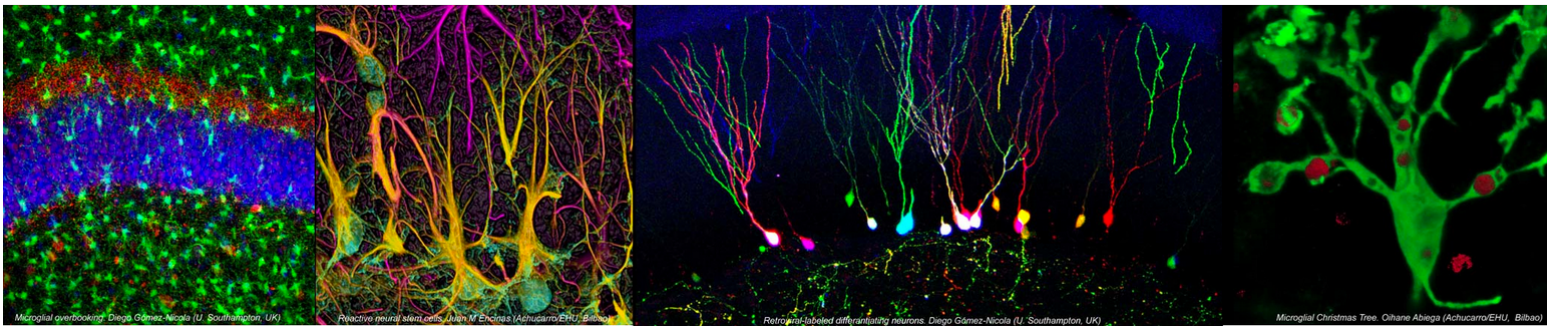

XI meeting of the Red Glial Española

3rd of November

Lleida 2021

RED GLIAL ESPAÑOLA
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XI meeting of the Red Glial Española
Lleida 2021

Wednesday 3rd of November (Secondary Room 1)

8:30-8:45 Welcome and inauguration.

8:45-09:30 Oral presentations.

8:45 ***Valerié Petegnief*** *(IDIBAP):* Characterization of microglia diversity through live cell image analysis.

9:00 ***Javier Palazuelos*** *(UCM):* CB1 receptors deficiency in oligodendrocyte precursors disrupts postnatal oligodendrogenesis and causes hypomyelination in mice.

9:15 ***María Gamarra*** *(Achucharro Center):* Contribution of astrocytes to local translation in neurons.

9:30-10:00 VI Laia Acarín Award: ***Beatriz Fernández-Gómez*** *(Hospital Nacional de Parapléjicos- SESCAM):*

Myeloid-derived suppressor cells support remyelination in a murine model of multiple sclerosis by promoting oligodendrocyte precursor cell survival, proliferation, and differentiation.

10:00-11:00 Plenary Lecture **Dr. Maarten Kole** (*Netherlands Institute for Neuroscience):*

Interneuron myelination, same wrapping but different function.

11:00-11:30 Coffe break.

11:30-12:30 Poster session (*Exhibition Hall*).

12:30-13:00 Assembly.

Venue:

Palau de Congressos de Lleida "La Llotja" Av. de Tortosa, 6-8, 25005 Lleida. Tel. 973221155.

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Leyre Mestre, Instituto Cajal (CSIC)

ORAL PRESENTATIONS

CO1. CHARACTERIZATION OF MICROGLIA DIVERSITY THROUGH LIVE CELL IMAGE ANALYSIS

V. Petegnief[1], A. Martinez[1], A. Bosch[2], M. Calvo[2], C. Tischer[3] , J.K. Hériché[3] & A.M. Planas[1].

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Microglia are very sensitive to changes in the environment and respond through morphological transformation, phagocytosis and metabolism adaptations. The characterization of microglia heterogeneity is still a challenge since microglia can adopt multiple morphologies and it is not easy to categorize them, particularly *in vitro*. Though it is well-known that morphology is usually related to function, we are still unable to interpret the meaning of a change in shape. In order to depict microglia behavior in healthy and pathological conditions, we developed image analysis programs to quantify neuronal death, microglia morphologies and phagocytosis. Primary mice neuron-glia cultures, in which microglia express the tdTomato protein, were exposed to excitotoxic or excitotoxic+inflammatory challenges and analyzed 8h later in time-lapse acquired in a confocal microscope. Neuronal death was assessed by SYTOX staining of nucleic debris and phagocytosis through the engulfment of green SYTOX positive particles in red microglia. We identified 7 morphologies (amoeboid, hypertrophic, fried-egg, bipolar and 3 “inflamed” morphologies) and found the morphometric features able to describe them. Through machine learning, we generated a classifier able to separate them and assign one of the 7 classes to microglia in sample images. In control or excitotoxicity-treated cultures, ameboid and hypertrophic morphologies were the most abundant and did not show changes in the distribution of the populations, or in phagocytosis. Conversely, excitotoxicity+inflammation decreased the amoeboid and hypertrophic populations, induced the appearance of inflamed morphologies and significantly increased the percentage of phagocytosing microglia. Our data suggest that *in vitro* accumulation of dead cells is not sufficient at least in our model to significantly modify microglia behavior at early time-points (up to 12h) and that inflammation is critical to promote phenotypical changes in microglia. The tools we generated can be useful to correlate microglia behavior with environmental changes and characterize the phenotype of disease-associated microglia.

Acknowledgment: This project is funded by a MICINN grant (PID2020-113202RB-I00) and has received funding from the European Union’s Horizon 2020 research and innovation program under grant agreement No 654248.

CO2. CB1 RECEPTORS DEFICIENCY IN OLIGODENDROCYTE PRECURSORS DISRUPTS POSTNATAL OLIGODENDROGENESIS AND CAUSES HYPOMYELINATION IN MICE

T. Aguado, A. Sánchez de la Torre, A. Huerga-Gómez, S. Santamaría, J.C. Chara, C. Matute, K. Monory, B. Lutz, S. Mato, M. Guzman, I. Galve-Roperh & J. Palazuelos

Complutense University Biochemistry and Molecular Biology.

Exogenous and endogenous cannabinoid molecules have been shown to modulate oligodendrogenesis and developmental myelination. However, the cell-autonomous action of these compounds on oligodendrocyte precursor cells (OPC) in vivo has never been explored. Here, by using OPC-specific genetic mouse models we show that selective CB1 cannabinoid receptor depletion in OPC prevented cell differentiation and perturbed oligodendrogenesis and postnatal myelination. Moreover, early postnatal CB1 depletion in OPC caused hypomyelination and motor alterations at adult ages in mice. Conversely, CB1 receptor pharmacological activation promotes oligodendrocyte development and CNS myelination in wild type but not in OPC-CB1 -null mice. Overall, this study addresses a cell-autonomous role for CB1 receptors in OPC modulating oligodendrogenesis that may help in understanding the complex network of signaling molecules that drives CNS myelination

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CO3. CONTRIBUTION OF ASTROCYTE EXTRACELLULAR VESICLES TO LOCAL TRANSLATION IN NEURONS

M. Gamarra[1,2], E. Gonzalez[3], M. Azkargorta[3], J.M. Falcón[3,4], F. Elortza[3], J. Baleriola[1,2,4]

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Local protein synthesis is a conserved mechanism by which mRNAs are localized to the cell periphery and proteins are synthesized at target sites. Local translation is especially relevant in polarized cells like neurons so that neurites can rapidly react to changes in their environment. For instance, the exposure of isolated axons to β-amyloid oligomers (Aβo), central to Alzheimer's disease, induces local protein synthesis and mediates neurodegeneration contributing to the disease. However, axons are not isolated in the nervous system but surrounded by other compartments or non-neuronal cells. Our laboratory is interested in the contribution of glial cells to local translation in neurons. Others have reported that extracellular vesicles (EVs) secreted by astrocytes are involved in the regulation of different neuronal functions. Based on these data, we hypothesize that astrocyte-derived EVs are delivered to neurons to modulate local protein synthesis in physiological and Aβo-induced conditions.

To assess the relevance of astrocytes in the neurons local proteome, we isolated somata and neurites from primary cortico-hippocampal neurons cultured in Boyden chambers in absence/presence of astrocytes and analysed the extracted proteins by LC-MS/MS. Results show that the presence of astrocytes in control conditions changes the neuritic proteome. Gene Ontology analyses show that proteins significantly regulated in the presence vs absence of astrocytes are mainly involved in RNA binding, processing and translation. In Aβo-induced conditions, astrocytes also change neuritic proteins compared to only-neuron cultures, with translation-involved proteins among them. To determine whether these proteins are locally synthesized in neurites, we have selected 176 and analysed their corresponding transcripts in somatic and neuritic compartments.

We have also assessed if EVs are directly involved in translation regulation. Isolated EVs from neuron-astrocyte cultures increase translation levels in neurites, suggesting that EVs are relevant for local protein synthesis in neurons. We are deeply studying EVs by LC-MS/MS to search for translation regulator.

Altogether, our data provide a new mechanism of local RNA translation regulation in which astrocyte-derived EVs could play an important role.

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POSTERS

A01. CORTICAL ASTROCYTES EXHIBIT FUNCTIONAL HETEROGENEITY TO DISCRIMINATE SENSORY MODALITIES

C. Miguel-Quesada*, M. Zaforas, S. Herrera, E. Fernández-López, E. Alonso-Calviño, J. Aguilar, JM. Rosa.

Experimental Neurophysiology and Neuronal Circuits Group, Hospital Nacional de Parapléjicos, SESCAM. Toledo. Spain.

