGF-IR in orexin neurons showed differential mood and cognitive responses to chronic exercise, including reduced running and coping with stress.

P4.

Importance of IL-6 trans-signaling in an animal model of Alzheimer's disease

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Strong evidences support the hypothesis that inflammatory processes are involved in the pathogenesis of Alzheimer's disease (AD). It has been de-

scribed that the cytokine IL-6 and especially its trans-signaling pathway, are involved in several pathologic states, and that blocking IL-6 trans-signaling with sgp130 alleviates many of the detrimental effects of this cytokine, even though its effects on AD remain unknown. In this study, we intend to characterize how IL-6 trans-signaling can modify the behavioural and histopathological phenotype of a mouse model of AD, the Tg2576 mice. Bigenic mice (named Tg2576/GFAPsgp130 mice) were generated with astrocyte-targeted production of human sgp130-Fc and coproduction of the human APP695 with the Swedish K679N/M671L mutation. In 16-17 months old mice, behavioural characterization indicated that blocking IL-6 trans-signaling has little, if any, effects on the hyperactivity, increased exploratory behavior and decreased anxiety elicited by the amyloid cascade. In contrast, it seems to be involved in some aspects of spatial learning and memory. Histopathological analysis evidenced a strong decrease on amyloid plaques in Tg2576/GFAP-sgp130 compared to Tg2576 mice. The findings indicate that blocking of IL-6 trans-signaling with sgp130 may be an effective treatment to alleviate many of the detrimental effects of the general rise on IL-6 that appears in AD. *Canal C & Escrig A contributed equally.*

Molecular basis of CNS function and disease

P5.

Effects of interleukin-6 trans-signalling in a mouse model of Alzheimer's disease and high fat diet-induced obesity

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Alzheimer's disease (AD) is one of the most common neurodegenerative disorders with clear signs of neuroinflammation, including increased interleukin-6 (IL-6) levels. It courses with a progressive cognition impairment due to the extracellular accumulation of β-amyloid protein and intracellular deposits of hyperphosphorylated tau protein. A loss of weight is also present in AD patients, being a big issue for patient's and caregiver's quality of life. Obesity and insulin resistance have been identified as risk factors for AD. Acute hyperinsulinemia produces beneficial effects on cognition, promoting a role in synaptogenesis and synaptic remodelling. However, chronically elevated insulin and insulin resistance down-regulate insulin receptors at the brain, reducing central insulin functions. Chronic inflammation is also present in obesity and IL-6 is one of the implicated cytokines. IL-6 deficient mice develop mature onset obesity, glucose intolerance, insulin resistance and increased liver inflammation in response to a high-fat diet, although contradictory results have been found. IL-6 has a complex signalling pathway, the pro-inflammatory functions are mediated via so called IL-6 trans-signalling, where IL-6 binds to a soluble form of IL-6R to activate gp130. Due to the ubiquitous expression of gp130, each cell in the body can be stimulated by this process. IL-6 trans-signalling pathway is specifically inhibited by the soluble form of gp130, sgp130. The main aim is to study the function of IL-6 trans-signalling in AD and high fat diet-induced obesity. We bred a widely used Alzheimer's animal model, 3xTg-AD (+/+) (APPKM670/671NL, MAPTP301L, PSEN1M146V) with a mouse that produces sgp130-Fc specifically in the central nervous system, GFAP-sgp130 (+/–), to obtain two genotypes: 3xTg-AD (+/–)(–/–), 3xTg-AD/GFAP-sgp130 (+/–)(+/–). We also bred wild-type (WT) animals with GFAP-sgp130 (+/–) to obtain: WT (–/–)(–/–) and GFAP-sgp130 (–/–)(+/–). The study was started with 17-month-old male and female mice. Mice were monitored under control diet for two months, followed by a high-fat diet for two additional months. Weight and energy intake were analysed weekly during all the experiment. Insulin tolerance test and oral glucose tolerant test were performed prior to and during high-fat diet. 3xTg-AD mice showed a lower body weight compared to control mice, when fed the control diet and also with high-fat diet, in both sexes. Blocking of IL-6 trans-signalling showed a sex-dependent effect, decreasing high-fat diet-induced obesity in female 3xTg-AD and WT mice, and the opposite in male 3xTg-AD but not WT mice. In general, insulin sensitivity was higher in 3xTg-AD mice. Blocking of IL-6 trans-signalling did not modify insulin sensitivity in females, but in 3xTg-AD males there was a clear trend to return it to WT levels. 3xTg-AD mice also showed a better response in the oral glucose tolerant test in mice fed the control but not the high-fat diet; blocking the IL-6 trans-signalling had no effect.

