Neuro gune

Iruña / Pamplona
July 1st
2022

Abstract Book

Acknowledgements

Many thanks and recognition to

Sara Martorell San Nicolás (Clínica Universidad de Navarra)

for her continuous support and help

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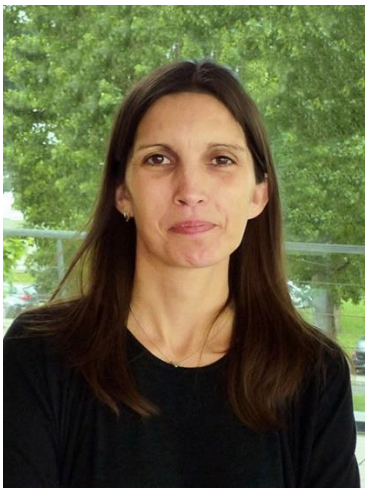
Opening Keynote

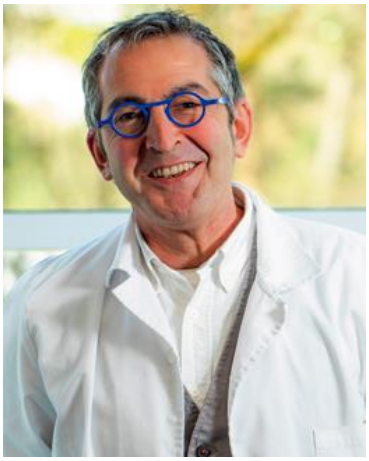


Aude PANATIER
INSERM, Bordeaux

"D-serine: a metabolic key factor in declining memory"

Chair:
Edgar Soria-Gómez (UPV/EHU & Achucarro, Leioa)

Closing Keynote



Pablo MARTÍNEZ LAGE
CITA Alzheimer, Donostia - San Sebastian

"Biomarker achievements for Alzheimer's diagnosis and prognosis... so now what?"

Chair:
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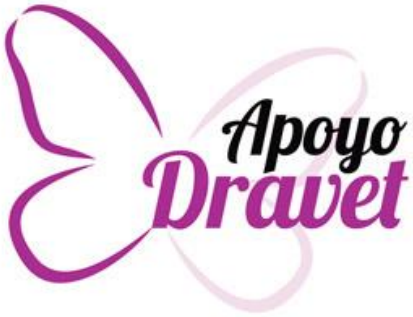

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TRACK 1: Cellular and Molecular Neuroscience / Physiology

ORAL PRESENTATIONS

Moderator: **Amaia Arranz Mendiguren (Achucarro, Leioa)**

Oral Communication: T1-OC1

ASTROCYTIC GLUT1 ABLATION IMPROVES SYSTEMIC GLUCOSE METABOLISM AND MEMORY RESILIENCE THROUGH ENHANCED INSULIN-STIMULATED ATP RELEASE

Aims: Brain glucose supply is controlled by glucose transporter GLUT1, highly expressed in astrocytes. Ablating vascular GLUT1 leads to brain hypometabolism and impaired cognition, but this approach cannot discriminate between insufficient glucose supply and vascular breakdown-derived effects. Such question is the focus of the present work, which aims to elucidate the relevance of astrocytic GLUT1 to glucose metabolism and cognition.

Methods: In vitro, cellular metabolism was examined using an extracellular flux analyzer (Seahorse). In vivo, astrocyte-specific gene ablation was performed using tamoxifen-inducible Cre/LoxP approaches. 18F-FDG PET, glucose tolerance, insulin secretion and fasting-induced hyperphagia were characterized. Memory was assessed using the Novel Object Recognition and Morris Water Maze tasks. Purinergic agonists or antagonists were intracerebroventricularly administered before each task. ATP levels were determined using microdialysis.

Results: GLUT1-ablated astrocytes featured reduced glucose uptake and glycolysis, but intact ATP production. Unexpectedly, mice subjected to astrocytic GLUT1 deletion (GLUT1ΔGFAP) showed increased CNS glucose utilization and improved metabolic status, being more efficient at suppressing hyperphagia and readjusting systemic glucose levels after hyperglycemia. Moreover, GLUT1ΔGFAP mice performed memory tasks adequately. Remarkably, GLUT1-ablated astrocytes showed enhanced insulin receptor (IR) expression and insulin-stimulated ATP release. Blocking brain purinergic signaling was sufficient to prevent GLUT1ΔGFAP-induced metabolic and cognitive abilities. Concomitant ablation of GLUT1 and IR in astrocytes exerted the same effect, which could be rescued upon brain purinergic stimulation.

Conclusion: Astrocytic GLUT1 ablation drives brain and systemic metabolism towards a more efficient glucose-handling phenotype and promotes memory resilience, requiring enhanced astrocytic IR-dependent ATP release for these features.

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Oral Communication: T1-OC2

BENEFICIAL EFFECTS OF ASTROCYTIC GLUT1 ABLATION IN ALZHEIMER'S DISEASE

Aberrant brain bioenergetics is proposed as one of the underlying mechanisms in Alzheimer's disease (AD) pathology. Indeed, a prominent drop in brain glucose uptake is observed prior to the manifestation of AD symptoms. Glucose is the main energy source of the brain, and its supply from the blood to the brain is controlled by the glucose transporter GLUT1, highly present in astrocytes. Thus, astrocytes are located at the interface between vessels and neurons, putting them in a privileged position to control brain glucose uptake. In the present work, we hypothesized that astrocyte-specific GLUT1 deletion could accelerate the onset of AD.

In our hands, Aβ-treated GLUT1-ablated cultured astrocytes exhibited lower maximal respiration and glycolytic rate, but unchanged total ATP production, thus being as able as control astrocytes to meet energy needs upon Aβ challenge. Furthermore, astrocytic Aβ clearance was analyzed using immunoblotting and immunofluorescence, finding that GLUT1 KO astrocytes are as capable as controls to degrade Aβ.

Surprisingly, postnatal GLUT1 deletion in glial fibrillary acidic protein (GFAP)-expressing astrocytes (GLUT1ΔGFAP) decreased the elevated mortality of APP/PS1 mice. Moreover, astrocytic GLUT1 ablation rescued the cognitive impairments shown by APP/PS1 mice in several behavioral memory tests. These effects were unrelated to Aβ pathology, as both amyloid plaques and Aβ levels were unchanged. Noteworthy, astrocytic Gi pathway activation by hM4Di chemogenetic manipulation completely blunted the beneficial effects of astrocytic GLUT1 ablation.

In conclusion, GLUT1-ablated astrocytes maintain the ability to metabolically cope with Aβ challenge, and far from worsening AD pathology, astrocyte-specific GLUT1 ablation prevents both the increased mortality and impaired cognition exhibited by APP/PS1 mice.

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Oral Communication: T1-OC3

IMPACT OF OLIGODENDROCYTE STIMULATION ON BEHAVIOURAL PROCESSES

Mature oligodendrocytes (OLs) are neuroectoderm-derived cells specialized to serve as electric insulators of axons, by wrapping them with fat-enriched myelin membranes. OLs play pivotal roles in CNS function allowing saltatory conduction and promoting the energetic support and survival of axons. However, few studies have analyzed the impact of mature oligodendrocyte stimulation in neuronal function. We have generated a inducible transgenic mice (tamoxifen-dependent) expressing hM3Dq receptor in PLP+ OLs and Schwann cells, which could be selectively activated by the exogenous ligand clozapine N-oxide (CNO). Selective stimulation of OLs, in the absence of neuronal stimulation, increases myelination and lactate supply to axons, resulting in an increase

in conduction velocity and in axon excitability. We then tested the hypothesis that direct OL stimulation could influence behavior and cognitive processes. Acute CNO stimulation induced acute hypolocomotion in the open-field test, an effect exacerbated in females. Chronic CNO treatment (15 days) induced a complex phenotype characterized by reduced motor activity and weight loss, accompanied by lowered body temperature and hunched posture. Moreover, we could trace the presence of dizziness, hypersalivation and bruxism, all symptoms peaking within 5 days and reversing by the end of the treatment. Washout from chronic CNO treatment quickly reversed hypolocomotion (open-field, elevated plus maze and light-dark boxes tests), and the fifth day after the last CNO injection we eventually tested visuospatial learning and memory in a Barnes maze 5-days paradigm. Chronic CNO stimulation did not alter the efficiency in both short- and long-term learning, e.g. the time spent and errors made to find the escape box along the same day and along the 5 days, in both sexes. However, we observed that different spatial strategies to solve the task are used by males or females, and interestingly the chronic CNO stimulation reversed such behavioral sexual dimorphism. Therefore, we conclude that stimulation of PLP+-myelinating cells has unpredicted and profound influences over animal behaviors. Further studies to dissect the central and peripheral components of these effects, as to determine the mechanisms involved are currently in progress.

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Oral Communication: T1-OC4

PHAGOCYTOSIS-INDUCED STRESS TRIGGERS TRANSCRIPTIONAL, METABOLIC AND MITOCHONDRIAL ADAPTATIONS THAT SUSTAIN LONG-TERM MICROGLIAL FUNCTION

Microglia, the resident immune cells of the central nervous system (CNS), surveil the parenchyma and phagocytose debris and apoptotic cells. Apoptotic cells are generated throughout life and their clearance by microglia avoids the spillover of toxic content and exerts immunomodulatory effects. In physiology, microglia are very efficient phagocytes, whereas they are impaired in diseases like epilepsy or ischemia. As phagocytosis modulators with therapeutic potential are developed, it is necessary to understand how phagocytosis affects microglial physiology and function. To analyze the impact of phagocytosis on microglia we have developed an in vivo model of “superphagocytosis” by low cranial irradiation (2Gy), which induces apoptosis of dentate gyrus newborn cells by 6h. At this early time point, microglia become “superphagocytic”, engulfing several apoptotic bodies and clearing them by 24h. This model did not induce microglial DNA damage nor monocyte infiltration, allowing us to specifically study post-phagocytosis events in microglia. We first analyzed transcriptional changes in post-phagocytic microglia using single-cell RNA-seq analysis. We observed changes in the size and signature of the different clusters detected, which we are currently validating using RNAscope and immunofluorescence. Moreover, post-phagocytic microglia were reduced

in number at 24h and started to proliferate 3 days after phagocytosis, suggesting that these cells were stressed. To explore the underlying mechanism of this stress, we used an in vitro model and metabolomics to analyze cellular metabolism. We observed a reduction in mitochondrial metabolism and no compensatory increase glycolysis in phagocytic microglia. The mitochondrial impairment was related to a remodelling of the mitochondrial network, with fewer and less complex mitochondria. Moreover, we detected alterations in the levels of polyamines, which are closely linked to cell proliferation. Finally, to test if post-phagocytic microglia recovered their function in vivo, we performed an experiment using two sequential doses of irradiation, 7 days apart. After the second exposure, microglia were able to phagocytose and clear the apoptotic cells, suggesting a functional recovery after the phagocytosis-induced stress. Overall, we show that phagocytosis is not a terminal process that ends with debris removal. Rather, phagocytosis induces microglial stress, triggering a series of transcriptional, metabolic, and mitochondrial adaptations, which ultimately lead to apoptosis and proliferation, supporting the long-term functionality of microglia.

Authors

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Oral Communication: T1-OC5

ASTROCYTIC LIPID METABOLISM DETERMINES SUSCEPTIBILITY TO DIET-INDUCED OBESITY

Hypothalamic astrocytes play pivotal roles in both nutrient sensing and the modulation of synaptic plasticity of hypothalamic neuronal circuits in control of feeding and systemic glucose and energy metabolism. Here, we show the relevance of astrocytic fatty acid (FA) homeostasis under the opposing control of angiopoietin-like 4 (ANGPTL-4) and peroxisome proliferator-activated receptor gamma (PPARgamma) in the cellular adaptations of hypothalamic astrocytes and neurons to the changing metabolic milieu.

We observed that increased availability of FA in astrocytes induced by cell- and time-selective knock down of Angptl4 protected against diet-induced obesity, while cell- and time-selective knock down of Angptl4-regulated PPARgamma lead to elevated susceptibility to obesity. Overall, our results unravel a novel role for astrocytic FA metabolism in central control of body weight and glucose homeostasis.

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POSTERS

Reference number: T1-P01

(T1-P01) ***GENERATION OF HUMAN PLURIPOTENT STEM CELL DERIVED ASTROCYTES TO MODEL ALZHEIMER´S DISEASE***

Recent studies highlight the importance of glial cells on the pathogenesis and progression of Alzheimer´s disease (AD). Among glial cells, it is well established that astrocytes undergo profound alterations in gene expression, morphology and function during the course of AD. However, such changes are still poorly defined and mostly unknown in the case of human astrocytes. To analyze human astrocyte responses and their contribution to AD we are using the stem cell technology and establishing in vitro models in which human pluripotent stem cell (hPSC)-derived astrocytes are exposed to various AD-related challenges. Through various innovative assays we are analyzing human astrocyte reactivity and neurotoxic profiles and their capacity to uptake and degrade amyloid beta (Aβ).
In sum, our approach allows exploration of human astrocytes on an AD context and will provide insights into their contribution to the pathophysiology of AD.

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Reference number: T1-P02

(T1-P02) ***ASTROCYTES AND MICROGLIA DISTINCTLY REGULATE MRNA AND PROTEIN METABOLISM IN NEURITES***

Proteins that support axonal, dendritic or synaptic functions in the brain typically reach their final destination by active transport. However, protein delivery to distinct cellular compartments can also rely on the transport of the mRNA that is then locally translated at target sites. Although once considered heretical, the mechanism of localizing RNAs has proven to be highly conserved in eukaryotes. Indeed, recent estimates suggest 50% of neuritic proteins are produced through local translation1.

Despite neuronal local translation being widely accepted by the scientific community there are still several aspects of this phenomenon that are still under debate. For instance, it is unclear whether local protein synthesis is fully cell-autonomously regulated or if it relies upon non-neuronal cells (e.g glia) surrounding the neurons. Our lab is interested in studying the contribution of glial cells to local translation in neurites (specifically in axons and presynaptic terminals). To this end we have analyzed the neuritic proteome of neurons cultured in the absence or presence of astrocytes or microglia. Our results show that the presence of either glia increases synthesis of neuritic and axonal proteins. Proteomic analyses have revealed the identity of specific proteins regulated solely by the presence of astrocytes or microglia or regulated by both

kinds of glia. We clustered these proteins using GO analyses and functional enrichment analyses and found that ribosomal proteins and other involved in RNA transport were affected by the presence of astrocytes but not by microglia. Coversely, components of the spliceosome and the proteasome were changed in neurites when cells were cultured in the presence of microglia but not in the presence of astrocytes. Our results so far indicate the astrocytes and microglia distinctly influence the neuritic proteome and regulate proteins involved in different steps of RNA and protein metabolism.

1 Zappulo, A. et al. Nat Commun 8, 583 (2017).

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Reference number: T1-P03

(T1-P03) ***ASTROCYTE METABOLIC REPROGRAMMING IN MULTIPLE SCLEROSIS***

Astrocytes are the most abundant cell population in the central nervous system (CNS) and regulate numerous physiological functions. These cells rely on intracellular Ca2+ rises for intracellular signaling and provide glycolic lactate to sustain neuronal activity. Astrocytes undergo a pronounced transformation during CNS pathology whereby they acquire important disease-promoting functions. Recent advances in the field suggest the existence of a subset of neurotoxic reactive astrocytes that exhibit transcriptional programs destructive to synapses and oligodendrocytes in response to pro-inflammatory signals. These cells are abundant in demyelinating lesions of multiple sclerosis (MS) patients and proposed to play a relevant pathogenic role during disease progression.

Astrocytes depend on metabolic reprogramming to meet their bioenergetic demands during activation and current studies suggest that astrocytic metabolic switch accelerates neurotoxicity. However, the metabolic signature of neurotoxic astrocytes has not been investigated in detail. This study addresses astrocyte metabolic reprogramming as pathogenic mechanism in MS, through a combination of bioenergetic, Ca2+ imaging and gene expression studies applied to rodent astrocytes in vitro. Cells purified from the mouse forebrain were maintained in a serum free culture system and activated to a neurotoxic phenotype by incubation with the pro-inflammatory factors (PIFs) IL-1α, TNFα and C1q. Astrocytes incubated with PIFs showed increased expression of neurotoxic phenotype related genes and down-regulated levels of genes related to neuroprotection and synaptogenesis supporting. In parallel, we measured attenuated Ca2+ responses to glutamate and ATP that were associated to changes in a number of Ca2+ handling genes in cells activated with PIFs. At the metabolic level, neurotoxic astrocytes displayed deficits in mitochondrial oxygen consumption rate (OCR) but increased basal extracellular acidification rate (ECAR) suggesting enhanced glycolytic activity, as supported by increased levels of extracellular lactate. Both compensatory ECAR and OCR/ECAR ratio were reduced in astrocytes incubated with PIFs. In summary, our ongoing research shows that astrocytes activated in vitro towards a neurotoxic phenotype display Ca2+ signaling and metabolic defects

that affect mitochondria-glycolytic interplay and that are likely to contribute to the pathophysiology of MS.

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Reference number: T1-P04

*(T1-P04)**APOE3 AND APOE4 HUMAN ASTROCYTES DIFFERENTIALLY AFFECT AB PATHOLOGY AND MICROGLIAL RESPONSES IN ALZHEIMER´S DISEASE CHIMERIC MICE***

Increasing evidence for a direct contribution of astrocytes to neuroinflammatory and neurodegenerative processes causing Alzheimer's disease (AD) comes from molecular studies in rodent models. However, these models may not fully recapitulate human disease as human and rodent astrocytes differ considerably in morphology, functionality, and gene expression. To address these challenges, we established an approach to study human astrocytes carrying different APOE variants within the context of the mouse brain by transplanting human induced pluripotent stem cell (hiPSC)-derived astrocyte progenitors into neonatal brains of suitable mice. APOE3 and APOE4 transplanted cells similarly differentiate into astrocytes that integrate functionally within the mouse host brain. Interestingly, in AD chimeric brains, APOE3 and APOE4 astrocytes show differences in ApoE expression and differentially affect amyloid-beta (Aβ) accumulation and microglial responses to Aβ plaques. In sum, we describe here a promising approach that allows studying the contribution of APOE3 and APOE4 human astrocytes to main pathological hallmarks associated to AD.

Authors
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Reference number: T1-P05

*(T1-P05) **MODULATION OF THE AXON TERMINAL TRANSLATOME BY EXTRACELLULAR VESICLES IN B-AMYLOID PATHOLOGY***

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.

Local translation allows the fast response of neuronal processes to environmental stimuli and contributes to the maintenance of axonal and dendritic homeostasis. Although the idea of local translation in neurons is getting established in the neuroscience field, it is still unclear whether neurons are the sole regulators of their own local proteome or if glial support it is required 1,2.

Interestingly, it has been suggested that extracellular vesicles (EVs) secreted by Schwann cells in the Peripheral Nervous System are capable of contributing to local protein synthesis and regenerate injured nerves 3. Nevertheless, although it is known that EVs delivered from oligodendrocytes support axonal transport and maintenance 4, it is least known whether glial cells are involved in local protein synthesis in the Central Nervous System neurons.

In Alzheimer´s Disease (AD) local translation is involved in the transmission of β-amyloid (Aβ) pathology from axons to somata, leading to pathological transcriptional changes that contribute to neurodegeneration in AD 5. Additionally, our research group has found presynaptically-translated proteins in synaptosomes isolated from Aβ-treated neurons. These results contribute to the growing body evidence that local translation in neurons contributes to neurodegenerative diseases.
Additionally, we have found evidence supporting that EVs secreted in the presence of astrocytes in the context of Aβ pathology modify the levels of translation in axons.
Based on these facts, our working hypothesis is that glial EVs contribute to the local translatome of axon terminals in physiological and in AD pathology. We have focused on studying translation in axon terminals of hippocampal neurons after treating them with EVs coming from neuron cultures, neuron-astrocyte co-cultures and glial cultures treated with Aβ or vehicle.

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5. Baleriola J et al. Cell, 158. 5 (2014)

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Reference number: T1-P06

(T1-P06) ***THE PRIMARY CILIUM AS AN ORGANELLE FOR ASTROCYTE-NEURON COMMUNICATION: AN IN-VIVO APPROACH.***

Primary cilia are microtubule-based organelles present in the plasma membrane of most cell types, including mature astrocytes and neurons. The primary cilium has emerged as a major signaling hub in the cell; however, little is known about the role of this organelle in the mature brain. Data from our lab show that neuronal primary cilia (nPC) is required for soluble amyloid beta oligomer signaling and modulation of autophagy, and that these events are modulated by physiological aging. Here, we hypothesize that, similarly to neuronal cilia, astrocytic primary cilium (aPC) senses and transduces extracellular signals and that it reacts to changes in neuronal cilium. We also hypothesize that aging might alter cilia-related events in old astrocytes. To test our hypothesis, we have studied how the loss of primary cilia in neurons induces changes in astrocytes, astrocytic primary cilia, and cilia-related autophagy. For that, we have studied by tissue immunofluorescence astrocyte reactivity and morphology in young and old IFT88::SLICK-H mice, a mouse model where cilia is lost in mature Thy1+ neurons. In these mice, we have also characterized astrocyte cilia presence and their morphology, as well as changes in the major autophagy markers. Moreover, we are working on the implementation of an in vitro model of PC-deleted human astrocytes and neurons, and a model of astrocyte-neuron co-culture, with the aim to study the dynamics of astrocyte and neuronal cilia changes between these two cell types. Overall, we aim to understand the role of mature astrocytic primary cilia in the brain, as well as its interplay with neurons and their possible changes during aging.

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Reference number: T1-P07

(T1-P07) ***GENERATION AND CHARACTERIZATION OF HUMAN PLURIPOTENT STEM CELL-DERIVED OLIGODENDROCYTES TO MODEL ALZHEIMER´S DISEASE***

Oligodendrocytes (OLs) are the glial cells that produce myelin within the central nervous system (CNS), which is crucial for a proper conduction of electrical signals. It is known that oligodendrocyte dysfunction and myelin degeneration occur in Alzheimer's disease (AD), but the specific mechanisms remain largely unknown. While animal models have contributed to unravel some of the altered mechanisms in AD, the species-specific complexity of human glial cells makes their study crucial in order to fully understand their role in the disease. In this sense, the human pluripotent stem cell (hPSC) technology constitutes a whole new tool to model AD.

We have differentiated two hPSC lines into oligodendrocyte precursor cells (OPCs): one line from a healthy individual and one line from a patient with sporadic AD. Cells have been characterized by immunocytochemistry at different stages of the protocol. At day in vitro 8, these cells already express progenitor markers such as Pax6, Sox2 or Nestin,

and at day 12 they begin to express the oligodendrocyte precursor markers Olig2 and NKX2.2. From day 12 to day 30 cells are grown in suspension, where Olig2-enriched aggregates are formed, and at day 30 spheres are replated and cells begin to migrate out of them. Between these migrating cells we found O4+ OPCs. O4+ cells obtained via this protocol can either be matured in vitro or isolated from day 75 on for molecular and functional analysis. By generating human OPCs, we aim to understand the pathophysiology of this specific cell type in AD.

Keywords: Alzheimer's disease, human induced pluripotent stem cells, oligodendrocyte, oligodendrocyte precursor cells

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Reference number: T1-P08

(T1-P08) ***CONTRIBUTION OF ASTROCYTES TO LOCAL TRANSLATION IN NEURONS***

Local translation is a mechanism specially relevant in neurons since it enables neurites to rapidly react to changes in the environment. For instance, the exposure of isolated axons to β-amyloid oligomers (Aβ), central to Alzheimer's disease, induces local translation 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. We are interested in the contribution of glial cells to local translation in neurons. Taking into account that extracellular vesicles (EVs) secreted by astrocytes are involved in the regulation of different neuronal functions, we hypothesize that astrocyte-derived EVs are delivered to neurons to modulate local translation in physiological and Aβ-induced conditions.

To assess the relevance of astrocytes in the neuritic proteome, we isolated neurites from primary neurons cultured in Boyden chambers in the absence/presence of astrocytes and analysed the extracted proteins by LC-MS/MS. Results show that the presence of astrocytes changes the neuritic proteome in both control and Aβ conditions. Gene Ontology analyses show that neuritic proteins regulated in presence of astrocytes are mainly involved in translation and are structural constituents of the ribosome. We have also addressed if EVs are directly involved in this regulation. Isolated EVs from Aβ-treated astrocytes and neuron-astrocyte cultures increase translation levels in neurites, suggesting that EVs are relevant for local translation in neurons. We have also studied the content of EVs released by neurons, neuron-astrocyte cultures and astrocytes by LC-MS/MS. Interestingly, ribosomal proteins are among the most enriched proteins.

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

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Reference number: T1-P09

(T1-P09) ***JANUS MAGNETIC PLASMONIC NANOPARTICLES: AFFINITY, SELECTIVITY, AND FUNCTIONAL EFFECTS IN MICROGLIA***

Janus nanoparticles (JNPs) made of gold and iron oxide combine remarkable optical and magnetic properties, including superparamagnetism, plasmonic behavior, and the ability to induce localized hyperthermal toxicity in tumor cells. However, their therapeutic potential in brain tumors or neurodegenerative conditions has not been explored. Before considering their therapeutic potential, their effect on microglia must be assessed because, as the brain professional phagocytes, microglia may have increased affinity for JNPs compared to other cell types. In addition, microglia would engulf dead tumor cells during phototoxic damage, leading to JNP accumulation within microglia. Since JNPs have a similar size to other nanoparticles that induce inflammatory effects, such as nanopollutants, the potential inflammatory effect of JNPs needs to be assessed prior to their use as therapeutic agents. In the present work, Au-Fe3O4 JNPs were synthesized via seed-mediated-growth method and their effects on microglia studied in primary cultures. First, microglia were treated in vitro with different concentrations of JNPs. We observed a strong cellular uptake of JNPs (1, 100µM, 6-24h) without affecting cell density, ruling out proliferation and cellular death. However, we observed morphological alterations that suggested a potential inflammatory response, a hypothesis that we are currently testing by challenging JNP-treated microglia with LPS (bacterial lipopolysaccharides). In addition, we are also testing the selectivity of microglia compared to other brain cell types using a triple co-culture model. Preliminary data suggest that nanoparticles have higher affinity for microglia, compared to neurons and astrocytes. These results set the ground for using JNPs as therapeutic agents in brain disorders, including brain tumors.

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Reference number: T1-P10

(T1-P10) ***LOCAL TRANSLATION IN MICROGLIAL PERIPHERAL PROCESSES***

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 a spatial and temporal manner to numerous environmental stimuli and it is extremely relevant in highly polarized cells. Although most of the work on local protein synthesis has been performed in neurons, 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 conditions1. More recently, the ability of peripheral astrocytic processes to translate proteins has also been described2.

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. Local translation in microglia has recently been described3. 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, the hypothesis we propose is that local translation in microglial peripheral processes plays an important role in brain function and dysfunction.

Previously, our group has obtained results indicating that LPS is able to induce changes in microglial local protein synthesis. Therefore, we studied the effect of inflammation in local translation of transcripts known to be locally translated in neuronal growth cones, in order to determine whether they play a role in microglial cell polarity and cytoskeletal rearrangements.

Specifically, β-actin and Pard3 RNA granules and translation events are increased in microglial peripheral processes when acutely treated with LPS, suggesting local translation of these two transcripts might be required for the inflammatory response in microglia.

Furthermore, we are currently modulating the localization of β-actin into microglial peripheral processes by silencing ZBP1, which has been described as the RNA binding protein (RBP) for this mRNA in neurons4.

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Reference number: T1-P11

(T1-P11) ***MORPHOLOGIC AND FUNCTIONAL CHARACTERIZATION OF ASTROCYTES IN PARKINSON'S DISEASE.***

Parkinson's disease (PD) is a chronic and multifactorial neurodegenerative disease with unknown etiology. PD patients can manifest clinical symptoms up to 20 years after neurodegeneration begins in substantia nigra and striatum. Currently, there is no cure for PD but only palliative treatments.

Genes known to have a causative role in the development of PD are expressed also in astrocytes and are relevant to astrocyte function. Thus, astrocytes and their role in supporting neuronal viability have been at the center of PD investigation. Recently, we demonstrated that human-induced pluripotent stem cells-derived astrocytes (hiAs) generated from PD patients are atrophic and have reduced mitochondrial activity as compared to astrocytes generated from non-PD donors. In this study, we have analyzed astrocyte morphology in post-mortem PD brain and confirmed a robust area and complexity drop in these cells in the subthalamic nuclei whereas we did not observed any significant reduction in the basal ganglia. To explore the impact of these morphological changes in astrocyte function we analysed the expression of key metabolic markers in hiAs by immunofluorescence, Western blot and semi-quantitative RT-PCR. We observed that PD hiAs expressed lower levels of EAAT2 (a glutamate transporter) and aquaporin 4 (a water channel) but higher levels of PTX3 (pentraxine 3, involved in the primary inflammatory response) respect to control hiAs. Altogether, our data show astrocytic atrophy as a pathological feature of PD brain and suggest that astrocytic asthenia may contribute to neuronal death through decreased homoeostatic support and failed neuroprotection.

Funded by BIOEF (EiTB Maratoia), CIBERNED.

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Reference number: T1-P12

(T1-P12) ***NEDDYLATION IS ESSENTIAL FOR OLIGODENDROCYTE DIFFERENTIATION AND MYELINATION***

The myelin sheaths that cover the axons are essential for the conduction of nerve impulses in the nervous system. In this regard, mature oligodendrocytes are the myelinating cells of the central nervous system (CNS). In recent decades, research on the mechanisms involved in myelination has generally focused on transcriptional level, whereas post-translational modifications remain mainly unknown. Among the latter, recent studies describe neddylation as a new mechanism analogous to ubiquitination, involved in numerous cellular functions. Yet, little is known about its role in the CNS. NAE1 is the initiating enzyme of the neddylation cascade, and is the target of the pharmacological inhibitor MLN4924 (MLN). On this basis, our main objective was to

study the role of neddylation in oligodendrocytes and, consequently, in myelination. First, we studied the effect of MLN during development by intraperitoneal injections into rats from postnatal day 7 to 15. In the brain and spinal cord of these rats, there was a significant impairment in the expression of proteins related to myelinating oligodendrocytes, such as MBP and MAG. Likewise, in MLN-treated cortical oligodendrocyte cultures, the inhibition of neddylation caused a significant decrease in cell viability and the expression of MBP and MAG, among others. Together, these results indicate that neddylation is essential for oligodendrocyte viability and maturation, and may negatively affect the formation and maintenance of myelin sheaths in the CNS. These findings shed light on the involvement of neddylation in myelination and lay the groundwork for future studies on the role of this mechanism in demyelinating diseases, such as multiple sclerosis.

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Reference number: T1-P13

(T1-P13) ***INCREASED SURFACE EXPRESSION OF P2X4 RECEPTORS IN MICROGLIA AMELIORATES EAE***

One of the main hallmarks of disease progression in MS is the decline in tissue repair. A key step in initiating myelin repair is the clearing of myelin debris by microglia and macrophages. We have previously proposed P2X4 as 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 selectivity of the pharmacological treatment and the cell-specific functions of P2X4 receptors contributing to the therapeutic effect. We used P2X4mCherryIN knock-in mice, in which P2X4 is substituted by a non-internalized P2X4mCherryIN (P2X4KI), leading to plasma membrane overexpression of P2X4 in all cells natively expressing P2X4. ATP-mediated inward currents in non-internalized P2X4KI microglia were higher confirming the increased surface density of functional P2X4 receptors in microglia. Importantly, lysosomes in P2X4KI microglia were bigger and have a lower pH than in WT microglia suggesting that P2X4 overexpression increases lysosomal function, a fact that could contribute to a more efficient myelin degradation. We therefore analysed the impact of increased surface P2X4 expression in the experimental autoimmune encephalomyelitis (EAE) model. Interestingly, P2X4KI mice showed an amelioration of the neurological symptoms at EAE peak and EAE chronic phase in female mice, but not in males. We further checked the specific contribution of microglial P2X4 using double transgenic mice expressing P2X4mCherryIN specifically in microglia/macrophages. CD11bCre-P2X4KI female, but not male, mice also showed an amelioration of neurological

symptoms after EAE induction. Our data demonstrate that increased surface P2X4 expression effectively promotes myelin recovery and that this therapeutic effect has a clear gender dimorphism. Further experiments are required to understand the differences in P2X4 expression, signalling or function between female and male microglia contributing to EAE outcome.

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Reference number: T1-P14

(T1-P14) ***ASTROCYTIC AND NEURONAL IGF-1 GLUCOSE REGULATION LOOP IN BRAIN***

While there is ample evidence of a wide neuroactive role of circulating insulin-like growth factor I (IGF-I), the physiological significance of the comparatively low amounts of locally produced IGF-I in the mammalian brain is slowly being unveiled. It has been described that IGF-I regulates glucose in brain in cooperation with insulin, as well as brain regulates glucose in other body targets, such as liver. The mechanisms involved in regulation of brain function by blood glucose still poorly understood, although it has been described that IGF-I cooperates to modulate glucose uptake by brain astrocytes.

To determine the implication of both astrocytes and neurons (together and separately) involved in brain glucose homeostasis, we studied glucose uptake (glucose capture assay), cell viability (MTT assay), glycogen accumulation and lactate release in astrocyte-neuronal co-cultures and monocultures exposed to different glucose concentrations (hypoglycemia: 5 mM, normal glucose: 25 mM and hyperglycemia: 50 mM).