Layer-specific activity of cortical circuits critically depends on the interplay among distinct cell types to shape sensory stimulus selectivity and control gain. In this complex network, astrocytes are crucial as they can sense the synaptic activity within the layering circuits to modulate the strength and timing of both excitatory and inhibitory neurons. However, cortical laminar distribution of astrocytes does not correspond to the six excitatory neuronal layers, which leads to the question on whether astrocytes across cortical layers may exhibit functional heterogeneity or engage in different interactions with neighbouring neurons to modulate neuronal dynamics of sensory processing. Here, we used a combination of in vivo electrophysiology, behavior and genetic tools to explore the layering functional organization of astrocytes as well as their ability to modulate the layer-specific neuronal circuitries. By using brain slices injected with the AAV5-gfaABC1D-cyto-GCaMP6f, we found that astrocyte activity is inherently distinct across cortical layers, with L2/3 astrocytes being less excitable and L5/6 exhibiting higher number of spontaneous oscillations. To determine whether such astrocyte functional diversity play a role in the neuronal sensory processing and stimulus selectivity, we used in vivo electrophysiology to record neuronal evoked-potentials across all layers and sensory behavioral tests while modulating astrocyte activity. Up-regulation of astrocyte activity using GFAP-hM3D(Gq)- DREADD decreased evoked-potential magnitudes in response to high-stimulus intensity in L4/5/6 neurons, which was accompanied by an increase threshold of paw withdrawal following thermal stimulation. On contrary, astrocytes down-regulation using IP3R2-/- mice line decreased L2/3/4 evoked-potential in response to low intensity stimulation and consequent increased threshold in response to tactile stimuli. Therefore, our data indicates that astrocytes work as a buffer of neuronal activity by plausible controlling E:I balance in a stimulus-dependent manner. In addition, astrocyte functional heterogeneity may serve to control stimulus sensitivity in a layer-dependent manner with consequences in behavior output.

A02. DIFFERENTIAL EFFECTS OF PARAQUAT IN HUMAN AND MOUSE ASTROCYTE'S MEMBRANES.

L. Sánchez-Sánchez[1,2], E. Astigarraga[1], D. Sánchez[2], M.D. Ganfornina[2], G. Barreda-Gómez[1].

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During last decades it has been observed an increasing prevalence in ageing concomitant diseases; a longer life expectancy due to an improvement of life conditions is the main factor of this augmentation. As astrocytes are responsible for proper neuronal function, their importance is clear to the maintenance of brain health. Metabolism homeostasis alterations, such as oxidative stress, can trigger these conditions, producing cellular pathological changes as lipid peroxidation, oxidative modification of proteins and DNA damage.

One of the main mechanisms involved in cellular oxidation is Reactive Oxygen Species (ROS) formation, key deregulators of cellular stability. ROS can be produced by mitochondrial electron transport chain dysregulation by external pro-oxidant compounds action, such as Paraquat.

To study sex influence, species and paraquat exposure on the activity of mitochondrial respiratory chain in astrocytes of different species, we developed microarrays using cell membranes isolated from a human astrocytic cell line (1321N1) and primary cultures of male and female mouse astrocytes. Subsequently, the superoxide formation capacity of each sample was determined on cell membrane microarrays using complex I substrate (NADH) combined with specific inhibitors of mitochondrial complex I (rotenone), complex III (antimycin A) and complex IV (azide). Paraquat treated astrocytes showed a higher superoxide formation when compared with control group, not only in basal condition but also in presence of respiratory inhibitors. A significant difference between male and female was also observed in mouse astrocytes primary cultures, while no difference was detected between human and mouse in male astrocytes.

Furthermore, the difference seen between control and paraquat-treated human astrocytes was not only due to mitochondrial electron transport chain, but also other NADH oxidoreductases might be implicated in ROS generation. Thus, further investigation with specific protocols will be necessary to elucidate it**.** How astrocytes manage oxidative stress and ROS formation is central to their neuroprotective responses.

A03. GENERATION AND CHARACTERIZATION OF HUMAN PLURIPOTENT STEM CELL (HPSC)- DERIVED ASTROCYTES TO MODEL ALZHEIMER´S DISEASE

M. Alfonso-Triguero[1,2, †], J. Cruz-Sesé[1,2, †], N. Galbis-Gramage[1], I. Jiménez-Ridruejo[1], E. Alberdi[1,2,3], A.M. Arranz[1,4]

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[2]Department of Neurosciences, Universidad del País Vasco (UPV/EHU), 48940 Leioa, Spain
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† These authors contributed equally.

Alzheimer disease (AD) is characterized clinically by memory loss and pathologically by amyloidβ (Aβ) accumulation, neurofibrillary tangle formation, extensive neuroinflammation, synaptic toxicity and neurodegeneration. Recent studies highlight the importance of glial cells on the pathogenesis and progression of AD. Among glial cells, astrocytes are fundamental for maintaining homeostasis and protecting neurons but, under different pathological conditions, when stimulated by specific factors, acquire different activation states that can be protective or harmful. While it is well established that astrocytes undergo profound alterations in gene expression, morphology and function during the course of AD, such changes are still poorly defined and mostly unknown in the case of human astrocytes.

To analyze human astrocyte reactive states in the context of AD, we are using the stem-cell technology to generate astrocytes derived from human pluripotent stem cells (hPSCs) and in vitro models of AD in which astrocytes are exposed to various Aβ challenges. Human astrocyte identity as well as reactivity after Aβ stimulation are being characterized at molecular and functional levels with various assays.

hPSC-derived astrocytes at day in vitro 90 express main markers of astrocytes (GFAP, S100, EAAT1, EAAT2, Vimentin and AQP4) without expression of neuron (MAP2) and oligodendrocyte (O4) markers. After stimulation with oligomeric Aβ, hPSC-derived astrocytes show a reactive profile that we are characterizing. In sum, our approach allows exploration of the human astrocyte reactivity on an AD context and will provide insights into the contribution of astrocytes to the pathophysiology of AD.

A04. ASTROCYTIC NETWORK HETEROGENEITY IN THE NUCLEUS ACCUMBENS

I. Serra, J. Esparza, C. Martín-Monteagudo, M. Navarrete

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Astrocytes have been traditionally studied as a homogeneous group, however, recent research has started to evidence their heterogeneity between different brain areas and within the same region. Our hypothesis is that specialized astrocyte subsets are responsible for the modulation of specific neuronal circuits. In the NAc converge different glutamatergic signals coming primarily from the medial prefrontal cortex (mPFC), basolateral amygdala (Amyg), and ventral hippocampus (vHip), providing us the perfect structure to study the presence of specialized astrocytic networks.

In this work, we analyze whether astrocytes establish segregated populations in the NAc with intrinsic properties and functional consequences for the circuit. To this end, we have used optogenetic manipulations to perform afferent-specific synaptic stimulation to the NAc, combined with a new adapted technique (CaMPARIGFAP, calcium-modulated photoactivatable ratiometric integrator under GFAP promoter) to specifically dissect the active astrocyte circuits with spatio-temporal precision.

We demonstrate that NAc astrocytes show pathway-specific interactions with the glutamatergic afferents coming from the mPFC, Amyg, and vHip, and that this activity unexpectedly does not correlate with glutamatergic innervation patterns, suggesting astrocytic connectivity, i.e. activation of a precise astrocytic population in response to specific glutamatergic inputs. Moreover, the activation of these defined spatial astrocytic networks are not influenced by alterations in astrocyte density or by uneven expression of mGluR5. Finally, we show that different sub-populations of astrocytes in both NAc regions receive and integrate signals arising from all the excitatory afferents. This work reveals astrocytic functional heterogeneity in the NAc regarding glutamatergic signaling, showing pathway-specific astrocytic responses mediated by mGluR5. Also, all these observations provide a potential explanation for comprehension of how NAc integrates information from multiple glutamatergic regions.

Supported by: Ministry of Science and Innovation (RYC-2016-20414 and RTI2018-094887-BI00) and Fondo Europeo de Desarrollo Regional (FEDER)

A05. *IN VIVO* ASTROCYTE ACTIVATION MODULATES SPONTANEOUS INHIBITORY ACTIVITY DURING SLOW WAVE OSCILLATIONS IN THE SOMATOSENSORY CORTEX

S. Herrera-Pérez *, **C.** Miguel-Quesada*, M. Zaforas, E. Alonso-Calvino, E. Fernández-López, J. Aguilar, J.M. Rosa.

Experimental Neurophysiology and Neuronal Circuits Group, Hospital Nacional de Parapléjicos, SESCAM, Toledo, Spain.

During sleep and anesthesia, cortical spontaneous activity is dominated by slow wave oscillations (SWO, <1.5 Hz) consisting of alternating synchronized network activity (up-states) and generalized neuronal silence (down-states). Several evidence indicate distinct excitatory and inhibitory neuronal mechanisms involved in the SWA regulation in rodents. Nonetheless, *in vitro* and *in vivo* data indicate astrocytes as regulators of cortical up-states by controlling both the initiation and the frequency of synchronized oscillations. However, whether astrocytes are also able to regulate neuronal excitability during up-states or are involved in the mechanisms of down- states is still unknown. Here, we investigated the role of astrocytes in the spontaneous cortical oscillatory activity by using a combination of *in vivo* electrophysiology and pharmacogenetics. For that, a 32-channel multielectrode was lowered into the somatosensory cortex to record spontaneous neuronal activity, while astrocyte modulation was achieved by using a hM3Dq-Gq-DREADD under the astrocyte GFAP promoter and acute i.p. injection of its ligand clonazepine (CNO). Neuronal recordings obtained from the same animals before and 1-hour after CNO application showed that astrocyte activation exhibited a clear effect on the SWO neuronal firing. While firing rate during down-states was significantly enhanced, up-states presented a reduced spontaneous activity. To determine the neuronal cell-type modulated by astrocytes under our conditions, we used a spike-sorting clustering algorithm based in three measurements (width, Trough-to-peak and after-high-hyperpolarization). Our analysis showed that CNO increased the number of putative inhibitory neurons during down-states, without affecting the number of putative excitatory clusters. Such apparent enhancement of inhibitory activity shorten down-state duration and therefore increasing SWO power as well as induced a slower down-to-up transition and decreased up-state amplitude. In conclusion, our findings indicate that down-states in SWO are directly modulate by astrocytes. In addition, the appearance of new putative inhibitory clusters would suggest the modulation of GABAergic interneurons by astrocyte activity.

A06. GENERATION AND CHARACTERIZATION OF HUMAN PLURIPOTENT STEM CELL (HPSC)- DERIVED ASTROCYTES TO MODEL ALZHEIMER´S DISEASE

M. Alfonso-Triguero[1,2, †], J. Cruz-Sesé[1,2, †], N. Galbis-Gramage[1], I. Jiménez-Ridruejo[1], E. Alberdi[1,2,3], A.M. Arranz[1,4]

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To analyze human astrocyte reactive states in the context of AD, we are using the stem-cell technology to generate astrocytes derived from human pluripotent stem cells (hPSCs) and in vitro models of AD in which astrocytes are exposed to various Aβ challenges. Human astrocyte identity as well as reactivity after Aβ stimulation are being characterized at molecular and functional levels with various assays.

hPSC-derived astrocytes at day in vitro 90 express main markers of astrocytes (GFAP, S100, EAAT1, EAAT2, Vimentin and AQP4) without expression of neuron (MAP2) and oligodendrocyte (O4) markers. After stimulation with oligomeric Aβ, hPSC-derived astrocytes show a reactive profile that we are characterizing. In sum, our approach allows exploration of the human astrocyte reactivity on an AD context and will provide insights into the contribution of astrocytes to the pathophysiology of AD.