P6.

Lack of microglial IL-6 ameliorates the brain's response to traumatic brain injury

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Traumatic brain injury has a relevant impact on the population's health in terms of disability and death. It implies different pathophysiological events at the lesion site, and minutes or days after, secondary injury, changes in intracranial pressure and blood perfusion could take place. Interleukin-6 (IL-6) is a pleiotropic cytokine that controls the immune system and influences the central nervous system both in normal and pathological conditions. IL-6 plays a critical role in the cold injury method, a murine model of traumatic brain injury, characterized by an increment of the blood-brain barrier permeability followed by vasogenic brain edema, and resulting in tissue damage with infiltration and activation of inflammatory cells and cell death. In fact, the inflammatory response is led by cytokines such as IL-6, as findings using *Il6*-deficient mice showed a significantly reduction of glia activation and macrophage recruitment as well as a lower number of neurons. Neurons, microglia, astrocytes and other central nervous system cells synthesize and respond to IL-6. However, little is known about how microglial IL-6 could contribute the pathophysiological response to traumatic brain injury. In this study, we used microglial knock-out *Il6* mice ($Il6^{Mic\Delta}$), by crossing $Il6^{lox/lox} \times CX3CR1^{CreER}$, which is tamoxifen-inducible model, and as a control, their $Il6^{loxflox}$ littermates. One month after tamoxifen injections (1 mg/day for five days), $Il6^{Mic\Delta}$ and $Il6^{lox/lox}$ (n = 4) were lesioned, under isoflurane anesthesia, in the right fronto-parietal cortex with size-controlled dry ice for 30 seconds. Furthermore, sham-operated $Il6^{Mic\Delta}$ and $Il6^{lox/lox}$ mice (n = 4) were also included. One day after, animals were euthanized and the ipsilateral cortex was dissected, snap-frozen in liquid nitrogen and stored at –80 °C. Total RNA was isolated using a kit RNAeasy Mini Kit and its concentration and dye incorporation was measured using a UV-VIS spectrophotometer. Then, hybridization to SurePrint G3 Mouse Gene Expression Microarray (ID G4852B, Agilent Technologies) was conducted following manufacturer's two-color protocol (Two-Color Microarray-Based Gene Expression Analysis v.6.5), and dye swaps (Cy3 and Cy5) were performed for RNA amplified from each sample. Microarray chips were then washed and immediately scanned using a DNA microarray scanner. We used a two-way ANOVA, with genotype and cryolesion as main factors, to detect differentially expressed genes. The differentially expressed candidate genes were additionally filtered with at least a log-2 fold change. Then, we carried out a gene ontology enrichment to identify the functional categories of these differentially expressed genes. We obtained 128 annotated genes significantly affected by the interaction ($p < 0.05$). We generated a heat-map

for the visualization of the gene expression per condition of these genes. Gene ontology analysis showed that cryolesion produced a clear induction of genes involved in immune system processes, inflammatory response, regulation of leukocyte and T cell migration, regulation of mononuclear cell proliferation, angiogenesis and oxidative stress. Microglial IL-6 deficiency in general reverted these responses. Our data provide evidence that microglial IL-6 could have a pro-inflammatory function and might take a decisive role during the first day of pathophysiological events.

P7.