As a result, glucose modulated in an IGF-IR-dependent fashion all the parameters studied at some extent. First, we confirmed that glucose modulates in vitro IGF-IR activity in neurons, which showed increased phosphor IGF-IR after 1 hour exposure to high glucose (50 mM) that was not observed in case of astrocytes. Moreover, astrocytes showed a 10% of cell viability decrease after exposure to higher concentrations (50 mM). However, when astrocytes and neurons were co-cultured, phosphoIGF-IR was also increased after high glucose, showing a higher phosphorylation level than the one obtained in neuron monocultures. Co-culture of astrocytes with neurons also elicited a significantly higher increased glucose uptake in response to 50 mM glucose as compared to astrocytes cultured alone. Interestingly, a higher basal glucose uptake was observed in astrocytes lacking IGF-IR (FIR astrocytes) as well as a lack of effect after dose-response IGF-I treatments, as WT astrocytes showed. Astrocytes grown with neurons also produced more glycogen when exposed to 50 mM glucose. However, when grown alone, astrocytes showed decreased glycogen synthesis in response to the same dose of glucose. Astrocytes also released more lactate at 25-50 mM glucose when cultured with neurons, but not when cultured alone. In neurons cultured alone, glycogen

content or lactate release were undetectable. In the absence of astrocyte IGF-IR, glycogen concentrations remained partially sensitive to changing glucose levels, whereas lactate release became independent of glucose levels.

Taken together, these results suggest that glucose regulates neuronal activity of brain IGF-I through this neuron-astrocyte crosstalk, which is affected in the glucose capture levels, glucogen accumulation and lactate release and mediated, at least partially, by IGF-IR to assure proper energy balance to neurons through lactate, a mechanism that accords to the “astrocyte-to-neuron lactate shuttle” proposal.

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Reference number: T1-P15

(T1-P15) ***MYELIN PHAGOCYTOSIS IN OLIGODENDROCYTES INCREASES THEIR PROLIFERATION***

Oligodendrocytes (OLs) are the myelinating cells of the central nervous system and the target of a dysregulated immune system in demyelinating diseases. In addition, OLs can play an active role in immune modulation in multiple sclerosis, acting as antigen-presenting cells. In order to broaden the knowledge on this topic, we analyzed whether OLs have phagocytic capacity. By time-lapse imaging, we observed that OL’s processes efficiently endocytose Alexa488-labelled myelin particles that are thereafter transported into the cytoplasm. Surprisingly, the addition of myelin to OL cultures induced an increase in the total number of OLs. Using lineage-specific markers, we detected an increase in the number of mature MBP+ OLs as well as NG2+ oligodendroglial progenitor cells. However, the ratio of NG2+/MBP+ cells was not altered, excluding an effect in OL differentiation. Myelination of nanofibers was also not significantly altered in the presence of myelin. However, we detected a significant increase in OL proliferation. We then performed stereotaxic injections of myelin in the cortex of adult mice. After 24 hours, Iba1+ microglial cells migrated towards the injection site to clean up myelin debris. As in vitro, myelin debris induced an increase in proliferation in both the core lesion and the perilesion. We conclude that OLs have phagocytic capacity and that myelin acts as a trophic factor promoting proliferation and/or survival of OLs.
Funded by MINECO, CIBERNED and Gobierno Vasco. C.P. holds a fellowship from MINECO.

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Reference number: T1-P16

(T1-P16) ***STEP-WISE COLONIZATION AND MATURATION OF MICROGLIA IN THE DEVELOPING HIPPOCAMPUS AND CEREBELLUM***

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. However, the molecular mechanisms by which microglia identity and function are established and maintained are largely unknown. We hypothesize that microglia progressively mature once they enter the brain parenchyma. To test this hypothesis, we focused on the hippocampus and the cerebellum, two structures whose development occurs largely at postnatal stages. First, we identify the critical window of microglia maturation by analyzing cell density, morphology and phagocytosis efficiency by confocal microscopy, and microglial process dynamics by 2-photon microscopy. We found that microglia progressively colonized the hippocampus and cerebellum from postnatal day 2 (P2) to P14. Then, they progressively acquired a branched morphology and achieved their highest efficiency of phagocytosis at P21. To further define the colonization process and correlate the different maturation parameters, we are developing a mathematical simulation to model the population dynamics. Additionally, as the critical window of microglia maturation is concurrent with the establishment of the hippocampal and cerebellar circuits, we are currently exploring the influence of the developing neuronal environment on microglia identity based on two scenarios: 1, disrupting the neuronal scaffolding using a mouse model of reelin depletion; and 2, inhibiting neuronal activity using designed receptors activated by designer drugs (DREADDS). Deciphering the microglial maturation program and its potential modulation by the developing neuronal connectivity is highly relevant because early changes could be genetically imprinted and lead to long-term functional alterations, impacting on many neurodevelopmental and neurodegenerative disorders.

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Reference number: T1-P17

(T1-P17) ***THE STIFFNESS OF THE SUBSTRATE AFFECTS MÜLLER CELL SURVIVAL, MORPHOLOGY AND REACTIVITY***

Müller cells, the main glial cell type of the retina, are key to retinal homeostasis, metabolism, neuronal support and signaling. They have been shown to be

mechanosensitive and thus respond to mechanical cues such as changes in the substrate (i.e. extracellular matrix) stiffness. These alterations are related with different diseases of the retina, such as proliferative vitreoretinopathy, age-related macular degeneration, diabetic retinopathy and glaucoma, where Müller cells intermediate filaments polymerization is enhanced by the stress forces generated by the environment. However, how these changes affect Müller cells are yet to be determined. Thus, our objective is to assess how different substrate stiffness affects Müller cells survival and behaviour in vitro.

In order to answer this objective, we performed primary cultures of rat Müller cells on acrylamide gels of 1kPa, 10kPa, 100kPa and 200kPa, respectively, and on a glass-slip as control. All culture dishes were treated with poly-L-Lysine and laminin. Then, Müller cells were labelled by immunocytochemistry with antibodies against vimentin for morphology, Ki67 for proliferation, GFAP for reactivity and the expression of pressure receptors were also analysed with antibodies against Piezo1 and TRPV4. Positive-labelled cells were counted and cell area was measured with ImageJ.

We observed that viability is severely reduced on softer gels (1 kPa 6%; 10 kPa 16%), while on stiffer gels, mainly 200 kPa, it closely resembles control conditions (94%). Likewise, there are significant variations in cell area and morphology; Müller cells grown on softer gels exhibit a round shape and reduced cell area compared to control (1 kPa 20%; 10 kPa 50%), as opposed to those grown on stiffer gels, which show a more elongated shape and a more similar area to the control ones (100 kPa 82%; 200 kPa 84%). Furthermore, more Müller cells grown on softer gels become reactive as shown by the increased expression of GFAP in the cells cytoplasm. However, cell proliferation does not seem to be affected by substrate stiffness.

In conclusion, Müller cells do not thrive in softer substrates as shown by their reduced viability, cell area, round morphology and increased reactivity. This suggests that the stiffness of the substrate in vitro, and in consequence the extracellular matrix stiffness, is determinant for Müller cell behaviour.
Supported by ELKARTEK (KK-2019/00086), MINECO-Retos (PID2019-111139RB-I00) and PIBA (2020_1_0026).

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Reference number: T1-P18

(T1-P18) ***TOWARDS PHARMACOLOGICAL MODULATION OF MICROGLIAL PHAGOCYTOSIS***

Microglia, the immune cells of the central nervous system, display a variety of functions to maintain homeostasis in the brain. As the brain professional phagocytes, they 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. Therefore, our goal is to find pharmacological modulators of microglial phagocytosis. Using a high throughput screening strategy, we tested 600 pharmacological 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 or inhibitors of 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 using two different approaches to validate phagocytosis modulators in vivo in physiological conditions: intraperitoneal delivery of the compounds and intrahippocampal delivery using osmotic pumps. Considering the lack of strategies to modulate microglial 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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Reference number: T1-P19

(T1-P19) ***ASSESSING THE ROLE OF OLIGODENDROCYTE CB1 RECEPTORS IN MULTIPLE SCLEROSIS***

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) and a main cause of disability among young adults. MS symptomatology is caused by the demise of mature oligodendrocytes (OLs) that produce and maintain CNS myelin and provide metabolic support to axons. These cells originate during development and adulthood from a population of oligodendrocyte precursor cells (OPCs) that enable myelin repair in MS.
The endocannabinoid system has protective effects in MS associated to the activation of CB1 receptors in neurons and CB2 receptors in microglia and peripheral immune cells. OLs and OPCs express low levels of CB1Rs that mediate protection from excitotoxicity and promote lineage progression in vitro. Pharmacological in vivo studies with cannabinoid agonists also suggest that the CB1R pool in oligodendroglia accelerates myelination and engages myelin repair in MS. However, bona-fide evidence supporting the involvement of CB1Rs expressed by OLs and OPCs in the myelin repair and regenerating effects of (endo)cannabinoids in MS is still lacking.

Here we investigated the role of oligodendrocyte CB1Rs in MS by addressing 1) the phenotype of conditional mutant mice lacking CB1Rs in OLs or OPCs in the experimental autoimmune encephalomyelitis (EAE) model of the disease, and 2) the effects of cannabinoid-modulating drugs on energy metabolism in these cells based on recent reports showing inhibitory activity of CB1Rs on mitochondrial respiration in astrocytes and neurons. Mice lacking CB1Rs specifically in OLs and OPCs were generated by

crossing CB1Rf/f mice with PLP-Cre and NG2-Cre mouse lines, respectively. PLP-CB1R-KO mice displayed slightly attenuated clinical scores during EAE progression. By contrast, NG2-CB1R-KO animals showed increased EAE severity as compared to their control littermates. On the other hand, metabolic analyses in rat oligodendrocyte cultures showed that the cannabinoid agonist ACEA and the endocannabinoid hydrolysis inhibitor JZL184 reduce mitochondrial oxygen consumption rate (OCR). Taken together, these preliminary results suggest a complex role of oligodendrocyte CB1Rs during autoimmune demyelination and point to the possibility that (endo)cannabinoids modulate oligodendrocyte energy metabolism.

Funded by FEDER and ISCIII (PI21/00629); Basque Government (PIBA19-0059), CIBERNED (CB06/0005/0076) and ARSEP Foundation.
Keywords: multiple sclerosis, CB1 receptors, oligodendrocyte, oligodendrocyte precursor, EAE, mitochondria

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Reference number: T1-P20

(T1-P20) ***DIFFERENCES IN LIPID ENVIRONMENT IN RAFT AND NON-RAFT MEMBRANES IN HUMAN ASTROCYTES TREATED WITH PRO-OXIDANT AGENT***

Aging related diseases have increased during the last decades due to an improvement of life conditions. To maintain a proper neural function, glial cells functions and specifically the correct functioning of astrocytes, is a key aspect to ensure brain health. Changes in cell homeostasis or the presence of external agents with oxidative properties can trigger these conditions, producing pathological changes such as the augmentation of oxidized products from lipids, sugars or nucleic acids. Changes in the lipid environment could imply the disruption of lipid rafts, microdomains that provide a platform for fast physiological regulation in response to metabolic conditions.

To study the influence of an external pro-oxidant agent, in lipid ordered membranes (LOM, or lipid rafts), and lipid disordered membranes (LDM, or non-raft membranes), we have developed microarrays of cell membranes isolated from a human astrocytic cell line (1321N1). For this purpose, MALDI mass spectrometry assays were performed in raft and non-raft membranes from control or Paraquat treated cells in positive and negative-ion mode. Significant differences between the lipid adducts presents in raft and non-raft membranes were observed in both conditions as well as the differences between control and paraquat treated astrocytes.

The presence of this compound entails the oxidation of lipid membranes, obtaining lipid adducts with more unsaturations or more oxygen molecules. Furthermore, these data are consistent with oxidative stress experiments performed previously. Our result point out that this technology can be useful not only to analyze the lipidic environment but also to perform different assays with little amount of sample, allowing the possibility of developing assays in human samples.

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Reference number: T1-P21

(T1-P21) ***MODIFICATION OF THE EXTRACELLULAR MATRIX IMPAIRS MICROGLIAL MOTILITY***

Beyond neurons and glia, the Central Nervous System (CNS) holds a plastic scaffold known as the extracellular matrix (ECM). Unlike the ECM from connective tissue, where collagen is the main unit, the neural interstitial matrix consists mostly on long chains of the glycan polymer hyaluronan. Protein components of the ECM bind to hyaluronan forming a self-assembled matrix that functions as structural framework and signalling hub. Microglia, the never-resting immune cell of the CNS, constantly surveys the brain parenchyma, interacting with neighbouring cells and the surrounding extracellular microenvironment. We have recently described in adult parkinsonian mice a bidirectional loop between hyaluronan and microglia, which affects both matrix structure and microglia reactive state, with consequences on neurodegeneration and tissue architecture (Soria et al., 2020, Nat Commun). Despite recent advances on matrix-glia interplay, it is unknown whether changes in the structural matrix affect microglial motility. Here we report on the interaction between microglia and hyaluronan, using an ex vivo approach to characterise microglia dynamics in response to matrix modification. Using 2-photon time-lapse imaging in acute slices of Cx3Cr1+/eGFP mice, we show that microglial motility, ramification and territory surveyed are reduced upon hyaluronan fragmentation ex vivo, with no changes when other matrix components are degraded instead. We also report alterations in directed motility upon laser ablation after hyaluronidase treatment. These results suggest impairment of microglial motility upon matrix modification and shed light on the dual role of hyaluronan as scaffolding polymer and pro-inflammatory signal in the CNS.
Funding: Ministry of Science and Innovation, Basque Government

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Reference number: T1-P22

(T1-P22) ***GENERATION AND CHARACTERIZATION OF HUMAN IPSC-DERIVED ASTROCYTES AS NOVEL MODEL OF MULTIPLE SCLEROSIS***

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS) caused by autoimmune responses against myelin followed by neuroinflammation and neurodegeneration. Astrocytes are crucially involved in MS initiation and progression and regarded as potential cellular targets for the development of novel therapies. To date, however, astrocyte pathophysiology in MS has been primarily studied in rodent models that do not recapitulate the complexity and specific functional features of human cells. Assessing the relevance of findings in animal models using human astrocytes is therefore crucial for better-adapted translational applications to treat MS.

Here we aim at differentiating astrocytes from MS patients and control subjects for functional studies using the human induced pluripotent stem cell (hiPSC) technology. In particular, we will generate astrocytes from 4 hiPSC lines with 2 different genotypes (TT and CC) of the polymorphism rs1800693 that affects the TNFRSF1A gene and confers an increased risk for MS. Differentiation of hiPSCs to astrocytes is carried out in 3 consecutive steps that include 1) generation of neural progenitor cells (NPCs), 2) astrocyte induction in the presence of EGF and LIF and 3) maturation of astrocyte progenitors in the presence of CNTF. NPCs generated from hiPSCCC and hiPSCTT MS lines were 9-98% positive for the neuronal progenitor markers Pax6, Sox2 and Nestin. We also corroborated that mature astrocytes differentiated from 1 hiPSCCC line display cytosolic Ca2+ transients in response to ATP. Our results support the utility of generating human astrocytes from MS patients as novel model to study disease pathogenesis and therapy.

Funded by FEDER and ISCIII (PI21/00629); Basque Government (PIBA19-0059), CIBERNED (CB06/0005/0076) and ARSEP Foundation.

Keywords: multiple sclerosis, astrocyte, hiPSC, calcium

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Reference number: T1-P23

(T1-P23) ***THE PRIMARY CILIUM AS AN ORGANELLE FOR ASTROCYTE-NEURON COMMUNICATION: AN IN VITRO APPROACH.***

Primary cilia are microtubule-based organelles located in the surface of different type of cells, including astrocytes. Primary cilia have a prominent role in cell signaling, although how cilia function is during aging is not well understood. Moreover, previous data from our laboratory show that amyloid beta modulates autophagy in a cilia- and age-

dependent manner in neurons. Here, we aim to understand how cellular senescence – one of the hallmarks of aging- modulates cilia function and its response to amyloid beta in astrocytes. For that purpose, here we show cellular senescence models in primary mouse astrocytes and in human immortalized astrocytes, as well as how we can impair primary cilia in astrocytes by using CRISPR/Cas9 system and lentiviral based shRNA gene silencing. These methodologies will help us studying the role of astrocytic cilia in cellular senescence, and if this organelle is essential for amyloid beta signaling in astrocytes.

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Reference number: T1-P24

(T1-P24) ***STAT3 INHIBITION PREVENTS THE TRANSFORMATION OF NSCS INTO REACTIVE-NSCS IN EPILEPSY***

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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Reference number: T1-P25

(T1-P25) ***MODELLING NEURODEGENERATIVE DISEASES WITH HUMAN BIOMODELS***

Most of current knowledge of brain physiopathology and pharmacological investigation on human brain is based on observational and functional studies on animal models. Nonetheless, several neuroprotective drugs have failed in human clinical trials despite promising pre-clinical data, suggesting that conventional cell cultures and animal models cannot precisely replicate human brain physiopathology. Although essential in many fields of neurodegenerative disease investigation, animal models can not fill the gap with human biology. The development of cell reprogramming with the generation of human induced pluripotent stem cells (hiPSC) opened the door to bridge this gap giving the opportunity to culture patient's neural cell in a dish. Here, we show how the Basque biomodel platform for human research (BBioH) can model the brain physiology and the main pathological hallmark of neurodegenerative diseases, mainly Parkinson's disease, using hiPSc-derived neural cells (astrocytes and neurons) and brain organoids. Especially, midbrain organoids recapitulate the cellular hallmarks of the actual human midbrain, with tyrosine hydroxylase positive neurons, astrocytes and oligodendrocytes. These organoids represent miniaturized and simplified versions of human midbrain converting them in patient's avatars worth to study the progression of neurodegeneration.

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Reference number: T1-P26

(T1-P26) ***DECELLULARIZED PORCINE ADIPOSE TISSUE HYDROGELS AND BIOINKS FOR CENTRAL NERVOUS SYSTEM***

Functional repair of injured tissue in the adult central nervous system (CNS) remains a big challenge for current biomedical research and its upcoming clinical translation. The axonal regeneration is generally low, and it is additionally restricted after injury by the presence of inhibitory molecules, generated by the glial scar.1 Extracellular matrix (ECM) scaffolds derived from mammalian tissues retain a number of bioactive molecules and their ability for CNS repair has been recognized.2 ECM materials represent structures very similar to those of the uninjured host tissue with complex natural composition, three-dimensional structure, retention of growth factors, and bioactive properties, including stimulation of angiogenesis and migration of endogenous progenitor cells or modulation of immune reaction.3 Previously described injectable ECM hydrogels prepared by decellularization of porcine brain, spinal cord, and porcine urinary bladder revealed in vitro neurotrophic properties.4,5 Other ECM materials such as decellularized muscle, have shown the capacity to enhance axon sprouting in an injured spinal cord.6 Here we describe an ECM material obtained by porcine adipose tissue decellularization,

post-processing techniques to create injectable hydrogel and bioink and the preliminary research of Mouse Neural stem cell (NE-4C) behavior after encapsulation and bioprinting. The decellularized adipose ECM material, meets the decellularization criteria established by the research community (absence of cell nuclei, 24.8 ± 2.05 ng/mg remnant DNA and effective lipid removal) while conserved almost all the ECM proteins (33 of 38 ECM-derived identified proteins).7 The composition of the decellularized adipose ECM material includes collagens, glycoproteins, proteoglycans, and basement membrane proteins such as collagen type IV, laminin, heparan sulphate proteoglycan-2 and nidogens. Decellularized adipose ECM material showed excellent compatibility with post-processing techniques. It can be easily fine-grained as a and optimally solubilized in acetic acid to obtain coatings or freeze-dried solid foams, with no cytotoxic, neither immunogenic response for stem cell8 and macrophage cultures.7 Additionally, In-situ polymerizable hydrogels and bioinks can be produce by acid-pepsin solubilization. Hydrogels formulated in the range of 0.3-1% (w/v) were used to encapsulate neural NE-4C stem cells and showed high viability and proliferation after 7 days in culture. Additionally, porcine decellularized adipose ECM formulated at 3% (w/v), resulted in a printable bioink able to combine with extrusion bioprinting technique and promote the orientation of the neurites toward the printing direction. Differentiated neurons were organized as neural clusters homogeneously distributed in the 3D culture which showed numerous and elongated projections of neurites. In conclusion, results showed a porcine decellularized adipose ECM material as a potential candidate for applications that require neural cell delivery and automated fabrication of 3D in vitro models to study biological processes of healthy brain and neurodegenerative diseases.

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ACKNOWLEDGMENTS

This work has been supported by the European Union's Horizon 2020 Research and Innovation Programme (H2020-FETOPEN-2018-2020, NeuroStimSpinal Project, Grant Agreement No. 829060).

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Reference number: T1-P27

(T1-P27) ***REACTIVE NEURAL STEM CELLS AND ABERRANT NEUROGENESIS IN A NEURON-SPECIFIC MODEL OF DRAVET SYNDROME***

Hippocampal neurogenesis (HN) is a form of neuroplasticity which implicates the generation of new neurons from neural stem cells (NSCs) in the dentate gyrus (DG). Although HN persists throughout adulthood, it reaches maximum values during early postnatal periods, when the population of NSCs is at its largest.
NSC activity and HN are particularly regulated by neuronal activity and severe alterations have been found in the hippocampal neurogenic niche in mouse models of epilepsy. Induction of reactive-like and gliogenic NSCs (React-NSCs) besides aberrant neurogenesis, defined by altered newborn neuron morphology, migration and functional properties, are induced by epileptic seizures.

We are thus interested in Dravet Syndrome (DS), a severe form of infant epilepsy characterized by the early onset (3-6 months of age) of seizures. DS is caused by mutations in the Scn1a gene encoding the α1 subunit of sodium channel Nav1.1, and provokes febrile seizures, hyperexcitability, neurological comorbidities and premature death. Therefore, we hypothesize that early seizures could have a greater impact and longer-lasting on the neurogenic niche in DS due to their early onset.

Through confocal microscopy imaging we are analysing the neurogenic niche of a novel inducible knock-in mouse model of DS (Syn-Cre/Scn1aWT/A1783V) at postnatal day 25 (soon after the onset of seizures) which consist in the neuron-targeted expression of a missense mutation (A1783V) in the Scn1a gene. We have observed the induction of React-NSCs, characterized by more and thickened branches plus overproliferation. We have also observed a strong induction of aberrant neurogenesis. Newborn immature neurons, identified by the expression of doublecortin are present in much higher numbers; migrate abnormally towards the hilus and the molecular layer; and have basal dendrites and V-shaped proximal apical dendrites. We are currently investigating other possible alterations such as cell death/survival, differentiation imbalance and changes in astroglia and microglia.

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Reference number: T1-P28

(T1-P28) ***PLCB1 REGULATES G1-PHASE PROGRESSION IN HUMAN NTERA-2 CELLS: IMPLICATIONS IN THE CONTROL OF CELL PROLIFERATION AND NEURONAL DIFFERENTIATION***

The involvement of PLCβ1 in cell cycle control and differentiation has been reported in various cell lines, including some neuronal cell lines (1, 2, 3). Human NTERA-2 (NT2) cells can be induced to differentiation into post mitotic NT2N neurons with retinoic acid (RA) or cytosine-β-D-arabinofuranoside (AraC) treatments, and this process is accompanied by an increase in PLCβ1 expression (4). Furthermore, AraC-induced neuronal differentiation is blocked by gene silencing of PLCβ1, and induced by transient overexpression of PLCβ1a/b in the absence of any other inducer, demonstrating that PLCβ1 is not only necessary but also sufficient to induce the differentiation process in NT2 cells (5). Trying to understand neuronal differentiation, this process has been linked many times, to the extension of the cell cycle and especially to the G1 phase (6, 7).

In this work, we investigated whether the basis of the mechanism to drive the process of NT2 cell differentiation of PLCβ1 is to induce changes in cell cycle kinetics. Specifically, we studied, by BrdU accumulation and pH3 labeling analysis, whether PLCβ1 expression downregulation in NT2 cells alters the duration not only of the entire cycle, but also of each of its phases.

The results showed that PLCβ1 expression downregulation significantly accelerates the cell cycle. Thus, after PLCβ1 siRNA cell-transfection the time to complete the entire cellular cycle was reduced by 20.11%, being the shortening of duration mainly in the G1 phase, where the decrease was a 33.7%. As a result, it may be thought that when PLCβ1 is expressed at basal level it should have the opposite effect, i.e. it may be responsible for maintaining a slower rhythm of the cellular cycle and this may, among other things, allows the differentiation process in the presence of different neurogenic stimuli.

This research was funded by the Basque Government (IT1230-19), MINECI, Spanish Ministry of Science, Innovation and Universities (CTQ2017-85686-R).

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Reference number: T1-P29

(T1-P29) ***HUMAN DENTAL PULP STEM CELLS EXPRESS THE ANGIOTENSIN-RENIN SYSTEM AND ITS STIMULATION INCREASES CELL PROLIFERATION, WHILE RETAINING THEIR NEURODIFFERENTIATION POTENTIAL***

Human dental pulp stem cells (hDPSCs) are a stem cell population derived from the neural crest which shares many characteristics with brain neural stem cells. Upon completion of neurodifferentiation protocols in serum-free media, hDPSC cultures generate cell populations expressing not only mature neuronal markers (NeuN, DCX, MAP2) but also mature astroglial (S100b, GFAP) and oligodendroglial (Olig2) markers. HDPSCs also express a large repertoire of physiologically active neurotransmitter and neurotrophin receptors, which generate intracellular calcium and sodium currents and regulate their stemness characteristics. Finally, hDPSCs are also able to differentiate to endothelial cells and pericytes, which can de novo generate blood vessels that may integrate into the brain vasculature. For all their remarkable properties, there is still a need for better control of the differentiation process of hDPSCs, to be able to finely regulate the generation of mature neuroglial and/or vascular cell populations. A fine control of hDPSC differentiation would be a powerful tool to improve the design of tailored personalized cell therapies. Furthermore, because most neurovascular differentiation protocols have to be carried out in the absence of fetal serum, there is also a need to explore new signaling pathways whose stimulation may promote the ex vivo expansion of hDPSCs, without negatively affecting their differentiation potential. One of the major challenges in stem cell therapies is to obtain a sufficient cell population without changing their neurodifferentiating capacity. Renin Angiotensin system (RAS) has been described to be involved in the control of cell differentiation and proliferation in several types of stem cells. However, its expression in hDPSCs has not been characterized yet. We hypothesized that RAS activity could also be found in hDPSCs, and its stimulation might be an alternative strategy to boost stem cell proliferation. In the present work, we describe RAS expression in hDPSCs. We show for the first time by both RT-qPCR and western blot the expression of key components the renin angiotensin system (RAS), including ACE2, ACE, AT1R, AT2R and MAS1 in hDPSCs, as well as the possibility of modulating its activity by means of agonist Angiotensin1-7 and antagonist A7774 of MAS1. By Ki67 immunostaining we determined an increase of

hDPSCs proliferation. Finally, differentiation of hDPSCs after RAS activation was not affected with regard to the expression of neuronal, astroglial and oligodendroglial markers, compared to standard protocols.
In conclusion, we describe here a new signaling pathway involved in the control of stemness and proliferation of hDPSCs. RAS stimulation allowed increasing the proliferation rate of hDPSC cultures without affecting their neuroglial differentiation capacity.
Funding: UPV/EHU (COLAB19/03; GIU20/050; GIU16/66; UFI11/44; IKERTU-2020.0155) Basque Government: GV/EJ; GV-GIC15/52; IT-927-16; IT831-13; Hazitek ZE-2019/00012-IMABI & ELKARTEK KK-2019/00093) and Health Department (Grant 2021333012), MICINN/AEI/10.13039/50110001103 (PID2019-104766RB-C21 & MINECO RYC-2013-13450). J.S. have an IKASIKER fellow (IkasC_2021_1_0258). B. Pardo-Rodriguez has a Predoctoral funding for non-doctoral Research Staff from the Basque Government (Ref. 2021_1_0112). I.M. have a UPV/EHU mod-III grant (PIFBUR21/05). Polimerbio and Y. P. have a Bikaintek PhD grant (20-AF-W2-2018-00001). I.G. have a Margarita Salas grant (MARSA21/41).

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Reference number: T1-P30

(T1-P30) ***USE OF BIORESORBABLE NANOPATTERNED POLYMER SCAFFOLDS AS A STRATEGY TO GUIDE THE MIGRATION OF NEURAL AND DENTAL STEM AND PROGENITOR CELLS***

Injuries in the central nervous system (CNS) and nerve lesions have a strong impact on high financial expenses and quality of life for the patients. We present a nanostructured polymer scaffold based on bioresorbable elastomeric co-polyesters functionalized with graphene derivatives to promote the attachment, alignment and migration of stem and progenitor cells of neural (murine origin, mNSC, control) or dental pulp stem cells (human origin, hDPSCs). hDPSCs present substantial advantages with respect to other types of stem cells for CNS therapy, as hDPSCs express neural markers and neurotransmitter receptors and have excellent capability to be differentiated towards neural lineage due to its neural crest origin. hDPSCs are resistance to hypoxic conditions & highly accessible, secrete neurotrophins and anti-inflammatory factors.

Scaffolds of lactide and caprolactone based copolyesters were first nanopatterned with gratings of 300 nm linewidth and subsequently functionalized with polydopamine, which acted as an adlayer for the final immobilization of graphene oxide (GO). mNSCs or hDPSCs were seeded and videorecorded on these scaffolds for 24h.

Both type of stem cells instead of grow forming neuro / dentospheres, sedimented attached and elongated following the nanograting axis generating chains of cellular migration. Immunohistochemistry analyses showed the persistence of both neuronal and glial markers when seeded on GO-functionalized nanostructured scaffolds compared with the control.

The combination of a nanostructured bioresorbable polymeric scaffold together with the functionalization of the surface with GO enables a simple and scalable method to align and guide the migration of neural and progenitor stem cells for future neuroregenerative therapies.

Funding: UPV/EHU (COLAB19/03; GIU20/050; GIU16/66; UFI11/44; IKERTU-2020.0155) GV/EJ (Dep. Salud 2021333012; GV-GIC15/52; IT-927-16; IT831-13;Hazitek ZE-2019/00012-IMABI & ELKARTEK KK-2019/00093), MICINN (PID2019-104766RB-C21), MINECO (RYC-2013-13450). Polimerbio and Y. P. have a Bikaintek PhD grant (20-AF-W2-2018-00001). B. Pardo-Rodriguez has a Predoctoral for non-doctoral research staff from de Basque Government (Ref. 2021_1_0112). I. Manero have a UPV/EHU mod-III grant (PIFBUR21/05).

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Reference number: T1-P31

(T1-P31) ***POTENTIAL NEUROPROTECTIVE ROLE OF LYSOPHOSPHATIDIC ACID RECEPTOR 1 OVEREXPRESSION BY HIPPOCAMPAL NEURONS IN A MODEL OF TEMPORAL LOBE EPILEPSY***

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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Reference number: T1-P32

(T1-P32) COMPUTATIONAL ANALYSIS OF CURRENT MAGNETIC STIMULATION PROCEDURES TO OPTIMIZE THE RECOVERY IN SPINAL CORD INJURED PATIENTS

Introduction: Spinal Cord Injury (SCI) produces the disruption of the top-down neural pathways, causing a permanent loss of motor and sensory function caudal to the level of injury. It has been demonstrated that magnetic stimulation at spinal cord level can induce recovery in SCI patients. Magnetic stimulation using commercial transcranial magnetic stimulators (TMS) and coils is becoming an established tool for the neurorehabilitation. However, when applied at the lumbar region it is not clear which neural structures are stimulated and if the spinal cord (SC) can be stimulated.
Aims: This study analyses current stimulating strategies using TMS coils for the stimulation of the spinal cord. The aim is to improve selectivity/precision and stimulation depth with the objective of maximizing neural activation and synaptic plasticity by studying the electromagnetic field distribution generated with the TMS coil and using 3D computational models of the spinal cord.
Methods: Sim4Life simulation platform, combined with computable human phantoms from the Virtual Population (ViP) of IT'IS foundation have been used to analyze the electromagnetic field distribution. In this study we have calculate the current density (J) distribution and magnitude under different output power levels of a commercial stimulator to describe the electromagnetic effects on the different tissues.
Results: The obtained results show that the spatial resolution of this technology is very poor to stimulate specific parts of the SC only. Although the stimulation aims at SC structures, we observed that most of the current does not reach SC, but the cerebrospinal fluid (CSF).
Conclusions: The potential of using computational simulation platforms with very realistic human models has been tested to optimize and improve current stimulation strategies using TMS for SCI patients.

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Reference number: T1-P33

(T1-P33) ***OPTIMIZATION OF NEURODIFFERENTIATION PROTOCOLS OF HUMAN DENTAL PULP STEM CELLS IN SERUM FREE MEDIA***

Human dental pulp stem cells (hDPSCs) are one of the most promising cells for neuroregenerative therapies, due to their unique features such as their shared neural crest origin with the peripheral nervous system and their capacity to secrete neuroprotective and anti-inflammatory factors and to express stem and mature neuronal and glial lineage cell markers (e.g GFAP, S100β, hNestin, NeuN, Tuj1, DCX and many neurotransmitter receptors and voltage gated channels markers). However, there is still no conclusive evidence of electrophysiological integration of hDPSCs into the synaptic network, and the protocols to optimize the ex vivo expansion of these cells and their differentiation into functional neurons must be refined.

In this study, we have compared two differentiation methods to establish the most suitable one for obtaining glial and neuronal like cells from hDPSCs. The presence of serum boosts in vitro proliferation of hDPSCs, at the expense of a loss of neural differentiation capacity. We also have assessed the reversibility of these changes when cells are switched from serum-containing to serum-free media.
We have observed that after inducing hDPSC neurodifferentiation in the same media these cells are morphologically different and show distinct marker expression patterns measured by immunocytochemistry when they are grown in a conventional medium with serum or in a specialized neural stem cell growth media without serum.