A07. NUCLEUS ACCUMBENS ASTROCYTES CONTROL THE COGNITIVE IMPAIRMENT DERIVED FROM CHRONIC EXPOSURE TO THC

C. Martín-*PhD Program in Neuroscience, Autonoma de Madrid University, Madrid, Spain 28029. [1*] , J. Esparza[1] , I. Serra[1] , N. Puentes[2] , P. Grandes[2] M. Navarrete[1]

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The nucleus Accumbens (NAc) is a key region of the reward system implicated in motivation, drug addiction, and in numerous neurological and psychiatric disorders. A remarkable feature of this nucleus is the integration of motor and limbic information from glutamatergic inputs. Due to the relevance of this communication, it is crucial the maintenance of glutamate homeostasis, which is altered by addictive drugs. Moreover, there is solid evidence that the modulation of synaptic transmission is mediated by activation of cannabinoid receptors type I (CB1Rs) in astrocytes, suggesting that astrocytic CB1Rs are involved in glutamate homeostasis and modulate long-distance communication between neuronal populations. However, the functional role of astrocytes in alterations derived from chronic drug exposure is not fully understood.

In this study, we have analyzed the role astrocytes play in alterations produced by tetrahydrocannabinol (THC), the psychoactive constituent of marijuana. For that purpose, we have removed specifically the protein p38αMAPK, which mediates exocytic release of glutamate, from NAc astrocytes (Navarrete et al., 2019). First, using fiber photometry in vivo we analyzed glutamate dynamics and astrocytic activity in NAc after 1mg/kg THC chronic administration in a wildtype (wt) and p38αMAPK-/- (Astrop38α) mice. Then, we performed behavioral tests to assess whether THC had reinforcing properties or affected learning and memory. Furthermore, using a chemogenetic approach (DREADDs) we activated NAc astrocytes to analyze the behavioral implications. And finally, we performed electrophysiology experiments to analyze synaptic plasticity. We observed: 1)THC administration increases astrocytic calcium activity in wt and Astrop38α; 2)THC administration induces glutamate release in NAc in wt, which is not present in Astrop38α; 3)Astrocyte signaling mediated by CB1R induces NMDAR-LTD at NAc; 4)NAc astrocytes are involved in learning; and 5)Removal of p38αMAPK in NAc astrocytes restores the cognitive impairment derived from THC treatment.

Altogether, our results reveal astrocytes as critical elements for the maintenance of glutamate signaling, with a significant role in drug-consumption related alterations.

*PhD Program in Neuroscience, Autonoma de Madrid University, Madrid, Spain 28029. Supported by: RYC-2016-20414, MINECO (RTI2018-094887-B-I00) and FPU19-01667

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A08. ADDRESSING ASTROCYTIC CALCIUM DYNAMICS AND THEIR MODULATION BY CB1 RECEPTORS IN MULTIPLE SCLEROSIS

A. Moreno-García[1,2,3], T. Colomer[1,2], A. Bernal-Chico[1,2], A. Ruiz[1,2], C. Utrilla[1,2], U. Skupio[3], R. Serrat[3], C. Matute[1,2], G. Marsicano[3], S. Mato[1,2]

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Multiple sclerosis (MS) is a chronic demyelinating disease initiated by pathogenic immune responses against myelin followed by a broader inflammatory and neurodegenerative process. Astrocytes physiologically respond to endocannabinoids and other synaptically released neurotransmitters with cytosolic Ca2+ elevations that engage intracellular signaling and fine-tune intercellular communication. MS induces a pronounced transformation of astroglial cells whereby they acquire a variety of diseasepromoting functions. In particular, accumulating evidence supports the existence of a subset of neurotoxic reactive astrocytes that exhibit transcriptional programs destructive to synapses and oligodendrocytes in response to pro-inflammatory signals. Aberrant Ca2+ signals in reactive astrocytes are closely related to disease severity in a number of neurological disorders. However, the Ca2+ handling properties of astrocytes in the context of autoimmune demyelination remain to be investigated. In this study we analyzed astrocyte Ca2+ dynamics and their modulation by CB1 receptors in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS as well as in neurotoxic astrocytes induced in vitro. Systemic administration of Δ9 -THC increased the amplitude of astrocytic Ca2+ transients in the somatosensory cortex of freely moving mice carrying astroglial GCaMP6. Cannabinoid-induced increase of astroglial Ca2+ levels was mediated by the population of CB1 receptors present astrocytes as determined using GFAP-CB1-KO conditional mutant mice. EAE induced a shift in spontaneous astrocytic Ca2+ activity that correlated to disease symptomatology and attenuated Ca2+ responses mediated by Δ9 -THC. Astrocytes purified during acute EAE exhibited deregulated gene expression of several membrane receptors coupled to intracellular Ca2+ regulation as well as changes affecting Ca2+ signaling/homeostatic toolkits. These observations were partially mimicked by neurotoxic activation of cultured astrocytes and associated to aberrant cytosolic Ca2+ responses by ATP and glutamate. Our results suggest deficits in spontaneous and pharmacologically induced astrocytic Ca2+ activity during autoimmune demyelination that may reflect and/or contribute to MS disease severity.

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A09. ASTROCYTIC GLUT1 ABLATION IMPROVES SYSTEMIC GLUCOSE METABOLISM AND PROMOTES COGNITION

C.G. Ardanaz[1] , M.J. Ramírez Gil[1] , C. Smerdou[2] & M. Solas[1]

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[2]Center for Applied Medical Research (CIMA), Pamplona, Spain

Glucose supply from the blood to the brain is controlled by the glucose transporter GLUT1, highly expressed in astrocytes, which coordinate brain glucose supply, metabolization and storage. Ablating GLUT1 at the blood-brain barrier (BBB) endothelial cells leads to BBB breakdown, brain glucose hypometabolism and impaired cognition, but this approach cannot discriminate between insufficient glucose supply and BBB breakdown-derived effects. Such question is the focus of the present work, which aims to elucidate the relevance of astrocytic GLUT1 to cellular, brain and systemic glucose metabolism, and to cognition.

To address these questions, GLUT1 was ablated from primary astrocytes. Cellular metabolism was examined using an extracellular flux analyzer (Seahorse). In vivo, astrocytic GLUT1 was ablated using a tamoxifen-inducible Cre/LoxP approach (GLUT1$^{\Delta GFAP}$ mice). ^{18}F-FDG PET, glucose and insulin tolerance and insulin secretion were characterized. Recognition and spatial memory were assessed using Novel Object Recognition and Morris Water Maze tasks.

GLUT1-ablated astrocytes showed reduced glucose uptake and glycolysis, although preserving total ATP production. Unexpectedly, postnatal astrocytic GLUT1 deletion increased CNS glucose utilization. GLUT1$^{\Delta GFAP}$ mice showed an improved metabolic status from which obese animals especially benefited. Specifically, GLUT1$^{\Delta GFAP}$ mice were more efficient readjusting systemic glucose levels after hyperglycemia, exhibiting marked increase in insulin secretion. In parallel with this improved systemic homeostasis, GLUT1$^{\Delta GFAP}$ mice performed both recognition and spatial memory tasks properly, even outperforming control mice. Noteworthy, those effects could be due to higher astrocytic ATP release. Indeed, central administration of a purinergic receptor antagonist (PPADS) could reverse improvements in metabolic and cognitive behaviors in mice with astrocyte GLUT1 knockout.

Overall, this study demonstrates that astrocytic GLUT1 ablation impairs astrocytic glucose availability but enhances brain glucose utilization, reprograms systemic glucose metabolism towards a more efficient glucose-handling phenotype and promotes cognitive abilities, which could be a key factor in neurodegenerative diseases such as Alzheimer's disease

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A10. ASTRO-LIGHT: A NEW TOOL FOR MODULATION OF SPECIFIC ASTROCYTIC NETWORKS.

I. Serra, C. Martín-Monteagudo, L. Delgado and M. Navarrete[+]

Cajal Institute, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain

Unravelling the principles of information processing in complex cell circuits requires techniques capable of target and modulate specifically the activity of those elements involved. Although it has been demonstrated that astrocytes play an active role in neuronal transmission, the evolution of genetic tools to study and control these circuits has focused mainly on neuronal activity. Currently, there are available techniques to modulate astrocytic activity with precise temporal control (optogenetics), or in a sustained activation period (chemogenetics). Nevertheless, these tools act on the whole astrocytic population and it remains challenging to target gene expression or activity in specific populations of astrocytes, leaving the role of these cells in neural circuits or behaviour still unclear.

In this study, we present a new tool to translate the activity-mediated calcium signals of astrocytes into gene expression in a light-dependent manner, i.e. Astro-Light. Using a combination of electrophysiology, molecular, pharmacological and behavioural techniques, we have tested Astro-Light capacity to modulate the activity of specific astrocytic networks with implications in animal behaviour.

First, we engineered Astro-Light vectors under GFAP promoter and characterized Astro-Light expression after viral infection in the mouse Nucleus Accumbens (NAc). We apply Astro-Light to label astrocytes in the NAc that are activated during optogenetic stimulation of long-range excitatory inputs thought to modulate motivated behaviors. Finally, we tested the ability of Astro-Light to modulate animal behaviour.

Our results reveal Astro-Light as a functional and powerful tool for studying astrocyte-neuron interactions and enables dissection of astrocytic circuits underlying complex behaviors with high spatiotemporal precision

A11. CELL TO CELL COMMUNICATION MEDIATES NEURODEGENERATION CAUSED BY GLIOBLASTOMA

S. Casas Tintó

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Glioblastoma (GB) is the most aggressive and frequent primary brain tumor. Current treatments include radio-, chemotherapy and surgical resection of the solid core of the tumor. However, GB infiltrative cells cause that almost 100% of the patients undergo relapses and cause death in 16 months. GB cells produce cellular protrusions known as Tumor Microtubes (TMs) or cytonemes, which facilitate tumor expansion and cellular interaction among GB cells and with healthy surrounding neurons.