The CREB-regulated transcription coactivator-1 (CRTC1) regulates synaptic plasticity through glutamate receptors

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The capacity of neuronal activity to modify the strength or efficacy in synaptic transmission at preexisting synapses is known as synaptic plasticity. Long-term potentiation is the most studied cellular form of synaptic plasticity and considered the molecular basis of learning and memory. Activation and synaptic recruitment of glutamate N-methyl-D-aspartate receptors (NMDARs; GluN) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs; GluA) in the hippocampus play key roles on synaptic plasticity and memory. Activity-dependent gene expression mediated by synapse-to-nucleus signaling plays also a central function in long-term synaptic plasticity and memory by linking activation of synaptic NMDA receptors to gene transcription at the nucleus. How synapse-to-nucleus signaling mediates potentiation of GluNs back at stimulated synapses is still unclear. In this study, we used biochemical and cellular techniques and gain and loss of function gene expression approaches to elucidate the role of the synapse-to-nucleus factor CREB-regulated transcription coactivator-1 (CRTC1) in synaptic plasticity and glutamatergic neurotransmission. Our results show that CRTC1 plays essential roles on long-term memory and dendritic spine morphology favorable to enhance synaptic plasticity. We found that CRTC1 overexpression or silencing does not affect levels of total and phosphorylated GluA1 and GluA2 subunits in the adult mouse hippocampus. By contrast, CRTC1 increases GluN1 subunit phosphorylation, but not total levels, and CRTC1 silencing reduces total and phosphorylated GluN1 levels. These results indicate that CRTC1-dependent synapse-to nucleus signaling regulates structural synaptic plasticity by acting on excitatory glutamate receptors. *This study was supported by grants from Ministerio de Economía, Industria y Competitividad (MINECO) SAF2016-80027-R and Instituto Carlos III (CIBERNED) CB/06/05/0042.*

P8.

Expression analysis of aging-suppressor factors in cortex and hippocampus from mouse models of neurodegenerative diseases

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α*Klotho* (KL) is a gene expressed mainly in proximal tubules in the kidneys and choroid plexus of the brain. It is composed by five exons, presenting two major splicing variants: a long one that transcribes for a transmembrane protein (m-KL) composed by two similar extracellular domains (KL1 and KL2), and a short one which generates a secreted protein (s-KL), containing just KL1 domain and a short 5'end specific sequence. *Klotho* anti-aging properties were discovered when mutations reducing this gene expression generated an early-aging phenotype in mice. Interestingly, *Klotho* expression was seen to be decreased during non-pathological aging and during Alzheimer disease's progression. Symptoms of Alzheimer disease's mouse model were improved after a *Klotho* overexpression treatment in the brain using gene therapy, showing *Klotho*'s therapeutic potential. To date no KL receptor has been identified for s-KL, and little is known about which domain/isoform of the protein is involved in the antiaging properties. Expression of other genes implied in aging-suppression events were also studied, both implied in *Klotho* signalling pathway, like *FOXO3* or *PPARγ*, or acting though other yet unknown mechanisms. Both s-KL and m-KL transcripts were analysed to detect possible changes specifically in one of the isoforms, which could be disease-specific. Gene expression was studied in cortex and hippocampus samples obtained from mouse models for the study of different neurodegenerative diseases. Expression of different genes was seen to be altered mainly in hippocampus of the studied mouse models, whereas *Klotho* isoforms and *FOXO3* presented higher changes in the cortex area. Some of the expression changes could be related to the symptoms observed in these mouse models, and could also be explained as compensatory mechanisms to decrease stress generated by the pathology. *This project was supported by the 'Plan Nacional I+D+I 2013-2016, Instituto de Salud Carlos III-Subdirección General de Evaluación y Fomento de la Investigación, cofinanced by Fondos FEDER (the European Regional Development Fund) (grant numbers ISC-III PI15-01270); and by the 'Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) of the Generalitat de Catalunya, Spain (grant number LLAVOR 2016LLAV00033).*

P9.

Molecular basis for cannabinoid CB1 receptor activation, agonist binding and lipid allosteric modulation