These results highlight the importance of choosing an appropriate protocol and give us a better knowledge of the potential use of hDPSCs in neuroregenerative cell therapies.

Funding: UPV/EHU (COLAB19/03; GIU20/050; GIU16/66; UFI11/44; IKERTU-2020.0155) Basque Government: GV/EJ; GV-GIC15/52; IT-927-16; IT831-13; Hazitek ZE-2019/00012-IMABI & ELKARTEK KK-2019/00093) and Health Department (Grant 2021333012), MICINN/AEI/10.13039/50110001103 (PID2019-104766RB-C21 & MINECO RYC-2013-13450). B. Pardo-Rodriguez has a Predoctoral funding for non-doctoral Research Staff from the Basque Government (Ref. 2021_1_0112). Polimerbio and Y. P. have a Bikaintek PhD grant (20-AF-W2-2018-00001). I.M. have a UPV/EHU mod-III grant (PIFBUR21/05). I.R have a Margarita Salas Grant (MARSA21/60).

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Reference number: T1-P34

(T1-P34) ***GRAPHENE DERIVATIVES AND CERIUM OXIDE NANOPARTICLES BASED HYDROGELS AS THREE-DIMENSIONAL SUBSTRATES FOR NEURAL STEM CELL DIFFERENTIATION TOWARDS ASTROGLIAL, NEURONAL AND OLIGODENDROGLIAL LINEAGES***

One of the most promising approaches for the regeneration of the nervous system relies on the use of stem cells. A major challenge in utilizing stem cells for regenerative therapies is the poor control over their survival, differentiation and functional integration when transplanted. The combination of stem cells with scaffolds has been proposed to overcome this limitation. In the present work, graphene derivatives-based hydrogels were developed as a substrate for the adhesion and neurodifferentiation of neural stem cells (NSCs). These hydrogels were further decorated with cerium oxide nanoparticles, providing a multifunctional platform that combined the intrinsic physico-chemical, electrical and mechanical cues provided by the graphene derivatives with the antioxidant and cytoprotective properties derived from cerium oxide nanoparticles.

Graphene-based hydrogels bearing cerium oxide nanoparticles were fabricated via self-assembly, using ascorbic acid (AsA) as a reducing agent at 1:1, 1:4 and 1:10 ratios. Their mechanical properties, electrical conductivity, antioxidant capacity, morphology (scanning electron microscopy-SEM) and physico-chemical properties (Raman spectroscopy and X-ray diffraction) were characterized. Increasing the amount of AsA resulted in a more collapsed structure with smaller pore size and higher shear modulus and electrical conductivity. Besides, the incorporation of cerium oxide nanoparticles conferred the hydrogels with antioxidant properties and reduced both their shear modulus and electrical conductivity.

NSCs were seeded on the hydrogels and fixed for immunostaining at different time-points (1, 3, 7 and 14 days). Several markers (Nestin, MAP2, NeuN, DCX, S100B, GFAP, Olig2) were used to distinguish between the undifferentiated and the differentiated cell populations found in the central nervous system (CNS). NSCs were able to attach and integrate into hydrogels without the need of extracellular matrix-like compound coating. Those hydrogels having smaller pores and higher shear modulus and electrical conductivity favored the astroglial differentiation of the NSCs, while those having larger pores with lower shear modulus and electrical conductivity promoted neuronal differentiation. Remarkably, the incorporation of cerium oxide nanoparticles promoted the differentiation of NSCs towards neuronal, astroglial and oligodendroglial lineages.

In this study, we present a simple and versatile method for the fabrication of graphene-based hydrogels with tunable mechanical, electrical, physico-chemical and morphological properties, supporting the adhesion and neurodifferentiation of NSCs, thus constituting a promising tool for future cellular therapies in nerve tissue regeneration.
Funding: Achucarro Seed-Funds (Achu-Seed003). UPV/EHU (COLAB19/03; GIU20/050; GIU16/66; UFI11/44; IKERTU-2020.0155) Basque Government: GV/EJ; GV-GIC15/52; IT-927-16; IT831-13; Hazitek ZE-2019/00012-IMABI & ELKARTEK KK-2019/00093) and Health Department (Grant 2021333012), MICINN/AEI/10.13039/50110001103 (PID2019-104766RB-C21 & MINECO RYC-2013-13450). B. Pardo-Rodriguez has a Predoctoral funding for non-doctoral Research Staff from the Basque Government (Ref. 2021_1_0112). I.M. have a UPV/EHU mod-III grant. Polimerbio and Y. P. have a Bikaintek PhD grant (20-AF-W2-2018-00001).
Topic: Neurogenesis

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Reference number: T1-P35

(T1-P35) ***COMPLIANT SUBSTRATES DOWNREGULATE NEURONAL ELECTRICAL ACTIVITY BY RESHAPING CHOLESTEROL HOMEOSTASIS IN PRIMARY HIPPOCAMPAL CULTURES***

The extracellular environment provides mechanical cues relevant to cell and tissue physiology. Neuronal cells actively sense and adapt to these stimuli and translate mechanical signalling into changes in functional behaviours. Increasing attention has been drawn to the ECM/substrate stiffness, but the exact mechanism involved in neuronal signalling regulation in the CNS remains poorly understood. Here, we investigated how stiffness impacts neuronal behaviour, attempting to shed some light on the relationship between mechanical and electrical adaptation. Our data showed that compliant substrates downregulate neuronal electrical activity. A similar effect was obtained by perturbing the mechano-sensing pathway in hippocampal neurons grown on stiff substrates by molecular inhibition of actomyosin contractility. We probed whether a possible alteration of plasma membrane lipid composition, specifically cholesterol, occurs. We discovered a progressive increase of cell plasma membrane cholesterol in neuronal cultures that perceive a compliant surrounding environment.

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Reference number: T1-P36

(T1-P36) ***CONSERVED CELL TYPES IN THE EARLY EMBRYONIC BRAIN ACROSS VERTEBRATES***

Organogenesis is the most evolutionarily conserved developmental stage when comparing morphology and whole-body transcriptome across vertebrates. During this phylotypic stage, body axes are defined and cell types are given their spatial identity. Due to its physiological importance, these segmentation and patterning genes (e.g. HOXs) are thought to be evolutionary conserved. However, across vertebrates, there is yet no evidence of transcriptomic conservation in essential organs as the brain, nor identification of homologue neural cell types in its initial neurodevelopmental bauplan. Thus, by performing single cell RNAseq and in situ hybridization assays, we obtained cellular and molecular atlases of the early developing brains of five vertebrate species: chicken, gecko, mice, zebrafish, and human. These single cell atlases allowed us to identify the neuroanatomical identities that naturally segment different vertebrate early brains and the genes that pattern these regions. Secondly, to unbiasedly evaluate the transcriptional conservation of these cell types across species, we performed three complementary methods: correlation of gene specificity indexes, datasets integration ("RPCA") and label transference. All approaches proved a high transcriptional conservation of equivalent morphogenic organizers and neuromeric identities among these vertebrate species, specially at transcription factor level. These results confirm the existence of a common phylotypic brain as well as the conservation of homologue neural cell types mastering its underlying bauplan. Therefore, this bauplan conservation is essential to stablish the foundations for assembling vertebrate brain structures, but also it sets the diversity boundaries within which these brains were allowed to evolve. Such an important constrain that early embryonic vertebrate brain has barely changed despite 500 million years of evolution.

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Reference number: T1-P37

(T1-P37) ***GENERATION AND CHARACTERIZATION OF HUMAN MIDBRAIN ORGANOIDS***

Introduction. Brain organoids are tridimensional cultures that imitate the spatial and cellular organization of a particular brain region and mimic its function. Organoids can be generated from Induced Pluripotent Stem Cells (iPSC) derived from patients' skin fibroblasts, which makes them useful tools for studying the development, physiology and pathology of human brain tissue, within the genetic context of the patient and as a proxy of human diseases. Parkinson's disease is a progressive neurodegenerative

disorder which results from the destruction of dopaminergic neurons in the midbrain. By combining specific small molecules, neural precursors can be differentiated into astrocytes, oligodendrocytes and neurons with midbrain-specific properties. However, the ectodermal origin of neural precursors prevents the development of microglia, which plays an essential role homeostasis, tissue organization and immune response.

Aims. The aim of our research is the generation and characterization of midbrain organoids and the optimization of a method for the introduction of functional microglia.

Results. Midbrain organoids were generated activating the floor plate precursor cells by the progressive addition of specific small molecules. We evaluated growth, differentiation and spatial organization of organoids by widefield, confocal and electron microscopy. Midbrain-differentiation level and patterning of the organoids was tested at different time points by immunofluescence with Lmx1 (marker for midbrain development), GFAP (marker for astrocytes), Olig2 (marker for oligodendrocytes development), MAP2 (marker for neurons) and tyrosine hydroxylase (marker for dopaminergic neurons). Microglia was included into the organoids by co-culturing immortalized human microglia with precursor cells in immature organoids (spheroids). Corresponding effectiveness was evaluated by immunofluorescence and electronic microscopy whereas live imaging in brightfield revealed highly motile cells within the organoid.

Conclusions. We present an effective method to generate brain organoids that fully resemble human midbrain. We also propose the microglia as an important actor for the physiology of the organoid. Further experiments are needed to develop a more physiological method that simulate the migration of macrophage precursor cells into the CNS, as observed during the human development.

Funded by BIOEF (EiTB Maratoia), CIBERNED, ASAP/MJF

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Reference number: T1-P38

(T1-P38) ***IMMUNODENSITY OF DOPAMINE D2 AND CANNABINOID CB1 RECEPTOR COMPLEXES IN POST-MORTEM PREFRONTAL CORTEX OF SUBJECTS WITH SCHIZOPHRENIA***

Background: Dysregulation of dopamine D2 (D2R) and cannabinoid CB1 (CB1R) receptors may contribute to the pathophysiology of schizophrenia (SZ). GPCR activities largely rely on their ability to form oligomeric structures with other receptors, changing their pharmacological features. The potential role of D2R and CB1R complexes in SZ brain has been barely studied. Our hypothesis is that D2R and CB1R complexes, rather than total protein expression, are altered in SZ prefrontal cortex.

Methods: Brain specimens from the dorsolateral prefrontal cortex (DLPFC; Brodmann's area 9) of subjects with SZ (n=27) and sex-, age-, and post-mortem interval-(PMI) matched controls (n=27) were obtained at autopsies performed in the Basque Institute of Legal Medicine. SZ cases were divided into antipsychotic-treated (AP+; n=20) and antipsychotic-free (AP–; n=7) groups, according to blood toxicological assessments at death. Cortical amounts of D2R and CB1R protomers and complexes were resolved by conventional SDS- and blue native-(BNP) PAGE, respectively, followed by quantitative immunoblotting, using knockout-validated antibodies. In GPCR oligomer analyses, the models were adjusted by the corresponding protomer amounts.

Results: The immunodensity of D2R protomers did not differ significantly between SZ cases and controls. However, protomeric CB1R cortical amounts were significantly lower in SZ DLPFC (–24%, $p<0.01$). Regarding antipsychotic treatment (APT), CB1R downregulation reached significance in AP+ cases only (–18%; $p<0.05$), while D2R cortical expression was not influenced by the medication. In BNP experiments, native brain D2R were revealed as three sharp immunoreactive bands of approximately 700-, 800- and 1,000-kDa, whereas CB1R antibody detected a diffuse band mixture between 800- and 1,000-kDa, which was quantified as one single heterocomplex. Adjusting by total protomeric D2R amounts, immunodensity of the 700-kDa D2R complex, but not that of 800- or 1,000-kDa species, was significantly lower in SZ subjects (–49%, $p<0.01$). Stratifying by toxicological data, AP+ cases displayed reduced levels of the 700- (-51%, $p<0.001$) and 1,000-kDa (-38%, $p<0.05$) D2R complex. Controlling for total CB1R levels, no alterations in the CB1R complex were found in SZ brains, regardless of the APT.

Discussion: The present results indicate that D2R ability to form specific receptor complexes is impaired in SZ brain. In addition, the deficit in the 700- and 1,000-kDa complex might be attributable to APT. On the other hand, APT may reduce cortical CB1R amounts, although no significant effects were observed in CB1R complex formation. Future studies would be required to determine the subunit composition and stoichiometry of those complexes found altered in SZ brains, as they could represent interesting targets for future drug design.
Supported by MCIU/AEI/ERDF (Grant RTI2018-094414-A-I00).

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Reference number: T1-P39

(T1-P39) ***GLUTAMATERGIC AND GABAERGIC RECEPTOR MODULATION PRESENT UNIQUE ELECTROPHYSIOLOGICAL FINGERPRINTS IN A CONCENTRATION-DEPENDENT REGION-SPECIFIC MANNER***

Brain function depends on complex circuit dynamics between excitatory and inhibitory neurons embedded in local and long-range networks. Systemic GABA receptor and NMDA receptor pharmacological modulation alter the excitatory/inhibitory (E/I) balance (EIB) which can be measured with electroencephalography (EEG) across species. However, these EEG signatures are complex in their localization and spectral composition. We developed novel analytical tools to investigate the periodic and aperiodic EEG features changed by the EIB modulators MK801 (NMDA-antagonist) and diazepam (GABA receptor positive allosteric modulator). We investigated how, across brain regions, specific EEG features would be linked to the different EIB modulators. We found seven clearly defined frequency bands for each condition in the periodic component. In addition, the aperiodic component was also different between compounds and brain regions. Importantly, the parametrization into periodic and aperiodic components unveiled correlations between quantitative EEG and plasma concentrations of pharmacological compounds. MK-801 exposures were positively correlated with slope and offset of the aperiodic component and peak amplitude of low-gamma activity in the parietal cortex. In the frontal cortex, the concentration of MK-801 also positively correlated to low-gamma peak. In contrast, correlations to diazepam exposures were mainly found over the primary auditory cortex. Diazepam exposure positively correlated with slope and offset of the aperiodic component and with high-gamma AUC, while negatively with theta AUC, theta modal frequency, and low-gamma modal frequency. In conclusion, correlations between exposures and pharmacodynamic effects can be better understood thanks to the parametrization of EEG features into periodic and aperiodic components.

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Reference number: T1-P40

(T1-P40) ***CROSSTALK BETWEEN S1P AND ENDOCANNABINOID SYSTEMS IN THE RAT CNS***

Sphingosine 1-phosphate (S1P) and endocannabinoid (eCB) are lipid-based neurotransmission systems whose main receptors, S1P1 and CB1, respectively, are some of the most abundant in the central nervous system (CNS). Both systems are

related with neurodegenerative diseases and they share metabolic pathways and localization in CNS, suggesting a crosstalk between them.

In this study, we describe the crosstalk between S1P and eCB systems. The affinity of S1P (agonist of S1P1) and NIBR-0213 (selective antagonist) for the binding sites of [3H]CP55,940 (CB1/CB2 radioligand) were analyzed in cell membrane homogenates of rat cerebral cortex and cell membranes overexpressing CB1 receptors. The affinity of CP55,940 or SR141716A (selective antagonist of CB1) for the binding sites of [3H]CS1P1 (S1P1 radioligand) were analyzed in cell membrane homogenates of rat cerebral cortex and cell membranes overexpressing S1P1 receptors.

[3H]CP55,940 binding was totally displaced by S1P (log Ki = -4.79) and NIBR-0213 (log Ki = -4.25) in rat cortex membranes. S1P displaced 50% of [3H]CP55,940 binding (log Ki = -6.34) and NIBR-0213 totally displaced it (log Ki = -4.60) in CB1 overexpressing cell membranes. CP55,940 displaced binding of [3H]CS1P1 20% in rat cortex membranes (log Ki = -9.30), while SR141716A did not. Interestingly, CP55,940 did not displace [3H]CS1P1 in S1P1 overexpressing cell membranes.
Further studies, including receptor activity assays using [35S]GTPγS in rat cortex membranes and brain slices, will be conducted to clarify the crosstalk between both systems, which is of great interest for the development of new treatments for neurodegenerative disorders, including Alzheimer´s disease and multiple sclerosis

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Reference number: T1-P41

(T1-P41) ***MODELING DIFFUSION IN SUPER-RESOLVED IMAGES OF BRAIN EXTRACELLULAR SPACE***

The structure and dynamics of the brain extracellular space (ECS) has long remained inaccessible for experimental studies, and we have a poor understanding of how these shape signaling and metabolite clearance on the microscale. This bottlenecks our understanding of phenomena occurring primarily in the ECS, such as extra-synaptic volume transmission and metabolite clearance via the glymphatic system.

We recently developed super-resolution shadow imaging (SUSHI) as a new microscopy approach to image and resolve the ECS in live mouse brain slices, thereby allowing us to analyze its structure and dynamics at unprecedented detail. Here, we introduce a computational diffusion model that allow us to simulate diffusion in SUSHI images. We plan to apply this model to understand how ECS structure and dynamics shape point-source diffusion to model signaling, as well as distributed diffusion to identify putative metabolite clearance pathways in dense parenchyma.
Our preliminary modelling suggest that ECS diffusion is highly anomalous on sub-micron scales, much more so than suggested by conventional volume-averaging techniques.

This provides us a new tool to understand the functional roles of the brain ECS in health and disease.

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Reference number: T1-P42

(T1-P42) ***PLA2G4E AS AN ESSENTIAL PROTEIN FOR PROPER SYNAPTIC FUNCTION AND BRAIN DEVELOPMENT***

PLA2G4E is a neuronal poorly characterized cytosolic phospholipase that has been found to play a role in memory and synaptic plasticity (Pérez-González et al., 2020). Despite these results, its molecular mechanism remains unknown. Furthermore, we have found that the expression of neuronal PLA2G4E peaks during postnatal period and it is maintained at lower levels in adult brain. In order to identify the role of this enzyme in synaptic plasticity and in brain function we have generated in vivo and in vitro neuronal models lacking PLA2G4E.

Pla2g4e-/- knock out (KO) mice showed impaired fear memory and an excessive self-grooming behavior compared to aged-matched wild type mice. Interestingly, during brain development synaptic plasticity markers were strongly decreased in different brain areas of the KO mice. Regarding in vitro models, primary neuronal cultures were treated with lentiviral particles containing a specific shRNA against Pla2g4e or an sh-control for RNAseq analysis. Accordingly, with Gene Ontology definitions, transcriptomic analysis data highlighted a predisposition to alterations in neuronal development, morphology and functionality, dendritic spines and synapses function and organization in neurons lacking PLA2G4E. RNASeq data were also analyzed using the Human Phenotype Ontology in order to get insight on the possible role of PLA2G4E in different disease and, surprisingly, “Autistic Behavior”, “Intellectual Disability” and other behavioral alterations were predicted. These results suggest that the lack of PLA2G4E is associated with Neurodevelopmental Disorders, which is in accordance with data observed in Pla2g4e-KO mice.

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Reference number: T1-P43

(T1-P43) ***A-SYNUCLEIN OVEREXPRESSION IN DOPAMINERGIC AND NON-DOPAMINERGIC PROJECTIONS TO THE HIPPOCAMPUS IS ASSOCIATED WITH EARLY SYNAPTIC IMPAIRMENT IN A RAT MODEL OF PROGRESSIVE PARKINSONISM***

Loss of dopaminergic neurons and aggregation of α-synuclein in intracytoplasmic Lewy bodies are key pathological features of Parkinson´s disease (PD). Although considered a predominantly motor disorder, PD patients often present cognitive deficits, which could be related to alterations in limbic brain regions. The aim of our study was to evaluate α-synuclein pathology within the mesolimbic pathway from the VTA to the hippocampus and to evaluate hippocampal synaptic function at different time-points of the neurodegenerative progress using an animal model of progressive parkinsonism. Rats were inoculated in the substantia nigra compacta with adeno-associated viral vectors coding for A53T mutated human α-synuclein (AAV-hαsyn) or empty viral vector as control group (AAV-EVV). Colocalization of hαsyn within dopaminergic, glutamatergic, and GABAergic pathways was studied by immunofluorescence in the VTA and hippocampus. Synaptosomes were isolated from hippocampus and synaptic protein expression was assessed by SWATH-MS proteomics, whereas synaptic plasticity was evaluated by chemical stimulation of long-term potentiation (cLTP), staining of postsynaptic GluA1 and presynaptic Neurexin1β, and flow cytometry analysis. The effect of pramipexole (PPX) and L-DOPA on cLTP were also tested. Hαsyn was found in the VTA and hippocampus from 1 week (w) post-inoculation (p.i.) onwards, colocalizing within dopaminergic, glutamatergic, and GABAergic projections. The proteomic study identified a total of 7958 proteins, of which 131 were differentially expressed in the AAV-hαsyn group. Deregulated proteins were related to synaptic structure and transport at early time points (1w and 2w p.i.), and related to membrane potential and plasticity at later time points (16w p.i.). AAV-hαsyn animals showed a significant inhibition of cLTP since 1w p.i. ($p<0.01$) coinciding with hαsyn expression. Incubation with PPX partially recovered cLTP in AAV-hαsyn animals at all time points ($p<0.05$), whereas L-DOPA only recovered cLTP at 4w p.i. ($p<0.05$). Of note, PPX partially inhibited hippocampal cLTP in AAV-EVV animals ($p<0.05$). Our results indicate that hαsyn is present in dopaminergic as well as non-dopaminergic mesolimbic projections and is associated with impaired synaptic function in the hippocampus of parkinsonian rats, which could be partially functionally recovered by dopaminergic treatment.

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Reference number: T1-P44

(T1-P44) ***IMPAIRED STRIATAL PLASTICITY AND DENDRITIC SPINE REMODELING IN THE PREMOTOR STAGE OF EXPERIMENTAL PROGRESSIVE PARKINSONISM***

Parkinson's disease (PD) is characterized by a progressive loss of dopaminergic neurons in the substantia nigra compacta (SNc) and accumulation of α-synuclein (α-syn), which underlies complex functional and structural changes in striatal spiny projection neurons (SPNs). Although a reduction in SPNs dendritic spine density has been reported in either postmortem studies of advanced PD patients or rodent models of parkinsonism, it is unknown if these alterations occur since the onset of the nigrostriatal dopaminergic degeneration. Thus, our aim was to study the temporal sequence of functional and structural changes in striatal dendritic spines. For that purpose, animals were inoculated in the SNc with an adeno-associated viral vector coding for A53T mutated human α-syn (hα-syn) and evaluated at 72h, 1, 2 and 4 weeks post-inoculation (p.i.). Synaptic plasticity by chemical stimulation of long-term potentiation LTP (cLTP) in isolated striatal synaptosomes and measurement of striatal dopamine by HPLC were assessed. The analysis of the density and morphology of SPNs dendritic spines was performed by microinjection and high-resolution confocal microscopy. The hα-syn group showed an inhibition of cLTP ($p<0.001$) and a decrease in dopamine content ($p<0.01$) since 72h p.i. even before the presence of hα-syn in the striatum and significant dopaminergic neurodegeneration. These functional alterations are associated with a dendritic spine remodeling, as observed by a significant loss of thin spines at 4 weeks p.i., along with an increase in the head volume of mushroom spines and ultrastructural alterations. These structural changes occur before the development of parkinsonian motor signs and could represent a compensatory mechanism to enhance the function of existing spines, balancing the observed decrease in spine turnover. Thus, our results indicate dysfunctional neurotransmission and impaired striatal synaptic plasticity at very early time points, leading to dendritic spine remodeling in later time points, before the manifestation of motor impairment.

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Reference number: T1-P45

(T1-P45) ***THE ROLE OF PROTEIN 4.1N IN SYNAPTIC PLASTICITY AND MEMORY***

During Long Term Potentiation (LTP) AMPA receptors (AMPARs) are inserted into the plasma membrane. This form of synaptic plasticity is crucial for many aspects of brain function, including learning and memory. Protein 4.1N (EPB41L1) is a neuronally

enriched member of the protein 4.1 family. In primary hippocampal cultures, 4.1N is enriched at the discrete sites of synaptic contact, co-localizing with the postsynaptic density proteins, suggesting a potential functional role for 4.1N as a component of the cytoskeletal architecture of excitatory synapses. 4.1N also directly interacts with the GluR1 subunit of the AMPARs and co-localizes with AMPARs at excitatory synapses.

There is evidence from behavioral and cellular studies pointing to AMPAR trafficking as an underlying mechanism for a variety of learned responses. This work focuses on the role of 4.1N during LTP and tries to enhance AMPAR trafficking in specific neurons that participate in memory and learning processes. As a final goal, the overexpression of 4.1N aims to improve cognitive abilities in mice.

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Reference number: T1-P46

(T1-P46) ***THE INTERPLAY BETWEEN NEURONAL FUNCTION AND FTO EXPRESSION.***

As DNA and proteins, RNA harbors the potential of being dynamically and reversibly regulated by adding and removing distinct chemical moieties, forming what is called the 'epitranscriptome'. Epitranscriptomics is an emerging field in biology as post-transcriptional RNA modifications represent novel mechanisms discovered recently and they are a vital process governing RNAs' fate and function. These modifications extend the RNA repertoire and alter its chemistry in various ways. N6-methyladenosine (m6A) methylation is one of the most abundant and functionally relevant RNA modifications. m6A methylation plays a critical role in many biological processes and a variety of diseases. The degree and pattern of m6A modifications on mRNAs can affect their splicing, transport, storage, stability, translation, and decay. Several enzymes catalyze the m6A modifications and are termed writers (m6A methyltransferases), erasers (m6A demethylases), and readers (m6A-binding proteins). FTO is an ‘eraser’ (demethylase) that removes methyl groups from mRNA molecules in a stimulus-dependent process. We aimed to establish the role of FTO in neurons and the ways to control its expression. We first found that FTO is widely expressed in the mouse brain and that FTO expression increases as neurons mature. We cloned FTO, overexpressed it in primary neurons, and studied how different drug treatments affected FTO. Our results suggest that the modulation of neuronal activity significantly alters the expression of FTO.

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TRACK 2: Cellular and Molecular Neuroscience / Pathology

ORAL PRESENTATIONS

Moderator: **Ana García Osta (UNAV & CIMA & CUN, Pamplona)**

Oral Communication T2-OC1

ENDOCANNABINOID MODULATION OF THE INFLAMMATORY REACTION IS NEUROPROTECTIVE IN THE CHRONIC MPTP MOUSE MODEL

Parkinson´s disease is a motor disorder characterized by the selective loss of dopaminergic neurons in the substantia nigra (SN), the presence of Lewy bodies and neuroinflammation. Current therapies are directed towards the motor symptoms of the disease, but are unable to halt the neurodegenerative process. Different lines of evidence indicate that the endocannabinoid system (ECS) modulates the inflammatory reaction that occurs in the brain under pathological conditions suggesting that it could be a therapeutic target. Our group has demonstrated that monoacylglycerol lipase inhibition with JZL184 is neuroprotective in the chronic MPTP mouse model and we proposed that glial cells were involved in the mechanism of action of this drug. In this study, we hypothesized that cannabinoid receptors type 2 (CB2) expressed in immune cells are involved in the neuroprotective effect exerted by JZL184. For this purpose, JZL184 (8 mg/kg) was administered to mice chronically treated with MPTP and the immune cell infiltration was analyzed in the midbrain and in the striatum by flow cytometry. Our results show that JZL184 reversed the parkinsonian motor deficits, was neuroprotective and blocked the CD4+ T cell infiltration in the midbrain. Using the CB2egfp/f/f mouse it was possible to determine that, under parkinsonian conditions, CB2 expression was associated to microglia and lymphocytes but not to astrocytes. When JZL184 was administered to the CB2 knock-out mice, it failed to improve the motor deficits and did not prevent CD4+ T cell extravasation into the midbrain. Next, we administered the selective CB2 receptor agonist JWH133 (0.2 mg/kg) to the chronic MPTP mice. JWH133 restored motor impairment but it was unable to protect the nigrostriatal pathway. In addition, the CB2 expression analyzed in brain human samples showed a specific decrease in the substantia nigra of PD patients, whereas it remained constant in the putamen and the globus pallidus. All together, these results suggest that CB2 activation blocks the extravasation of CD4+ T cells into the midbrain under parkinsonian conditions that may be necessary to preserve the nigrostriatal pathway.

Authors

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Oral Communication T2-OC2

ALTERED MYELIN REGULATORY FACTOR PROCESSING MODIFIES OLIGODENDROCYTE DIFFERENTIATION IN ALZHEIMER´S DISEASE MODELS

Oligodendrocyte and myelin dysfunctions are early events in Alzheimer's disease (AD). Oligodendrocyte maturation and myelination are strictly controlled by transcription factors (TF) such as myelin regulatory factor (MYRF). Self cleaved N-MYRF fragment translocates from the ER into the nucleus, where it induces expression of myelin genes. Subsequently, this fragment is phosphorylated by GSK3 and degraded via ubiquitin-proteasome system. Despite the relevance of MYRF in oligodendrocytes and myelin, little is known about its role in AD.

Here, we report increased numbers of newly generated mature oligodendrocytes in 3xTg-AD mice, as revealed by EdU incorporation assays. Similarly, an intracerebral injection of amyloid β oligomers (Aβo) also promoted oligodendrocyte differentiation and maturation in WT mice. In addition, Aβo increased nuclear MYRF levels in oligodendrocytes both in vivo and in in vitro. By luciferase assays performed in primary oligodendrocytes, we revealed that Aβo promote myelin gene expression, an effect that was reverted by Myrf siRNA. Mechanistically, we found a slower degradation of N-MYRF by a cycloheximide chase assay, increased levels of the inhibitory phosphorylation of GSK3, and reduced levels of phosphorylated N-MYRF in Aβo-treated oligodendrocytes. Furthermore, an RNA-Seq analysis revealed alterations in ubiquitin-proteasome system- and glial cell differentiation-related genes in oligodendrocytes isolated by MACS from 3xTg-AD mice.

Altogether, these results suggest that enhanced oligodendrocyte maturation in AD may be mediated by altered MYRF degradation.

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Oral Communication T2-OC3

TREG DEPLETION DECREASES THE INFILTRATION OF IMMUNE CELLS INTO THE MIDBRAIN AND IS NEUROPROTECTIVE IN THE A-SYNUCLEIN OVEREXPRESSION MOUSE MODEL

Inflammation is a common feature in neurodegenerative diseases that contributes to the process of neuronal loss. Understanding the specific neuroinflammatory reaction triggered by each condition is necessary to propose therapeutic interventions directed towards the modulation of the immune system. Our group demonstrated that, under physiological conditions, the basal inflammatory tone is different between brain regions, and that in response to a systemic inflammatory stimulus, these regions generate diverse responses. Interestingly after lipopolysaccharide administration, microglia and infiltrated immune cells presented an anti-inflammatory phenotype in the midbrain compared to the striatum. Thus, we questioned whether the inflammatory reaction generated in Parkinson´s disease (PD) by dopaminergic neuron degeneration would also trigger a differential inflammatory reaction in these two regions. Dopamine neuron death was induced by the stereotaxic administration of an adeno-associated virus serotype 9 (AAV9) that overexpressed α-synuclein and mCherry independently in the mouse substantia nigra. Control animals received another AAV9 that only overexpressed the mCherry protein. Microglia and astrocytes were purified from the midbrain and the striatum for RNA sequencing. Midbrain microglia presented a phagocytic and anti-inflammatory phenotype with elevated MHC-II expression, while astrocytes participated in the pro-inflammatory response through pathways that involve the activation of IFNγ signaling. By contrast, striatal microglia, but not astrocytes, were involved in the pro-inflammatory environment of this region. Flow cytometry and immunohistochemical studies revealed a specific extravasation of CD4+ lymphocytes and myeloid cells from the peripheral immune system, being very prominent in the midbrain. The analysis of CD4 T cell infiltration showed a polarization towards Th1 in detriment of a Th17 response. Although the number of regulatory T cells (Tregs) was increased, the ratio of Tregs with respect to CD4 T cells remained constant. Depletion of Tregs using Foxp3DTR mice resulted in neuroprotection of the nigrostriatal pathway. Ablation of Tregs for 2 weeks induced blood lymphocyte proliferation and CD4 T cell overexpression of the immunosuppressive molecule PD-1. Under these conditions, the infiltration of CD4+ and myeloid cells into the brain was reduced, and the infiltrated CD4 T lymphocytes produced less TNFα. Our results suggest that Tregs within the brain parenchyma can modulate immune cell infiltration into the brain, and that the reduction of immune cell infiltration and CD4 cytotoxicity may constitute a neuroprotective strategy for the treatment of PD.

Authors

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Oral Communication T2-OC4

DYSREGULATION OF RNA DYNAMICS AND TRANSLATIONAL ACTIVITY OF OLIGODENDROCYTES AND MYELIN IN ALZHEIMER´S DISEASE MODELS

Oligodendrocyte dysfunction, myelin degeneration and alterations in the white matter structures are early events in Alzheimer's disease (AD) that might lead to cognitive deficits. One of the hallmarks of AD is the presence of extracellular aggregates of amyloid beta peptide (Aβ), and Aβ oligomers (Aβo) have been proposed to induce changes in oligodendrocytes and myelin. Here, we report modifications of RNA transport and localized protein synthesis in oligodendrocyte in AD models.

As hippocampus is one of the earliest affected brain regions in AD we analyzed oligodendrocytes and myelin in 6-month-old 3xTg-AD mouse, in which Aβo are detected. First, we observed an upregulation of the major and essential proteins of myelin MOBP and MBP, however, transcriptomic analysis of isolated oligodendrocytes and myelin did not showed significant expression changes of these genes. Thus, we hypothesized that translation activity could be altered in oligodendrocytes and myelin in the AD mouse. According to that, RNA-seq analysis revealed upregulation of genes related to ribosomes and protein synthesis in isolated oligodendrocytes of 3xTg-AD mice. Next, using the SUnSET method, we validated the increased translational activity in oligodendrocytes of dentate gyrus in the 3xTgAD. To gain a mechanistic understanding or these cellular processes, we treated cultured primary oligodendrocyte with Aβo and granules containing Mbp and Mobp mRNAs associated with a subset of heterogeneous nuclear ribonucleoproteins (hnRNPs) were studied. Immunofluorescence analysis along with RNA-immunoprecipitation showed an increase in total transport granule number and active granules, together with a higher association between Mbp and Mobp mRNA and hnRNPs. Besides, direct visualization of newly synthesized proteins by Puro-PLA method confirmed that Aβo increased local translation in oligodendrocytes. We further show that protein synthesis is also increased in vitro.
Taken together, these results suggest that Aβo could alter the proteostasis of oligodendroglial maturation and myelin, with potential consequences in axonal conduction.