We use a Drosophila melanogaster GB model to study glia-neuron cellular interactions that contribute to the neurodegeneration induced by GB signals. TMs expand through the brain and connect GB cells, and with the healthy neurons through synapses.

As a consequence of GB-neuron interaction, WNT pathway and Insulin Receptor (InR) signaling attenuation play a central role in the neurodegeneration associated to GB. TMs accumulate specific Frizzled receptors that contribute to the depletion of WNT from surrounding neurons. This imbalance in WNT pathway causes JNK pathway activation and Matrix Metalloproteases (MMPs) secretion, MMPs degrade extracellular matrix and facilitates further TMs expansion. In consequence of WNT depletion, neurons undergo synapse loss and neurodegeneration that contribute significantly to the premature death caused by GB.

Besides, GB cells also produce ImpL2, an antagonist of the Insulin receptor known as IGFBP7 in humans. ImpL2 is secreted and impact on neighboring neurons, in consequence Insulin pathway is repressed, causes mitochondrial defects and synapse loss. Restoration of InR signaling in neurons counteracts neurodegenerative effects of GB.

Therefore, we propose that TMs mediate cell to cell communication and promote GB expansion; in consequence, GB progression causes neurodegeneration in healthy surrounding cells

A12. MODULATION OF THE PRESYNAPTIC TRANSLATOME BY ASTROCYTIC EXTRACELLULAR VESICLES IN ALZHEIMER'S DISEASE

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Neurons are highly polarized cells with an asymmetric morphology, thus implying an asymmetric distribution of proteins. Protein synthesis is vital to guarantee the correct neuronal function. Under physiological conditions, proteins need to be appropriately sorted to the target cellular compartment where they elicit their function. Noteworthy, protein synthesis is not always carried out by the classical translation pathway, in which proteins are synthesized in the rough endoplasmic reticulum and after maturation, proteins are transported to the target compartment. Protein translation can also be executed by another way based on the delivery of the mRNA to the target site, where mRNAs will be locally translated into proteins. This process is known as local protein synthesis. Neuronal local translation allows for a faster reaction of neural processes in response to environmental cues and contributes to the maintenance of axonal and dendritic homeostasis. In the Peripheral Nervous System, it has been described that extracellular vesicles (EVs) secreted by Schwann cells are capable of contributing to local protein synthesis and regenerate injured nerves. Nevertheless, it is so far unclear whether glial cells are involved in local protein synthesis. Recent evidence show that in Alzheimer´s disease (AD) pathology local protein synthesis is involved in the transmission of β-amyloid pathology from the axons to the soma. In this way, the retrograde transport of proteins synthesized in the axon in response to amyloid peptide leads to pathological transcriptional changes that contribute to neurodegeneration in AD. Furthermore, previous results of our research group have found evidences supporting that EVs secreted in presence of astrocytes modifies the levels of translation in axons of the Central Nervous System in vitro, both under physiological and AD conditions. Based on these facts, the working hypothesis is that astrocytes contribute to presynaptic translatome through the transfer of EVs in physiological and AD conditions.

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A13. ROLE OF ASTROCYTE-NEURON SIGNALING IN MAJOR DEPRESSIVE DISORDER

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Major depressive disorder (MDD) is a severe and debilitating mental illness with a very large socioeconomic impact worldwide (1). The neurobiology of this disease has been studied for a long time, focused on neuronal alterations; however, the underlying etiology is not yet fully understood. Astrocytes, a glial cell type, have been shown to play relevant roles in synaptic transmission and plasticity, with significant impact on behavioral responses (2). Evidence collected during the past two decades have shown that astrocytes might contribute to the pathophysiology and pathogenesis of MDD (3). Therefore, we aim to investigate the role of astrocyte-neuron signaling in this mental disease.

Here, we used a corticosterone treatment approach as depressive-like mouse model to evaluate the role of astrocyte-neuron signaling in medial prefrontal cortex (mPFC) from naïve and MDD mice. Ca2+ imaging techniques in vivo and ex vivo, and behavioral test have been performed.

Results: 1- In vivo spontaneous and behaviorally-related astrocyte calcium signaling was altered in MDD mice. 2- Serotonin-evoked astrocyte calcium dynamics were reduced in mPFC slices from MDD mice. 3- Selective chemogenetic activation of astrocytes by Gq-DREADDs in mPFC was able to restore the behavioral deficits of MDD mice.

Although additional experiments are required, these results reveal the potential impact of astrocyte signaling in the pathophysiology of MDD.

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A14. HIPPOCAMPAL-TARGETED, CELL TYPE-SPECIFIC MANIPULATION OF NFKB ACTIVITY TO TREAT BRAIN INJURIES AND DISEASES

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NFκB is a major transcription factor that regulates a large number of genes during various biological processes, such as early development, cell survival, synaptic plasticity, memory functions, and various diseases, including brain damage, neuroinflammation and neurological diseases. In the central nervous system, many of those processes, such as memory formation, learning, control of anxiety, and cognitive functions, depend on the hippocampus. This brain region is profoundly affected in mesial-temporal lobe epilepsy (MTLE) and traumatic brain injury (TBI) models, where hyperexcitation and neuronal excitotoxicity cause gliosis, cell death, and aberrant neurogenesis. In those models, increased NFκB expression levels have been detected. In other models, as ischemic animal models, downregulation of NFκB activity reduces brain damage, whereas inhibition of NFκB activity promotes the severity of disease expression in models of spinal cord injury.

In order to evaluate the importance of NFκB in diseases that cause hyperexcitation in the hippocampus, we have developed adeno-associated viruses equipped with tetracycline controlled genetic switches selectively targeting astrocytes and neurons. By cell type specific inducible control of NFkB gene expression, we aim to investigate the role of NFkB in disease onset and progression, and possibly also protection.

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A15. THE NEUROPROTECTIVE LIPOCALIN APOLIPOPROTEIN D INTERACTS WITH SPECIFIC SUBTYPES OF DETERGENT-RESISTANT MEMBRANE DOMAINS IN A BASIGIN-INDEPENDENT MANNER.

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Repair mechanisms of cell membranes are critical for maintaining their roles as selective barriers and for an efficient communication and transduction of biological messages between and within cells. Plasma and lysosomal membranes contain specialized detergent-resistant domains (DRMs) rich in sphingomyelin, cholesterol and gangliosides. Maintenance of these membranes is of special relevance for the nervous system, where most neurons are long-lived cells, with a membrane-centered physiological role, and continuously challenged by oxidative stress and toxic catabolites.

The Lipocalin Apolipoprotein D (ApoD) is expressed by glial cells, secreted to the extracellular milieu, internalized by glia and neuronal cells, and targeted in a finely controlled way to the subset of lysosomes most sensitive to oxidative stress.

In this work we use membrane and isolated DRM preparations from whole brain, primary astrocytes, and glial and neuronal cell lines. Binding of purified ApoD to membranes of non-expressing neurons *in vitro* and ApoD detection in DRMs allow us to assay biochemical parameters on which ApoD-membrane interactions depends. We use fluorescence immunocytochemistry and confocal microscopy to test protein subcellular localization and the MTT assay to quantify cell viability.

We demonstrate that ApoD is stably associated to a particular subset of DRMs with specific buoyancy properties, co-fractionating with both plasma and lysosomal membrane markers. The association of ApoD with isolated neuronal and glial membranes is stable under metabolic and acute oxidative stress conditions. We have tested if Basigin (Bsg), a transmembrane glycoprotein reported to be an ApoD receptor, is required for ApoD-membrane association and endocytosis-dependent uptake. Using a Bsg-KO astrocytic cell line, we conclude that neither ApoD interaction with DRMs, nor its internalization, are dependent on Bsg in astroglial cells. Our current analysis centers on the dependency of membrane lipid composition, since the molecular nature of ApoD-membrane interaction is an important issue to fully understand its neuroprotective mechanism.

M01. *IN VIVO* AND *IN VITRO* STUDIES REVEAL A SEX-DEPENDENT ROLE FOR THE INSULIN DEGRADING ENZYME (IDE) IN MEMORY TASKS AND IN MICROGLIAL CELLS

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The insulin-degrading enzyme (IDE) is a metalloprotease highly expressed at major sites of insulin degradation, but surprisingly also markedly expressed in the brain. IDE has been described to cleave not only insulin but also amyloid beta (Aß) peptides, which makes this enzyme a good candidate acting as a pathophysiological link between Alzheimer's disease and Type 2 diabetes.

To address the role of IDE *in vivo* we performed a comprehensive analysis of metabolic, behavioral and molecular parameters on a cohort of 12-month-old wild-type, heterozygous and knockout mice for the *Ide* gene. The open field test indicated that the partial or total absence of IDE does not produce significant abnormalities in the behavior of mice, while memory tests revealed sex- and genotype-dependent differences. We are currently performing a histological analysis to assess gliosis in the hippocampi of these mice and a multivariable analysis integrating all variables measured to construct a model that accounts for differences between genotypes. We then moved to *in vitro* studies to decipher the role of IDE specifically in primary microglial cells, master regulators of the neuroinflammatory response associated with brain degeneration and the main Aß-degrading cells. IDE absence significantly decreased microglial proliferation and delayed its response to the mitogen M-CSF. Cytokine production by Luminex assay revealed that IDE-KO microglial cells have impaired polarization under both pro-/anti-inflammatory stimuli, are more sensitive to oxidative stress, and exhibit a sex-specific pro-inflammatory response to Aß oligomers. Regarding Aß managing, amyloid phagocytosis was unchanged, but Aß degradation was diminished in IDE-KO microglia.

Our results indicate that IDE plays significant sex-dependent roles in memory tasks, and in microglial cells. IDE shows prominent functions in inflammatory polarization, microglial proliferation and Aß oligomers degradation, which makes IDE a potential therapeutic target for neurodegenerative processes.