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Understanding the molecular mechanisms of G protein-coupled receptor (GPCR) function is a major goal for the improvement of drug design. In fact, GPCRs constitute the target of one third of currently marketed drugs. In recent years, molecular dynamics simulations have become a powerful tool to analyze transient and intermediate states of the receptor that are not easily observed by experimental methods. Here, we use published X-ray crystal structures of cannabinoid receptor 1 (CB1) to study its activation mechanism by performing long-timescale molecular dynamics simulations, totaling 36 μs, starting from the inactive conformation of the CB1 receptor bound to potent and efficacious agonists (CP-55940 and HU-210). Receptor activation was characterized by the approach between Y294(5.58) and Y397(7.53) and by the rigid body movement of transmembrane helix 6. A higher efficiency in receptor activation was observed for CP-55940, which triggered the W356(6.48)/F200(3.36) rotameric switch and thus facilitated the breakage of interactions between transmembrane helices 3 and 6. The observed receptor conformational changes allowed Gs protein docking although, due to the lack of this signaling protein in the molecular dynamics systems, fully active-like states of the receptor were not stabilized. Additionally, the possible presence of membrane lipid-mediated allosteric modulation was evaluated for the net negatively charged 1,2-dioleolyl-sn-glycero-3-phosphoglycerol (DOPG), which has been reported as a positive allosteric modulator of the homologous β_2-adrenergic receptor. DOPG seemed to exert a weak positive allosteric effect in CB1 receptor activation through the stabi-

lization of intracellular loop 3 in an outward conformation, which may have favored the outward movement of transmembrane helix 6. We expect that our results may provide a useful basis for drug design and optimization.

P10.

In silico investigation of the allosteric modulation of adenosine A2A receptor by phospholipids

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G protein-coupled receptors (GPCRs) regulate many cellular responses and they are one of the major targets for currently marketed drugs. Molecular dynamics simulations have been successful in providing accurate molecular features of GPCR conformational space. Currently, the common behaviour of the majority of GPCRs, when they are simulated using molecular dynamics simulation, is to fluctuate between its inactive and intermediate(s) states and not been able to stabilize or induce the active conformation state. Therefore, one of the current challenges in molecular dynamics simulations is to understand the molecular basis for the agonist-induced transition of membrane receptors into the active state. Interestingly, a recent experimental study pointed to negatively charged lipids as allosteric modulators of the β_2-adrenergic receptor, stabilizing the active conformation and facilitating the activation of the agonist-bound receptor, while zwitterionic lipids inactivate the receptor. These differences were suggested to be related to lipid headgroup charge distribution and hydrophobicity, and may be important to uncover the GPCR activation process. The adenosine A2A receptor (A2AR), a class A GPCR, has attached significant attention regarding ligand binding and activation process. On the one hand, molecular dynamics simulations have shown that the active state of the agonist bound-receptor cannot be fully achieved. On the other hand, it has been suggested that A2AR can be modulated by lipids. Protein-lipid interactions seem to play an important role in the modulation of this receptor. The protein-lipid interactions mainly involve H-bond formation (or lack thereof) between the protein and phospholipid headgroups. To accurately examine the process of activation in A2AR, we performed microsecond time scale of molecular dynamics simulations starting from the inactive conformation with the crystalized endogenous ligand adenosine embedded in two homogenous lipidic environments: 1,2-dioleoyl-sn-glycerol-3-phosphoglycerol (DOPG) and 1,2-dioleoyl-sn-glycerol-3-phosphocholine (DOPC). The 'ionic lock' (a receptor feature associated to the inactive receptor state) broke during the simulations when the receptor system included an agonist and DOPG lipid, which show the relevance of allosteric lipid effects in receptor activation.

P11.

Cell-specific mitochondrial proteomic analysis in a mouse model of mitochondrial disease

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Leigh syndrome (OMIM #256000) is the most common pediatric presentation of a defined mitochondrial disease. This progressive neurodegenerative disorder is characterized by a rapid deterioration of cognitive and motor functions, in most cases resulting in death due to respiratory failure. To date, no general curative treatment is available for this devastating disorder. Strikingly, mitochondrial disease neuropathology presents a remarkable selectivity for certain neuronal populations, which may likely be driven by differential mitochondrial protein content in neurons. Using a mouse model of mitochondrial disease that is a correlate of the human Leigh syndrome, we isolated cell-type specific intact mitochondria and protein alterations were estimated by using protein- and peptide-based approaches and LC-MS/MS methods. These novel approaches will have a high impact in mitochondrial diseases, with the overarching goal of identifying novel therapeutic targets that may lead to effective treatments for mitochondrial diseases.

P12.