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Oral Communication T2-OC5

GENETICALLY-MODIFIED MACROPHAGES ACCELERATE MYELIN REPAIR

Preventing neurodegeneration-associated disability progression in patients with multiple sclerosis (MS) remains an unmet therapeutic need. As remyelination prevents degeneration of demyelinated axons, promoting this process in patients might halt the development of permanent disability. In demyelinating mouse lesions, local overexpression of Semaphorin 3F (Sema3F), an oligodendrocyte progenitor cell (OPC) attractant, increases OPC recruitment and remyelination. However, molecular targeting to MS lesions is a challenge because these are disseminated in the central nervous system.

We hypothesized that a clinically-relevant paradigm to deliver Sema3F to demyelinating lesions and increase OPC recruitment may be to use blood-derived macrophages as vehicles. Thus, we chose transplantation of genetically-modified hematopoietic stem cells (HSCs) as means of obtaining circulating monocytes that overexpress Sema3F. We first demonstrated that the supernatant from Sema3F-lentiviral vector transduced HSCs stimulates OPC migration in Neuropilin 2 (Nrp2, Sema3F receptor)-dependent fashion. We then investigated whether OPCs remain responsive to Sema3F with age. While Sema3F expression in the lesions of middle-aged and old mice (characterized by decreased efficiency of OPC recruitment and remyelination) was decreased, middle-aged OPCs retained Nrp2 expression and migrated in response to both recombinant Sema3F and Sema3F-transduced cell supernatant in vitro. We then investigated whether blood cells engineered to overexpress Sema3F can target demyelinating CNS lesions and improve remyelination. Thus, we transplanted Sema3F-transduced HSCs and obtained chimeric mice (with Sema3F overexpression in blood cells), in which we induced demyelinating spinal cord lesions. Transgene-carrying cells, predominantly macrophages, quickly infiltrated lesions in both control and Sema3F chimeras. While infiltration of Sema3F-expressing cells did not alter the inflammatory status of the lesions nor OPC survival, it increased OPC recruitment, which accelerated the onset of remyelination.

Our results provide a proof-of-concept that blood cells, particularly monocyte-derived macrophages, can be used to deliver pro-remyelinating agents “at the right time and place”, suggesting novel means for remyelination-promoting strategies.
Funding: This work was supported by the French National Institute of Health and Medical Research (INSERM), French National Research Agency (ANR, project Stemimus ANR-12-BSV4-0002-02), the European Leukodystrophy Association (ELA, project 2016-004C5B), NeurATRIS, the program “Investissements d'avenir” (ANR-10-IAIHU-06), CIBERNED (CB06/0005/0076), and Gobierno Vasco (IT1203-19). VT was a recipient of the Spanish Ministry of Economy Young Investigator Grant (SAF2015-74332-JIN).

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POSTERS

Reference number: T2-P01

(T2-P01) SEX-DEPENDENT EFFECT OF KETAMINE AND OTHER ANTIDEPRESSANTS ON MITOCHONDRIAL ENERGY METABOLISM

Depression is a mental disorder characterized by anhedonia and is one of the leading causes of disability worldwide. There are interesting differences in the prevalence rate, symptoms, and efficacy of antidepressants regarding patient's sex. Current antidepressants show a delayed response and, in many cases, a lack of effect, whereas intravenous administration of ketamine has a rapid and sustained antidepressant effect over time. The basis of this treatment is due to changes in mitochondrial activity, since its mechanism of action involves activation of mTOR signaling, affecting the mitochondrial energetic system.

The aim of this study is to determine the activity of mitochondrial transport chain complexes in male and female mice and to quantify the effect of antidepressants on superoxide formation. For this purpose, we developed Cell Membrane Microarrays (CMMAs) from different brain areas related to depression. NADH dehydrogenase activity was determined in presence of different antidepressant drugs: ketamine, escitalopram, and fluoxetine. The main results show alterations in superoxide formation, mediated by NADH dehydrogenase, in males with respect to females. Ketamine inhibited the superoxide formation and significant differences were observed in medial prefrontal cortex, dorsal and ventral hippocampus brain areas. Fluoxetine showed the same tendencies in females, at high concentrations, except in locus coeruleus. Nevertheless, it stimulated the superoxide formation at low concentrations in males. In contrast, escitalopram stimulated the production in both sexes, with greater disparity observed in males

These data indicate that mitochondrial activity and the effect of antidepressants is conditioned by sex. This fact could be related to the differences in depression prevalence and response to treatment observed between men and women.
Keywords: depression, mitochondrial activity, superoxide, microarray, ketamine, antidepressant.

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Reference number: T2-P02

(T2-P02) ROLE OF USP9X IN REGULATING PREFRONTAL CORTEX EXCITABILITY AND ITS POTENTIAL IMPLICATIONS FOR ANGELMAN SYNDROME

The lack of functional ubiquitin E3 ligase UBE3A in the brain leads to the rare neurodevelopmental disorder Angelman Syndrome (AS), associated with alterations in prefrontal cortex (PFC) function and cognitive impairment. Protein ubiquitination, however, is not only modulated by E3 ligases, but also by deubiquitinating (DUB) enzymes. Identifying and characterizing DUBs responsible of counteracting UBE3A could lead to therapeutic targets that could ameliorate AS symptoms. A previous study from our lab demonstrated that USP9X –a DUB associated with X-linked intellectual disability– counteracts UBE3A mediated ubiquitination. We aim to characterize the role of USP9X in regulating cortical excitability, found to be altered in AS mouse models, and shed light on the potential therapeutic value of USP9X inhibitors. We performed in vitro patch clamp recordings in cortical slices of C57BL/6 mice focusing on pyramidal cells of layer V in the medial PFC (mPFC), one of the main output neuron types of the PFC. USP9X activity was blocked using the two specific inhibitors. Blocking USP9X led to changes in several parameters of neuronal excitability, the most prominent being an increase in the sag ratio and in the medium after hyperpolarization (mAHP) of mPFC layer V pyramidal neurons. Our results suggest that UPS9X regulates the activity or expression of different ion channels that contribute to intrinsic properties regulating basic membrane properties and synaptic integration. Following these results, we will examine whether blocking USP9X activity ameliorates changes observed in AS mouse models, to further investigate the therapeutic potential of USP9X inhibitors.

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Reference number: T2-P03

(T2-P03) GABAB RECEPTORS IN OLIGODENDROCYTE PROGENITOR CELLS TO REACH REMYELINATION

Oligodendrocytes (OLs) drive myelination in the central nervous system and remyelination following demyelinating insults, such as in multiple sclerosis (MS). For that, OLs must differentiate from oligodendrocyte progenitor cells (OPCs) in a process guided by many factors, including γ-aminobutyric acid (GABA). Recently, we have demonstrated that GABAB receptor (GABABR) selective agonist baclofen promotes myelin protein generation in cultured OPCs, whereas GABABR antagonist CGP55845 reverts this effect. Here, we first delved into this event using polycaprolactone nanofibers to assess myelin sheath formation by OPCs after treatment with baclofen or CGP55845. Given the little information available describing GABABR-mediated mechanisms in oligodendroglia, we also analyzed phospho-kinase arrays to measure the activation state of relevant signaling molecules and evaluated GABABR-induced calcium activity in OPCs, using conditional knockout mice for GABAB1 subunit in NG2-positive cells. On the other hand, baclofen is administered to MS patients as a spasticity

treatment but its role on neuroprotection or remyelination has not been assessed yet. Therefore, we checked its remyelinating potential by intraperitoneal injection of baclofen to adult mice following lysolecithin-induced spinal cord demyelination. In this model, we observed a significant acceleration of myelin regeneration within the lesions. Finally, using SIMOA technology, we determined changes in axonal damage and inflammation biomarkers in plasma samples of MS patients clinically treated or not with baclofen. Overall, our results provide relevant information about GABABR contribution to OPC functionality and identify baclofen as a powerful candidate for remyelinating therapies in MS.

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Reference number: T2-P04

(T2-P04) ***IDENTIFYING ALPHA-SYNUCLEIN INTERACTOME MAP IN A MOUSE MODEL USING PROXIMITY-BIOTINILATION.***

The loss of dopaminergic neurons in the substantia nigra (SN) that project to the striatum causes Parkinson´s disease (PD). The abnormal increase of pre-synaptic alpha-synuclein (aSyn) protein levels or aSyn mutations are one of the main drivers of PD and other synucleinopathies. In spite of the extensive research on this topic, the molecular mechanisms whereby quantitative/qualitative aSyn changes trigger neurodegeneration remain largely unclear. In this study, our working hypothesis is that mutations and/or early increases of aSyn protein levels could facilitate pathological protein-protein interactions (PPIs). The identification and modulation of aSyn PPIs could provide new therapeutic approaches to treat PD and other synucleinopathies. Here, we aimed to identify the early aSyn interactome in a mouse model using a methodology that identifies PPIs through proximity biotinylation. By expressing aSyn fused to a promiscuous biotinilase (BioID2 or TurboID) and adding biotin, proximal stable and transient interacting partners become biotinylated. Biotinylated substrates are affinity-purified and identified by mass spectrometry (MS). BioID2 and TurboID-tagged aSyn proteins recapitulate aSyn-dependent neurotoxicity. TurboID-tagged proteins, however, displayed a more efficient biotinilase activity. Thus, adeno-associated viral (AAV) vectors encoding TurboID and aSynTurboID were generated and stereotaxically injected into the SN of mice. AAV9-SynTurboID mice showed aSyn increased levels in the SN and the striatum, loss of dopaminergic striatal terminals and motor deficits for AAV9-TurboID mice. Pull-down experiments from injected and biotin-treated mice, as well as MS analysis, are ongoing to identify aSyn PPIs from dopaminergic striatal terminals and SN somas.

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Reference number: T2-P05

(T2-P05) ***MAGNETIC FIELD IN THE EXTREME LOW FREQUENCY BAND PROTECTS NEURONAL CELLS FROM OXYGEN-GLUCOSE DEPRIVATION IN-VITRO***

Ischemic stroke consists of a rapid loss of cerebral functions as consequence of brain vessel obstructions, followed by damages to the neighbouring tissue. The cerebral tissue surrounding the lesions becomes irreversibly damaged, yet, brain parenchyma peripheral to the clogged vessel (ischemic penumbra) is potentially recoverable during the initial phases after the stroke. In this study we tested the effect of Extreme Low Frequency Electromagnetic Stimulation (ELF-EMS) on oxygen-glucose deprivation (OGD) induced cellular death in primary neuronal and microglial cultures.
Primary cultures were subjected to an in vitro stroke model, OGD in absence and presence of increasing concentrations of iodoacetic acid (IAA 20 or 50 μM), a glycolysis inhibitor. After 1h OGD the cultures were magnetically stimulated in a solenoid designed and characterized with SIM4LIFE (ZMT Zurich MedTech AG) to generate a homogenous magnetic field (MF) on the cell plate. At the end of the incubation period, viability and microglial proinflammatory shift were measured with CalceinAM fluorometric assays and immunocytochemistry.
The magnetic stimulation under basal non-OGD conditions did not induce any effect in cell survival. However, ELF-EMS stimulation significantly reduced cell death in OGD conditions in both cell types. Moreover, ELF-EMS on microglia showed a tendency to activate the expression of both pro- and anti-inflammatory factors, accompanied by an amoeboid morphological shift.
This preliminary study suggests potential benefits in the application of MF in the ELF band to limit ischemic irreversible damages in stroke conditions, encouraging in vivo preclinical validations of ELF-EMS as a potential therapeutic strategy for ischemic conditions.to the clogged vessel (ischemic penumbra) is potentially recoverable during the initial phases after the stroke. In this study we tested the effect of Extreme Low Frequency Electromagnetic Stimulation (ELF-EMS) on oxygen-glucose deprivation (OGD) induced cellular death in primary neuronal and microglial cultures.
Primary cultures were subjected to an in vitro stroke model, OGD in absence and presence of increasing concentrations of iodoacetic acid (IAA 20 or 50 μM), a glycolysis inhibitor. After 1h OGD the cultures were magnetically stimulated with an homogenous magnetic field (MF) of 1 mT and 50 Hz for 24h. At the end of the incubation period viability and microglial proinflammatory shift were measured with CalceinAM fluorometric assays and immunocytochemistry.
The magnetic stimulation under basal non-OGD conditions did not induce any effect in cell survival. However, ELF-EMS stimulation significantly reduced cell death in OGD

conditions in both cell types. Moreover, ELF-EMS on microglia showed a tendency to activate the expression of both pro- and anti-inflammatory factors, accompanied by an amoeboid morphological shift.
This preliminary study suggests potential benefits in the application of MF in the ELF band to limit ischemic irreversible damages in stroke conditions, encouraging in vivo preclinical validations of ELF-EMS as a potential therapeutic strategy for ischemic conditions.

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Reference number: T2-P06

(T2-P06) THE OLFACTORY TRACT PROTEIN DYSHOMEOSTASIS HIGHLIGHTS SEXUALLY DIMORPHIC MECHANISMS IN HUMAN ALZHEIMER´S AND PARKINSON´S DISEASES

Smell impairment is one of the earliest features in Alzheimer's (AD) and Parkinson's diseases (PD). However, the underlying molecular mechanisms associated to the olfactory dysfunction are poorly understood. We applied sequential window acquisition of all theoretical fragment ion spectra mass spectrometry (SWATH-MS) in 57 postmortem olfactory tracts (OT) derived from non-demented (n=6F/11M), AD (n=4F/13M) and PD (n=7F/16M) subjects. From the 1835 quantified proteins, around 11% varied between groups. Interestingly, 35 and 20 proteins were commonly deregulated across both sexes in AD and PD, respectively. Our preliminar data point out sex-dependent differences in terms of olfactory proteostasis, pathway modulation and protein networks. Our workflow is currently being complemented with secretability and neuropathological stage-dependent analysis in order to propose potential sex-specific fluid biomarkers and to increase our knowledge about the AD and PD progression at olfactory level in women and men.

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Reference number: T2-P07

(T2-P07) ***SYNTHESIS OF FLUORINATED SMART NANOPROBES FOR MMP-2/9 DETECTION IN STROKE BY 19F MRI***

Magnetic resonance imaging (MRI) is a non-invasive imaging technique for diagnostic purposes. In particular, 19F-MRI takes advantage of the lack of endogenous fluorine to produce images without interference from the background. This provides unambiguous detection suitable for quantification. To improve the information provided by this technique often a probe or contrast agent is used. Among the different types of contrast agents, smart OFF/ON probes are of particular interest as they are systems in which the OFF signal is switched to an ON state in response to an external stimulus. This selective stimulus is triggered, for example, by the presence or dysregulation of enzyme activity, which may act as of pathological processes. Matrix metalloproteinases (MMPs) are proteolytic enzymes that degrade all components of the extracellular matrix, and are involved in many of these processes. They produce deleterious effects during the early ischemic stage, but are beneficial in the recovery stage. We propose the synthesis smart probes based on gelatin nanoparticles (NPs) sensitive to matrix metalloproteinases (MMP-2/9) that encapsulate fluorine-labelled NPs detectable by 19F MRI. We hypothesise that when the probe is encapsulated in gelatin NPs, the 19F-MRI signal is turned OFF, but this signal is turned ON when disassembly of the gelatin NPs occurs, caused by digestion of the gelatin by MMPs. Optimisation of the synthesis and characterisation of gelatin and fluorinated NPs are key points for the design of a bioresponsive probe. On the one hand, gold NPs with an organic coating based on polyethylene glycol (PEG) ligands will be synthesised due to their solubility in water and good MR properties for 19F-MRI. On the other hand, the two-step desolvation method using EDC/NHS as crosslinking agents will be used for gelatin synthesis. The characterisation of these NPs will be performed by transmission electron microscopy (TEM), Dynamic light scattering (DLS), inductively coupled plasma-mass spectrometry (ICP), etc. The design of these probes with OFF/ON characteristics shows the potential use of fluorinated NPs with applications in detection of MMP activation following stroke using 19F-MRI.

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Reference number: T2-P08

(T2-P08) ***BIOMARKER IDENTIFICATION OF PARKINSON´S DISEASE IN A RODENT MODEL BASED ON THE OVEREXPRESSION OF ALPHA-SYNUCLEIN IN THE SUBSTANCIA NIGRA***

Parkinson´s Disease (PD) is a neurodegenerative disorder characterized by neuron loss in different brain areas, specially dopaminergic neurons in the Substancia Nigra (SN). The alterations cause motor symptoms such as tremor, rigidity and bradykinesia among others However, it is not until these symptoms appear that the pathology can be diagnosed, when the neuron loss in SN is over 60%, because there is a lack of early stage

biomarkers that allow the diagnosis. The trigger of the degeneration remains unknown but some features have been associated with neuron death, such as the presence of Lewy bodies. These structures are formed by the accumulation of alpha-synuclein and play a key role in neurodegeneration since its presence could disturb the mitochondrial function, leading to the formation of reactive oxygen species (ROS), that induce oxidative stress and cell death.
The aim of this study is to identify PD biomarkers on different kinds of central and peripheric tissue samples. In order to achieve this purpose, we have stablished a rodent model based on the overexpression of alpha-synuclein in the SN. We have performed a behavioral evaluation of our model testing motor and motor behavior including general activity and anxiety. We also collected blood and brain samples and fabricated cell membrane microarrays to analyze the activity of the mitochondrial electron transport chain. Even thought, significant changes were not noticed in behavioral evaluation, alterations on mitochondrial activity have been observed in blood samples. With the current studies we are aiming to identify early stage PD biomarkers that allow a sooner diagnosis of the disease.

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Reference number: T2-P09

(T2-P09) ***GLIOBLASTOMA CELL LINES RECAPITULATE CONVERTASES EXPRESSION IN A BIOMIMETIC STIFFNESS-BASED PLATFORM.***

Glioblastoma (GBM) is the most frequent and aggressive malignant brain tumor in adults, with an average survival time of just 15 months. It is characterized by infiltrative growth of malignant glioma cells in the surrounding brain parenchyma, striking cellular heterogeneity, and the presence of glioma stem cells (GSC), which are involved in imperative processes of tumor growth, chemo- and radiotherapy resistance, and tumor relapse.

Convertase proteins are proteases involved in activating a wide variety of proteins, such as metalloproteases, growth factors, and adhesion molecules that have been proven to facilitate tumor formation and progression. In silico analysis, we observe increased

expression of convertases such as Furin and PCSK9 in cells expressing CD133, a cancer stem cell marker, relative to GBM tumors.

Even though it has been long understood that GBM tissue is mechanically stiffer than the healthy brain stroma, the effect of mechanical signal over the GBM microenvironment is still a pending issue.

We designed a PDMS-based material that mimics GBM stiffness to understand the effect of the mechanical properties of the tumor microenvironment on GBM biology. We cultured U87, A172 and, U251 GBM cell lines for six days in non-physiological 2D culture plates (Emod = 107 KPa), and in the PDMS-based platform (Emod = 7.09 ± 1.16 kPa). We found that cells cultured in substrates mimicking GBM stiffness increased the stemness markers SOX2 and NANOG, and Furin and PACE-4 protein convertases.

In conclusion, culturing GBM cells under physiological stiffness provides expression levels comparable to native tumors from patients. The use of relevant biomimetic cancer models gives us relevant information that can be translatable to the clinic better than traditional culture plates.

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Reference number: T2-P10

(T2-P10) ***Immunoanalysis of membrane-bound tyrosine hydroxylase and dopamine transporter in a monkey model of Parkinson disease***

Parkinson's disease (PD) is known as the second most common neurodegenerative disorder whose main characteristic is the loss of dopaminergic neurons in the substantia nigra (SN). This loss of dopamine is responsible for the altered neuron activity in the basal ganglia, that triggers the cardinal motor symptoms in the patient. The neurotoxin MPTP produces similar symptomatology to the one observed in PD, so its use in animal models has been extensively studied.
The neurodegeneration observed in the SN translates into a decrease in the dopaminergic markers such as tyrosine hydroxylase (TH) and dopamine transporter (DAT). However, the appearance of aberrant forms like insoluble oligomers may contribute to increase this degeneration. Therefore, our purpose was to determine the amount of membrane-bound tyrosine hydroxylase (mbTH) and DAT in MPTP treated monkeys as PD animal models where, the MPTP-treated model besides causing a dopaminergic loss, may induce TH dysregulation. To achieve this, we developed Cell Membrane Microarrays (CMMAs) of 9 monkey brain areas treated with and without MPTP to determine the expression of DAT and mbTH.

Thus, immunofluorescence assays showed an increase in mbTH/protein ratio in the SN of MPTP monkeys, whereas in the striatum this ratio was decreased. Likewise, in DAT expression followed the same pattern as the mbTH. These results suggest that the loss of the viability of dopaminergic neurons in the SN could be related to an initial increase of mbTH, which also correlates with DAT expression. Although further experiments are required for confirming this hypothesis, it is likely that increased in mbTH expression would eventually generate aggregates leading to neuronal death in the SN and loss of dopaminergic innervation in the striatum.

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Reference number: T2-P11

(T2-P11) ***STUDY OF TISSUE-SPECIFIC REACTIVE OXYGEN SPECIES FORMATION BY HUMAN CELL MEMBRANE MICROARRAYS TO EVALUATE THE POTENTIAL TOXICITY OF BRAIN-TARGETED DRUGS***

Mitochondria are responsible for generating most of the cellular energy as well as one of the main sources of reactive oxygen species (ROS). Some neuromodulator drugs, in addition to acting on the corresponding pharmacological targets, also interact with the different mitochondrial complexes generating oxidative stress, which is implicated in many of the drug side effects. Therefore, the identification of those compounds during the drug discovery process is a key aspect for the optimization of pharmacological research.

For this purpose, cell membrane microarrays isolated from rat brain areas were developed and superoxide production evoked by NADH dehydrogenase activity were assayed in the presence of specific inhibitors: rotenone, antimycin A and azide. Once the protocol was stabilized, the effect of a series of compounds with antidepressant, antipsychotic, anticholinergic, narcotic and analgesic properties were evaluated using cell membrane microarrays extracted from a collection of human tissues. All the compounds analyzed promoted specific actions on superoxide formation, being the liver and heart the organs that reached the highest rates of superoxide formation. In addition, nefazodone, an antidepressant drug withdrawn from the market because of its mitochondrial hepatotoxic effects, produced the highest rate of superoxide formation in human liver cell membranes.

In conclusion, this methodology provides relevant data on oxidative stress triggered by a set of brain-targeted drugs in a collection of human tissues, which are related to certain adverse effects as in the case of nefazodone. Thus, it allows the identification of possible drug side effects in early stages of the drug discovery, optimizing the screening and improving patient safety.

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Reference number: T2-P12

(T2-P12) ***INSIGHT INTO THE KEY FEATURES OF A NEW METHOD ALLOWING THE SPONTANEOUS FORMATION OF BONA FIDE RECOMBINANT PRIONS IN VITRO WITHIN MINUTES***

Spontaneous misfolding of recombinant prion protein in vitro, one of the most useful tools to study the main event underlying Transmissible Spongiform Encephalopathies of sporadic and genetic origin, has always rendered highly variable results. The different methodologies developed to achieve the spontaneous generation of infectious prions in vitro have given rise to a large spectrum of misfolded proteins and amyloid aggregates with distinct properties. From those unable to propagate and cause disease in vivo or those requiring overexpressing animal models and multiple serial inoculations, to infectious prions with high titers, able to cause disease in wild-type animals. Despite the success of some procedures, such as the PMCA using recombinant mouse PrP and polyanionic cofactors, generation of synthetic bona fide prions was inconsistent, obtaining on other occasions non-infectious products with the same method.
Herein, we break down a novel and easily scalable methodology that consistently leads to the spontaneous misfolding of recombinant PrP into infectious, high titer, bona fide prion preparations, allowing the understanding of the minimal requirements for such an event. Using recombinant PrP from bank vole complemented with dextran as substrate, we have optimized the Protein Misfolding Shaking Amplification (PMSA) to obtain infectious synthetic prions within minutes. Additionally, it allows the formation of a variety of strains with specific and differential features. Thus, through fine-tuning of PMSA operational conditions, we can consistently produce highly infectious recombinant prions in a spontaneous manner, offering an invaluable tool to study every aspect contributing to spontaneous prion formation.

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Reference number: T2-P13

(T2-P13) ***CROSS-TALK BETWEEN NEUROMELANIN AND SYNUCLEIN IN PARKINSON'S DISEASE***

Neuromelanin accumulates in dopaminergic neurons during normal aging in humans. In Parkinson's disease (PD), melanized dopaminergic neurons in the substantia nigra pars compacta (SNpc) are those that selectively degenerate. Intraneuronal neuromelanin could play a protective role during its synthesis but it seems evident that when the accumulation of NM exceeds a pathological threshold, it can trigger the degeneration of dopaminergic neurons, characteristic of PD. In contrast to humans, rodents, commonly used as PD animal models, lack NM. In order to mimic an age-dependent accumulation of neuromelanin within nigral dopaminergic neurons, Carballo-Carvajal et al. (2019) successfully injected an adeno-associated viral vectors carrying the gene that codes for human tyrosinase (AAV-hTyr) in the rat substantia nigra.
Pathological α-syn and NM are two prominent hallmarks in this selective and progressive neurodegenerative disease. In order to study the interaction between NM and □-synuclein in PD, we used the approach of Carballo-Carvajal et al. (2019) and we injected an AAV-hTyr into the SN of a knock-in mice for human □-synuclein. Our results showed that AAV-hTyr -injected mice showed a clear motor phenotype characteristic of the disease and the corresponding striatal dopaminergic denervation due to the loss of dopaminergic neurons in the SN. Our results indicate that NM accumulation in dopaminergic neurons in this animal model is an appropiate approach to study the interaction between NM and synuclein in the development of PD.

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Reference number: T2-P14

(T2-P14) ***A NOVEL METHOD FOR NEURONAL SPIKE DETECTION AND THEIR CLASSIFICATION FROM EXTRACELLULAR ELECTROPHYSIOLOGICAL RECORDINGS OF ANIMAL MODELS OF PARKINSON´S DISEASE.***

Neuronal oscillations and their synchronization are crucial for efficient brain communication and an abnormal activity was described in Parkinson's disease (PD) which is the first major motor disabling neurodegenerative brain disorder. In addition, to dopamine, serotonin and noradrenalin monoaminergic systems are known to be affected by the disease. Previous to the motor symptoms, in the early stages of PD, many patients present non-motor symptoms, such as anxiety and depression. Indeed, more than 60% of the PD patients present these comorbid symptoms that do not respond to treatment, however, the molecular mechanisms that trigger these comorbidities are not understood. In the present work, we carried out extracellular

single-unit and Local Field Potential (LFP) recordings in both DRN and LC together with electrocorticogram (ECoG) in transgenic models of PD based on the accumulation of truncated human α-synuclein that and pitx3-/- mice. All recordings were performed under urethane anesthesia and electrical signals were recorded using Cambridge Spike2 software. We aimed to develop a signal-processing algorithm for MATLAB that simultaneously analyzes all registered signals and autonomously computes the spike detection by combining information concerning from both LFPs and ECoGs. The quantification of neural information from the spike detection in extracellular recordings is crucial for understanding the functional impairment and the mechanisms that underlies processing in the central nervous system. We have already observed that these models display an impairment of electrophysiological properties in agreement with the establishment of motor-disabilities.
Funded by Spanish Ministries of Science and Innovation (PID2019-111693RB-I00) and UE (H2020-SC1-BHC-2018-2020, grant agreement n° 848002) and by NextGeneration EU/PRTR (MICIN/CSIC/PTI+ NeuroAging). SVS has a MS from UPV/EHU and JDC-f fellowship.

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Reference number: T2-P15

(T2-P15) ***EVALUATION OF THE DELETERIOUS EFFECT OF HYPERGLYCEMIA IN EXPERIMENTAL STROKE: ROLE OF THE FACTOR INDUCIBLE BY HYPOXIA (HIF).***

INTRODUCTION
Ischemic stroke is a current important public health problem due to its high incidence and the worsening of years of healthy life due to its sequelae. Hyperglycemia during stroke has been related to a worse prognosis, but the mechanisms involved are still unknown (1-2). As in other brain pathologies, the imbalance between O-glycosylations/phosphorylations of different proteins could be involved (3), and since hypoxia-inducible factor (HIF) is one of the transcription factors that are induced during ischemia (4-5), changes in its post-translational modifications would be key.

MATERIAL AND METHODS
Adult male Sprague-Dawley rats has been assessed to investigate the effects of hyperglycemia for 75-min middle cerebral artery occlusion (MCAO). Hyperglycemic and normoglycemic rats received either dextrose (25%; 2.5 mL) 30 minutes before MCAO (326±37 mg/dL at the occlusion; n=11) or vehicle (2.5 mL water; 217±31 mg/dL; n=7). Neurofunctional impartment including motor, sensory and reflex deficits was evaluated with the 9-neuroscore test at 3 and 24 hours after MCAO in hyperglycemic and normoglycemic rats. Post-mortem brains were subjected to Cresyl violet and Fluoro-Jade (neurodegeneration) stainings to measure cell viability at 3 and 24 hours after ischemia.

In addition, the GYSC-GST probe was used to visualize glycogen storage in astrocytes by immunofluorescence.
In vitro model of ischemia-reperfusion injury was developed by immortalized human astrocyte cultures both in normoxia and hypoxia (1% O2; 6 hours) in combination with normoglycemia (11 mM Glucose) or hyperglycemia (25 mM glucose). Cell viability has been determined by Trypan-Blue as well as glycogen labeling by GYSC-GST probe. The expression of HIF-1 alpha was measured by RT-PCR under normoxic conditions and by Western-Blot in normoxia and hypoxia.

RESULTS/DISCUSSION
Hyperglycemia increased neurodegenerative neurons using Fluoro-Jade staining at 24 hours after cerebral ischemia onset. Besides, hyperglycemic rats showed a worsening in neurofunctional recovery.

In vitro ischemia showed that hyperglycemia decrease cell viability respect hypoxic conditions with normoglycemia. Hypoxia induced a decrease in the nuclear glycogen stores that is maintained after 24 and 48 hours in normoxic conditions (reperfusion). However, hyperglycemia caused a significant increase in these deposits at 48 hours after the low oxygen stimulus. After 6 hours of hypoxia the expression of HIF-1 alpha was higher than in normoxic conditions measured by Western-Blot.

Finally, the pattern of O-glycosylation of HIF-1 alpha in astrocytes could be affected by nuclear glycogen deposits changes causing a less beneficial response of this transcription factor against hypoxia and, therefore, a loss of the protective functions of this cell type.

CONCLUSIONS
Hyperglycemia worsens the neurological outcome and neuronal degeneration at 24 hours after cerebral ischemia in rats. Nuclear glycogen stores increase with hyperglycemia at 48 hours and HIF 1-alpha expression increases after 6 hours of hypoxia in cultures of immortalized human astrocytes.

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Reference number: T2-P16

(T2-P16) ***OPPOSITE ASSOCIATIONS BETWEEN TAU PHOSPHORYLATION AND CORTICAL ACTIVATION OF MAJOR TAU KINASES IN ELDERLY PARTICIPANTS OF A LARGE AGING STUDY***

Introduction: Together with amyloid deposition, tau phosphorylation and concomitant accumulation into neurofibrillary tangles is the major pathological hallmark of Alzheimer's disease (AD). Three major kinases have been described to induce tau hyperphosphorylation in AD brains: cyclin-dependent kinase-5 (CDK5), glycogen synthase kinase-3beta (GSK3□), and the extracellular signal-regulated kinase 1/2 (ERK1/2). While the neuronal cofactor p35, and its truncated form p25, are responsible of CDK5 (hyper) activation, GSK3□ and ERK1/2 stimulation is regulated via phosphorylation. Previous work analyzing activated/inhibited forms of these tau kinases (tauK) in AD postmortem brains revealed contrasting results, and small sample cohorts were assessed in each report. The present study analyzed the associations between cortical activation of all three tauKs and multiple phosphotau species, along with other neuropathologic and cognitive outcomes, in a large sample from well-characterized participants of the Rush Memory and Aging Study (MAP).

Methods: Postmortem brain specimens from the dorsolateral prefrontal cortex (DLPFC) were obtained at autopsies from 150 MAP participants experiencing no-(NCI, n=51) or mild-(MCI, n=42) cognitive impairment, or clinical dementia (DEM, n=57) by the time of death. Antemortem cognitive and postmortem neuropathologic assessments were performed according to standard criteria. NFTs were examined by both Bielschowsky impregnation and AT8 immunohistochemistry. Additionally, DLPFC amounts of seven phosphopeptides within the tau protein sequence, and their corresponding nonphosphorylated proteoforms, were quantified by SRM proteomics. The immunodensities of CDK5, p35/p25, phosphoinhibited (and total) GSK3□ and phosphoactivated (and total) ERK1/2 were quantified by Western blotting with selective antibodies in the same cortical samples.