M02. TOWARDS PHARMACOLOGICAL MODULATION OF MICROGLIAL PHAGOCYTOSIS

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Microglia, the immune cells of the central nervous system, display a variety of functions to maintain homeostasis in the brain. Microglia are professional phagocytes and remove apoptotic cells to prevent the spillover of toxic components. Although phagocytosis is a key process to maintain brain homeostasis and is very efficient in physiological conditions, little is known on how to modulate microglial phagocytosis when it is impaired or exacerbated, such as in epilepsy or schizophrenia, respectively. Therefore, our goal is to find pharmacological modulators of microglial phagocytosis. Using a high throughput screening strategy in primary cultures of microglia, we tested 600 compounds from the Prestwick library, already approved by the Federal Drug Administration (FDA) and the European Medicines Agency (EMA) to be used in humans, in an *in vitro* model of phagocytosis. We found a subset of drugs that could be classified as promoters of phagocytosis (pro-phagocytosis drugs) or inhibitors (anti-phagocytosis). To validate the phagocytosis modulators in a more complex system, we used organotypic cultures and confirmed that some compounds promoted phagocytosis, while others blocked it.

Currently, we are validating the compounds in vivo in two different models: anti-phagocytosis drugs are tested against physiological phagocytosis of apoptotic newborn cells in the adult hippocampal neurogenic niche; pro-phagocytosis drugs are tested against the pathological phagocytosis impairment induced in a model of epilepsy by intrahippocampal administration of kainic acid. Considering the lack of strategies to modulate phagocytosis, our compounds may represent a new therapeutical strategy to restore brain parenchyma homeostasis in pathologies where phagocytosis is impaired or exacerbated.

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M03. MICROGLIAL LOCAL TRANSLATION IN AB-INDUCED PATHOLOGY

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Protein synthesis is essential for the maintenance of cellular proteostasis. Neural cells (e.g neurons, astrocytes, oligodendrocytes) are highly polarized and therefore their proteins have to be asymmetrically distributed to fulfil their function. This distribution occurs through two different mechanisms: 1) the classical pathway where proteins are synthetized in the perinuclear region and delivered to their target site after maturation and/or 2) through local translation, where mRNAs are transported to the target site in a repressed state to be locally translated into proteins. Local translation allows cells to react in spatial and temporal manner to numerous stimuli. Most data regarding local translation have been obtained in neurons. However, there is evidence that local translation plays a crucial role in other CNS cell types too. For instance, local translation of MBP in oligodendrocytes has been described in neurodegenerative conditions. More recently, the ability of peripheral astrocytic proteins to translate proteins has been described. Microglia, although not being of neural origin, are the resident immune cells of the nervous system, and show a morphology as equally complex as neurons and neuroglia. In microglia, local translation has newly been described. Nonetheless, the involvement of local translation in these cell types in physiology and pathology is still to be elucidated. Taking into account that glial cells might be active participants in neurodegenerative diseases and based both on the literature and recent results of our group, our hypothesis is that local translation in microglial peripheral processes is involved in neurodegenerative diseases. Previously, our group has obtained results supporting the idea that local translation in microglia is altered in the context of inflammation. It is well stablished that neurodegeneration and neuroinflammation are strictly related. Thus, we are currently analysing the effect of both parameters combined and their relation to changes in local translation of microglial peripheral processes.

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M04. BRAIN ISCHEMIA INDUCES AN IFN-MEDIATED RESPONSE IN MICROGLIA OF MICE AND HUMANS

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Brain ischemia causes necrotic cell death, microglia reactivity and blood-brain barrier breakdown. Danger signals – including nuclear proteins, nucleic acids and heat-shock proteins – are released from injured cells and trigger immune responses by activating pattern recognition receptors (PRRs). Microglial cells are equipped with PRRs, can sense danger signals in the environment and induce inflammation. Our aim was to investigate the inflammatory response of microglia to ischemia in mice and humans. We induced cerebral ischemia in mice by 45-min middle cerebral artery occlusion followed by reperfusion. We compared the transcriptomic profile of microglia isolated from brain tissue of control and ischemic mice using fluorescence activated cell sorting followed by RNA-Seq. Enrichment analysis showed a strong anti-viral response induced by ischemia in microglia, highlighted by upregulation of type-I interferons (IFNs) including *Ifnb* and many IFN-stimulated genes (ISG). Accordingly, in whole brain tissue, ischemia increased *Ifnb*, *Ifna7* and *Ifna9* mRNA expression, as well as many ISG, including *Dhx58*, *Cxcl10*, *Irf7, and Irg15*. A time course-study in brain tissue samples obtained between 1h and 7 days post-ischemia showed ISG expression increase from about 16h post-ischemia, reaching a plateau at 4-7 days. We also detected enhanced expression of ISG in post-mortem human brain tissue of ischemic stroke patients. To find out the contribution of microglia to gene expression in whole brain tissue, we depleted microglia in mice with a CSF1R inhibitor (PLX5622). Ischemia-induced expression of *Ifnb, Ifna7*, or *Ifna9* mRNA was not reduced after microglia depletion. This implies that cells other than microglia are the main source of IFNs in the injured brain. In contrast, we found that microglia depletion reduced cerebral ISG expression. The results show that type I IFNs generated after ischemia in cells other than microglia activate type I IFN receptors in microglia inducing ISG expression and triggering a specific transcriptional program.

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M05. APOLIPOPROTEIN D FUNCTION IN MICROGLIAL RESPONSES TO OXIDATIVE STRESS AND AMYLOID BETA-TRIGGERED DAMAGE

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The brain is surveyed by microglia, resident phagocytes that show complex phenotypes. Microglia degrade injury-related cell debris, and their secreted factors modulate the immune response and tissue repair. Apolipoprotein D (ApoD) is secreted by astrocytes and myelinating glia upon injury, altered proteostasis or oxidative stress (OS). ApoD helps to maintain lysosomal functional integrity, contributes to cell survival, and optimizes macrophage phagocytosis.

Microglial cells do not express ApoD, neither in homeostatic conditions nor upon experimental OS. By adding exogenously ApoD, we found that it rapidly internalizes into BV2 microglial cells and exerts a pro-survival effect upon acute challenges of OS or β-amyloid oligomers. Following internalization, ApoD locates in vesicular cell compartments. We found a partial colocalization of ApoD with the lysosomal/endosomal marker Lamp-2, prompting further investigations on the protein traffic within microglial cells and its function in phagocytosis. We evaluated the phagocytic activity of BV2 cells upon exposure to myelin purified from wild type and ApoD-KO mouse brains. The presence of ApoD in phagocytosed myelin, or pre-exposure of microglia to exogenous ApoD, conditions phagocytosis efficiency and rate of myelin degradation, which was quantified by immunofluorescence, flow cytometry and immunoblot. ApoD influences the process in different ways depending on whether the cell is already “primed” with internalized ApoD, or ApoD enters the cell associated to the phagocytosed myelin. Multiplex analysis of cytokine production by primary microglial cells reveals that ApoD stimulates TNFα response to OS and Aβ oligomers, but not to LPS. Modulation of IL-4 production is stimulus- and sex-dependent. ApoD inhibits IL-4 secretion by male microglia in control and OS situation, but not upon Aβ exposure, while it has no influence on IL-4 secretion by female microglia. Understanding how ApoD acts on microglia, modulating its polarization and phagocytic activity upon disease-related stimuli is key to assess its neuroprotective potential.

M06. RNA LOCALISATION AND LOCAL TRANSLATION IN MICROGLIAL PERIPHERAL PROCESSES

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Local RNA translation allows the cells to respond fast and efficiently to environmental stimuli. Local translation is especially important in highly polarized cells, such as neurons, because it provides axons and dendrites a means for an accurate response to fast environmental changes. Although most of the work on local protein synthesis in brain cells has been performed in neurons, we now know this phenomenon is not restricted to these cell types. For instance, local translation has been described in peripheral astrocytic processes. In astrocytes local protein synthesis is essential to be involved in synapsis (Sakers et al, 2017). Furthermore, in oligodendrocytes it has been seen that MBP is translated locally in neurodegenerative conditions and in differentiation processes, giving an important role to local translation in oligodendrocytes (Quintela-Lopez et al., 2019). However, neither physiological nor pathological localized protein in microglia has been stablished. Thus, we propose that local translation in microglia also plays an important role in brain function and dysfunction. Therefore, we worked with different stimulus, like LPS, ATP, Aβ and MCSF, and analysed how they affect local translation in microglial peripheral processes. LPS is the only stimulus inducing changes in local protein synthesis, as well as inducing changes in global RNA production and in the interface between microglial lamellae and filopodia. Even more, ACTB transcripts are increased in microglial lamellae and filopodia when they are treated with LPS. However, ACTB transcripts are reduced in microglial periphery processes when they are treated with Aβ.

Our results indicate that local protein synthesis might be required for the inflammatory response in microglia cells. Moreover, we wanted to analyse the localization of transcripts known to translate locally into protein in the growth cones and in order to determine whether they play a role in cell polarity and cytoskeletal rearrangements in microglial cells.

M07. REPOPULATED MICROGLIAL CELLS AFTER DEPLETION IN OLD MICE MAINTAIN THE AGING FEATURES BUT ARE PROTECTIVE IN BRAIN ISCHEMIA

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Microglial cells show a differential transcriptional profile and manifest signs of dysfunction in the elderly. Impaired microglial function may contribute to worse neurological outcome following brain injury. Survival of microglial cells depends on colony-stimulating factor 1 receptor (CSF1R) signaling, and CSF1R inhibitors allow rapid microglial depletion. Repopulation of microglia takes place when treatment is discontinued. We used this strategy aiming to rejuvenate microglia and ameliorate stroke outcome. Exposure to a diet containing CSF1R inhibitor (PLX5622) for 2 or 3 weeks led to over 90% microglia depletion relative to animals receiving control diet. We used female C57BL/6 mice aged 3 months (young) or 21 months (old). Three, 7 or 21 days after interruption of the PLX5622 diet, we induced brain ischemia in mice by 45 min-middle cerebral artery occlusion followed by reperfusion for 4 days. Using fluorescent reporter chimeric mice, we found that microglial cells repopulating the injured brain tissue derived from brain cells and not from peripheral hematopoietic cells. Mice with repopulated microglia showed smaller ischemic lesions. In old mice, repopulation was accompanied by marked improvement of stroke-induced neurological deficits. The transcriptomic profile of microglia after ischemia was different in old versus young mice. However, repopulated microglia in old mice acquired a transcriptional profile similar to the original microglia. We conclude that old age confers changes in microglia that are transferred to novel microglia through cell division. In spite of this, novel microglia provide some environmental change that is able to reduce the neurological deficit following stroke.