Deciphering neuronal contribution in mitochondrial disease

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Leigh syndrome (OMIM #256000) is the most frequent pediatric mitochondrial disorder characterized pathologically by the presence of bilateral symmetrical lesions in the brainstem and basal ganglia. Animals lacking the complex I subunit Ndufs4 recapitulate the classical signs of Leigh syndrome, presenting ataxia, motor dysfunction, reduced body weight, breathing alterations, seizure activity and premature death. Our previous results have shown the primary role of the central nervous system in the pathology. Accordingly, Ndufs4-deficient mice (Ndufs4KO) present prominent brain lesions and gliosis in discrete regions, namely the olfactory bulb, cerebellum, inferior olive and vestibular nucleus. To define the molecular identity of the susceptible neuronal populations we generated animals lacking Ndufs4 in glutamatergic (Vglut2-cre, Ndufs4KO mice) or GABAergic neurons (Gad2-cre, Ndufs4KO mice). With this approach we have been able to dissect specific roles for these cell types in the development of the fatal phenotype of Ndufs4KO. While Ndufs4 deletion in either neuronal population leads to reduced body weight and a drastic reduction in the lifespan of mice, histopathological and clinical alterations are markedly different. Ablation in glutamatergic neurons (Vglut2-cre, Ndufs4KO mice) leads to brainstem inflammation, reduced motor activity and respiratory deficits. On the other hand, animals with deletion in GABAergic neurons (Gad2-Cre, Ndufs4KO mice) present inflammation predominantly in the basal ganglia without respiratory and motor affectation, but accompanied by epileptic episodes resulting in a premature death and higher sensitivity to thermal-induced seizures. This work provides a cellular dissection of the neuropathological mechanisms of Leigh syndrome.

P13.

Influence of MT-1 and MT-2 metallothioneins in the experimental autoimmune encephalomyelitis

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Metallothioneins (MTs), constitute a superfamily of proteins unusually rich in cysteine residues and high content of metals, implicated in metal homeostasis, transport and storage of essential heavy metals (zinc and copper) and detoxification of non-essential ones (cadmium or mercury). In mammals, MTs are divided in four subfamilies, with a single member per subfamily in the mouse, MT-1 to MT-4 isoforms, constituted by 61 to 68 amino acids. MT-1/2 are widely expressed and highly induced in a coordinated manner by stimuli such as metals, hormones, cytokines, inflammation and stress. Essentially both isoforms have been considered physiologically equivalent but recent studies based in metal binding abilities rather than structure, indicated that MT-1 and MT-2 could differ in biological functions. MT-1&2 KO mice (given the lack of mutants for either MT-1 or MT-2), provided relevant information about the antioxidant, anti-inflammatory and neuroprotective effects of these isoforms in brain injury and neurodegenerative diseases animal models, such as, experimental autoimmune encephalomyelitis (EAE), a well-known model of multiple sclerosis. In the present study the exogenous admin-

istration of MT-1 and MT-2 isoforms in EAE mice, revealed that MT-2 had a robust effect reducing the severity of the symptomatology and increasing the grade of remission of the disease compared to vehicle group. Mice injected with MT-1 showed similar but not as potent an effect as with MT-2 isoform. In line with clinical evaluation, histological analysis showed significantly less demyelination and less load of CD3+ infiltrates in MT-2 injected mice group. Furthermore, MT-2 had increasing levels of anti-inflammatory cytokines in splenocyte cultures stimulated with MOG35-55. However, at peripheral level, MT-1-treated group significantly decreased most of the analysed cytokines in the serum. In conclusion, the results suggest that MT-1, but principally MT-2 isoform, are involved in the recovery of an inflammatory process, moderating the demyelination and activating predominantly anti-inflammatory cytokines.

P14.