Results: The main outcome of this study revealed an opposite association between several tau phosphopeptides and the activation of different tauKs: (1) positive correlation with phosphoinhibited GSK3□ ($r = 0.20$ to 0.36, $p<0.05$)□□□□□□inverse correlation with p35 and its truncated form p25 ($r = -0.19$ to -0.37, $p<0.05$), and (3) a lack of association with phosphoactivated ERK1/2. Cortical NFT counts were not associated with the activation status of the tauKs studied, although greater p25 immunodensity correlated with lower numbers of diffuse ($r=-0.26$, $p<0.01$) and neuritic plaques ($r=-0.17$, $p<0.05$). Furthermore, accounting for demographic variables, greater cortical density of p25 (Std□=0.16, $p<0.05$), but not p35 or CDK5, was associated with better cognitive function in MAP participants. Among the five domains building global cognitive function, working and semantic memories were the strongest correlates of the CDK5 cofactor p25.

Conclusion: The present results suggest that none of the three major tauKs described so far might be involved in tau phosphorylation, at least in the DLPFC. Future studies will aim at evaluating other possible mechanisms behind cortical NFT formation (e.g. phosphotau propagation).

Supported by Margarita Salas Program (Ministerio de Universidades; Plan de Recuperación, Transformación y Resilencia; NextGenerationEU) with participation of the University of the Balearic Islands (postdoctoral fellowship to EHH), and by MCIU/AEI/ERDF (Grant RTI2018-094414-A-I00).

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Reference number: T2-P17

(T2-P17) ***BRAIN ANGIOGENESIS INDUCED BY NON-VIRAL GENE THERAPY LEADS BRAIN DAMAGE RECOVERY FOLLOWING EXPERIMENTAL ISCHEMIC STROKE***

Introduction:

Stroke remains a major health problem worldwide, and is the leading cause of serious long-term disability and mortality. Hence, emerging therapeutic strategies to recover cerebral vasculature after cerebral ischemia through the enhance of angiogenesis might stimulate endogenous stroke recovery mechanisms1. For this reason, this study aims to evaluate a novel gene therapeutic approach by the intracerebral injection of nioplexes based on VEGF plasmid combined with Niosomes in the rat brain after cerebral ischemia2.

Methods:

The synthesis of Nioplexes were obtained and characterized as previously described2 and including Niosome (GPxT-CQ) + Plasmid PVEGF-GFP. The therapeutic properties of intracerebral injection of Nioplexes or control buffer after middle cerebral occlusion (MCAO) in rats was evaluated by using neurological test, in vivo (Tomato Lectin DsRed) and ex vivo (α-GFP (PVEGF-GFP) immunofluoresce and FluoroJade staining to evaluate the stroke outcome, volume vessel formation and neurodegeneration at day 7 after ischemia onset.

Results:

This study showed that the intracerebral administration of Nioplexes efficiently delivered the plasmid containing VEGF-GFP in ischemic penumbral neurons resulting in the increase of blood vessel formation in comparison to control ischemic animals at day 7 after stroke. Likewise, Nioplexe-treated ischemic rats showed a reduction of infarct volume together with a significant neurofunctional improvement and a decline of neurodegeneration at day 7 after MCAO.

Conclusions:
Our results show for the first time the usefulness of strategies promoting angiogenesis and shed light on the therapeutic potential of nioplexes based on VEGF plasmid and Niosomes for ischemic stroke recovery.

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Reference number: T2-P18

(T2-P18) ***DELETION OF MONOCARBOXYLATE TRANSPORTER 2 IN OLIGODENDROCYTES LEADS TO DEMYELINATION WITH OLIGODENDROGLIAL PRESERVATION***

Monocarboxylates (ketone bodies, pyruvate, and lactate) are alternative energy fuels that can be used by cells either constitutively, in addition to glucose, or under specific circumstances (hypoglycemia, etc). It is known that ketone bodies represent an important energy source for the brain during lactation, and lactate has been shown as a preferred energy substrate for neurons to maintain synaptic activity.

Monocarboxylate trafficking into/outside the cells is achieved through monocarboxylate transporters (MCTs). While initial studies of MCT expression in the CNS showed that MCT2, a high affinity monocarboxylate transporter, is the predominant neuronal MCT, more recent studies of gene expression showed that this transporter is also expressed by microglia and cells of oligodendroglial lineage. Here, we have investigated MCT2 expression on oligodendroglial cells in the adult CNS. RNA sequencing data showed increased MCT2 expression in oligodendroglial cells with age. Protein expression of MCT2 was detected in the brain and spinal cord oligodendrocytes at different ages.

To investigate the function of MCT2 in oligodendrocytes, we injected a Cre-GFP- or GFP-expressing oligodendrotropic AAV in the spinal cord white matter of wildtype (wt) or MCT2lox/lox mice. MCT2lox/lox mice injected with the Cre vector showed myelin loss

and alterations associated with a mild increase in inflammation, without death of transduced oligodendrocytes. Instead, recombined oligodendrocytes showed a decrease in the expression of enzymes associated with lipid synthesis.
Our results suggest that MCT2 expression in oligodendrocytes plays a role in lipid synthesis, likely by mediating monocarboxylate import.
This work has been supported by MINECO Young Investigator Grant to VT and Basque Government Grant IT1203/19 to CM. LI is a recipient of the Basque Government PhD studentship.

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Reference number: T2-P19

(T2-P19) ***A TIGHTLY-CONTROLLED INDUCIBLE SYSTEM FOR HIPPOCAMPAL-TARGETED, CELL TYPE-SPECIFIC MANIPULATION OF NFKB ACTIVITY TO TREAT BRAIN INJURIES AND DISEASES***

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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Reference number: T2-P20

(T2-P20) ***METABOLIC DYSHOMEOSTASIS INDUCED BY SARS-COV-2 STRUCTURAL PROTEINS REVEALS IMMUNOLOGICAL INSIGHTS INTO VIRAL OLFACTORY INTERACTIONS***

One of the most common symptoms in COVID-19 is a sudden loss of smell. SARS-CoV-2 has been detected in the olfactory bulb (OB) from animal models and sporadically in COVID-19 patients. To decipher the specific role over the SARS-CoV-2 proteome at olfactory level, we characterized the in-depth molecular imbalance induced by the expression of GFP-tagged SARS-CoV-2 structural proteins (M, N, E, S) on mouse OB cells. Transcriptomic and proteomic trajectories uncovered a widespread metabolic remodeling commonly converging in extracellular matrix organization, lipid metabolism and signaling by receptor tyrosine kinases. The molecular singularities and specific interactome expression modules were also characterized for each viral structural factor. The intracellular molecular imbalance induced by each SARS-CoV-2 structural protein was accompanied by differential activation dynamics in survival and immunological routes in parallel with a differentiated secretion profile of chemokines in OB cells. Machine learning through a proteotranscriptomic data integration uncovered TGF-beta signaling as a confluent activation node by the SARS-CoV-2 structural proteome. Taken together, these data provide important avenues for understanding the multifunctional immunomodulatory properties of SARS-CoV-2 M, N, S and E proteins beyond their intrinsic role in virion formation, deciphering mechanistic clues to the olfactory inflammation observed in COVID-19 patients.

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Reference number: T2-P21

(T2-P21) ***CHARACTERISATION OF MYOTONIC DYSTROPHY TYPE I CELL CULTURE MODELS BY IN-CELL WESTERN TECHNOLOGY AND DIGITAL DROPLET PCR***

Myotonic dystrophy type I (DM1) is a complex disease characterized by multisystemic impairment, such as the central nervous system, skeletal and smooth muscle, eye or heart. The wide range of symptoms suggests a broad tissue affectation, leading to multiple cell type candidates for pre-clinical drug evaluation, such as patient-derived fibroblasts and myoblasts. Nevertheless, DM1 hallmarks are not homogenous among tissues, which requires the most suitable well-characterized model for each screening. The objective of this work is to evaluate fibroblasts and myoblasts suitability for pre-clinical assays through a novel assessment platform.

Myoblots and fibroblots are assays for protein quantification in microplates based in the In-Cell Western method that we have adapted to the study of DM1-relevant proteins in myoblast and fibroblast cultures, respectively. They allow studying the expression of several proteins with many biological and technical replicates per experiment, resulting in high reproducibility and a throughput similar to ELISA applications. In addition, we have optimised a digital droplet PCR (ddPCR) protocol for the quantification of the mRNA expression of the same proteins to assess the consequences of MBNL1 sequestration in the DMPK mRNA repeats at post-transcriptional level.
Optimisation of myoblots and fibroblots allowed us to accurately quantify different proteins in both cell models. We observed differences in protein expression among DM1 and control groups in myoblasts, and variations of these differences between myoblast and fibroblast cultures. Also, different patterns were shown at RNA level. In addition, we treated cultures with well-known small molecules that have been tested in pre-clinical assays for DM1 and quantified their effect in the expression of target proteins.
Combination of myoblots/fibroblots and ddPCR analysis of DM1 samples allows a highly reproducible and less laborious characterization of DM1 cultures suitable for evaluation of potentially therapeutic compounds in DM1. Also, we describe different protein expression patterns between fibroblasts and myoblasts cultures, suggesting the need for testing potential treatments in more than one cell model

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Reference number: T2-P22

(T2-P22) ***ANHEDONIA IN THE VGLUT1 +/- MOUSE MODEL OF DEPRESSION DOES NOT CORRELATE WITH CHANGES EITHER IN THE SEROTONERGIC CELL POPULATION OR IN SEROTONERGIC FIBER DENSITY IN THE DORSAL RAPHE NUCLEUS IN FEMALE MICE.***

Major depressive disorder is a mental disorder whose hallmark symptom is anhedonia, that is, an extremely low mood and inability to experience pleasure in pleasant activities. The decrease in serotonin in the prefrontal cortex (PfC) is a characteristic feature of depressive patients. The PfC receives serotonergic projections from the dorsal raphe nucleus (DRN) and in turn controls serotonergic function in the DRN through cortico-raphe projections expressing the vesicular glutamate transporter 1 (VGlut1).

Heterozygous mice for VGlut1 (VGlut1 +/-) express ≈ 50% of VGlut1 in the whole brain, and this deficit is associated to a marked anhedonia and desensitization of 5HT1A presynaptic autoreceptors; for this reasons the VGlut1+/- mouse is considered a good model of depression. The enzyme that synthesizes 5HT is the tryptophan hydroxylase enzyme (Tph) which has two isoforms, Tph1 and Tph2 in DRN. The goal of this work is to investigate if the differences between wildtype and heterozygous mice may be due to changes in the total number of serotonergic neurons in DRN, or to changes in the expression of the Tph enzyme, considering both Tph1 and Tph2 isoforms. Female mice were used to determine the total neuronal population in the DRN, the total DRN volume and the neuronal density of Tph1 and Tph2 cells, using stereological methods. No significant differences were found between WT and Vglut1 +/- mice in mean total number of Tph2-cells, total DRN volume or TPh2 cell density. Similarly, preliminary results in the Tph1 cell population revealed no significant differences either in the mean number of total Tph2 neurons or Tph2 cell density in the DRN. We also compared Tph1 and Tph2 fiber density in dually labeled immunofluorescence sections of wt versus Vglut1 +/- mice both in the total DRN nucleus as well as in the individual DR subnuclei (rostral, ventral, lateral wing and caudal). No statistically significant differences were found in either Tph1 or Tph2 density in the total DRN or in the different subnuclei.
In order to determine if there are differences related to sex, the same experiments will be carried out in male mice. In addition, non-serotonergic cell populations will be studied next in this experimental model of depression.

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Reference number: T2-P23

(T2-P23) ***CALCIUM IMAGING IN EPILEPSY: AN IN VIVO AND IN VITRO APPROACH***

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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Reference number: T2-P24

(T2-P24) ***CR3 INHIBITION REDUCES MICROGLIA MIGRATION AND AB INTERNALIZATION, AND INDUCES TRANSCRIPTIONAL MODIFICATIONS.***

Complement receptor CR3 participates in diverse inflammation processes, and both of its subunits CD11b and CD18 are elevated in the cortex of AD patients. Genetic ablation of CD11b reduces uptake of fibrillar Aβ1-42 (Fu et al., 2012; Czirr et al., 2017), and also diminishes microglia-mediated synaptic elimination (Hong et al., 2016; Merlini et al., 2019). These studies support the crucial role of CR3 in AD progression and, consequently, its potential as therapeutic target. To explore further this premise, we evaluated the relationship between CR3 and microglial functions.

As a tool to analyze how CR3 could modulate microglial functions, we used XVA143, an allosteric CR3 antagonist that binds to CD18 subunit.
CR3 inhibition altered cellular morphology, reduced migration capacity and induced gene expression changes in microglia in vitro. Interestingly, inhibition of CR3 with XVA143 impaired microglial internalization of Aβ42 oligomers and reduced extracellular levels of Aβ42 in vitro and ex vivo. Finally, XVA143 modulated the expression of complement components C1q and C4 in vitro and ex vivo.
In conclusion, our results support a key role of CR3 in microglial response to injury and disease, and indicate that CR3 could be involved in modulation of complement component C1q, a novel discovery that needs further investigation.
Funding: CIBERNED, MINECO, Basque Government, UPV/EHU, Fundación Tatiana Pérez de Guzmán el Bueno.

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Reference number: T2-P25

(T2-P25) ***UBE3A-INDUCED UBIQUITINATION CHANGES IN THE BRAIN REVEAL THE MOLECULAR COMPLEXITY OF ANGELMAN SYNDROME***

Angelman Syndrome (AS) is a neurodevelopmental disorder with complex symptomatology caused by the loss of maternal allele expression of one single gene in

the brain, the ubiquitin E3 ligase UBE3A. The underlying genetic basis of AS, and the phenotypes observed in both humans and in animal models of AS, have previously been extensively described. However, the molecular mechanisms regulated by UBE3A ubiquitination in the brain remain highly elusive. Previous studies have reported a number of proteins whose abundance or activity are altered in AS models, implicating various signalling pathways in the physiopathology of AS. But the identified pathways could well be altered further downstream of UBE3A ubiquitination events. Here we provide the first proteomic report of UBE3A-mediated ubiquitination events in a mammalian brain. For this we have used both our bioUb mouse model and a new mouse strain that only slightly elevates UBE3A levels. Several proteins known to be involved in the trafficking and maintenance of neurotransmitter receptors as well as proteins relaying the signals of these synaptic receptors are shown here to be ubiquitinated by UBE3A. The identified proteins have roles in higher mental function, long term potentiation, seizures and neurodevelopmental disorders, being involved in the BDNF, RAS/ERK and TSC/mTOR signalling pathways. A reduced ubiquitination of these proteins is expected when UBE3A levels are lower, in Angelman patients; so their identification could be key to opening novel therapeutic strategies for treating AS. Further work will be required to characterize how UBE3A orchestrates each of these multiple regulatory events in the human brain.

Authors

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Reference number: T2-P26

(T2-P26) ***GSS A117V IN TRANSGENIC MICE EXPRESSING VOLE PRPC: A FAST AND VERSATILE MODEL OF HUMAN PRION DISEASE WITH EARLY BIOMARKERS***

Gerstmann-Sträussler-Scheinker (GSS) syndrome is a rare genetic form of prion pathology characterized by a longer disease course and clinical and neuropathological manifestations differing from those of Creutzfeldt-Jakob disease (CJD). It can be caused by a number of different mutations in the PRNP gene, the most common of which is P102L. GSS was long thought to be a non-transmissible proteinopathy given the low capacity of these prions to infect wild-type and human PrPC-expressing transgenic animal models. However, it was later proven that the infectiousness of a particular prion isolate depends also on the model chosen. For example, GSS was found to be highly

transmissible to bank voles, causing a neurological disease and the accumulation of PrPSc in the brain.
In this study, we present a new model of prion disease consisting of bank vole PrPC-expressing mice (TgVole) at approximately physiological levels (1x) experimentally infected with one isolate of GSS linked to the mutation A117V. GSS A117V transmits by intracerebral route to TgVole (1x) with high efficiency and extremely short incubation periods of 67 ± 1 dpi. Additionally, GSS A117V transmits by intraperitoneal route to TgVole (1x) with a period of 72 ± 3 dpi, being nearly the same as the intracerebral route. To further characterize the prionopathy triggered by GSS A117V prions in the aforementioned TgVole (1x) murine model and to identify biomarkers in easily accessible body fluids, we performed a kinetic study by collecting blood samples along the incubation period of the animals and analyzed two biomarkers: neurofilament light chain (NfL) and β-synuclein (β-syn). The combination of the brief incubation period by intracerebral and, importantly, intraperitoneal route with the possibility to track the disease progression by means of serum biomarkers postulates this new TgVole model as an ideal in vivo approach for preclinical studies on future treatments for human transmissible spongiform encephalopathies.

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Reference number: T2-P27

(T2-P27) ***KETAMINE AND CERTAIN ANTIDEPRESSANTS ENHANCE THE MITOCHONDRIAL ETC ACTIVITY IN RAT HIPPOCAMPUS.***

Major depression disorder (MDD) is a common mental disease and one of the leading causes of disability worldwide. Globally, it is estimated that 5.0% of adults suffer from this disease.
One of the difficulties in treating depression is the limited efficacy of antidepressant drugs. This is the reason of increasing interest in the mechanism of action of rapid-

acting antidepressant drugs (RAADs), such as ketamine. Although the processes involved in the effects of ketamine are not fully known, evidence supports the association of ketamine and other antidepressant drugs with the effects on mitochondrial function. Here we studied the antidepressant-like effects in the rat of the acute treatment with five antidepressants: fluoxetine (SSRI), reboxetine (NARI), venlafaxine (SNRI), PF-4778574 (positive allosteric modulator of AMPA receptors), and ketamine (NMDA receptor antagonist) on the forced swim test (FST). In addition, we also examined the effects of the same treatments on the mitochondrial respiratory chain capacity. For this purpose, we evaluated the superoxide production in the hippocampus, prefrontal cortex, and midbrain membranes isolated from rats using cell membrane microarrays. To characterize the electron transport chain activity the microarrays were incubated with either rotenone (complex I inhibitor) or sodium azide (complex IV inhibitor) in the presence of NADH and decylubiquinone at the optimized time. An increase in mitochondrial activity was observed in the hippocampus of the ketamine-treated group. In addition, a kinetic study of superoxide production was performed using hippocampal membrane homogenates, and the velocities of its production in each animal were determined under the same conditions by spectrophotometry. All drugs showed antidepressant-like effects measured as immobility time in FST and increased superoxide production velocity in membrane homogenates from the hippocampus. These findings suggest an increase in the capacity of the electron transport chain production after antidepressant treatments.
Keywords: depression, mitochondrial ETC, hippocampus, ketamine, antidepressants

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Reference number: T2-P28

(T2-P28) ***GABAR AGONISTS MUSCIMOL AND BACLOFEN PROTECT CULTURED RAT OLIGODENDROCYTES FROM EXCITOTOXICITY INDUCED BY AMPA***

Oligodendrocytes are the myelinating cells of the central nervous system (CNS). Excitotoxic damage produced by overactivation of glutamate receptors (GluR) triggers oligodendrocyte death and highly contributes in the pathogenesis of neurological disorders including neurodegenerative diseases, such as multiple sclerosis. In addition to GluRs, oligodendrocytes express GABA receptors (GABARs) that are involved in their survival and differentiation. The interaction between Glu and GABAergic systems are well-documented in neurons but this potential cross-talk in oligodendrocytes has not been studied in depth yet. Here, we evaluated the protective effect of GABAR agonists baclofen (GABAB) and muscimol (GABAA) against AMPA-induced excitotoxicity in cultured rat oligodendrocytes. First, we observed that both baclofen and muscimol reduced cell death and caspase-3 activation after AMPA insults, proving their oligoprotective potential. Interestingly, the analysis of cell-surface expression of calcium-impermeable GluR2 subunit in oligodendrocytes revealed that GABAergic agonists significantly reduced GluR2 internalization induced by AMPA. Furthermore, we detected that baclofen and muscimol also impaired AMPA-induced intracellular calcium increase and subsequent events as mitochondrial membrane potential alteration, ROS generation and calpain activation. Finally, we evaluated the activation state of relevant

signaling molecules involved in oligodendroglial functionality, as Src, Akt, JNK and CREB, and detected significant changes induced by AMPA exposure. However, these AMPA-triggered modifications were not affected in the presence of baclofen or muscimol. Overall, our results suggest that GABAR activation initiates alternative molecular mechanisms that attenuate AMPA-mediated apoptotic excitotoxicity in oligodendrocytes by interfering with GluR subunits membrane expression and with calcium-dependent intracellular signaling pathways. Together, these findings provide evidence of the potential of GABAR agonists as oligodendroglial protectants in central nervous system disorders.
Funded by Basque Government, MINECO and CIBERNED.

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Reference number: T2-P29

(T2-P29) ***MICROGLIAL PHAGOCYTOSIS DYSFUNCTION DURING STROKE IS PARTIALLY PREVENTED BY RAPAMYCIN***

Microglia are key players in mayor neurological disorders such as stroke, but they have been largely analyzed at the gene expression level, focusing on inflammatory polarization. In contrast, here we study a critical but widely overlooked microglial function: apoptotic cell phagocytosis, which prevents the spillover of cytotoxic contents from dying neurons and limits the inflammatory response. To analyze apoptotic cell phagocytosis in situ, we use as a model the neurogenic niche of the hippocampus, wherein we can establish baseline phagocytosis levels of naturally dying newborn cells by microglia. We show that microglial phagocytic function was impaired after ischemic stroke using an in vivo rodent model of transient medial cerebral artery occlusion (tMCAo) as well as an in vitro oxygen and nutrient deprivation model (OND) in hippocampal organotypic slices and primary microglia. We identified several mechanisms as drivers of the microglial phagocytic blockade after OND, including impaired microglial process motility, lysosomal alterations and the induction of an adaptive autophagy response. Autophagy inhibition had a deleterious effect on microglial phagocytosis and viability, determined using pharmacological (ULK1/2 inhibitor MRT68921) and genetic (ATG4B KO) approaches. In contrast, the autophagy inducer rapamycin prevented to some extent the microglial phagocytic blockade induced by tMCAo in vivo, although it did not significantly affect microglial phagocytosis in vitro. These results suggest that rapamycin partially prevents stroke-induced microglial phagocytic impairment possibly through an indirect, non-cell autonomous mechanism and pave the way to develop more specific strategies for the modulation of microglial phagocytosis.

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Reference number: T2-P30

(T2-P30) ***TOOLS FOR THE STUDY OF UBIQUITINATION IN RARE NEUROLOGICAL DISEASES***

The coordinated activity of ubiquitin E3 ligase (E3) and deubiquitinating (DUB) enzymes is crucial for maintaining the appropriate balance of protein ubiquitination required for cellular homeostasis. In fact, deregulation of many of these enzymes is implicated in a number of diseases, including cancer and rare neurological diseases. Therapeutic strategies that can modify the biological activity of these enzymes and thus, restore appropriate ubiquitination levels of cellular proteins are therefore currently emerging; in particular, those targeting DUB enzymes. However, due to the insufficient understanding of the role of protein ubiquitination in neurons, the development of selective drugs for therapeutic purposes has been limited.
Our lab has developed different strategies for the isolation and identification of ubiquitinated proteins in vivo. Using these approaches, we have reported basal ubiquitin landscapes under physiological conditions in the nervous systems of flies and mice, as well as identified several E3 ligase enzymes' substrates. Moreover, we have identified the substrates of several DUBs, an information that we have integrated in a newly developed interactive database (DUBase: Deubiquitinating Enzyme's Substrate Database), together with another 650 high-confidence manually reviewed DUB substrates. All in all, we have proved that our strategies are very efficient for the analysis of the cellular ubiquitome from different tissues, organisms and conditions. Therefore, the next step would be to apply them into human neurons from patients with rare neurological diseases. For that, we plan to implement all these approaches into human Dental Pulp Stem Cells (DPSC)-derived neurons.

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Reference number: T2-P31

(T2-P31) ***DEEP PHOSPHOPROTEOME LANDSCAPE OF INTER-HEMISPHERIC FUNCTIONALITY OF NEUROANATOMICAL REGIONS OF THE HUMAN BRAIN***

Post-translational modifications (PTMs) have been found to enhance the understanding of disease biology and aid in advancing diagnostic strategies. Despite the widespread research findings in neuroproteomics, one of the key drawbacks has been the lack of knowledge of healthy brain regions in MS-based proteomics level. We have investigated the proteome level expression in different neuroanatomical regions under the Human Brain Proteome Project (HBPP) and developed the global inter-hemispheric brain proteome map (Brainprot) earlier. Further, this study has extended to decipher the phosphoproteome map of human brain interhemispheric regions through high-resolution mass spectrometry (HRMS). The phosphoproteomics examination of 12 unique interhemispheric neurological brain regions using Orbitrap fusion LC-MS/MS provided comprehensive coverage of 996 phosphoproteins, 2010 phosphopeptides and 3567 phosphosites. In addition to this, the study has verified the phosphosignatures using the spectrum data and has been demonstrated in contrast to the Human Protein Atlas-mapped regional brain transcriptomes (HPA). Moreover, interhemispheric phosphoproteome profiling has been categorized according to synaptic ontologies and interhemispheric expression to understand the functionality. Finally, we have integrated the phosphoproteome signatures under PhosphoMap section in the Inter-Hemispheric Brain Proteome Map Portal (IBPM) (www.brainprot.org) for advancement and support of the ongoing neuroproteomics research worldwide.

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Reference number: T2-P32

(T2-P32) ***GALECTIN-1 O-GLCNACYLATION CONTROLS MESCS RESPONSES TO AMYLOID B PEPTIDE***

Protein O-GlcNAcylations are dynamic post-translational modifications (PTM) in serine and threonine residues of many nucleocytoplasmic proteins. In fact, as many proteins

have been described to be O-GlcNAcylated, these PTMs control many important biological responses. In fact, disruptions in the O-GlcNAcylations have been associated with cancer, neurodegeneration and neurodevelopmental disorders. Some proteins have shown to be hyper-O-GlcNAcylated in AD (e.g. APP and c-Fos), others show decreased levels of O-GlcNAcylation (e.g. tau protein). Among all those proteins, some play key roles in the progression of AD. In our laboratory, we have identified by LC-MS/MS 60 O-GlcNAcylated proteins that were exclusively present in the Aβ1-42 oligomers-stimulated lysates. Galectin 1 (Gal-1) was one of them, this is a key protein in the regulation of cell responses, controlling different intracellular and extracellular responses. Galectin-1 (Gal-1) presented two different fragments or peptides that were O-GlcNAcylated. Both peptides contained the same single residue, the serine (Ser) 8, that could be O-GlcNAcylated.
In order to investigate the role of the O-GlcNAcylated Gal-1 in cell biology, we generated using CRISPR-Cas9 gene editing two mESC lines: clones S8A and S8C. The first one, represents the impossibility of O-GlcNAcylation of S8A whereas the second one the same protein is permanently O-GlcNAcylated.
With the 2 clones of mESCS and the mESCs wt, we first examined the migration pattern in mESCs in the presence and in the absence of Aβ1-42. Our results showed different patterns in migration between the 3 lineages and different response to the Aβ1-42 oligomers stimulation. In addition, we examined whether Gal-1 O-GlcNAcylation affected to mESCs differentiation to astrocytes. Preliminary results shown that Gal-1 O-GlcNAcylation controls mESCs differentiation to astrocytes from mESCs in the 3 clones, whoever, we observed differences in the GFAP expression, as astrocytic marker, and in the cell area. Alltogether, our results sugest that the Gal-1 O-GlcNAcylation is S8 is key for for mESCs. Further experiments will be conducted in order to determine the role of O-GlcNAcylation in this process.

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Reference number: T2-P33

(T2-P33) ***HYALURONAN ACCUMULATES IN CORTICAL AREAS IN THE AGEING BRAIN: INTERPLAY WITH MICROGLIA***

Beyond neurons and glia, the Central Nervous System (CNS) holds a plastic scaffold known as the extracellular matrix (ECM). In the brain, the interstitial matrix consists mainly of long chains of the glycan polymer hyaluronan. Protein components of the ECM bind to hyaluronan forming a self-assembled matrix that functions as structural framework and signalling hub. Microglia, the never-resting immune cell of the CNS, constantly survey the brain parenchyma, interacting with cells and the surrounding extracellular microenvironment.
Current data suggest a link between hyaluronan and neuroinflammation, although most results derive from in vitro studies and there is scarce information on the microglia-matrix interplay in vivo, especially in ageing. Here, we report on the interaction these components in the mouse brain of 1, 12 and 18 months. Using high-resolution confocal microscopy and image analysis, we describe structural alterations and changes in the distribution pattern of the hyaluronan matrix as well as a significant accumulation of this

glycan polymer in ageing. By analysing the spatial relation between hyaluronan and glia, we gain insight on the turnover of this glycan polymer by either astrocytes or microglia, and the effect of its accumulation on the reactive status of these cells. These results shed light on the structure of the extracellular matrix in ageing and its interplay with glial cells, a critical stepping-stone towards exploring this duo in CNS disorders.
Keywords: hyaluronan; microglia; ageing; image analysis
Funding: This work has been supported by the Spanish Ministry of Science.

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Reference number: T2-P34

(T2-P34) ***ANALYSING THE OVEREXPRESSION OF ALPHA-SYNUCLEIN IN A RAT MODEL OF PARKINSON'S DISEASE***

Parkinson's disease (PD) is an age-related neurodegenerative disorder characterized by a progressive loss of dopaminergic neurons in the substantia nigra (SN). Surviving neurons contain alpha-synuclein-positive intracellular inclusions, mainly in nerve terminals. The aim of the present work is to investigate the effects of alpha-synuclein (alpha-syn) in the SN and striatum and its role on pathogenesis and symptomatology of PD.

We used a rat model for Parkinson's disease based on overexpression of alpha-syn with adeno-associated viral vectors. 1 µl of rAAV9-CMVie/SynP-wtsyn-WPRE (wt alpha-syn) was injected bilaterally in SN of 8 weeks Sprague Dawley male rats. After 4 months of alpha-syn overexpression, animals were perfused and immunohistochemistry with Thyroxine Hydroxylase (TH) was performed to evaluate the loss of dopaminergic fibers and the presence of axonal swellings in the striatum, the loss of dopaminergic fibers in the substantia nigra pars reticulate (SNpr) and the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc).

TH immunostaining revealed a decrease of striatal optical density accompanied by the presence of axonal swellings in the striatum, and a significant reduction of TH + neurons in the SNpc and TH + fibers in the SNpr in alpha-syn animals.
These findings support the effectiveness of alpha-syn model to reproduce an animal model of PD. Therefore, the morphological description of this model could be a promising tool to investigate new strategies to implement against PD.

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Reference number: T2-P35

(T2-P35) ***ALTERED ELECTROPHYSIOLOGICAL ACTIVITY OF LOCUS COERULEUS AND DORSAL RAPHE NUCLEUS IN PITX3-/- MICE***

Although Parkinson´s Disease (PD) is still considered as a paradigmatic motor disorder, non-motor symptoms appear in early pre-symptomatic stage and are both common and disabling. Apart from the dopaminergic system, monoaminergic systems also became affected during PD and this heterogeneous and progressive neurodegeneration may explain the diverse symptomatology observed. Two of the first areas undergoing degeneration are the locus coeruleus (LC) and dorsal raphe nucleus (DRN) and their alteration have been linked with the non-motor symptoms such as anxiety and depression which are present at least in 50% of patients with PD. In this work, we used the pitx3-/- aphakia mice, a genetic model that mimicked mild-mid stages of PD with the aim of understanding the contribution of the LC and DRN in the non-motor symptoms. To do that, we performed extracellular single-unit recordings and local field potential (LFP) from the LC and DRN together the electrocorticogram (ECoG) from the motor cortex in urethane-anaesthetised mice. The results suggest that after dopaminergic decline, the noradrenergic LC neurons and serotonin DRN neurons presented altered electrophysiological properties and oscillatory activity of LFPs and ECoG and abnormal synchronization with the ECoG. Altogether, these findings suggest that the electrical activity of both LC and DRN seems to be compromised by the dopaminergic decline observed in pitx3-/- mice.

Funded by Spanish Ministries of Science and Innovation (PID2019-111693RB-I00), UE (H2020-SC1-BHC-2018-2020, grant agreement n° 848002), nextGeneration EU/PRTR (MICIN/CSIC/PTI+ NeuroAging), the Basque Government (PIBA19-0038; PUE21-03), the University of the Basque Country (COLAB20/07; GIU19/339) and Transborder Joint Laboratory (LTC) “non-motor CoMorbidities in Parkinson’s Disease (CoMorPD)”. SVS has a MS from UPV/EHU and JDC-f fellowship.

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Reference number: T2-P36

(T2-P36) ***CHARACTERIZATION OF RETINAL GANGLION CELLS SUBTYPES IN THE RAT RETINA.***

Retinal ganglion cells (RGCs) are a heterogeneous population of neurons that propagates visual information from the retina to the brain. Glaucoma is a disease characterized by the selective death of RGCs which induce a progressive and irreversible loss of vision. Increasing evidence suggests that in glaucoma not all RGCs subtypes are

affected equally, with peripheral RGCs being most affected. Indeed, several studies have reported that some RGCs subtypes are more vulnerable than others. Moreover, some antibodies against different RGC subtypes have recently been discovered. However, a rigorous characterisation has not been fully described. Therefore, the aim of this work is to fully characterize and quantify different RGCs subtypes at different localizations in rat retinas.

For this purpose, RGCs were labelled by immunochemistry in control rat whole-mount retinas. Images were captured using the optic disc as a reference for the different retinal zones (centre, middle-centre, middle-periphery, and periphery). RGCs were labelled with an antibody against RBPMS which is considered the 100% of RGCs and co-labelled with subtype-specific antibodies against: CART, Islet1/2, TBR2, SPP1. After quantification, the percentage of RGCs of each subtype were calculated in relation to the total RBPMS positive cells in the different retinal areas.