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M08. ENDO-LYSOSOMAL DISRUPTION DRIVES MICROGLIAL PHAGOCYTOSIS DYSFUNCTION IN STROKE

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Microglial phagocytosis of apoptotic cells is an essential process to maintain tissue homeostasis and avoid the spillover of the cytotoxic content that results from the cell death induced by excitotoxicity and/or inflammation. We have shown that microglial phagocytosis is chronically impaired in models of neurodegenerative diseases such as epilepsy and stroke, where microglial phagocytosis is blocked as early as 6 hours after transient Medial Cerebral Artery occlusion (tMCAo), a model of stroke. Here, we hypothesize that microglial phagocytosis impairment in tMCAO was related to an energetic failure and used different in vitro systems to test the role of oxygen and nutrient deprivation (OND). To assess the effect of OND on phagocytosis we first used hippocampal organotypic cultures and observed a similar defect in apoptotic cell phagocytosis, which was related to a reduced microglial surveillance and process motility by 2- photon microscopy, likely related to the generalized energetic failure that takes place in stroke. The OND-induced phagocytosis reduction rapidly recovered after reperfusion suggesting that, in addition to the acute energetic failure, a more complex mechanism is responsible for the longterm impairment of phagocytosis found in tMCAo mice. We then treated primary microglial cultures with OND and observed phagocytosis deficits, in particular, a reduced degradation of apoptotic cells. This reduction was related to an increased lysosomal pH, possibly as a consequence of alterations in energy-dependent proton pumps that lead to a deficient enzymatic activity. The energetic dysfunction not only affected phagocytosis but also autophagy, another endosomal pathway that converges in the lysosome. We assessed autophagy using electron microscopy and found an increase in autophagy-like vesicles, presumably due to stalled autophagosomes related to a deficient lysosomal degradation. In order to revert the phagocytosis impairment, we tested the autophagy inductor rapamycin, both in vitro and in vivo, to restore the autophagy flux and the altered endo-lysosomal pathway, and hence, recover the phagocytic activity. Thus, the microglial phagocytic potential opens a novel approach to accelerate the recovery of the ischemic brain by harnessing microglial phagocytosis of apoptotic cells through the stimulation of the autophagy.

M09. INCREASED SURFACE P2X4 RECEPTOR EXPRESSION PREVENTS NEUROLOGICAL DAMAGE IN EAE

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One of the main hallmarks of disease progression in MS is the decline in tissue repair. One of the key steps involved in initiating myelin repair is the clearing of myelin debris by microglia and macrophages. We have previously proposed that P2X4 could be a therapeutic target for promoting remyelination and thus improving recovery in the chronic phase of multiple sclerosis. Thus, pharmacological activation of the P2X4 receptor by the P2X4 positive modulator ivermectin ameliorates EAE symptoms and promotes remyelination. However, for future transferral of these findings, it will be important to define more accurately the specificity of the pharmacological treatment and the different cellular compartments of P2X4 receptors contributing to the therapeutic effect. To corroborate the therapeutic potential of P2X4 we have used a P2X4mCherryIN knock-in mice, in which P2X4 is substituted by a non-internalized P2X4mCherryIN, leading to plasma membrane overexpression of P2X4 in all cells natively expressing P2X4. We first analyse the impact of P2X4 overexpression in microglial cells. Membrane resting potential and input resistance were not altered. However, we observed an increase in ATP-mediated inward currents in non-internalized P2X4KI microglia confirming the increased surface density of functional P2X4 receptors in microglia. Confocal analysis did not reveal significant changes in microglia morphology (Sholll analysis) or in the expression of proinflammatory markers. We then compare neurological score in P2X4 WT and P2X4mCherryIN MOG-injected mice. Importantly, P2X4mCherryIN mice showed a significant amelioration of the neurological symptoms at EAE chronic phase. This data indicate that increased surface P2X4 expression effectively prevent demyelination and axonal damage

M10. BORDER-ASSOCIATED MACROPHAGES INFLUENCE THE ENDOTHELIAL RESPONSE TO BRAIN ISCHEMIA

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Brain perivascular macrophages are resident in the perivascular space of arterioles and venules between the vasculature and the glia limitans and they are surrounded by basal lamina. Together with meningeal and choroid plexus macrophages they remain for long in the brain since early stages of development and display low or negligible exchange with the periphery during the lifespan under steady state conditions. Thus, brain macrophages reside at the edges of the brain parenchyma in strategic locations for communication with the periphery. To emphasize such features these cells have been termed border-associated macrophages. In this study we hypothesized that BAMs interact with the vasculature and affect the response of vascular endothelial cells after brain ischemia. In order to investigate the role of BAMs we took advantage of the fact that subsets of these macrophages express CD169 (Siglec1) under steady-state conditions. We obtained CD169-DTR mice, which express the diphtheria toxin receptor under the promoter of CD169. Administration of Diphtheria toxin (DTx) i.p. at days 1, 3 and 5, caused a strong reduction of BAMs, as assessed by immunofluorescence and cell counting. Treatment controls were CD169-DTR mice that received saline injection at the same time points. At day 8 after the first DTx administration, ischemia was induced by middle cerebral artery occlusion for 45 min followed by 24h reperfusion. At 24h, mice received an MRI scan and the brain was processed for fluorescence activating cell sorting in order to isolate $CD31^+$ endothelial cells from the cerebral tissue. We then obtained endothelial mRNA to study gene expression. Ischemia induced upregulation of NOX-2 (*Cybb*) and Mcp-1 (*Ccl2*) mRNA in the vascular endothelium, according to previous results of our group. Our preliminary results in the BAM-depleted mice show an increased expression of CCL2 and some reduction of NOX-2 in the endothelium that we are currently validating.

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M11. STUDY OF BONE MARROW-DERIVED MICROGLIAL CELLS IN A MODEL OF SELECTIVE NEURODEGENERATION

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The Purkinje Cell Degeneration (PCD) mouse presents a mutation in the Ccp1 gene that produces the selective post-natal death of Purkinje cells. Along with this neuronal loss, a strong microgliosis takes place in the cerebellum, but it is not fully understood whether it plays a beneficial or a harmful role in the development of the pathology. In this sense, it is also unknown if this gliosis is a direct consequence of the neurodegeneration, or if, by contrast, the pcd mutation itself causes an aberrant microglial behavior. Therefore, the direct effect of the pcd mutation on the functioning of the microglia was studied using cell cultures, without the influence of a neurodegenerative environment.

For this purpose, hematopoietic cells were isolated from the bone marrow of both wild-type and PCD mice and differentiated into microglia. Subsequently, immunofluorescence techniques and qPCR analyses were performed to characterize microglia by studying different markers and gene expression. Likewise, the viability of these cells was studied by means of a proliferation essay with Alamar Blue.

The preliminary results obtained suggest that the hematopoietic cells of PCD mice differentiated into microglia have a predominant polarization towards an anti-inflammatory phenotype. Besides, a differential gene expression has been observed for all the analyzed genes. Finally, the Alamar Blue essay demonstrated that PCD cells show a higher proliferation than the wild-type cells.

Therefore, it can be concluded that the mutation of the Ccp1 gene affects some microglial features related to cell morphology and neurochemical, gene expression and proliferation.

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M12. MICROGLIA GRADUALLY ACQUIRE THEIR MATURE PHENOTYPE IN THE DEVELOPING HIPPOCAMPUS

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Microglia originate from yolk sac progenitors and invade the brain at embryonic stages to progressively become integrated in the parenchyma, presenting a brain-specific phenotype that distinguish them from other tissue macrophages. During the first postnatal weeks of development, microglia go over a period of high transcriptional heterogeneity before their mature phenotype is settled. However, the molecular mechanisms by which microglia identity and function are established and maintained are largely unknown. We hypothesize that microglia morphology and function progressively mature once they enter the brain parenchyma. To test this hypothesis, we focused on the hippocampus because it develops its cellular network during the first postnatal weeks, when microglia heterogeneity is at its peak. We analyzed microglial morphology and phagocytosis efficiency by confocal microscopy, and microglial dynamics by 2- photon microscopy. We found that microglia progressively invaded the hippocampal dentate gyrus from postnatal day 2 (P2) to P10. We are currently exploring two possible routes of colonization: one resembling that of neural precursors, based on the co-localization with the reelin scaffold; and another one related to meningeal macrophages, which decrease over time as microglia increase in the parenchyma. Then, microglia progressively acquired a branched morphology and achieved their highest efficiency of phagocytosis at P14. Hence, they invaded the hippocampus in the first postnatal days, and subsequently acquired their characteristic morphology, dynamics, and mature function. The concurrent maturation of microglia and the hippocampal structure in the first postnatal days suggests the intriguing hypothesis of an active role of the brain environment. Deciphering the microglial maturation program is highly relevant because early changes could be genetically imprinted and lead to long-term functional alterations, which could have an impact in many neurodevelopmental and neurodegenerative disorders.

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O01. Proteomic Profile and Clonal NG2-Glia Morphometric Response in Two Experimental Models of Multiple Sclerosis

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Multiple sclerosis (MS), an autoimmune and neurodegenerative disorder of the central nervous system (CNS), displays high variability in its pathophysiology, making its study difficult. Nevertheless, a more holistic comprehension of this disease is given by the use of different animal models. Here we have used both, the experimental autoimmune encephalomyelitis (EAE) and the toxin-induced demyelination by cuprizone (CPZ) models, simulating the relapsing-remitting and the primary progressive MS courses, respectively. NG2-glia, also known as oligodendrocyte precursor cells (OPC), are of great interest in demyelinating diseases due to their remyelination potential. Here we investigate their differential clonal response to CPZ and EAE brain lesions by combining the StarTrack, a genetic lineage-tracing tool, along with a single-cell morphometric cluster analysis. Data from the morphometric analysis allowed us to unravel different NG2-cells clusters categorized by morphological parameters related, not only to their ontogenic origin, but also to their changes due to the different demyelinating scenarios. In addition, to address the differential altered neural pathways, we compared the proteomic profile of EAE mice (during the disease peak), and CPZ mice (during the acute phase) in two different brain regions: somatosensorial cortex and spinal cord. Bioinformatic analysis revealed changes and protein regional differences between the EAE and CPZ models. In addition, the detailed morphometric analyses of NG2-glia clusters sheds light on the NG2-glia heterogeneity, relevant to deciphering the physiological role of these cells in response to MS.