Interleukin-4 and interleukin-13 induce different transcriptomic profiles in macrophages and microglia after spinal cord injury

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Spinal cord injury elicits an inflammatory response produced mainly by peripheral blood-derived macrophages and microglia. These two cells can exert detrimental or beneficial role depending on their activation state. Pro-inflammatory macrophages and microglia are related with cytotoxicity and tissue damage. In contrast, anti-inflammatory macrophages and microglia are associated with repair and remodeling events after injury. Unfortunately, macrophages and microglia acquire a pro-inflammatory phenotype after spinal cord injury. Approaches aimed at driving macrophages and microglia towards an anti-inflammatory state are expected to minimize secondary tissue damage and functional impairments. Interleukin-4 (IL-4) and interleukin-13 (IL-13) are two cytokines that promote an anti-inflammatory phenotype of macrophages and microglia *in vitro*. When they are injected into injured spinal cord they increase the levels of the anti-inflammatory markers arginase-1 and CD206 in macrophages and microglia. However, only IL-4 improves the functional recovery. To explain these differences at functional level, we performed a cell-specific RNA sequencing study. We compared the transcriptome of macrophages and microglia treated with IL-4 or IL-13 after injury. We found more than 400 genes differentially expressed between these two treatments. In microglia, most of these genes were related to metabolic reprograming whereas in macrophages most of these genes were related to binding capacities. According to these results, IL-4 induces a switch in the metabolic profile of microglia, promoting oxidative phosphorylation. In the case of the macrophages, IL-4 promotes cell adhesion. The more efficient metabolism and the improved adhesion could explain the differences observed in the locomotor recovery. We also found that IL-4 induces a broad number of genes related with embryonic microglia. Altogether, these results show that the classical markers used to identify anti-inflammatory macrophages and microglia do not extrapolate to neuroprotection and functional recovery. The metabolic profile and the adhesion capacities should be taken into consideration as potential markers for anti-inflammatory microphages and microglia. Wide-analysis tools such as RNA sequencing are determinant to study these characteristics.

P15.

A caloric-restricted diet and polyphenol-supplementation based approach to revert obesity in a rodent model

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Obesity has become a worldwide health problem. Therefore, its treatment has become a priority for public health systems and a challenge for science. Leptin has emerged as a major regulator of energy balance through its actions in the hypothalamus. Furthermore, this adipokine seems to be involved in the dysregulation of sensorial and reward brain systems occurring during obesity, such us sweet taste and preference. Some of the most realistic treatments for obesity pathology is the administration of low-caloric diets and the use of specific bioactive compounds. The aim of our study was to develop an intervention based on a caloric-restricted diet and, in addition, to analyse the possible effects of the supplementation with a natural bioactive compound named oleuropein on that restricted diet. Oleuropein is a polyphenolic compound obtained from green olives and olive leaves and it has been previously described to have significant effects on body weight loss and adipose tissue mass. We used 40 male Sprague-Dawley rats aged 21 days at the start of the experiment. The animals were divided in two diet groups for eight weeks: a standard (STD) group (n = 10), fed with standard chow ad libitum, and a cafeteria diet (CAF) group (n = 30), fed with cafeteria diet (composed by: bacon, carrots, muffins, cookies, pâté, cheese and sugared milk) ad libitum in order to induce obesity. After eight weeks, the CAF group was divided into three subgroups (n = 10 per group) until the end of the experiment at week 22: the CAF group remained with the same diet; a cafeteria restricted-diet group (CAFr), fed a 30% of caloric-restricted diet; and the oleuropein group (CAFrO), fed the restricted diet supplemented with oleuropein 25 mg/kg/day. Several biometric parameters were measured in order to characterize the effects of the dietary intervention. Moreover, a set of plasmatic parameters are being performed from blood samples to determine the concentrations of glucose, triacylglycerides, total cholesterol, non-esterified fatty acids, insulin-resistance, leptin and adiponectin. As expected, CAF diet induced a significant increase of body weight and body weight gain compared to STD diet. Also, both CAFr and CAFrO interventions induced a soft increase of body weight. Adiposity levels were higher due to the CAF diet, whereas in the restricted-diet groups they were intermediate between those in CAF and STD groups. Regarding food intakes, both CAF and CAFr diets induced a significantly higher caloric intake compared with the STD diet. In addition, the protein and simple sugar intakes were also higher in these groups than in the STD. CAF diets induced a higher interscapular brown adipose tissue mass and also higher subcutaneous and visceral white adipose tissues. The CAFr diet reduced the visceral fat compared to the CAF diet. Lastly, oleuropein supplementation did not exert any additional effect over caloric restriction alone on those parameters. The analysis of all the parameters obtained during the experiment is expected to provide us a deeper insight on the mechanisms underlying obesity and dietary intervention.