In the rat retina, the density of RGCs was 1,853/mm2 in centre, 2,041/mm2 in middle-centre, 1,760/mm2 in middle-periphery and 916/mm2 in periphery. CART showed perinuclear labelling and it was expressed in 31.2% of the RGCs from the centre, in 32.1% from the middle-centre, in 33.3% from the middle-periphery and in 27.8% from the periphery of the retina. Islet1/2 showed nuclear labelling and it was expressed in 72.4% of the central RGCs, in 79.3% from the middle-central RGCs, in 77.8% from the middle-peripheral RGCs and in 72.4% from the peripheral RGCs of the retina. TBR2 showed also nuclear labelling and it was expressed in 11.4% of the RGCs from the centre, in 13.3% from the middle-centre, in 12.8% from the middle-periphery and in 16.1% from the periphery of the retina. SPP1 slightly marked the soma of almost 100% of total RGCs.

In conclusion, there is a characteristic pattern for each RGCs subtype in the rat retina. Therefore, we will compare these results with the same analysis in a rat glaucoma model allowing us to elucidate which RGCs subtypes are most susceptible to damage with the aim to purpose different therapeutic strategies to protect the most vulnerable RGCs and thus preventing and treating visual impairment caused by glaucoma.
Supported by ELKARTEK (KK-2019/00086), MINECO-Retos (PID2019-111139RB-I00) and PIBA (2020_1_0026).

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Reference number: T2-P37

(T2-P37) ***A PROXIMITY BIOTINYLATION ASSAY TO IDENTIFY PATHOGENIC ALPHA-SYNUCLEIN INTERACTOME.***

Increased levels or point mutations of α-synuclein (aSyn) cause neurodegeneration (synucleinopathies) but the underlying molecular mechanisms of aSyn-dependent toxicity remain largely unclear. To quantitatively score aSyn toxicity and study its

pathogenic mechanisms, we established a neuronal model in which longitudinal survival analysis can be performed by tracking individual primary neurons expressing fluorescently tagged aSyn variants. By Cox regression analysis, we quantified the influence of aSyn pathological events leading to neuronal death. Importantly, this model recapitulated the aSyn dose-dependent toxicity, and the gain of toxicity mediated by point mutations, being the pathological E46K aSyn mutation the most toxic variant among the ones tested. This model led us to establish that the aSyn N-terminus, most likely through its acetylation, determines aSyn protein levels and toxicity. We hypothesize that protein-protein interactions (PPIs) favored by increased protein levels, pathological mutations or N-terminal acetylation modulate aSyn toxicity. To further identify aSyn-dependent PPIs we set up an assay based on proximity biotinilation using the biotin ligase (BioID2). When expressed as a fusion protein, BioID2 biotinylates by proximity stable and transient interacting partners, allowing their identification by mass spectrometry. We expressed BioID2-tagged aSyn and performed an analysis of the aSyn interactome in HEK293 cells. At least six WT and E46K aSyn protein specific interactors were found, one of them mediated by N-terminal acetylation. We have addressed its effect in aSyn-dependent neuronal death by longitudinal survival analysis using genetic or pharmacologic modulation. Finally, their expression is being evaluated in a humanized aSyn transgenic model. Disruption of toxic aSyn PPIs could serve to develop therapeutic approaches to treat synucleinopathies.

Authors

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Reference number: T2-P38

(T2-P38) ***TEMPORAL SEQUENCE OF GLIAL ACTIVATION, NIGROSTRIATAL DEGENERATION AND MOTOR IMPAIRMENT INDUCED BY ALPHA-SYNUCLEIN AGGREGATION IN NIGRAL DOPAMINERGIC NEURONS***

Misfolding and aggregation of alpha-synuclein (α-syn) are specific features of Parkinson's disease (PD) and other synucleinopathies. In fact, PD progression has been correlated with the formation and extracellular release of α-syn aggregates. Therapeutic interventions in the onset of PD require a clear understanding of the mechanisms by which α-syn aggregation induces the nigrostriatal degeneration and/or neuroinflammation that precede motor symptomatology. We aimed to investigate the temporal relationship between synucleinopathy, glial activation, nigrostriatal degeneration and motor impairment in a mouse model of PD. Mice received uni or bilateral intranigral injection of adeno-associated viral vectors (AAV) or encoding human α-syn (AAV-hα-syn) and were sacrificed 15, 60 or 120 days post-injection. At these time points, motor behavioural tests (open field and wire hang test) and

immunohistochemistry for α-syn, p-α-syn, nigrostriatal degeneration (TH) and glial activation (microglia (Iba-1) and astrocytes (GFAP)) were carried out.

Uni or bilateral intranigral injection of AAV-hα-syn led to nigral α-syn aggregation that was significant at day 15 post-surgery, reaching the peak at day 60. Significant reductions in striatal and nigral TH+ fibres were observed 60 and 120 days post-surgery, whereas the number of nigral TH+ cells was unchanged. Behavioural analysis revealed that motor impairments were significant at 120 days post-surgery in uni and bilateral models. Also in both models, a significant nigral microglial activation was observed 60 and 120 days post-injection, but no striatal or nigral astroglial activation was found. Although these preliminary data indicate that the viral-vector mediated overexpression of α-syn in the mouse substantia nigra is appropriate to model PD-like pathology, additional time points need to be explored to elucidate whether the neuroinflammation contributes to DA degeneration.

Supported by grants from the Basque Government (PIBA 2019-38; PU21-03) and the University of the Basque Country (COLAB20/07; GIU19/092). This research was conducted in the scope of the Transborder Joint Laboratory (LTC) “non-motor CoMorbidities in Parkinson’s Disease (CoMorPD)”. No conflict of interest. MZ is supported by UPV/EHU fellowship.

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

ORAL PRESENTATIONS

Moderator: **Naroa Ibarretxe Bilbao (U. Deusto, Bilbao)**

Oral Communication T3-OC1

CHRONIC CLOZAPINE AND MINOCYCLINE TREATMENTS IN A DOUBLE-HIT MOUSE MODEL OF SCHIZOPHRENIA: EFFECTS ON BEHAVIOR AND GENE EXPRESSION

Background: Cognitive and negative deficits are core features of schizophrenia not fully addressed by current antipsychotic treatments. Altered peripheral and central inflammatory signaling has been shown in schizophrenia and associations between inflammatory markers and cognitive/negative deficits have been reported. The inflammatory activity inhibitor minocycline has been investigated as an add-on treatment to antipsychotics in schizophrenia. Among schizophrenia rodent models, maternal immune activation (MIA) has been shown to increase the vulnerability of the offspring to develop neuroimmune and behavioral abnormalities in response to stressful events later in puberty. We have previously developed a double-hit animal model of schizophrenia based on MIA followed by social isolation in peripuberty. This model showed schizophrenia-like negative and cognitive deficits and alterations in the expression of the neuroinflammatory signaling protein NF-κβ and its repressor IκBα. Objective: We aimed to assess in a double-hit model of schizophrenia the effect of chronic treatment with the atypical antipsychotic clozapine, minocycline, and the combination of both drugs, on cognitive and social deficits and in the expression of brain NF-κβ and IκBα.

Methods: Clozapine (10 mg/kg/day) and/or minocycline (30 mg/kg/day) were chronically administered (i.p., once daily, 3 weeks) to control and double-hit mice. After of 48 h of washout, cognitive and social performance were evaluated by the Novel Object Recognition Test (NORT) and the Social Preference Test (SPT), respectively. Gene expression of NF-κβ and IκBα was evaluated in the frontal cortex of these mice by RT-qPCR assays. Data are expressed as mean±SEM, and were analyzed by unpaired Student's t- or two-way ANOVA tests.

Results: Double-hit mice showed worse NORT Discrimination Index (DI) (0.03±0.03) and social exploration times (SET) (59.42±3.78 s) than controls (ControlDI: 0.23±0.12; ControlSET: 71.6±4.66 s) (DI: t=1.42, p=0.18. SET: t=1.98, p=0.05). In control mice, NORT DI and SPT Social Index (SI) were not altered by any of the pharmacological treatments [DI: FCLOZAPINE(1,23)=0.51, p=0.48; FMINOCYCLINE(1,23)=0.08, p=0.78; FINTERACTION(1,23)=1.53, p=0.23], [SI: FCLOZAPINE(1,70)=2.13, p=0.15; FMINOCYCLINE(1,70)=0.14, p=0.71; FINTERACTION(1,70)=1.26, p=0.26]. In double-hit mice, NORT DI was significantly increased by minocycline treatment [FCLOZAPINE(1,23)=1.36, p=0.25; FMINOCYCLINE(1,23)=7.82, p<0.05; FINTERACTION(1,23)=1.68, p=0.21]. In the SPT, neither clozapine nor minocycline

showed a significant effect in the SI [FCLOZAPINE(1,58)=0.02, p=0.89; FMINOCYCLINE(1,58)=1.85, p=0.18]. However, combination of clozapine and minocycline significantly decreased SI [FINTERACTION(1,58) =6.07, p<0.05]. In double-hit mice, NF-κβ and IκBα gene expression were not modulated by any of the treatments [NF-κβ: FCLOZAPINE(1,27)=0.0002, p=0.99; FMINOCYCLINE(1,27)=0.14, p=0.71; FINTERACTION(1,27)=0.30, p=0.59], [IκBα: FCLOZAPINE(1,28)=0.48, p=0.50; FMINOCYCLINE(1,28)=2.25, p=0.14; FINTERACTION(1,28)=0.31, p=0.58].

Conclusion: These results indicate that chronic administration with the inflammatory inhibitor minocycline is able to revert cognitive deficits related to schizophrenia in the double-hit model. However, combination of clozapine and minocycline worsen schizophrenia-like social impairment. Gene expression of inflammatory signaling proteins NF-κβ and IκBα seems not to be related to treatment-dependent behavioral effects. Thus, this model shows predictive validity, and might be a useful translational tool to develop new pharmacological approaches for the treatment of schizophrenia cognitive and negative deficits.

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Oral Communication T3-OC2

ALPHA-BAND OSCILLATIONS REFLECT TACTILE ATTENTION VIA THE ENGAGEMENT OF OCCIPITAL REGIONS IN EARLY BLINDNESS

Improved attentional selectivity developed through continuous practice has been suggested to underlie the superiority of early blind (EB) population on certain somatosensory tasks. Specifically, alpha-band activity is thought to contribute to the cueing of attention through the engagement of task-relevant neural populations. However, the role of such mechanisms is still controversial and different functions have been attributed to posterior alpha rhythms in blindness. Therefore, we used high-density electroencephalography to answer whether alpha oscillations reflected a differential recruitment of task-relevant regions between expected and unexpected conditions in two (texture and shape discrimination) haptic tasks. The time frequency analysis showed that pre-stimulus alpha oscillations and post-stimulus alpha suppression in parieto-occipital sites was significantly reduced in EB individuals and that group-differences were similar on both tasks. The source reconstruction analysis revealed that the origin of the group differences was located in the middle occipital lobe. Here, expected trials evoked higher alpha desynchronization than unexpected trials in the EB group. Our results support the role of alpha rhythms in the recruitment of task-relevant areas and we show for the first time that the posterior alpha activity in blindness is not task independent. Our findings suggest that alpha activity may be involved in tactile attention in blind individuals, maintaining the function proposed for visual attention in sighted population -but switched to the tactile modality- and that these attentional mechanisms may contribute to the superiority of unsighted individuals

on some haptic tasks. Altogether, our results bring a new understanding to the role that alpha oscillatory activity plays in blindness.

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Oral Communication T3-OC3

IN VIVO MULTIMODAL IMAGING OF ADENOSINE A2A RECEPTORS IN NEUROINFLAMMATION AFTER EXPERIMENTAL STROKE

Introduction

Adenosine receptors have become of interest due to their expression in both the innate and adaptive immunity, suggesting a possible role of these receptors in the neuroinflammatory response (Martín et al., 2018). Particularly, a neuroprotective role of adenosine A1 receptors after ischemia has been recently described (Joya et al., 2021). However, the role of adenosine A2A receptors (A2ARs) after ischemia is still largely unknown. Therefore, the present study aims to analyse for the first time the spatial and temporal expression of A2ARs in the healthy and ischemic brain and to evaluate the possible role of A2ARs on the inflammatory response using in vivo imaging techniques.

Methodology

The expression of A2ARs was evaluated using in vivo positron emission tomography (PET) with [11C]SCH442416 before and at 1, 3, 7, 14, 21 and 28 days after transient a middle cerebral artery occlusion (tMCAO) in rats (n=6). Likewise, magnetic resonance imaging (MRI-T2W) studies were carried out at 24 hours to assess the extent of brain damage. PET signal uptake was quantified by means of %ID/cc in different brain regions and radiotracer kinetic values were obtained using the Simplified Reference Tissue Model (SRTM) (Lammertsma & Hume, 1996) using the cerebellum as the reference tissue. Additionally, the characterisation of A2ARs expression was evaluated before and after ischemia by immunohistochemistry (n=12) and the possible role of A2ARs on the ischemic response was analysed by MRI and PET with [18F]DPA-714, after the treatment with an agonist of A2ARs (vehicle, n=5; CGS-21680, n=6).

Results

In healthy conditions, [11C]SCH442416-PET signal showed that A2ARs expression was restricted to the striatum. These findings were supported by the SRTM, showing an increase of non-displaceable binding potential (BPnd) values in this brain region. In contrast, other brain regions including the cortex showed negligible BPnd despite they showed higher %ID/cc values as a result of non-specific binding. At day 1 after stroke,

MRI-T2W showed infarction affecting cortico-striatal regions leading a PET signal uptake (%ID/cc) decrease in only the ischemic striatum followed by a slight signal recovery at day 3 and a progressive decline later on. These results were confirmed by SRTM, showing a striatal increase of BPnd at day 3 after stroke. Moreover, our results demonstrated an increase of microglia/macrophages expressing A2ARs at day 3 after stroke in relation to control (day 0) and day 1 in both the penumbra and infarcted striatal brain regions. Additionally, [18F]DPA-714-PET imaging showed that ischemic animals treated with A2ARs agonist displayed a significant increase of inflammatory reaction in the area of the striatum.

Conclusion

The present study shows that A2ARs are mainly expressed in the healthy rat striatum and describes for the first time the temporal expression of A2ARs following cerebral ischemia. Moreover, these findings demonstrate that the increase of the BPnd observed in the striatum at day 3 in relation to day 1 might be related to the increase of microglia/macrophages expressing A2ARs. Currently, we are conducting western blotting analysis to semi-quantify the concentration of A2ARs before and after ischemia and immunohistochemical studies to validate in vivo [18F]DPA-714-PET findings.

Acknowledgement

The authors would like to thank A. Lekuona, X. Ríos, V. Salinas for technical support in radiosynthesis and technical assistance in the PET studies. This study was funded by Spanish Ministry of Education and Science (RYC-2017-22412, PID2019-107989RB-I00, PID2020-117656RB-100).

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Oral Communication T3-OC4

FATAL FAMILIAL INSOMNIA IN THE BASQUE COUNTRY: DESCRIPTION OF A CASE SERIES

Fatal familial insomnia (FFI) is an uncommon genetic prionopathy characterized by sleep disturbances and dysautonomia, together with motor and cognitive dysfunction and

neuropathological manifestations of severe atrophy, neuronal loss, and gliosis in the thalamus. It is caused by a change of Aspartic acid into Asparagine at residue 178 of the prion protein accompanied in cis by a methionine at the polymorphic 129 position.
In this study, we retrospectively examined the FFI cases diagnosed in the Basque Country from 2010 to 2021, including a total of 16 (12 male and 4 female) genetically and neuropathologically confirmed FFI patients. Epidemiological and clinical data, as well as complementary tests, including polysomnography (PSG), brain imaging, neuropathological, biochemical and genetic information were analyzed.
The mean age of onset was 54 years (range 36-70) and the disease course of 11 months (range 4-24). Insomnia (first symptom in 33% and present during disease in 94%), rapid progressive dementia (87%), myoclonus (75%) and cerebellar signs (69%) were the most frequent signs. The brain MRI (88%) and the electroencephalography (EEG) (81%) were usually normal, whereas polysomnography was pathological in 92% of the cases. Most (75%) of the patients were homozygous for Met129 and in the remaining 25%, the mutation was in cis with Met variant. There was no family history in 25% of the cases. Basal ganglia gliosis, astrocytosis and neuronal loss was present in the whole series. Therefore, while the value of the new markers (total tau and RT-QuIC) is still pending for evaluation, polysomnography is, together with the genetic study, the test with the highest diagnostic value in FFI.

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Oral Communication T3-OC5

IMPLANTED NEURAL INTERFACE FOR STROKE REHABILITATION

Rehabilitation studies leveraging non-invasive brain-machine interface (BMI) technology have demonstrated clinical improvements with systems where changes in decoded brain activity coincide with peripheral feedback through the movement of an orthosis. In these systems, the decoded movement intention from brain signals is translated into movement of the peripheral orthosis, giving patients feedback about their brain activity. This allows them first to refine their neural activity patterns to be more specific for a given movement, and second leverages the endogenous sensorimotor learning system to link these neural signals and the activation of the peripheral nervous system. So far, studies have been limited to linking patterns from non-invasively acquired brain signals to single movements such as only grasping or only reaching. One challenge with using non-invasive brain signals in these systems is the low number of movements that can be

reliably decoded on a single-trial basis. This restriction limits the feedback available to the patient about how to refine their neural activity patterns, as well as the number of trained movements these systems can reinforce. Thus, one way to improve upon current state-of-the-art is to use a neural recording modality that allows for accurate, single-trial decoding of greater number of movements, and deliver feedback of these decoded movement intention signals through an orthosis with multiple degrees of freedom.

Hemiplegic stroke patients may not exhibit overt movements, but some patients have subthreshold muscular activations during intended movements that can be detected with surface electromyography (EMG). Such activity can be used to discriminate differing movement intentions.

In this work, we present a novel brain-machine interface rehabilitation system for hemiplegic chronic stroke patients that leverages a temporarily implanted intracortical multi-electrode array and a seven degree-of-freedom arm and hand orthosis. Previous work has demonstrated successful use of neural signals acquired from implanted multi-electrode arrays for closed-loop control of multi degree-of-freedom robotic arms. Further, we introduce a novel method of shared control of the orthosis where decoded neural activity signals are fused with decoded movement intention signals from surface EMG. This fusion is hypothesized to accelerate the formation of the neuro-muscular link by reinforcing the co-activation of motor-related neural circuits and target muscles. We demonstrate the long-term (3 years) usability of this system with a severely paralyzed chronic stroke patient.

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Reference number: T3-P01

(*T3-P01*) *STUDYING THE ROLE OF CB1 RECEPTORS ON MEMORY AND NAVIGATION IN MALE AND FEMALE MICE*

Hippocampal circuits play a crucial role in cognitive processes (e.g., navigation strategies). Furthermore, cortical areas participate in decision-making and emotional processes shaping the performance of the animals during memory tasks. In this work, we want to dissect the specific and sex-dependent role of CB1 signaling in different circuits on memory and navigation.
Methods: We use behavioral genetics and pharmacology in C57BL6 and CB1-flox male and female mice carrying a Cre-mediated CB1 deletion. Viral vectors (AAV-Cre) were injected into the hippocampus and the prefrontal cortex. We use the Barnes maze test to assess cognitive performance (e.g., memory, navigation). Furthermore, a battery of anxiety-like behavioral protocols is performed prior to cognitive evaluation to rule out potential confounding factors.
Results: intraperitoneal injection of THC immediately after each day of acquisition in the Barnes maze impairs memory consolidation in males but not in females. Interestingly, THC-treated mice specifically show a decrease in spatial strategy, suggesting a direct effect of the pharmacological treatment on hippocampal circuits. On the other hand, male and female CB1-flox mice carrying a hippocampal or prefrontal CB1 deletion present no significant changes in the learning processes compared to control littermates. However, CB1 deletion on male mice produced a specific decrease in the spatial strategy. Strikingly, such changes were not found on female mice.
Conclusions: These results show that CB1 receptors in the hippocampus and the prefrontal cortex are necessary for developing and consolidating navigation strategies in a sex-dependent way.

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Reference number: T3-P02

(*T3-P02*) *PHONEMIC VERBAL FLUENCY FACILITATION BY TRANSCRANEAL RANDOM NOISE STIMULATION OVER THE LEFT PREFRONTAL CORTEX*

In recent years, several studies have analyzed the effects of transcranial direct current stimulation (tDCS) on verbal fluency tasks in a non-clinical population. Nevertheless, the reported effects on verbal fluency are diverse and inconsistent. In addition, the effect of other noninvasive stimulation techniques such as transcranial random noise stimulation (tRNS) on verbal fluency has been scarcely studied. Therefore, the aim of the present study is to explore the effects of tRNS on verbal fluency tasks in healthy individuals. To this end, a double-blind, counterbalanced, sham-controlled trial was conducted. Fifty healthy native Spanish-speaking participants, aged 18-47 years old, were randomly

assigned into two groups: tRNS and placebo-control group. The electrodes were placed over the left dorsolateral prefrontal cortex (L-DPFC) and the left inferior frontal gyrus (L-IFG), as these areas have been closely associated with speech and language processing in several studies. All participants performed phonemic and semantic verbal fluency tasks before, during (online), and immediately after (offline) the stimulation. In the active condition, a 1.5mA current (100–500 Hz) was delivered for 20 minutes. The results showed significant differences in phonemic fluency between the groups during the online ($p = .014$) and offline ($p = .008$) phases. However, there were no differences between conditions in the online ($p = .41$) and offline ($p = .15$) semantic tasks. The results obtained in this study suggest that the excitatory effects of tRNS over the left prefrontal cortex area (L-DLPFC and L-IFG) could contribute to the improvement of performance in phonemic verbal fluency tasks, but not in semantic skills in healthy individuals.

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Reference number: T3-P03

(T3-P03) WIN 55,212-2 MODULATES RECOGNITION MEMORY AND CB1 RECEPTOR ACTIVITY IN A RAT LESION MODEL OF CHOLINERGIC IMPAIRMENT

The impairment of basal forebrain cholinergic neurons (BFCN) is key to developing Alzheimer's disease (AD) and these pathways are modulated, among others, by the endocannabinoid (eCB) system. In this study, we evaluated the effect of cannabinoid agonist WIN 55,212-2 in learning and memory in a rat lesion model of BFCN degeneration.

192-IgG-saporin was infused bilaterally to rats in the BFCN (SAP group); control group received vehicle without toxin (aCSF group). Additional control and SAP rats received either 0.05 mg/kg or 0.5 mg/kg of WIN 55,212-2, or vehicle, for 5 days. Learning and memory were evaluated by novel object recognition task (NORT) 3h and 24h after learning. The CB1 receptor activity was analyzed by functional [35S]GTPyS autoradiography.

SAP group failed to explore the new object more than the old one, as opposed to aCSF group (54.85% ± 3.93 vs 70.81% ± 2.42), 24h after learning. After treatment with 0.5 mg/kg of WIN 55,212-2 (SAP+WIN0.5 group) for 5 days, memory was restored and rats explored the new object more (60.37% ± 6.36). However, this dose had the opposite effect in control rats, as it impaired their memory. Regarding the low dose of 0.05 mg/kg, it also improved recognition memory (67.42% ± 8.35, new object exploration) in lesioned rats (SAP+WIN0.05 group), while no effect on memory was observed in control rats (60.29% ± 2.14).

These results suggest that low doses of cannabinoid agonists might be a plausible treatment for the learning and memory impairment derived from BFCN degeneration in AD patients.
Acknowledgements: Technical and human support was provided by the General Research Services SGIker [University of the Basque Country (UPV/EHU)], the Ministry of Economy and Competitiveness (MINECO), the Basque Government (GV/EJ), the European Regional Development Fund (ERDF), and the European Social Fund (ESF). J.M.-G. is the recipient of Margarita Salas fellowship funded by the European Union-Next Generation EU.

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Reference number: T3-P04

(*T3-P04*) *IN VIVO/IN VITRO NEUROCHEMICAL AND BEHAVIOURAL CHARACTERISATION OF THE PSYCHEDELIC 5HT2AR AGONIST PSILOCYBIN IN MICE*

Background: The psychedelic 5-HT2A agonist (5HT2AR) psilocybin (or the active metabolite psilocin) has emerged as a potentially useful drug for various neuropsychiatric diseases such as depression, with a rapid onset of therapeutic activity. However, the mechanisms responsible for these therapeutic effects remain incompletely characterised. In addition, the relationship between psychedelic experience and antidepressant response has not been fully elucidated. Animal studies suggest that antidepressant effect may be independent to its serotonergic 5HT2AR-mediated hallucinogenic action.

Aim: The aim of the present study was to characterise the acute psychoactive effects of psilocybin in mice through head-twitch response (HTR), and to evaluate the role of 5HT2AR, 5HT2CR and 5HT1AR on such response. We also aimed to study in vivo neurochemical effects after acute administration of psilocybin. In addition, long-lasting antidepressant/anxiolytic effects of psilocybin and the role of 5HT2AR were assessed.

Methods: Psilocybin (0.125/0.25/0.5/1/3 mg/kg i.p.) or saline (5 mL/kg i.p.) (n=7-14) was administered to male C57BL/6J mice and the HTR was evaluated for 20 minutes. Selective antagonist of 5HT2AR, MDL11939 (1 mg/kg i.p.) (n=11), 5HT2CR antagonist SB242084 (0.1 mg/kg) (n=9), or 5HT1AR antagonist WAY100635 (1 mg/kg i.p.) (n=6), was administered 30 minutes prior to psilocybin to evaluate the role of such receptors on the HTR. In another set of animals, the effect of systemic administration of psilocybin and blockade of 5HT2AR on extracellular monoamine concentration in the frontal cortex (FC) was studied by microdialysis. Dialysates were collected every 12.5 minutes and extracellular noradrenaline (NA), dopamine (DA) and serotonin (5-HT) concentrations were quantified on basal conditions and post-systemic MDL/vehicle + psilocybin/saline administration (n=4-12) using UHPLC. Finally, the role of 5HT2AR was evaluated in the

long-term behavioural and neurochemical effects of psilocybin: MDL11939 (1 mg/kg i.p.) or vehicle was administered 30 minutes prior to psilocybin (1 mg/kg i.p.) or saline (n=12). Subsequently, mice underwent a set of behavioural tests in the following days post-treatment: forced-swimming test (FST) 24 hours later and on day 8, elevated plus maze (EPM) on days 3 and 10, and tail-suspension test (TST) on day 7. Animals were sacrificed on day 13 post-treatment. The content of tissue NA, DA and 5-HT was evaluated in cortex (Ctx) using HPLC.

Results: Psilocybin induced a dose dependent response of HTR (maximal effect 17.07±1.31 at 1 mg/kg) that was completely suppressed by the 5HT2AR antagonist MDL11939 (1 mg/kg). A higher dose of psilocybin (3 mg/kg) induced a lower HTR compared to 1 mg/kg (9.00±0.53). 5HT2CR antagonist SB242084 (0.1 mg/kg) potentiated HTR exerted by 3 mg/kg of psilocybin. 5HT1AR antagonism did not have an effect on psilocybin-induced HTR. Extracellular NA, DA and 5-HT were not affected by acute administration of psilocybin, antagonist or antagonist + psilocybin compared to control. In the behavioural assessment, psilocybin did not show any antidepressant or anxiolytic effect in the FST, EPM and TST on naïve mice, regardless of pre-treatment with MDL11939. NA and 5-HT content were increased by psilocybin treatment in Ctx, regardless of pre-treatment with MDL11939 (NA: FSal/Psil[1,90]=4.45, $p<0.05$) (5-HT: FSal/Psil[1,91]=6.36, $p<0.05$).

Conclusions: Acute psychedelic effect of psilocybin in mice is mainly mediated by 5HT2AR activation and modulated by 5HT2CR activation. Such effect does not seem to be linked to changes in synaptic release of monoamines in the FC. However, administration of a single dose of psilocybin is able to induce long-term selective increases of NA and 5-HT tissue content in Ctx by a 5HT2AR-independent mechanism. In summary, data suggest that hallucinogenic action and long-term modulation of monoaminergic circuits induced by psilocybin may be mediated by different mechanisms of action.

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Reference number: T3-P05

(*T3-P05*) *BIOMARKERS OF MOTOR FUNCTION AND RECOVERY IN THE MUSCLE SYNERGIES OF CHRONIC STROKE PATIENTS*

Background. Stroke affects the expression of muscle synergies underlying motor control, most significantly in patients with more affected motor function. Most studies have conventionally approached the analysis of muscle synergies by assuming alterations in their inner structures after stroke. Although different features extracted from synergies following this assumption have to some extent described pathological

mechanisms in post-stroke neuromuscular control, a biomarker that reliably reflects motor function and recovery is still missing.
Methods. Based on the theory of muscle synergies, we alternatively hypothesized that functional synergy structures are physically preserved and we computed the temporal correlation between the temporal recruitment profiles of healthy structures by paretic and healthy muscles, a feature hereafter reported as the Functional Synergy Recruitment Index (FSRI). We measured clinical scores and extracted the muscle synergies of both upper limbs of 18 chronic stroke survivors from the electromyographic activity of eight muscles during bilateral movements before and after 4 weeks of non-invasive Brain-Machine Interface (BMI)-controlled robot therapy and physiotherapy. We computed the FSRI as well as conventional features quantifying inter-limb structural differences and finally evaluated the correlation of all these synergy-based measures with clinical scores.

Results. Correlation analyses revealed weak relationships between conventional features describing inter-limb synergy structural differences and motor function. In contrast, FSRI values during datasets including single or various movements significantly correlated with upper limb motor function and recovery scores. Additionally, we observed that BMI-based training with contingent positive proprioceptive feedback led to improved FSRI values during the specific trained finger extension movement.
Significance. We demonstrated that FSRI can be used as a reliable physiological biomarker of motor function and recovery in stroke, which could be targeted via BMI-based proprioceptive therapies and adjuvant physiotherapy to boost effective rehabilitation.

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Reference number: T3-P06

(*T3-P06*) *CURCUMIN NANODELIVERY EFFECTS ON MK-801 INDUCED SCHIZOPHRENIA-LIKE BEHAVIOURS*

Curcumin as an antioxidant natural herb has shown neuroprotector and anti-inflammatory effects and has been used for treatment of different pathologies. However, the poor bioavailability of curcumin is a significant pharmacological barrier for its activities. Schizophrenia is a disabling psychiatric disorder that implies dysfunction in GABAergic neurotransmission and N-methyl-D-aspartate receptors (NMDAR) hypofunction. The aim of this study is to evaluate the beneficial motor and cognitive

effects of short treatment with curcumin-loaded nanofibers in early adulthood (P55-P73) of MK-801 animal model of schizophrenia.

35 Long Evans male rats were segregated into 4 groups: C (n=9 controls treated with saline); NF (n=10 treated with nanofibers); NFC (n=10 treated with curcumin-loaded nanofibers); MK-801-NFC (n=6 MK-801 rats treated with curcumin-loaded nanofibers). MK-801, a NMDAR antagonist, was intraperitoneally administered to rat pups once daily from P10 to P20 (0,5 mg/kg diluted in 0,9% NaCl). From P55 to P62, animals received a daily intraperitoneal dose of curcumin-loaded nanofibers (20 mg/kg). Motor and cognitive alterations were evaluated by Open Field, Novel Object Recognition, Object-in-Place and Elevated Plus Maze test.

The results showed differences in motor parameters, but no modifications were observed at cognitive level. Curcumin-loaded nanofibers enhanced mobility in healthy animals, while in MK-801 rats did not revert MK-801 adverse effects. This can suggest that curcumin-loaded nanofibers concentration is not enough to improve motor symptoms. Thus, it would be interesting evaluate the effects of curcumin-loaded nanofibers at different concentrations in MK-801 rat model. Moreover, a neurochemical study will be performed to correlate behavioral and histological alterations.

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Reference number: T3-P07

(T3-P07) ***OVEREXPRESSING HUMAN ASYNUCLEIN IN THE LOCUS COERULEUS AS A PRESYMTOMATIC ANIMAL MODEL OF PD TO ASSESS BEHAVIOURAL CHANGES IN RODENTS.***

Parkinson's disease (PD) is a neurodegenerative process characterized by the presence of Lewy bodies in different brain regions, affection of many neurotransmitter systems and manifestation of motor and nonmotor symptoms. These latter ones often appear before the motor symptomatology and highly impact on the life quality of the patients. Already in the presymptomatic phase, the noradrenergic nucleus locus coeruleus (LC) shows Lewy bodies and PD-like pathology, being one of the first nuclei that undergoes neurodegeneration. This nucleus has a critical role in stress response, emotional memory and control of motor, sensory and autonomic function and can even modulate dopamine homeostasis. The aim of this study is to investigate how the LC participates in pathogenesis and symptomatology in PD. For that, we studied the impact of alpha-synuclein overexpression in the LC in a pre-symptomatic rodent model of PD analysing the molecular and behavioural changes overtime. To do so, we performed a battery of behavioural tests that evaluate the anxiety and depressive-like behaviour, olfactory deterioration, nociception and locomotion. Although alpha-synuclein is already expressed in the first month after surgery, behavioural changes seem to appear 3 months after injection. These preliminary data will contribute to better understanding

the role of the noradrenergic system in the early phases of the disease, and to identify novel therapeutic strategies to dampen PD progression.