O02. CLEMASTINE IMPAIRS MYELIN DEVELOPMENT

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Abnormalities in myelination are associated to behavioral and cognitive dysfunction in neurodevelopmental psychiatric disorders. We detected a severe but transient reduction of myelination during development in both white and gray matter in a contactin-associated protein-like 2 (Caspr2) deficient mouse model of autism. Thus, therapies to promote or accelerate myelination could potentially ameliorate symptoms in mental illness. Clemastine, a histamine H1 antagonist with anti-cholinergic properties against Chmr1, is the most promising drug with promyelinating properties today (Mei et al., 2014). Clemastine penetrates the blood-brain barrier efficiently and promotes remyelination not only in animal models of multiple sclerosis, but also in patients. Here, we studied the role of clemastine in oligodendrocyte lineage during development. Chronic treatment of mice with clemastine induced an increase in oligodendrocyte differentiation in corpus callosum and cerebral cortex. However, despite the increase in the number of oligodendrocytes, conduction velocity of myelinated fibers (N1) in the corpus callosum was significantly decreased in clemastine-treated mice. Confocal and electron microscopy studies showed a reduction in the number of myelinated axons and nodes of Ranvier as well as a reduction of myelin thickness in corpus callosum. To understand the mechanisms leading to the impairment of myelin formation in the presence of an excess of myelinating oligodendrocytes, we focused on the role of clemastine in microglial cells, also expressing Chmr1 receptors. Microglia displayed a more branched morphology and a reduced expression of the pro-inflammatory marker iNOS in clemastine-treated mice. Moreover, clemastine reduced the percentage of $CD11c^+$ microglia cells and the levels of IGF, pivotal for correct myelination during development. Altogether, these data suggest that clemastine is not a useful drug to promote myelination during development.

O03. CHEMOGENETIC STIMULATION OF MATURE OLIGODENDROCYTES DRIVES MYELIN-AXON METABOLIC COUPLING AND PREVENTS AXONAL DAMAGE

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Oligodendrocytes make myelin and support axons metabolically with lactate. Experience and neuronal activity can induce dynamic changes in myelination during development and in adult life, suggesting a new form of plasticity to adapt brain function to environmental stimuli. Myelin remodeling is driven mainly by newly-formed oligodendrocytes from precursors cells. However, the role of mature oligodendrocytes in plastic changes of myelin is practically unknown. We have generated transgenic mice, using the CreERT2-lox technology, overexpressing the DREADD receptor hM3Dq under the PLP promoter, specific of mature oligodendrocytes. Chronic stimulation of hM3Dq receptors induced an increase in myelination of axons in cerebral cortex and corpus callosum, and consequently, an increase in axonal conduction velocity of interhemispheric callosal connections. Importantly, acute stimulation of $hM3Dq^{+}$ activates metabolism in oligodendrocytes. We detected an increase in glycolytic rate and in lactate production and release. Moreover, these higher metabolic coupling between oligodendrocytes and axons maintained axonal function under high frequency stimulation and prevented axonal damage secondary to oxygen glucose deprivation. We then tested the impact of mature oligodendrocytes stimulation to promote remyelination and protect axons in demyelinating disease models. Preliminary data show that chemogenetic oligodendrocyte stimulation ameliorates motor symptoms of mice with experimental autoimmune encephalomyelitis, a model of multiple sclerosis. Taken together, these findings indicate that this chemogenetic mouse line is a very useful tool to elucidate the contribution of mature oligodendrocytes to myelin remodeling in physiological and pathological conditions, and reveals a novel role of myelin-axon lactate shuttle in axonal protection.

O04. Δ9-TETRAHYDROCANNABINOL PROMOTES FUNCTIONAL REMYELINATION IN THE MOUSE BRAIN

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Δ9 -Tetrahydrocannabinol (THC), the most prominent active constituent of the hemp plant Cannabis sativa, confers neuroprotection in animal models of multiple sclerosis (MS). However, the possible effect of THC on oligodendrocyte regeneration and myelin repair has never been studied. Here, by using oligodendroglia-specific reporter mouse lines in combination with 2 models of toxin-induced demyelination, we show that THC administration enhanced oligodendrocyte regeneration, white matter remyelination, and motor function recovery. Interestingly, THC also promoted axonal remyelination in organotypic cerebellar cultures ex vivo. THC remyelinating action relied on the induction of oligodendrocyte precursor cell cycle exit and differentiation via CB1 cannabinoid receptor activation. Overall, our study identifies THC administration as a promising pharmacological strategy aimed to promote functional CNS remyelination in demyelinating disorders as MS.

O05. ADAPTIVE MYELIN PLASTICITY LINKED TO INCREASED NEURONAL EXCITABILITY IN THE SOMATOSENSORY CORTEX FOLLOWING CENTRAL SENSORY DEPRIVATION

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The neocortex is organized in vertical columns responsible to integrate and compute signal information. In addition to that, distinct layers within the vertical organization projects horizontally to neighbouring columns in order to distribute activity between cortical areas. Interestingly, recent anatomical data shows that horizontal axonal projections, specially the longones in L2/3, exhibit increased myelin segments elongation following monocular deprivation. Such adaptive myelin plasticity may improve synaptic efficacy of corticocortical connections with a possible role in mediating the reorganization of cortical maps after sensory deprivation. Here, we explored the relationship between axonal myelination and the level of cortical reorganization in distinct layers of the somatosensory cortex following central sensory deprivation. Furthermore, we also investigated the ability of astrocytes, known to provide trophic factors for myelinating glia, to influence the adaptive myelin plasticity. Complete thoracic spinal cord was used to induce sensory deprivation of the cortical areas receiving information from hindlimbs. Fifteen-to-thirty days later, injured and control animals were subjected to electrophysiological recordings using a vertical array lowered into the hindlimb cortex to record evoked potentials in response to contralateral forelimb stimulation. Our experimental design aimed to determine the strength of the synaptic connectivity between the deprived and intact cortex. Our data showed that sensory deprivation enhanced L2/3 corticocortical connectivity observed as increased magnitude and slope of deprived neurons. Next, we explored whether the increased L2/3 synaptic efficacy was associated to myelin remodelling. While immunostaining against the neurotrophic factor-oligo2 showed no overall changes in both deprived and intact cortices, myelin basic protein staining showed increased intersections and longitudinal myelination patterns. These changes were not observed in IP3R2-/- mice exhibiting deficient astrocyte activity, suggesting that astrocytes directly impact myelination. Overall, our data indicate a positive correlation among neuronal excitability and adaptive myelin plasticity that may mediate cortical reorganization through increased L2/3 corticocortical connectivity

N01. MONITORING OF ABERRANT NEUROGENESIS IN HIPPOCAMPUS DURING IN VITRO EPILEPTOGENESIS

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Epilepsy is among the most common serious neurological disorders, yet epileptogenesis is poorly understood. The epileptic focus in temporal lobe epilepsy is frequently found in the hippocampus, where it causes sclerosis and putatively upsets neurogenesis in the dentate gyrus. Our goal here is to understand what happens in the neurogenic niche during epileptogenesis.

Organotypic hippocampal cultures are a widely used in vitro model of temporal lobe epilepsy, and offers unique optical access to the hippocampal circuit over days and weeks in vitro. The first task of this project is to establish an experimental framework for microscopically monitoring neurogenesis in organotypic hippocampal cultures at regular intervals during culturing. For this, hippocampal slice cultures from mice will be maintained as interface cultures, and at early time points infected with a retroviral vector to label newly born cells. Hereafter, the slice cultures are submitted to wide-field or 2- photon microscopy on a daily to weekly basis, allowing us to follow the emergence and fate of newborn neurons. In one set of cultures we will add an epileptogenic agent (the GABA-A receptor antagonist picrotoxin), while other cultures will serve as controls.

We hypothesize that neuronal hyperexcitation translates into aberrant functional responses in NSCs and astrocytes, aggravating the course of epileptogenesis in a spiraling cascade. In this scenario, it is essential to determine the level at which the neuronal hyperexcitation triggers the response of NSCs and astrocytes to understand the underlying mechanisms of epileptogenesis and ultimately to identify new therapeutic targets.

We have successfully established a model of organotypic hippocampal slice cultures and retro viral vector-based cell labeling of newborn neurons. By combining this with an epileptogenic agent and immunolabelling, we have been able to assess aberrant neurogenesis after different treatments.

We will continue the project by comparing neurogenesis and astrogliosis in control and epileptiform conditions, to unravel novel mechanisms of epileptogenesis

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N02. POTENTIAL NEUROPROTECTIVE ROLE OF LYSOPHOSPHATIDIC ACID RECEPTOR 1 OVEREXPRESSION BY HIPPOCAMPAL NEURONS IN A MODEL OF TEMPORAL LOBE EPILEPSY

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Lysophosphatidic acid receptor 1 (LPA1) is G-protein coupled receptor involved in cell proliferation, survival differentiation and other biological processes. In the adult rodent brain, LPA1 specifically labels hippocampal neural stem cells (NSCs) which generate newborn neurons throughout postnatal and adult life in most mammals.

Interestingly, LPA1 also labels Reactive-NSCs (React-NSCs). The reactive glia-like counterparts of NSCs induced by seizures and that abandon neurogenesis to transform into reactive astrocytes and contribute to gliosis.

Further, using a transgenic mouse line in which the enhanced green fluorescent protein is expresses under the regulatory elements of LPA1 (LPA1-GFP) we have stablished that React-NSCs lose LPA1 expression several weeks after seizures, as they differentiate into reactive astrocytes. In parallel, neurons of the granule cell layer start to express LPA1 gradually in the epileptic brain and maintain its expression in the long term.

Using confocal microscopy imaging of control and epileptic LPA1-GFP mice we are currently evaluating whether LPA1 expression promotes the survival of neurons in granule cell layer. In addition, we are using hippocampal NSC-derived neuronal cultures to activate or inhibit LPA1 inducing cell death to better asses it potential role in neuroprotection.

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N03. CALCIUM IMAGING IN EPILEPSY: AN IN VIVO AND IN VITRO APPROACH

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Epilepsy is a neurological disorder characterized by recurrent epileptic seizures generated by disfunctions in the neuronal circuitry. Notably, hippocampus is especially vulnerable to epilepsy due to its complex cytoarchitecture and recurrent circuitries which potentiate and perpetuate the neuronal hyperexcitation generated by seizures. As a consequence, hippocampal circuitry plays a key role in epilepsy, especially in the temporal lobe epilepsy (MTLE). Seizures trigger a vast cascade of event which generate gliosis, inflammation and neuronal desynchronization. All these processes generate a positive feedback in which the effect derive from seizures provoke new seizures.

