Neurogune 2024

Centro Ignacio Mª Barriola

Donostia – San Sebastián
28th June

Book of Abstracts

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1. ORGANIZATION

Scientific Committee:

- Ainara Vallejo (UPV/EHU, Biogipuzkoa)
- Adolfo López de Munain (Biogipuzkoa / HU Donostia)
- Patxi Gil (Biogipuzkoa)
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- Javier Ruiz Ederra (UPV/EHU, Biogipuzkoa)
- Jaime Sagarduy (Achucarro)
- Ainara Vallejo (UPV/EHU, Biogipuzkoa)

2. SCIENTIFIC PROGRAMME

08.30 - 09.00	**Registration and Poster arrangement**
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11:15 - 11:30	***María Rodríguez Hidalgo - IIS Biogipuzkoa*** WGSprisma: A new tool to support variant filtering and prioritization in whole genome sequencing data
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11:45 - 12:00	***Adhara Garminde - UPV/EHU, Achucarro*** Amyloid-β increases MBP and MOBP translation in oligodendrocytes through dysregulation of hnRNP A2 dependent RNA dynamics
12:00 - 12:15	***Andrés Jiménez Zúñiga - IIS Biogipuzkoa*** Single-cell RNAseq in Drosophila models of ALS to decipher non-cell autonomous mechanisms of neurodegeneration
12:15 - 12:30	***Jorge Juan Bravo - CIMA, Universidad de Navarra*** Identification of a subcellular-dependent alpha-synuclein interactome in a mouse model of Parkinson´s disease by proximity-biotinylation.
12.30 - 14.30	**Posters and networking session (with lunch)**
14.30 - 15.20	***Closing Keynote by Dr. Rubén Fernández Busnadiego - Göttingen University, Germany*** Unraveling the structure of toxic protein aggregates in situ
15.20 - 15.30	**Closure**

3. KEYNOTES

Opening Keynote:

Nanoparticles for optical stimulation and sensing of neuronal activity

Dra. Blanca del Rosal

RMIT University, Melbourne, Australia

Closing Keynote:

Unravelling the structure of toxic protein aggregates in situ

Rubén Fernández Busnadiego

Gottingen University, Germany

4. SPONSORS

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5. ORAL PRESENTATIONS

O1

Age Matters: Hippocampal Microglia Dynamics in Mouse Brain Slices

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Alterations in microglia are implicated, among others, in the pathogenesis of age-related diseases, including Alzheimer's disease (AD). These changes are well characterized at the molecular and morphological levels, but not extensively at the dynamic level. In the mouse retina, microglial dynamics are known to change in an age-dependent fashion (Damani et al., 2011), with microglia maintaining a 'steady-state' but with quantitatively diminished dynamics. Similarly, studies in the mouse brain using in vivo cranial windows (Hefendehl et al., 2014) have shown age-related reductions in microglial process motility. To explore these dynamic changes further, we used acute brain slices. Our results demonstrate that the observed age-dependent decline in microglial dynamics aligns with previously reported trends, confirming that aging significantly reduces the motility and process extension-retraction activity of microglia. These findings underscore the importance of microglial dynamic alterations in the aging process and contribute to the understanding of microglial behavior in the context of neurodegenerative disease progression.

O2

WGSprisma: A new tool to support variant filtering and prioritization in whole genome sequencing data

Rodríguez-Hidalgo, M *1,2; Bruijn, SE de 3; Rodenburg, K 3; Elorza-López, G 1; Sarasola-Gastesi, M 1,2; Lara-López, A 4; Corominas-Galbany, J 3; Gilissen, C 3; Irigoyen, C 1,5; Cremers, FPM 3; Roosing, S 3; Ruiz-Ederra, J 1,2*

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As the implementation of whole genome sequencing (WGS) is increasing, it is desired to standardize and simplify data analysis pipelines. We have developed an application, coined WGSPrisma, for automated variant filtering and prioritization in inherited retinal dystrophies (IRD). We developed a Rstudio shiny application that assigns a color code to each WGS variant based on its type and in silico prediction score. To select the best combinations of in silico prediction tools, we studied seven programs (CADDv1.6, Grantham, PhyloP, Revel, SIFT, PolyPhen-2 and PROVEAN) using 1,810 curated variants (333 likely pathogenic and 1,477 likely benign) from the VKGL-datashare-database. This workflow was validated using WGS data from 61 solved IRD individuals (validation cohort). Finally, WGS-data from 190 genetically unexplained IRD-individuals (discovery cohort) were analyzed using WGSprisma. Development of WGSprima showed that CADDv1.6 (>15) and Revel (>0.5) provided the optimal combination to detect missense variants. WGSprisma also identified 93% of causative variants in our validation cohort. Moreover, we solved 35% (67/190) and likely solved 18% (34/190) of the individuals in the discovery cohort. The use of this automatized process reduced filtering and prioritization time by 70%. Furthermore, it provided a user-friendly interface, producing a visual prioritization of variants. WGSprisma provides an efficient strategy for WGS-data variant filtering and prioritization, which aids in determining genetic diagnosis in unsolved cases.