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Reference number: T3-P08

(*T3-P08*) *MULTI-FIBER PHOTOMETRY TO RECORD NEURONAL ACTIVITY IN A RODENT MODEL OF ALPHA-SYNUCLEIN AGGREGATION.*

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease and is characterized by the degeneration of dopaminergic neurons in the substantia nigra and the pathological accumulation of misfolded α-synuclein protein in Lewy Bodies. During the course of the disorder, different structures and neurotransmitters systems progressively degenerate and according the Braak's hypothesis, the dorsal raphe nucleus (DRN) is one of the earliest affected in the time course of PD but the region where the degeneration initiates is still unknown. Due to the main projections from DRN to other brain regions, the alteration in the DRN signaling may be affected in early stages of the disease. In this work, we try to elucidate main alterations in this signaling using a transgenic rodent model of PD based on the inclusions of the truncated form of the human α-synuclein which mimics the pathological accumulation of this protein in the human brain. To do that, we first lesioned with 6-hydroxydopamine (6-OHDA) neurotoxin the DRN to accelerate the death of dopaminergic neurons of this region. Next, we injected fluorescent genetically encoded dopamine (DA) or norepinephrine (NE) sensors in combination with in vivo multi-fiber photometry technique, which allows to measure DA or NE neurotransmitters release on neuronal circuitries arising from the DRN in order to establish a timeline of neural degeneration as well as defining the neuronal circuits from the DRN are involved in PD.

Funded by Spanish Ministries of Science and Innovation (PID2019-111693RB-I00) and UE (H2020-SC1-BHC-2018-2020, grant agreement n° 848002) and by NextGeneration EU/PRTR (MICIN/CSIC/PTI+ NeuroAging) to RM. CS has a FPI fellowship and SVS has a MS from UPV/EHU and JDC-f fellowship.

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Reference number: T3-P09

(*T3-P09*) *COMBINED INTERVENTION OF COGNITIVE REHABILITATION IN PATIENTS WITH PARKINSON'S DISEASE AND PSYCHOEDUCATION IN THEIR FAMILY CAREGIVERS*

Background
Parkinson's disease (PD) is a neurodegenerative disorder characterized by the presence of motor and non-motor symptoms that affect PD patients and their families. As the disease progresses, the care for PD patients becomes essential, leading to physical, emotional and psychosocial difficulties in caregivers. Cognitive rehabilitation is effective for the improvement of cognitive functions in PD. However, there is limited research analyzing the impact of different interventions in family caregivers.
This study aims to examine the efficacy of a combined intervention of cognitive rehabilitation for PD patients and psychoeducation for their family caregivers.
Methods
Sixty-seven PD and their 67 family caregivers were assigned to combined [cognitive rehabilitation in PD patients + psychoeducation in family caregivers], single [cognitive rehabilitation in PD patients + no intervention in family caregivers)], or control group [no intervention in PD and family caregivers]. Participants underwent a neuropsychological, mood, functional disability, quality of life and caregiver burden evaluation at baseline and post-treatment. Forty-six PD patients [combined (n=20), single (n=14) and control group (n=12)] and 46 family caregivers [combined (n=20), single (n=14) and control group (n=12)] completed post-treatment assessment. Differences in change scores were assessed by analysis of covariance (ANCOVA) in PD patients (combined, single and control groups) and family caregivers (combined group and single + control groups), including change score as the dependent variable and baseline performance as a covariate.
Results
PD patients of combined group showed significant improvements in verbal memory ($F=4.88$; $p=.012$) compared to single ($p=.027$) and to control group ($p=.002$) after cognitive rehabilitation. No significant differences were observed in the rest of the measures. Post-hoc analyses revealed that combined group also showed significant improvements in facial emotions recognition ($p=.029$) and general health ($p=.018$) compared to control group.
Regarding family caregivers, those that participated in psychoeducation program revealed significant improvements in general health ($F=7.21$; $p=.014$) and anxiety scores ($F=4.45$; $p=.029$) compared to those who did not participate in psychoeducation.
Conclusions
Findings suggest that PD patients of combined group showed improved verbal memory, facial emotions recognition and general health after cognitive rehabilitation. Family caregivers who participated in psychoeducation improved in general health and anxiety scores. The combined application of cognitive rehabilitation in patients and psychoeducation in their family caregivers can provide a comprehensive approach to PD patients and their families, promoting the benefits of cognitive rehabilitation in PD and providing family caregivers with the necessary techniques to promote a better quality of life.

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Reference number: T3-P10

(*T3-P10*) *THERAPEUTIC POTENTIAL OF SIRT2 INHIBITION BY THE COMPOUND 33I IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE*

Sirtuin 2 (SIRT2) has been associated with aging and neuroinflammation, which are essential mechanisms for the progression of Alzheimer's disease (AD). In this study, we analyzed the potential effect of SIRT2 inhibition (by 33i compound) on a transgenic mouse model of AD, the APP/PS1 model.

33i administration reverted cognitive impairment in Morris Water Maze and restored long-term potentiation compared to APP/PS1-vehicle mice. These benefits were further confirmed at the molecular level where amyloid pathology and neuroinflammation were reversed. However, an increase of pro-inflammatory markers on the periphery was observed suggesting that systemic inhibition of SIRT2 could have detrimental long-term consequences. In line with this hypothesis, the inhibition of SIRT2 only at the periphery (by AGK-2 compound) increased inflammatory cytokines and worsened the memory of the animals.

These results establish a potential target to treat AD; however, further studies are needed to understand the specific roles of SIRT2 in different cell types.

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Reference number: T3-P11

(*T3-P11*) *THE MEDIATION ROLE OF GLOBAL COGNITION IN THE RELATION BETWEEN DEPRESSION SYMPTOMS AND THEORY OF MIND*

Objective
In Parkinson´s disease (PD) patients, theory of mind (ToM), which is an important social-cognitive skill that involves the ability to think about mental states, has shown to be aggravated by depression symptoms and cognitive deficits individually. It is thought that a common non-motor symptom such as cognitive impairment may play an important role in the relation among depression and ToM. Thus, the aim was to explore the role of

global cognition as mediator in the relation between depression symptoms and ToM in PD patients.

Participants and Methods
Forty-eight PD patients (n=20, <5 years of disease evolution; and n=28, >5 years of disease evolution) and 37 healthy controls (HC) underwent a neuropsychological battery including Montreal of Cognitive Assessment (MoCA) for the global cognition screening, Happé´s Strange Stories Test for the assessment of ToM, and the Geriatric Depression Scale (GDS-15) for the assessment of depression symptoms. First, Pearson´s r was carried out to analyze the correlation between the three variables and then, regression analyses were executed in order to observe whether global cognition explains or not the relation between depression symptoms and ToM. Significance level of 0.05 was set for all the statistical analysis and contrasted with two-tailed tests. Bootstrapping strategy was used to investigate the significance of the indirect effect of global cognition in the relation of depression symptoms and ToM.

Results
Significant correlations were found among MoCA, Happé´s Stories and GDS-15 results in PD patients. In the HC group only significant correlation between MoCA and Happé´s Stories was found. Therefore, regression analyses were carried out in the PD group. Analyses showed significant relationship between GDS-15 and Happe´s Stories (B = -.211; p = .022) and between GDS-15 and MoCA (B = -.374; p = .032). Mediation analysis showed that MoCA fully mediates the relationship between GDS-15 and Happe's Stories (B = .225; p = < .001) and it explained 47.96% of the relation among GDS-15 and Happé's Stories scores in the PD group. Additionally, when separating the PD group according to the disease´s years of evolution, in those who suffered it for more than five years, MoCA contributed significantly to the relation between GDS-15 and Happé's Stories (B = .253; p = .001). In this case, MoCA explained 46.47% of the relation. Conversely, in those with less than five years of disease evolution, no significant correlation was found between the three variables, so regression analysis could not be carried out. Bootstrapping method determined that the coefficient interval 95% did not contain zero which means that the indirect effect of MoCA was significant in the PD group (-0.095, 95% [-0.232, -0.0232]) and in patients suffering for more than five years of disease evolution (-0.132, 95% [-0.345, -0.0265]).

Conclusion
Findings suggest that global cognition plays an important role in the relation between depression symptoms and ToM impairment in PD. It has been demonstrated that as the disease evolves depression symptoms and cognitive deficits worsen the ability to think in other´s mental states, understood as ToM. This study showed how connected non-motor symptoms are in PD and, considering the bidirectionality among cognitive and clinical symptoms, it may help to design a more integrative and personalized treatment.

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Reference number: T3-P12

(*T3-P12*) *THERAPEUTIC EFFECT OF A7 NICOTINIC RECEPTOR MODULATION AFTER CEREBRAL ISCHEMIA IN RATS*

Introduction

The activation of α7 nicotinic receptors (nAChRs) has a well-known modulator effect in neuroinflammation (Colás et al., 2018; De Jonge & Ulloa, 2007; Wang et al., 2003). Despite this, the therapeutical effect of α7 nAChRs activation after stroke has been scarcely evaluated to date. For this reason, the present study aims to determine the therapeutic potential of α7 nAChRs after ischemic stroke through the evaluation of microglial/infiltrated macrophage activation, matrix metalloproteinases (MMP) activity, blood brain barrier disruption (BBBd) and neurofunctional outcome.

Methods

The role of α7 nAChRs in ischemia was evaluated with a daily administration of the α7 agonist PHA 568487 (1,25 mg/Kg) during the following 7 days after reperfusion (n=24). The effect of α7 nAChRs modulation on neuroinflammation was assessed with positron emission tomography (PET) radioligands, [18F]DPA-714 (TSPO) and [18F]BR-351 (MMPs) at days 1, 3 and 7 after transient middle cerebral artery occlusion (MCAO) in rats. The assessment of brain oedema, BBBd and neurofunctional progression after treatment was evaluated with T2 weighted and dynamic contrast-enhanced magnetic resonance imaging (T2-W and DCE-MRI) and behavioural evaluation. In addition, the in vivo findings were supported by fluorescence immunohistochemistry, gel zymography and Fluoro Jade staining at day 7 after stroke.

Results/Discussion

The activation of α7 nAChRs resulted in a decrease of ischemic lesion and cell neurodegeneration from days 3 to 7 after ischemia by in vivo T2W-MRI (brain oedema) and ex vivo Fluoro Jade (neurodegeneration) staining. Besides, the treatment improved the neurofunctional outcome by means of motor, sensory and reflex responses from days 1 to 7 after stroke onset. Treated ischemic rats showed a significant [18F]DPA-714-PET uptake reduction in brain cortex at day 7 together with a decrease of activated microglia/infiltrated macrophages expressing TSPO. Likewise, the modulation of α7 receptors displayed an increase of [18F]BR-351-PET signal in the cerebral cortex from days 3 to 7, which resulted from the overactivation of MMP-2 by gel zymography. Finally, the effect of PHA 568487 on BBB integrity by DCE-MRI showed a significant decrease in BBB disruption at day 7 in the cerebral cortex of treated rats, together with the preservation of structural integrity of the vascular unit in treated rats observed by VE-Cadherin inmunofluorescence.

Conclusions
Our findings support the neuroprotective effect displayed by α7 receptors on brain infarction evolution, neurodegeneration, inflammation, BBB integrity and describe for the first time, its modulatory activity on MMP2 activation (MMP with a beneficial role) after cerebral ischemia in rats. Therefore, these data shed light on the therapeutic potential of treatments based on the modulation of α7 nAChRs.
Acknowledgement
The authors would like to thank A. Lekuona, X. Ríos, V. Salinas for technical support in radiosynthesis and technical assistance in the PET studies. This study was funded by Spanish Ministry of Education and Science (RYC-2017-22412, PID2019-107989RB-I00, PID2020-117656RB-100), the Basque Government (BIO18/IC/006) and Fundació La Marató de TV3 (248/C/2020).
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Reference number: T3-P13

(T3-P13) ***PLASTICITY-DRIVEN CROSS-MODAL REORGANIZATION AND HEARING EXPERIENCE: EVIDENCE FROM CHILDREN WITH HEARING LOSS***

Cross-modal reorganization (CMR) is a manifestation of brain plasticity documented in individuals with hearing loss (HL). This reorganization is manifested as brain activity in response to non-deprived sensory modalities (e.g., visual stimuli) in brain areas that typically activate to auditory stimulation. HL can be treated using hearing devices such as a Cochlear Implant (CI), which electrically stimulates the cochlea creating a percept of sound. CIs facilitate speech processing enabling spoken language acquisition, but the variability in language outcomes of CI recipients is high. Previous studies in adults have shown that part of this variance can be explained by CMR, and that it negatively influences oral language outcomes. In young children with CI, the impact of CMR on oral language outcomes is still unclear. Given the high brain plasticity present in young

children, studying CMR in this population is crucial to understand the brain's capacity to adapt to HL and later integrate the degraded signal given by the CI.

This study measured CMR in children before and after cochlear implantation (after 6 months) in order to assess the relation between individual CMR and later oral language outcomes. Here, we present pre-implantation measures of CMR in children with HL, comparing them to age-matched typically-hearing (TH) controls. Due to hearing deprivation in children with HL, we predict greater CMR compared to TH controls, as an index of pre-implantation brain plasticity.

Data collection for this longitudinal study is currently in progress, so we will present preliminary data for 16 children: eight children with profound HL before CI implantation (average age: 25.47 months) and eight age-matched TH controls. Brain activity was measured using functional Near-Red Spectroscopy (fNIRS), a neural imaging technique that uses optical signals to capture oxygen concentration changes on the cortical area of the brain. Children were presented with visual-only stimuli consisting of 40-second silent videos of moving colourful shapes (preceded and followed by 20-second intervals without stimuli, i.e., black background). An optode design of 16 sources and 16 detectors was placed around temporal and pre-frontal areas. Children with HL are expected to show higher CMR than TH children, showing higher amplitude of the hemodynamic response around the temporal brain areas in response to visual-only stimulation.

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Reference number: T3-P14

(*T3-P14*) *"OLDER CIRCUITS GET RICHER": LINKING HUBS, EMBRYONIC NEUROGENESIS, TRANSCRIPTOMICS AND DISEASES IN HUMAN BRAIN NETWORKS*

Understanding the architectural principle that shapes the topology of the human connectome at its multiple spatial scales is a major challenge for systems neuroscience. This would provide key fundamental principles and a theory for browsing brain's networks, to ultimately generate hypothesis and approach to which extent key structures might impact different brain pathologies. In this work, we propose the hypothesis that the centrality of the different brain nodes in the human connectome is a product of their embryogenic age, and accordingly, early-born nodes should display higher hubness, and viceversa for late-born nodes. We tested our hypothesis by identifying and segmenting eighteen macroregions with a well-known embryogenic age, over which we calculated nodes' centrality in the structural and functional networks at different spatial resolutions. First, nodes' structural centrality correlated with their embryogenic age, fully confirming our working hypothesis. However, at the functional level, distinct trends were found at different resolutions. Secondly, the difference in embryonic age between nodes inversely correlated with the probability of existence and the weights of the links. This indicated the presence of a temporal developmental gradient that shapes connectivity and where nodes connect more to nodes with a

similar age. Finally, brain transcriptomic analysis revealed high association between embryonic age, structural-functional centrality and the expression of genes related to nervous system development, synapse regulation, and human neurological diseases. Overall, these results support the hypothesis that the embryogenic age of brain regions shapes the topology of adult brain networks. Our results show two key principles, “preferential age attachment” and “older gets richer” on the wiring of the human brain, thus shedding new light on the relationship between brain development, transcriptomics, node centrality, and neurological diseases.

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Reference number: T3-P15

(*T3-P15*) *VIRAL-MEDIATED FLUORESCENT LABELLING OF THE EXTRACELLULAR MATRIX FOR LIVE BRAIN TISSUE IMAGING.*

In the Central Nervous System (CNS), neurons and glia are surrounded by a dynamic compartment, the brain extracellular space, which contains a plastic scaffold known as the extracellular matrix (ECM). Unlike the ECM from connective tissue, abundant in fibrous proteins, the neural interstitial matrix relies mostly on the glycan polymer hyaluronan (HA). Proteoglycans bind to hyaluronan forming a self-assembled matrix that functions as scaffold and signalling hub. Although several staining protocols exist for ECM in fixed tissue, there are no reliable matrix labels for live cell imaging. Here we report an adenoviral-mediated fluorescent probe that binds to hyaluronan and tags the brain ECM in vivo and ex vivo permanently. The vector codifies the HA binding domain from Neurocan fused to GFP and an externalization tag (AAV-Ncan-GFP), so transduced cells export this fluorescent hyalectan to the extracellular space and label membrane-

bound hyaluronan. We defined a transduction protocol in rat organotypic cortical cultures for stable expression of the probe, validated HA labelling by immunofluorescence and confocal microscopy, and analysed cell motility and migration along with HA context by live cell imaging. These results highlight AAV-Ncan-GFP as a useful probe for ECM labelling and imaging in living tissue.
Funding:
-Aligning Science Across Parkinson's (ASAP)/M.J.Fox Foundation.
-Ministry of Science and Innovation (MICIIN).

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Reference number: T3-P16

(*T3-P16*) *EVALUATION OF NOVEL LIPID BIOMARKERS FOR NEUROINFLAMMATION IN ISCHEMIC STROKE USING MALDI IMAGING*

Introduction
The neuroinflammatory response that occurs after cerebral ischemia is one of the most detrimental processes during secondary ischemic damage1. Certain classes of lipids including phospholipids have been described as promising biomarkers for neuroinflammation in neurodegenerative diseases2. However, the lipid fingerprint of inflammation after cerebral ischemia has not been described yet. Likewise, the evaluation of novel lipid-based biomarkers for neuroinflammation might be essential for the establishment of promising imaging and therapeutic targets for ischemic stroke. For that reason, the aim of this study is to evaluate the changes in cerebral lipid expression after brain ischemia using Lipid Imaging Mass Spectrometry (LIMS).

Methodology
The lipid fingerprint of the rat brain was analised at days 1, 3 and 7after a transitory middle central artery occlusion (tMCAO), using ex vivo Matrix Assisted Laser Desorption Ionization-Imaging Mass Spectrometry (MALDI-IMS). Spatial resolution was set at 100 micron/pixel and mass resolution at 60,000 in negative-ion mode using DAN as matrix. At this spatial resolution it was possible to identify the main brain areas affected by the cerebral ischemia. To compare the changes in lipid expression between ipsi and contralateral hemispheres, a region of interest was defined and the average mass spectrum was extracted. Identification of the lipid species in the mass spectrum was carried out by direct comparison between the m/z values in the spectrum and the lipids in our software database. The assignments were validated using the data from HPLC-MS obtained using lipid extracts of the same samples. A MALDI-LTQ Orbitrap XL (ThermoFisher) was used to record IMS data, while HPLC-MS data were recorded using an HFX Orbitrap (ThermoFisher). Finally, the comparison with immunohistochemistry (IHC) data allowed us to evaluate the role of the glial cells in the inflammatory response after ischemic stroke.

Results/Discussion
MALDI-IMS data showed a particular distribution of lipids defining the ischemic area at day 1 after stroke. Results also demonstrated that although a variability of lipid distribution was observed among different rats along the following days after ischemia, all animals showed a progressive increase of the relative intensity of certain lipid classes from day 1 to 7, following a similar trend to the progression of inflammation. Additionally, immunohistochemical studies showed the presence of lipid droplets (LDs) in activated microglia/ infiltrated macrophages from days 3 to 7 after ischemia onset.
Conclusions
The present study suggests that there is a characteristic lipidic response in the inflammatory process after stroke. Moreover, our findings confirm that an accumulation of LDs exists in the activated microglia/ infiltrated macrophages during the first week of evolution of the cerebral infarction. Hence, our results shed light on the potential of lipids as novel biomarkers for diagnosis and therapeutic modulation of neuroinflammation in ischemic stroke.

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Reference number: T3-P17

(*T3-P17*) *METHOD DEVELOPMENT FOR THE CENSUS OF RELEVANT NEURONAL POPULATIONS IN THE HUMAN BRAIN: APPLICATION IN LOCUS COERULEUS*

The study of human brain is a priority as demonstrated by the several National and international initiatives that have appeared in the last years. To fully understand the brain, it is necessary to develop tools able to map the different brain areas with (at least) cellular resolution. Lipid imaging mass spectrometry (LIMS) has the potential to become

a tool of reference, as it is able to build lipid distribution maps directly from tissue sections without any previous labelling. Furthermore, as each cell type in a given metabolic state presents a characteristic lipid fingerprint, this tool can be used to map cell populations and subpopulations in the brain, as a previous step to unravel the metabolic alterations in the context of different pathologies. As a proof of concept, we chose the locus coeruleus (LC) as it is a challenging bluish noradrenergic nucleus, whose cellular heterogeneity is known to be vast. It plays a critical role in core physiological processes, such as the sympathetic nervous system control, the regulation of mood or sleep cycles. Moreover, LC has been involved in pathologies like Parkinson´s and Alzheimer´s disease.
Approx. 700 sections of 16 micron-thick were obtained from human LC and 6 of each batch of 100 were selected for LIMS. The samples were covered with DAN with the aid of an in-house designed sublimator and explored in negative-ion mode with an MALDI LTQ-Orbitrap XL. The modification performed on the mass spectrometer source allowed us to scan the samples at 10 micron/pixel without oversampling. The combination of IMSL with immunohistochemistry and bright field microscopy
images allowed us to identify the different cell populations in the LC and to extract their lipid fingerprint. These results open the door to use LIMS in combination with immunohistochemistry for cell censing in human brain, as a preliminary evaluation of the lipid changes in the context of different brain diseases.

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Reference number: T3-P18

(*T3-P18*) *MULTIMODAL IMAGING EVALUATION OF THE ROLE OF HYPERGLYCEMIA IN EXPERIMENTAL SUBARACHNOID HAEMORRHAGE*

Introduction
Spontaneous subarachnoid haemorrhage (SAH) is a devastating cerebrovascular disorder due to the rupture of an intracranial aneurysm with high mortality and morbidity1. In this context, the occurrence of hyperglycemia has been linked with a worsen outcome after SAH2, and more specifically, with an increased blood-brain barrier disruption (BBBd) and oxidative stress3. Hence, the aim of this study is to

evaluate these changes in a preclinical model of SAH using Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET), and ex vivo studies.

Methods
In vivo MRI (T2W and Dynamic Contrast Enhanced (DCE)) and PET imaging studies ([18F]BR-351 and [18F]FSPG) were carried out at days 1 and 3 after SAH onset to evaluate the effect of hyperglycemia on the i) volume of haemorrhage, ii) volume of ischemia, iii) midline displacement, iv) activation of metalloproteinases (MMPs) and v) oxidative stress. Animals were sacrificed at day 3 and brains were processed for the ex vivo evaluation of MMPs activity and oxidative stress by gelatin zymography and immunohistochemistry (xc- system), respectively. Besides, the neurological outcome after SAH was measured using a neurological test based in a 0 (highest handicap) to 18 (normal) point-scale at days 1 and 3 after SAH.

Results/Discussion
Hyperglycemic animals (n=28 for severe and n=120 for mild hyperglycemia) showed lower survival rates than normoglycemic rats (n=94) at different days after SAH, evidencing the direct correlation between blood glucose levels and mortality. MRI studies showed an increase in haemorrhage, stroke and oedema volume after SAH together with a major neurofunctional impairment in hyperglycemic animals in comparison with normoglycemic rats. Moreover, PET analysis displayed a significant increase of MMPs activation in the ischemic hemisphere at days 1 and 3 in hyperglycemic (n=6) compared to normoglycemic rats (n=9). A slightly increase of the signal was observed in the area of haemorrhage, although not significant. Likewise, the effect of hyperglycemia on BBBd was supported by zymography studies and DCE-MRI analysis. Finally, PET imaging with [18F]FSPG and immunohistochemistry showed the increase of oxidative stress in hyperglycemia in comparison to normoglycemia after SAH.

Conclusions
These results confirm that high glucose levels worsen the pathological evolution of preclinical SAH increasing the mortality rate, volume of ischemia, oedema, BBBd, oxidative stress and neurofunctional impairment. Hence, these findings are in agreement with the effect of hyperglycemia after clinical SAH, on the increase in complications and the higher risk of death or functional disability.

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Reference number: T3-P19

(*T3-P19*) *A VERSATILE TOOLBOX FOR THE ANALYSIS OF NERVOUS TISSUE ORGANIZATION WITH LIGHT MICROSCOPY*

The brain is an exceptionally sophisticated organ consisting of billions of cells and trillions of connections that orchestrate our cognition and behavior. To decode its complex connectivity, it is pivotal to disentangle its intricate architecture spanning from cm-sized circuits down to tens of nm-small synapses.

To achieve this goal, we have developed CATS - Comprehensive Analysis of nervous Tissue across Scales, a toolbox for obtaining a holistic view of nervous tissue context with fluorescence microscopy.

CATS provides rich ultrastructural context by creating contrast between the intra- and extracellular space in a variety of samples types, including slice cultures, perfused brain tissue and clinical samples. It is compatible with common (super-resolution) fluorescence imaging techniques, such as STED and expansion microscopy, as well as labeling of molecular markers. We interface this toolbox with a state-of-a-art open-source machine-learning based analysis pipeline for segmentation and annotation.

CATS enables the analysis of key features of nervous tissue connectivity across scales, ranging from whole tissue organization down to synapse architecture. We present the potential of this novel toolbox by reconstructing neuronal in- and output fields in the mouse hippocampus, a feat that so far has only been achieved by electron microscopy. Further, we combine CATS with electrophysiological recordings to investigate structure-function relationships in the brain, apply it to human clinical samples to study tissue context and fully annotate a piece of cerebral organoid, thereby paving the way towards light microscopy-based saturated reconstruction of nervous tissue.

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Reference number: T3-P20

(*T3-P20*) *INCREASED BRAIN UPTAKE OF THE P2X7 RECEPTOR-SPECIFIC [18F]JNJ-64413739 RADIOTRACER FOLLOWING STATUS EPILEPTICUS ACCORDING TO SEIZURE SEVERITY IN MICE*

INTRODUCTION
The ATP-gated P2X7 receptor (P2X7R) is an important contributor to inflammatory processes and is increasingly recognized to play a key role during different pathological conditions of the central nervous system, including epilepsy. P2X7R expression is increased in the brain during epilepsy and P2X7R antagonism modulates seizure severity during status epilepticus (SE) and suppresses seizure frequency during epilepsy.

However, detection of P2X7R increases post-seizures via P2X7R-specific PET imaging and whether this may predict the underlying pathology has not been investigated to date.

METHODS
SE was induced via an intra-amygdala kainic acid (KA) injection in wild type (WT) mice (study group). Animals injected with physiologic solution (sham group) and P2X7-knock out (KO) mice were used as controls. For ex vivo studies, brain slices (48 h post-SE) were incubated with the P2X7R-specific radiotracer [18F]JNJ-64413739 [1] and imaged with PET. For in vivo studies, [18F]JNJ-64413739 was intravenously administered 48 h post-SE, and dynamic PET studies were conducted. Images were coregistered with a brain MRI atlas to obtain time-activity curves for different brain regions. Images obtained 40-60 min post injection were averaged to determine percentage of injected dose per cm3 (%ID/cm3). For the study group, uptake values were correlated to Racine score obtained during SE.

RESULTS
In vitro PET studies on brain slices showed statistically higher uptake values at the whole brain level for the study group compared to the sham group (Figure 1), while very low binding was observed for the KO group. Time activity curves obtained in PET studies for both the study and the sham group showed an initial increased uptake in the brain, followed by a progressive decrease which reached a plateau at t>40 min. Average values of %ID/cm3 obtained 40-60 minutes post injection for the study group were statistically equivalent to those obtained for the sham group, irrespective of the brain sub-region. However, radiotracer uptake in the whole brain, cortex, hippocampus, thalamus, and to a lower extent cerebellum correlated with seizure severity during SE (Figure 2), suggesting prognostic potential of P2X7R-based PET imaging.

CONCLUSIONS
This study provides the proof-of-concept that P2X7R-based PET imaging may offer a novel test for the diagnosis of seizure-induced pathology and for the identification of patients who may benefit from P2X7R antagonism-based treatment. So far, preliminary results on diseased human brain tissue have shown a similar trend on in vitro PET imaging with P2X7R-specific radiotracer [18F]JNJ64413739.

ACKNOWLEDGEMENTS
This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 766124 and the Spanish Research Agency (AEI; project code: PID2020-117656RB-100).

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Reference number: T3-P21

(*T3-P21*) *AHK1 EFFICACY IN DUCHENNE MUSCULAR DYSTROPHY: IN VITRO AND IN VIVO APPROACHES*

An abnormal elevation of intracellular calcium (Ca+2) levels is one of the pathological mechanisms that contributes to Duchenne Muscular Distrophy (DMD) disease progression. The Ryanodine receptors (RyRs) are responsible for sarcoplasmic reticulum (SR) Ca+2 release into the cytosol. In the mdx mouse model of DMD, there is a deficit in the interaction of FKBP12 with RyRs, leading to Ca+2 leakage through RyR channels into the cytosol. AHK1 compound is a novel FKBP12 ligand that acts as a calcium modulator by potentiating the interaction of FKBP12 with RyRs. In this work, we have performed a high- throughput in vitro approach using microarray plates to study the phenotype of dystrophin-deficient myoblasts concerning adhesion, proliferation, differentiation, and calcium handling. Our results demonstrate that this is a promising platform for drug screening of therapeutic candidates for DMD since it can detect subtle dystrophic phenotypes. As for in vivo studies in mdx mice, we have analyzed AHK1 efficacy in restoring cardiac deficits. We have used the beta-adrenergic agonist isoproterenol (ISO) to exacerbate the cardiac deficits of mdx mice and found that AHK1 treatment reduces ISO-induced cardiac damage. In particular, we found reduced fibrosis and serum cardiac troponin I concentration in treated mdx mice compared to non-treated animals. Our results demonstrate the efficacy of AHK1 in ameliorating cardiac deficits in the mdx mouse model of Duchenne.

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Reference number: T3-P22

(*T3-P22*) *OLFACTORY TRACT MALDI MASS-SPECTROMETRY IMAGING: A PILOT STUDY IN ALZHEIMER´S AND PARKINSON´S DISEASES*

The olfactory tract (OT) is one of the first site for the processing of olfactory information in the brain, and its deregulation is one of the earliest features of neurodegenerative diseases. For several decades, neuroanatomical, volumetric, and histological approaches have been the gold standard techniques for the characterization of the OT functionality. However, little attention has been given to the specific molecular landscape of the OT from the perspective of proteomics. Imaging mass spectrometry (IMS) using matrix-assisted laser desorption/ionization (MALDI) has emerged as a powerful tool for analyzing the spatial distribution of peptides and small proteins (among other

molecules) within the tissues. Here, we describe a protocol to analyze OT protein/peptide signals employing MALDI-Imaging. Briefly, the workflow consists in the creation of formalin-fixed paraffin embedded blocks from human OT, cut at 5um thickness, deparaffination, alcohol washes, in situ-digestion by trypsin and matrix deposition, using an automated sprayer for the last two steps. Preliminary results at mid lateral resolution (50um) point out the capacity of this approach to potentially detect differential features between control, Alzheimer´s and Parkinson´s diseases (AD and PD) at olfactory level.

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Reference number: T3-P23

(*T3-P23*) *A MULTIVARIATE METHOD TO FIND NEURAL SOURCES OF PEAK FREQUENCY SHIFTS CORRELATED TO FUNCTIONAL VARIABLES*

Neuroscientific signals are studied through multiple spatiotemporal scales, from milliseconds to hours depending on the experimental paradigm [1]. Temporal fluctuations of the peak frequency have been shown to be physiologically linked to different cognitive, psychological and motor states [2]. Studies are mostly performed at sensor level, however, peak frequency measured over the scalp cannot be understood in terms of sources of brain activity, and thus the neurophysiological interpretability of the results is very limited [3] .

Furthermore, sensor-based estimates are also very influenced by noise, which increases the variance of peak frequency estimates, additionally limiting the interpretation of the results. In this abstract we present a method, called Peak Frequency Decomposition (PFD), to find sources whose peak frequency is maximally correlated to a functional variable. PFD can be written as an optimization based on minimizing the difference between a projected estimate of the peak frequency in source space and a set of values representing a measure of interest (for example find which source relates age and alpha peak frequencies changes over time) [2].

We performed extensive simulations with artificial data using a realistic head model [5]. The results show that our method can recover with high accuracy the source whose peak frequency is correlated to an experimental variable. PFD has the potential to provide researchers with a tool that might shed new light into the role of peak frequency variations in neuroscientific experiments.

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Reference number: T3-P24

(*T3-P24*) *A MULTIMODAL MRI-EEG APPROACH TO UNRAVEL THALAMOCORTICAL INTERACTIONS*

The thalamus interacts with virtually the entire cerebral cortex and, in particular, first-order thalamic relay nuclei interact with sensorimotor cortical and cerebellar areas for processing visual and auditory information reaching our senses as well as for motor execution. Although refined neuroanatomical research has characterized the structural connections between i) the lateral geniculate nucleus (LGN) and visual cortex via the optic radiations, ii) the medial geniculate nucleus (MGN) and the auditory cortex via the auditory radiations, and iii) the ventrolateral nucleus (VLN) and the motor cortex via the motor radiations and between the VLN and dentate nucleus in the cerebellum via the dendatothalamic tract; current neuroimaging research has scarcely examined the involvement of these nuclei and their thalamocortical projections in cognitive operations requiring processing sensorimotor information. This is, in part, due to the lack of availability of accurate and reliable atlas of the human thalamus that could properly segment its different nuclei to study in vivo thalamic function and structure. We have recently developed the first probabilistic atlas of the human thalamus, based on high-resolution MRI and histology (Iglesias et al., 2018), and made its companion segmentation tool publicly available as part of the FreeSurfer package. Recently, we have also developed a reproducible protocol to obtain the four main tracts (optic radiations, auditory radiations, motor radiations, dendatothalamic tract) associated with these three first-order relay thalamic nuclei (Liu et al., 2022). Here, we move one step further with a multimodal MRI-EEG project aimed at mapping functional MRI (fMRI) and their associated cortical electrophysiological signatures in regard to first-order relay thalamic nuclei and thalamocortical interactions in the context of language systems requiring the participation of visual (reading), auditory (speech comprehension) and motor (verbal production) systems. Our goal is to identify EEG correlates of cortical fMRI activations associated with thalamic activity during reading, speech comprehension and verbal production by analyzing event-related potentials coupled with source separation algorithms maximally related to the expected cortical activations obtained by fMRI. We will also examine associations between the microstructural properties of the main

thalamocortical tracts of interest and the fMRI and electrophysiological signatures associated with these language systems. The relevance of the project is not only related to further unraveling thalamic contributions to significant language systems, but also to deliver multimodal methodology that will help scientists to exploit the spatial resolution of fMRI and the temporal resolution of EEG.