One of the main consequences of seizures is the alteration of the hippocampal neuronal circuitry inducing a desynchronization in the neuronal activity.

To study the effect of the hippocampal circuit desynchronization in vivo we will use live calcium imaging (using genetically encoded calcium indicators Gcamp6) by means of a miniscope to evaluate the differences in circuit in behaving WT and epileptic mice.

In-vitro we will use calcium imaging in developing hippocampal slices to study the impact of epilepsy on calcium dynamics and spontaneous synchronizations by means of a 2-photon microscopy.

Therefore, the study of the neuronal network is especially useful to understand the underlying mechanism in the generation of seizures and the possible therapeutic strategies derived from it.

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NP01. TRAUMATIC BRAIN INJURY INDUCES A BIPHASIC LONG-TERM EFFECT ON ADULT HIPPOCAMPAL NEUROGENESIS

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Several important cognitive functions affected by traumatic brain injury (TBI) depend on the hippocampus, which harnesses several forms of neural plasticity, among them adult neurogenesis, the generation of new neurons throughout life. Adult hippocampal neurogenesis is a process involved in memory, learning and control of anxiety, cognitive functions which result impaired after TBI. We hypothesize that TBI induces fast and long-term changes in both neural stem cells (NSCs) and newborn neurons which could subsequently alter hippocampal and brain functioning. Using a model of controlled cortical impact (CCI) we have found that TBI has a dual effect on neurogenesis: In the short term (up to two months) it causes an increase in the number of newborn neurons but with aberrant migration, increased soma size and altered electrophysiological properties; in the long term, neurogenesis results impaired by a reduction in the number of immature neurons. We also suggest that the alteration in the expression of Rho Family GTPase 2 (Rnd2) could be causing some of the morphological changes in the immature neurons as well as their aberrant migration and thus could be a target to prevent TBI-induced aberrant neurogenesis, a hypothesis that we are currently investigating at the cellular level. In addition, we have found that NSCs get activated in higher numbers early after TBI, a result that could explain the later reduction in neurogenesis

NP02. OPTOGENETIC MODULATION OF NEURAL PROGENITOR CELLS IMPROVES NEUROREGENERATIVE POTENTIAL

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Spinal Cord Injury (SCI) is a severely debilitating condition that causes motor, sensory and autonomic dysfunctions. Currently, SCI remains a worldwide problem due to its complexity, involving diverse biochemical and physiological processes [1]. Nowadays, neural progenitor cells (NPC) transplantation has been shown as a useful tool for treatment of SCI [2], demonstrating potential to recovery complex neurological functions after the injury, however, the limited cell survival rates and host circuit integration limits the extension of their capabilities [3]. Optogenetic modulation of NPCs is emerging as a new tool to modulate neural fate and improving therapeutics outcomes in vitro and in vivo [4]. Results from our group have shown that blue-light stimulation of NPCs engineered to ectopically express the excitatory light-sensitive protein channelrhodopsin-2 (ChR2-NPCs) prompted an influx of cations and a subsequent increase in proliferation and differentiation into oligodendrocytes and neurons. Furthermore, light-stimulated ChR2-NPCs triggered the polarization of astrocytes from a pro-inflammatory phenotype to a pro-regenerative/antiinflammatory phenotype with decreased activation of NF-κB. On the other hand, neurons derived from blue-light-stimulated ChR2-NPCs exhibited both, increased branching and axon length and improved axon growth in the presence of axonal inhibitory drugs such as lysophosphatidic acid. To further study this approach, ChR2-GFP-NPCs transplantation was performed in an in vivo subacute rat model of SCI by T8 hemisection. In vivo optogenetic activation was carried out using Neurolux spinal cord device which has tethered a blue μ-LED. Rats received 1h of stimulation every day for a total of 4 weeks at 20 Hz with 5 ms on and 45 ms off. We studied functional recovery, NPC engraftment and differentiation, phenotypic characterization of astrocytes, host neurons activation and tissue preservation, in respond to in vivo optogenetic activation in stimulated and non-stimulated rats.

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NP03. DERIVED CELL-PROGENY FROM SINGLE NEURAL PROGENITOR CELLS

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The assemble of the brain from a pool of Neural Progenitor Cells (NPCs) is a complex process. Increasing evidence supports the heterogeneity of NPCs across and within distinct brain regionsand their importance for the generation of the different neural types. Some studies suggest that progenitor diversity is more restricted to one specific lineage whereas others show a potential cell diversity depending on the spatio-temporal patterning. Neural stem cells give rise to transient progenitors termed neuroepithelial cells that generate Radial Glial Cells (RGC), multipotent progenitors that produce, in overlapping waves, neurons, astrocytes, NG2-glia and oligodendrocytes. However, although RGCs are the most known cortical NPCs, NG2-glia (or NG2- cells) is another remarkable cell type that can also act as a progenitor. In the adult mouse brain NG2-glia is able to generate OLs, Astrocytes or even Neurons. Moreover, our previous works revealed the existence of NG2-progenitors during development, enable to produce different neural cell lineages depending on the embryo stage. To elucidate the cell potential of single-NPCs, my lab developed the UbC-StarTrack a multicolor genetic tool that allow usto tag single progenitors with stable and heritable labelling.This strategy, based on PiggyBac system, consists of the integration (thanks to a hyperactive transposase PiggyBac -HyPBase) of twelve plasmids that codify up to six different fluorescence proteins (XFs) aim to cytoplasm or nucleus. To target single NG2 or GFAP-progenitor cells, we echanged the CMV-promoter of HyPBase in UbC-StarTrack for NG2 or GFAP-promoter in UbC-(NG2-PB)- StarTrack or UbC-(GFAP-PB)-StarTrack, respectively. After targeting NPCs at either E12, E14, E16 or P0, we performed a clonal analysis of the derived-cell progeny at P30. Data showed that GFAPand NG2- expressing progenitors produce distinct cell types and whose differentiation potential changes with time and space. Our results provide new data of the lineage cell potential of NG2 and GFAP-progenitors that strengthen the heterogeneity of NPCs during cortical development. Supported by research Grants from MICINN (PID2019-105218RB-I00) and Fundación Ramón Areces (CIVP9A5928)

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NP04. EFFECT OF THE SRC INHIBITORY PEPTIDE TAT-CX43266-283 ON NEURAL STEM CELLS WITH EGFR OVEREXPRESSION OR EGFRVIII MUTATION

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Glioblastomas are one of the most malignant tumours worldwide. Among the causes of such malignancy is a subpopulation of tumour cells with stem cell properties known as Glioma Stem Cells (GSCs). These cells are resistant to standard treatments, such as temozolomide, which causes tumour recurrence. Several studies have proposed Neural Stem Cells (NSCs) from the subventricular zone (SVZ) as a possible origin for GSCs. The transition of NSCs to GSCs frequently concurs with epidermal growth factor receptor (EGFR) overexpression or mutations, such as EGFRvIII. Our group designed a cell penetrating peptide based on connexin43 (TAT-Cx43266-283) that inhibits the activity of the oncoprotein c-Src and therefore targets GSCs, increasing survival rates in gliomabearing mice. Because Src is involved in EGFR signaling, we aimed to explore the effect of TAT-Cx43266-283 in the transition of NSCs to GSCs. For this purpose, we analysed the cell growth of SVZ NSCs (Control-NSCs), NSCs with EGFR amplification (EGFRwt-NSCs) and NSCs with the mutant EGFRvIII (EGFRvIII-NSCs). Our results show that TAT-Cx43266- 283 specifically inhibited the growth of EGFRwt-NSCs and EGFRvIII-NSCs, without significant effects in Control-NSCs. Importantly, we found that temozolomide and other control peptides did not affect the cell growth of any of these NSCs. To gain insight into the mechanism involved in the effect of TAT-Cx43266-283, we analysed the EGFR signaling pathway by Western blot. Our preliminary results show that TAT-Cx43266-283 decreased the activity of EGFR and EGFRvIII, as well as c-Src activity. So far, our results indicate that TAT-Cx43266-283 impairs EGFR signaling pathway with the subsequent reduction in the proliferation and survival of NSCs that overexpress or exhibit mutations in EGFR. These results stress the relevance of TAT-Cx43266-283 as a future therapy against glioblastoma, alone or in combination with temozolomide or other treatments that do not target stem cells with EGFR alterations.

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NP05. STAT3 INHIBITION PREVENTS THE TRANSFORMATION OF NSCS INTO REACTIVE-NSCS IN EPILEPSY

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Adult neurogenesis persists throughout adulthood in the hippocampus of most mammals because of a population of neural stem cells (NSCs) that remains in the dentate gyrus. The capability of NSCs to generate neurons is promoted by neuronal activity. However, hyperexcitation at the level of epileptic seizures induce NSCs to transform into reactive NSCs (React-NSCs), that become multibranched and hypertrophic and abandoning neurogenesis to enter massively in mitosis and transform into reactive astrocytes that contribute to gliosis. We are now exploring signaling mechanisms that control the transformation of NSCs into React-NSCs. One of the candidates is STAT3 (signal transducer and activator of transcription 3) which plays a critical role in astrogliogenesis and NSCs proliferation and differentiation. We have confirmed by quantitative rtPCR (Q-rtPCR) that STAT3 is overexpressed and by confocal microscopy that the phosphorylated form (P-SAT3) is increased in React-NSCs in a mouse model of mesial temporal lobe epilepsy (MTLE). Further we have stablished a model of React-NSCs in culture, which allows an easier manipulation of the STAT3 activity. We have confirmed also by Q-rtPCR and by confocal microscopy that these cultured React-NSCs also overexpress STAT3 and have more P-STAT3 when compared to control NSCs. We hypothesize that the inhibition of STAT3 activity will prevent the induction of React-NSCs. To test this hypothesis, we are using two strong inhibitors of STAT3 activity: pharmacological agent WP1066 and silibinin, the main component of silimarin which is isolated from the seeds of milk thistle (Sylibum marianum). Our preliminary results suggest that indeed the inhibition of STAT3 reduces the transformation of NSCs into React-NSCs, as it decreases their overproliferation as well as their morphological transformation

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