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Reference number: T3-P25

(*T3-P25*) *CHARACTERIZATION OF NOVEL FKBP12 LIGANDS WITH THERAPEUTIC POTENTIAL FOR NEUROLOGICAL DISORDERS*

Ryanodine receptors (RyRs) are calcium channels localized at the endoplasmic reticulum that are essential for proper calcium regulation. RyR2 is the predominant isoform in the brain, although all three isoforms are expressed. FKBP12, one of the most studied RyR modulators, coordinates cooperative gating of RyR subunits and prevents subconductance states by stabilizing the closed state of the channel. Mutations or post-translational modifications affecting FKBP12-RyR interaction in the muscle and central nervous system may alter calcium homeostasis, by inducing calcium leakage from the endoplasmic reticulum. This pathogenic mechanism has been involved in several neurological disorders, such as cognitive dysfunction, Alzheimer's and Parkinson's Diseases. AHKs are novel FKBP12 that are able to bind FKBP12 and modulate RyR activity through molecular reshaping. We have optimized novel high throughput methodological approaches to determine target engagement and in vitro efficacy of AHKs using HEK293 cell lines expressing wild type and mutant RyR2. Our results indicate that these approaches may be used for screening of novel compounds targeting FKBP12/RyR interaction.

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Reference number: T3-P26

(*T3-P26*) *NON-INVASIVE BRAIN-SPINE INTERFACE: CONTINUOUS CONTROL OF TRANS-SPINAL MAGNETIC STIMULATION USING EEG*

Brain-controlled neural interfaces have emerged as a potential technology to promote functional recovery in patients with motor paralysis. These interfaces favor neuroplastic mechanisms and could be used for restoring voluntary control of lower limbs. Towards a non-invasive approach for leg neurorehabilitation, we present a platform based on the continuous volitional control of trans-spinal magnetic stimulation (ts-MS) using electroencephalographic (EEG) activity. We engineered, for the first time, a non-invasive brain-spine interface (BSI) that contingently connects cortical activity encoding motor intentions with the activation of afferent (from the spine to the somatosensory cortex) and efferent (from the spine to the lower-limb muscles) pathways using ts-MS. Our BSI efficiently removed stimulation artifacts from EEG, regardless of the stimulation intensity used, allowing continuous monitoring of cortical activity and online decoding for real-time closed-loop control of ts-MS. We demonstrated the capability of our platform to engage the central and peripheral nervous system inducing afferent and efferent intensity-dependent evoked responses. The presented system represents a novel non-invasive means of brain-controlled neuromodulation and opens the door towards its integration as a therapeutic tool for lower-limb rehabilitation.

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Reference number: T3-P27

(*T3-P27*) *COULD WE DESIGN A NUCLEIC ACID THERAPY FOR A CNS DISORDER?*

Nucleic acid therapies are a new class of drugs that, until very recently, have been considered a niche for rare disease research. However, the advantages of being able to design a drug to target specifically a DNA or RNA sequence has opened its application to a wide range of disorders. There is still a large hurdle in the poor delivery of these new drugs to target tissues, but disorders of the CNS are a privileged group of diseases that have already solved some of those problems.

As coordinator of a large network of researchers interested in the application of nucleic acid therapeutics to clinical practice (www.antisenserna.eu), I would like to share with the audience the opportunities that lie in the development of these drugs and the tools that are at hand to embark in research in this field.

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Reference number: T3-P28

(*T3-P28*) *BRAIN STRUCTURE, PHENOTYPIC AND GENETIC CORRELATES OF READING ABILITIES*

Reading is an evolutionary new development that recruits and tunes brain circuitry connecting visual- and language-processing regions. We investigated the structural correlates of reading and whether genetics influence brain-reading associations. First, we identified left hemisphere cortical surface area (CSA) and cortical thickness (CT) correlates of reading in the large ABCD dataset (N=9,013) of 9-to-10-year-olds. Next, the heritability of cognitive and brain measures of interest was examined through complementary approaches. Last, shared genetic effects between reading, reading-related cognitive traits and reading-associated brain measures were examined by computing genetic correlations and polygenic score analyses, and through mediation analyses. Our results support that morphometric brain measures are related to reading abilities, and that the total left CSA in general, and left superior temporal gyrus CSA in particular, contribute to reading partially through genetic factors.

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Reference number: T3-P29

(*T3-P29*) *NEUROINFLAMMATION BIOMARKERS, DEPRESSIVE SYMPTOMS, AND COGNITIVE DYSFUNCTION IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS*

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease that affects multiple organs, including the peripheral and central nervous system. Its aetiology involves an interaction of genetic-environmental components, resulting in immune dysregulation. Among main clinical symptoms associated with SLE, neuropsychiatric disturbances are frequently reported. In fact, neuropsychiatric disorders encompass a heterogeneous cluster of symptoms, including headache, psychosis, anxious-depressive disorders and cognitive deficits. Previous studies on experimental models of SLE and depression suggest an increased expression of inflammatory biomarker levels, leading to an inflammatory response at the microglial level and associated astrocytic damage. Those enhanced cytokines have been widely

associated to neurotoxic effects on the brain by altering various neurotransmission systems.

The aim was to characterise depressive symptomatology in patients with SLE and its impact on cognitive functioning and functionality. We also analysed the presence of alterations of different components of inflammatory response and indicators of CNS damage in blood plasma. The sample consisted of four groups: patients with SLE (n=79), patients with SLE and comorbid mood disorders (SLE-MDD) (n=15), patients with major depressive disorders (MDD) (n=27) and control subjects (n=35). The relationship between main clinical and biological variables was evaluated. Statistical analysis was performed by U-Mann-Whitney test and clinical correlations by using the Spearman's rank correlation coefficient.

Following a comprehensive psychiatric evaluation of SLE patients on active follow-up (n=92), the most prevalent psychiatric diagnosis was mood disorders, comprising 16% (n=15) of the sample. We observed a heterogeneous profile of cognitive impairment in patients with SLE-MDD compared to patients with SLE, mainly in the working memory domain (U=170.00; p=0.037), learning and visual memory domain (U=119.00; p=0.002), attention (U=169.5; p=0.048) and speed of processing (U=113.50; p<0.001) domains. Similarly, we found significant differences between groups in plasma biomarker parameters related to glial and neuronal damage and inflammatory factors. Specifically, increased levels of anti-inflammatory cytokine IL-10 were found in the SLE and SLE-MDD groups (F[3,150]=14.51; p=0.0230) and decreased levels of HDL in MDD group.

Results indicate a lower prevalence of MDD than previously reported in the literature; however, MDD appears to play an important role in SLE management. It is essential to develop multidisciplinary teams for the clinical management and treatment of autoimmune diseases.

Authors

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Reference number: T3-P30

(T3-P30) ***ORAL HEALTH STATUS AMONG PEOPLE WITH SCHIZOPHRENIA IN BIZKAIA***

Patients with schizophrenia constitute a particularly vulnerable group for oral diseases. In fact, previous studies have shown higher dental caries and degree of periodontal diseases in this population and point to drug-induced xerostomia as an important risk factor for oral health deterioration. The aim of this study was to determine the oral and

dental health of people with schizophrenia attended in the Bizkaia Mental Health Network treated with antipsychotic medication. Additionally, we aim to characterize the possible correlations between impaired oral health and several factors such as medication, anticholinergic burden, life habits or hygiene habits.

To do so, a prospective multicentre descriptive study was carried out, including inpatients and outpatients with a diagnosis of schizophrenia treated with antipsychotics (n=115) from the Red de Salud Mental de Bizkaia (RSMB), and a control group of volunteers (non-schizophrenic) (n=127) attending the University of the Basque Country Dental Clinic and other private dental clinics. Dental condition was determined according to the standardized criteria of the Decayed, Missing and Filled teeth (DMFT) index, and Community Periodontal Index of Treatment Needs (CPITN) was applied to assess the periodontal status of the participants. In addition, salivary flow measurement was performed.

The results revealed that psychiatric patients had significant higher scores than the control group with respect to decayed teeth (1.7 vs. 1.0), missing teeth (9.4 vs. 5.5) and DMFT index (15.5 vs. 11.1). By contrast, schizophrenic patients had fewer score of filled teeth (2.4 vs. 3.4). Additionally, they had poor periodontal health, highlighting a higher percentage of patients exhibiting periodontal pockets >5.5 mm (11.1 % vs 0% in control group). On the other hand, although the mean salivary flow was similar in both studied groups, we detected significantly more cases of xerostomia among people with schizophrenia (41 % vs. 22%) Finally, a positive correlation was found between the CPITN index and the treatment with atypical antipsychotics ($p<0.001$), and between the DMFT index and both classes of antipsychotics ($p<0.05$).

The elevated anticholinergic burden observed in the schizophrenic patients, together with the higher consumption of sugary drinks and tobacco and poor oral hygiene could contribute to the deterioration of the oral health.

The findings of this study highlight the urgent need for a prevention/intervention program in our health system for patients with schizophrenia, including information on adequate dental care and access to oral health care services.

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Reference number: T3-P31

(*T3-P31*) *STUDY OF COMBINED KETAMINE AND FLUOXETINE ADMINISTRATION IN AN ANIMAL MODEL OF DEPRESSION: THE ROLE OF INTERNEURONS.*

Major depressive disorder (MDD) is a complex illness that is arising in developed countries. GABAergic interneurons (INs), mainly in medial prefrontal cortex (mPFC), are one of the most affected neurons in MDD, which are related to the control of pyramidal cells bursts, neuronal networks and basic microcircuit functions. Although serotonin selective reuptake inhibitors (SSRI) are prescribed as MDD treatment, several weeks are needed to achieve the therapeutic effect. New rapid antidepressants (RAAD) are emerging as promising therapy during last years and ketamine at sub-anaesthetic doses has shown very fast and sustained recovery. Ketamine blocks N-methyl-D-aspartate receptors (NMDA) resulting in an increase of neurotrophic factors levels and restoration of neuronal network. Indeed, this mechanism may induce rapid plasticity activation mediated by neurotransmitter homeostasis, synapse restoring and increased dendritic spines. The aim of the present work is to study the effects of intraperitoneal ketamine administration at sub-anaesthetic dose, as well as combined administration of ketamine and fluoxetine in an animal model of depression. For that purpose, 70 young male Sprague-Dawley rats between 240-320 g were submitted to different pharmacological treatments. Forced swimming test was used to evaluate the antidepressant effect of applied treatment and a stereological study of mPFC INs was realized to study changes in this neuronal population. In addition, the correlation of reestablished GABAergic inputs and antidepressant effects of administered treatment was also studied. Our preliminary results indicate that ketamine without fluoxetine may play a key role in restoring INs homeostasis burst in mPFC.

Authors

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Reference number: T3-P32

(*T3-P32*) *EPIGENETIC BIOMARKERS OF MAJOR DEPRESSION IN MONOCYTES AND T-CELLS*

Background: Epigenetics play a key role in the pathogenesis of major depression (MD) and its treatment. Changes in histone acetylation mediated by HDAC5 and SIRT2 can regulate the transcription of genes related to different cell functions such as neuronal plasticity [1]. Previous studies using peripheral blood mononuclear cells (PBMCs) [2] or prefrontal cortex (PFC) tissue from depressed patients show an increase of HDAC5 and

SIRT2. Further, murine models have shown an opposite regulation of these enzymes in the PFC by chronic stress and antidepressant treatment [3].
Moreover, it is known that many depressed patients suffer comorbid conditions together with mild chronic inflammation [4]. Studying the role of HDAC5 is interesting to understand epigenetic and inflammatory theories of depression [5].
Hypothesis and objectives: Our hypothesis is that HDAC5 and SIRT2 in three monocyte subsets and T-cells are peripheral biomarkers of depressive state. Here, we asked whether MD and/or symptoms severity influence the expression and function of these epigenetic enzymes.
Methods: A team of psychiatrists from different hospitals and health care centres from Pamplona (Navarra, Spain) recruited 56 depressed patients (Montgomery-Åsberg scale ≥20) and age and sex matched healthy controls (M- Åsberg<7). Blood (20 ml, heparine tubes) was obtained from each participant and monocytes (classic, intermediate and non-classic) and T-cells (CD3+) were isolated by fluorescence activated cell sorter (FACS). HDAC5 and SIRT2 immunofluorescence was carried out and nucleocytoplasmic distribution of these enzymes was calculated by confocal microscopy coupled to an Image J analysis plugin. In addition, classic monocytes and T-cells were incubated with the □-adrenoceptor agonist naphazoline and the antidepressant duloxetine and phosphorylation of HDAC5 at serine 498 was measured by immunofluorescence. Gene expression studies by RT-qPCR included HDAC5 and SIRT2 as well as other genes related to brain plasticity (BDNF), angiogenesis (KLF2, eNOS) and inflammation (IL-6, IL-10, TNF-α). In T-cells, the expression of some regulatory markers were also studied (FOXP3, PD-1, CTLA4). Subsequently, Full-length RNA Seq of classic monocytes was applied to a selected sample of 10 MD patients and 10 healthy controls.
Results:
Confocal microscopy studies showed a lower ratio (cytoplasm/nucleus) for HDAC5 and SIRT2 in MD patients. In addition, there was an inverse correlation between the severity of the disease (M-Åsberg scale) and HDAC5 cytoplasm/nucleus ratio. Interestingly, a higher percentage of healthy volunteers have shown increased P-HDAC5 induced by naphazoline and duloxetine in T-cells compared to the group of MD patients. Moreover, MD patients that did not respond to these drugs showed on average a significant more severe illness than those that did respond according to the M-Åsberg scale.
Interestingly, gene expression studies showed an increase of HDAC5 and a reduction of BDNF expression. In addition, in T-cells a significant decrease of CTLA-4 and KLF-2 expression was observed.
Further, data obtained by FACS showed that proportion of classic monocytes and activated T- cells is lower in depressed patients.
Conclusions: These studies suggest that changes in HDAC5 and SIRT2 function in monocytes and T-cells could be associated to major depression.
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Reference number: T3-P33

(*T3-P33*) *EVALUATION OF THE G PROTEIN SIGNALLING PATTERN AND POLYPHARMACOLOGICAL PROFILE OF DIFFERENT ANTIPSYCHOTIC DRUGS IN POST-MORTEM HUMAN NATIVE BRAIN TISSUE.*

Background: Biased agonism (or ”functional selectivity”) at G-protein-coupled receptors (GPCR) has attracted rapidly increasing interest as a means to improve drug discovery of more efficacious and safer pharmacotherapeutics. Ligands can induce G protein-coupled receptors to adopt a myriad of conformations, many of which play critical roles in determining the activation of specific signalling cascades associated with distinct functional and behavioural consequences.
Second generation antipsychotic medications, known to block dopamine D2-like and 5HT2ARs, also to bind to a several other GPCRs. The knowledge of the specific signalling pathways over which they act and if they do as agonist, antagonist or inverse agonist would help in the discrimination of those related to therapeutic vs adverse effect, contributing to the future design of better drug candidates with an improved therapeutic profile.
Objective: Characterization of the pharmacological profile of clozapine, risperidone and paliperidone over Gαi1 and Gαq/11 subunits in postmortem human brain tissue. Furthermore, the role of the different GPCRs in these effects will be described by pharmacological antagonism.
Methods: [35S]GTPγS binding assay coupled to immunoprecipitation with specific Gα-protein antibodies were carried out to determine the agonist, antagonist or inverse agonist properties of the different drugs to Gαi1- and Gαq/11-proteins. All drugs were tested at a single concentration (10 μM) in absence or presence of the different antagonist MDL-11,939, ketanserin, SB 242084, raclopride, atropine, cetirizine (1 μM) or phentolamine (10 μM) in order to confirm the involvement of 5HT2A/CRs, D2Rs, mAChRs, H1Rs or αARs in the observed effects of each drug.

Results: Clozapine, risperidone, and paliperidone significantly decreased basal coupling to Gαi1-proteins (Imax=26±4% to 12±1%, p<0.01 one-sample t-test vs 100%). An effect specifically blocked by the 5HT2AR antagonists (p<0.05 one-way ANOVA vs drug alone), suggesting inverse agonist properties over this receptor and pathway. On the other hand, risperidone (Imax=14±4%, p>0.05 one-sample t-test vs 100%) was the only drug with inverse agonist activity on Gαq/11-proteins. No effect over this reduction was observed by the co-incubation with any of the antagonist used with the exception of phentolamine (p<0.01 one-way ANOVA vs risperidone), suggesting a role of αARs, probably α1ARs, on this inverse agonism. Finally, clozapine showed an agonist profile (Emax=25±4%, p>0.05 one-sample t-test vs 100%) on the Gαq/11-protein that was not sensitive to any of the antagonist used with the exception of atropine (p<0.05 one-way ANOVA vs clozapine), suggesting a role of mAChRs, probably M1Rs, on this agonism.
Conclusion: As expected, a multi-target pharmacological profile was observed, and the present study demonstrates a differential targeting of G-protein signalling modulation by antipsychotic drugs, compatible with the existence of functional selectivity in their activity over different GPCRs in human native brain tissue. Although a further characterization of other different Gα subunits is necessary to better understand and discriminate those mechanisms specifically related with therapeutic and adverse effect, an interesting inverse agonist profile of the three antipsychotic tested on the pro-hallucinogenic Gαi1-protein-mediated 5HT2AR signalling pathway has been observed. In this context, the present study rises the relevancy of the inverse agonist effects through 5HT2ARs over the Gαi1-protein pathway as a promising mechanism involved in the antipsychotic effect.
inverse agonist profile of the three antipsychotic tested on the pro-hallucinogenic Gαi1-protein-mediated 5HT2AR signalling pathway has been observed. In this context, the present study rises the relevancy of the inverse agonist effects through 5HT2ARs over the Gαi1-protein pathway as a promising mechanism involved in the antipsychotic effect.
Supported by the Spanish R+D+i Programme and European Regional Development Funds (SAF2017-88126R), the Basque Government (IT-616-13, ELKARTEK-2019/00049, and predoctoral fellowship to IM-A), the European Eranet Neuron Programme (PSYBIAS Consortium, AC18/00030) and the Instituto de Salud Carlos III FEDER (PI18/00094).

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Reference number: T3-P34

(*T3-P34*) *IN VITRO GENOTOXIC ASSESMENT OF SERTRALINE, DULOXETINE AND FLUOXETINE BY THE COMET ASSAY*

Antidepressants are widely prescribed for the long-term treatment of major depressive disorder and other psychiatric conditions, being selective serotonine reuptake inhibitors and serotonine and noradrenaline reuptake inhibitors first-line drugs. Although the number of antidepressant prescriptions is increasing over the years, few studies evaluate the potential genotoxicity of these drugs. Here, we evaluated the potential of duloxetine, sertraline and fluoxetine to induce DNA stand breaks and oxidized bases on THP-1 cells (derived from acute monocytic leukemia patient) after 6 and 24 hours. For this purpose, therapeutic concentrations of fluoxetine (1 and 10 μM), duloxetine (0.43 and 4.30 μM) and sertraline (0.18 and 1.8 μM) were used and the standard and the formamidopyrimidine DNA glycosylase (Fpg)-modified comet assays were applied. Moreover, the vulnerability or resilience of antidepressant-treated cells to KBrO 3 , an oxidant agent, was studied by applying the Fpg-modified comet asay.
Results indicated that none of the antidepressants produce single stand-breaks or oxidized bases comparing to negative control (p>0.05).Moreover, none of the antidepressants alter the level of oxidized bases induced by KBrO 3.

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Reference number: T3-P35

(*T3-P35*) *INCREASED SEROTONIN 5-HT2AR RECEPTOR CONSTITUTIVE ACTIVITY ON GAI1- BUT NOT GAQ/11-PROTEINS IN POST-MORTEM HUMAN PREFRONTAL CORTEX OF SUBJECTS WITH SCHIZOPHRENIA*

Background: The serotonin 5-HT2A receptor (5-HT2AR) is able to activate both Gαq/11- and/or Gαi/o-proteins, depending on the drug binding properties. Activation of Gαi1-proteins by 5-HT2AR agonists has been proposed as a molecular fingerprint of hallucinogenic properties. On the other hand, a higher stimulation of Gαi1- but not Gαq/11-proteins by the hallucinogenic drug (±)DOI through the activation of 5-HT2AR has been described in prefrontal cortex of subjects with schizophrenia. If this supersensitivity is consequence of altered constitutive activity of 5-HT2AR in schizophrenia is still unknown. In order to clarify the existence of a hypothetical enhanced 5-HT2AR constitutive activity in native brain tissue, drugs behaving as inverse agonists would be necessary as pharmacological tools.
Based on previous studies, pimavanserin shows selective biased Gαi1-protein inverse agonism through 5-HT2AR with no effect on the Gαq/11-proteins. In contrast, volinanserin shows 5-HT2AR-mediated inverse agonism over both Gαi1- and Gαq/11-proteins.
Objective: The aim of the present study was to evaluate the existence of enhanced constitutive activity of 5-HT2AR through Gαi1- and/or Gαq/11-proteins in post-mortem human prefrontal cortex of subjects with schizophrenia and their matched controls
Methods: [35S]GTPγS binding assays associated to immunoprecipitation with specific antibodies for Gαi1- and Gαq/11-proteins were carried out with increasing

concentrations of the 5-HT2AR inverse agonists pimavanserin (10-10, 10-8 and 10-6 M) and volinanserin (10-9, 10-6.5 and 10-5 M). Post-mortem prefrontal cortex samples from 23 subjects with schizophrenia and 23 matched controls were analysed. In order to demonstrate that the observed effects were mediated by 5-HT2ARs, single concentration (10 μM) experiments were carried out in absence and presence of the 5-HT2AR selective and neutral antagonist MDL-11,939 (1 μM).
Results: Pimavanserin and volinanserin significantly decreased, in a concentration-dependent manner, basal 5-HT2AR coupling to Gαi1-proteins in both schizophrenia and control subjects. However, the inhibitory effect of pimavanserin and volinanserin on the basal [35S]GTPγS binding to Gαi1-proteins was higher in schizophrenia subjects (Imax=20±1 and 16±1%) compared to controls (Imax=14±1% and 10±1%).Volinanserin was also able to significantly decrease, in a concentration dependent manner basal 5-HT2AR coupling to Gαi1-proteins in both groups. In contrast, no differences in the inhibitory effects of volinanserin were observed on the canonical Gαq/11-protein signalling pathway of 5-HT2ARs (Imax=10±1% and 12±1%, in schizophrenia and control subjects, respectively). Pimavanserin and volinanserin activities on both Gαi1- and Gαq/11-proteins were sensitive to MDL-11,939, confirming the role of 5-HT2ARs.
Conclusions: These findings directly demonstrate a selective increase of the constitutive activity of 5-HT2ARs on the Gαi1-protein mediated pro-hallucinogenic pathway in prefrontal cortex of subjects with schizophrenia. No changes were found in the constitutive activity of the canonical 5-HT2AR coupling to Gαq/11-protein pathway in the same subjects. This enhanced coupling of 5-HT2ARs towards Gαi1-proteins in schizophrenia support the development of 5-HT2AR drugs with selective inverse agonist effect on the Gαi1-protein signalling pathway as suitable tools to improve the therapeutic efficacy and the adverse effect profile of antipsychotic drugs.
Supported by the Spanish R+D+i Programme and European Regional Development Funds (SAF2017-88126R), the Basque Government (IT-616-13, ELKARTEK-2019/00049, and predoctoral fellowship to IM-A), and the European Eranet Neuron Programme (PSYBIAS Consortium).

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Reference number: T3-P36

(*T3-P36*) *ASSOCIATIONS BETWEEN SEVERE PSYCHIATRIC ILLNESSES, PRESYNAPTIC FUNCTIONALITY, AND BDNF, COMT AND MIR137 POLYMORPHISMS: A POSTMORTEM BRAIN, MULTI-SAMPLE STUDY*

Background: Synaptic abnormalities may be a common hub in severe mental illnesses, including schizophrenia (SCZ), bipolar disorder (BPD) and major depression (MDD). On the other hand, these disorders were associated with polymorphisms in the brain-derived neurotrophic factor (BDNF), catechol-O-methyltransferase (COMT), and/or micro-RNA 137 (MIR137) coding genes, which in turn have important roles in synaptogenesis, neurotransmission, and synaptic protein homeostasis, respectively.

Using a large postmortem brain case-control sample, we tested the hypothesis that abnormalities in the presynaptic SNARE (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor) machinery could mediate the associations between BDNF, COMT and/or MIR137 risk variants and the corresponding severe mental illness.
Methods: Postmortem samples from the orbitofrontal cortex (OFC; Brodmann's area 10/47) of subjects with SCZ (n=70), BPD (n=34), and MDD (n=15), as well as sex-, age-, and postmortem interval-(PMI) matched controls (CTL, n=63) were obtained from the Stanley Foundation and the Macedonian/New York State Psychiatric Institute Brain Collection. Genotyping of select single nucleotide polymorphisms (SNPs) across the BDNF (11), COMT (9) and MIR137 (3) loci was performed by tetra-primer amplification refractory system (T-ARMS). Cortical amounts of eight presynaptic proteins were determined by ELISA, while SNARE PPIs were assessed by blue native-(BNP) PAGE followed by quantitative immunoblotting in the same postmortem OFC samples.
Results: We found no significant associations between SCZ and any of the studied SNPs. Interestingly, BDNF rs988748 (G) and rs7103411 (T) minor alleles were underrepresented in BPD ($p<0.0001$), while overrepresented in MDD ($p=0.001$) in a dose-dependent manner. Adjusting by sex, age and PMI, cortical amounts of vesicle-associated membrane protein (VAMP) were significantly lower in all three disorders, compared to controls ($p<0.05$). By contrast, SNARE PPIs were significantly increased in the same OFC samples from SCZ and MDD subjects ($p<0.01$). In turn, several synaptic proteins, including VAMP, were influenced by two particular COMT variants, rs737865 and rs2075507. Interestingly, greater density of SNARE PPIs was observed in the BDNF risk-allele carriers in BDP. Further logistic regression analyses showed that the effects of both SNPs (rs988748 and rs7103411) on BPD and MDD were indeed mediated by SNARE PPIs.
Discussion: While the present sample series is underpowered to perform genome association studies, the results suggest the possibility that BPD and MDD are respectively increased or decreased in carriers of two highly linked BDNF SNPs, rs988748 and rs7103411. Remarkably, these associations were mediated by a reduced (BPD) or increased (MDD) ability of the SNARE proteins to build functional complexes in cortical areas of the brain. Of note, dysregulation of SNARE PPIs may reflect synaptic dysfunction. COMT variants may also contribute to VAMP downregulation and synaptic dysfunction in MDD brains. While greater OFC amounts of SNARE PPIs were also associated with SCZ, larger numbers of samples will be needed to demonstrate whether the studied genes may contribute to presynaptic abnormalities and/or illness development.
Supported by MCIU/AEI/ERDF (Grant RTI2018-094414-A-I00).

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Reference number: T3-P37

(*T3-P37*) *ACTIVATING EPIGENETIC MODIFICATIONS ARE UPREGULATED IN SCHIZOPHRENIA SUBJECTS: EFFECT OF ANTIPSYCHOTIC TREATMENT*

Susceptibility to develop schizophrenia (SZ) is determined by complex interactions between genes and environment. Environmental risk factors may exert their negative effects at critical stages of brain development, possibly via epigenetic mechanisms. While most studies evaluated gene-selective epigenetic modifications, few studies reported SZ-associated alterations affecting epigenetic mechanisms globally. Here, we evaluated the global expression of histone posttranslational modifications (HPTM) in a case-control cohort of SZ subjects.
Dorsolateral prefrontal cortex samples of SZ subjects and age-, sex-, and post-mortem delay- matched controls were obtained at autopsies performed at the Basque Institute of Legal Medicine. SZ subjects were grouped into antipsychotic treated (AP-treated) or antipsychotic free (AP-free) groups, according to blood toxicological data. For this study, cortical amounts of gene expression activating (H3K9ac, H3K27ac, H3K4me3 and H4K16ac) and repressing (H3K9me3, H3K27me3 and H4K20me3) histone H3 and H4 PTM were quantified by Western Blot.
H3K9ac (+42%, $p<0.01$), H3K27ac (+28%, $p<0.05$) and H3K4me3 (+24%, $p<0.05$) were significantly increased in SZ subjects, compared to matched controls. Cortical immunodensities of all other HPTM did not show significant differences between SZ subjects and controls. Subgroup analyses in AP-free and AP-treated SZ subjects revealed that H3K27ac and H3K4me3 were specifically increased in AP-treated group. Pearson's correlation analyses showed overall positive associations between activating and, separately, between repressing HPTM. The expression of activating H3K4me3 also correlated with that of repressing H3K27me3.
In conclusion, upregulation of H3K9ac, H3K27ac and H3K4me3 may be associated with globally increased gene expression in SZ. AP treatment might enhance H3K27ac and H3K4me3 levels. Future studies should clarify whether the observed upregulation of cortical H3K27ac and H3K4me3 might be part of the mechanism of action of AP drugs, or due to a specific mechanism in SZ brain. Finally, the overall correlations between the HPTM species suggests that epigenetic mechanisms are tightly regulated.

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Reference number: T3-P38

(*T3-P38*) *EVALUATION OF GRM2 AND GRM3 EXPRESSION IN THE PREFRONTAL CORTEX OF SUBJECTS WITH SCHIZOPHRENIA.*

Metabotropic glutamate receptor 2 and 3 (mGlu2/3R) agonism showed promising results as a therapeutic strategy in animal models of schizophrenia (SZ), but clinical

studies reported controversial data when mGluR2/3 ligands where co-administered with other antipsychotic (AP) drugs1. On the other hand, brain expression of mGlu2R protein, and its coding gene GRM2, have been reported to be decreased in SZ, the reduction being larger in SZ subjects that received clozapine (CLO) as AP treatment2. The mechanism might involve 5-HT2A receptor antagonism by chronic CLO, which would increase HDAC2 transcription leading to decreased H3 acetylation at GRM2 promoter in prefrontal cortex (PFC) of SZ subjects3. In addition, mGlu3R coding gene GRM3 has been identified as a susceptibility gene in SZ4, and evaluation by our own group has shown a reduction in protein expression in SZ. Thus, this study aimed to evaluate GRM2/3 gene expression in the PFC of SZ subjects and the potential effect of AP treatment. The particular effect of CLO treatment, compared to other AP drugs, on GRM2/3 gene expression was also investigated.

Two cohorts of PFC specimens were obtained at autopsies in the Basque Institute of Legal Medicine. The first cohort consisted of 24 pairs of SZ cases and controls, matched by age, sex and postmortem-delay (PMD). SZ subjects were divided into AP-treated (n=12) or AP-free (n=12) subjects, according to the presence or absence of first-/second-generation APs in blood toxicological assessments. The second cohort consisted of 13 CLO-treated SZ subjects and 13 age-, sex- and PMD-matched SZ subjects treated with other APs. GRM2/3 gene expression was analyzed by qPCR on total RNA extracted from PFC samples, using SYBR green and specific primers. Gene expression was normalized using ΔΔCt method with GAPDH and RPS13 gene expression and an internal cDNA pool as a reference sample.

Comparison between SZ and matched control subjects did not show significant alterations in GRM2/3 gene expression. However, GRM3 was significantly overexpressed in AP-free (Δ=45±20%, n=10, p=0.034, two-tailed paired t-test), but not in AP-treated SZ subjects, when compared with their respective matched controls. In addition, preliminary results indicated that CLO treatment is associated with reduced cortical GRM2, but not GRM3, gene expression, compared to other AP drugs.

Altogether, the present results suggest that previous observations of reduced mGlu2/3R protein expression in SZ are not necessarily preceded by reduced GRM2/3 gene expression. Increased GRM3 gene expression in AP-free SZ patients may indicate a compensatory transcriptional mechanism in response to reduced mGlu3R protein expression in the same subjects. The absence of alterations in both GRM3 protein and gene expression in AP-treated SZ subjects suggests that AP drugs could return GRM3 expression to control values. Furthermore, the ability of CLO to reduce GRM2 gene expression is not shared by other AP drugs, and may define a CLO-selective mechanism of action.

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Authors

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Reference number: T3-P39

(*T3-P39*) *CHARACTERIZATION OF A TECHNIQUE FOR OBTAINING NEURONS AND GLIA FROM THE OLFACTORY NEUROEPITHELIUM OF LIVING SUBJECTS*

Introduction

The study of mental illnesses such as schizophrenia requires the use of their substrate tissue, that is, nervous tissue. Currently, the techniques used are far from being useful to provide a predictive, diagnostic or prognostic biomarker. Olfactory epithelial cells come from the same neuroepithelium of the embryonic neural tube, from which new neurons are continuously generated during adult life. The olfactory neuroepithelium contains pluripotential cells that can proliferate in vitro and differentiate into multiple cell types including neurons and glia, making it a unique resource for the investigation of intraneuronal molecular status and trait markers in neuropsychiatric diseases.

Hypothesis

For all these reasons, we propose a technique that will allow longitudinal studies to be carried out in living patients, being able to correlate the possible biomarkers with the evolution of the patient and the effect of the treatment.

Objective

Obtaining neurons and glia from the olfactory neuroepithelium of living patients for use in the search for biomarkers of mental illness.

Results

Differentiated neuronal and glial cultures have been separated from adherent cultures of the olfactory neuroepithelium and their subsequent qualitative and quantitative characterization.

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Neurogune 2022

July 1st, 2022

Museum of the University of Navarre
Iruña - Pamplona

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