O3

CD4+ T cell modulation is neuroprotective in an AAV9-mediated α-synuclein overexpression mouse model of Parkinson´s disease

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Neuroinflammation is a complex pathological process, which involves numerous cell types and inflammatory mediators that can promote cell death in many neurodegenerative diseases. Brains from Parkinson's disease (PD) patients and animal models that mimic PD neurodegeneration present T lymphocyte infiltration in the substantia nigra (SN). We hypothesized that lymphocyte infiltration could enhance the proinflammatory reaction taking place in the brain parenchyma, promoting neuronal loss and disease progression, and that modulation of their pathological phenotype may result in neuroprotection. Experimental parkinsonism was induced by overexpressing α-synuclein in the SN with an adeno-associated viral vector. Flow cytometry analysis revealed that this model presented a specific infiltration of myeloid and CD4+ T cells into the SN. These CD4+ T cells included an increase in regulatory T cells (Treg) and a polarization towards Th1 response. The selective depletion of Treg using the Foxp3DTR mice prevented α-synuclein-dependent degeneration of the nigrostriatal pathway. These animals presented a decreased accumulation of myeloid and CD4+ T cells in the SN, with a decrease in TNFα+ release. Thus, to prevent T cell extravasation pharmacologically into the brain, we selected the drug fingolimod, an agonist of sphingosine-1-phosphate receptors, that retains lymphocytes within secondary lymphoid organs. In the 2-week-duration α-synuclein overexpression model, fingolimod treatment effectively reduced T cells in blood and their infiltration into the brain, and improved motor deficits. However, fingolimod did not protect the nigrostriatal pathway after α-synuclein overexpression. Then, we tested long-term treatment with fingolimod in a chronic α-synuclein overexpression model. After 8 weeks, fingolimod protected SN dopaminergic neurons. These findings suggest that CD4+ T cells may actively contribute to α-synuclein dependent neurodegeneration and blocking their infiltration into the brain parenchyma could reduce neuronal loss and disease progression.

O4

Amyloid-β increases MBP and MOBP translation in oligodendrocytes through dysregulation of hnRNP A2 dependent RNA dynamics

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Oligodendrocyte dysfunction, myelin degeneration, and white matter structural alterations are critical events in Alzheimer's disease (AD) that contribute to cognitive decline. A key hallmark of AD, Aβ oligomers, disrupt oligodendrocyte and myelin homeostasis, but a comprehensive global analysis of the mechanisms involved is lacking. Here, transcriptomic profiling of Aβ-exposed oligodendrocytes revealed widespread gene expression changes, particularly affecting pathways related to RNA localisation. Among the genes identified, we focused on Hnrnpa2/b1, the gene encoding the hnRNP A2 protein, which is essential for RNA transport and translation of myelin proteins. We confirmed aberrant upregulation of hnRNP A2 in hippocampal oligodendrocytes from post-mortem human brains of early-stage AD patients, Aβ-injected mouse hippocampi and Aβ-treated disrupting cells in vitro. RIP-seq analysis of the hnRNP A2 interactome revealed attenuated interactions with Hnrnpk and Hnrnpa2/b1, while interactions with Mbp and Mobp were enriched, suggesting changes in RNA metabolism of molecules associated with mRNA transport of myelin proteins. Aβ increased the total number and dynamics of mRNA-containing granules, facilitating local translation of the myelin proteins MBP and MOBP and attenuating Ca 2+ signalling. These findings suggest that Aβ oligomers disrupt RNA metabolism mechanisms crucial for oligodendrocyte myelination through dysregulation of hnRNP A2 and myelin protein levels, potentially affecting oligodendroglia Ca2+ homeostasis.

O5

Single-cell RNAseq in Drosophila models of ALS to decipher non-cell autonomous mechanisms of neurodegeneration

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that rapidly affects motor neurons (MNs) in the brain and spinal cord. There is currently no cure for this disease. In about 90% of cases, the origin of the disease is unknown, but in 10% it is caused by Mendelian genetic mutations in more than 20 genes, including TARDBP, which encodes the TDP-43 protein. Recent evidence has questioned the neurogenic origin of the disease by suggesting that glial cells, the supporting cells of motor neurons within the neuromuscular junction (NMJ), contribute to MN toxicity. The aim of this study is to analyse how the ALS-associated pathological inputs from glial cells affect the MN transcriptome, in order to identify early changes for intervention. To this end, we have generated a Drosophila melanogaster model expressing human TDP-43 specifically in glial cells and showing a strong ALS-like phenotype. Using single-cell RNA sequencing technology, we are analysing the evolution of transcriptomic profile of brains over disease progression in this Drosophila model. This study reveals a significant reduction of glutamatergic and cholinergic neurons, suggesting a neurodegenerative process, together with the emergence of new glial and neuronal 'subtypes' over progression, which will allow us to identify disease modifying molecular targets to design new therapeutic interventions.

O6

Identification of a subcellular-dependent alpha-synuclein interactome in a mouse model of Parkinson´s disease by proximity-biotinylation.

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Parkinson´s disease (PD) is caused by the loss of dopaminergic neurons in the substantia nigra (SN) that project to the striatum. Current treatments are symptomatic and do not stop disease progression. Thus, effective PD treatments are required. Mutations and/or abnormal levels of the alpha-synuclein (aSyn) protein cause nigrostriatal degeneration in PD. Still, the molecular mechanisms whereby aSyn triggers this neurodegenerative process remain largely unclear. We hypothesize that the early increase in aSyn protein levels leads to i) a subcellular proteomic redistribution and to ii) pathological protein interactions. These events may eventually cause dopaminergic neuronal death. Nowadays, the combination of state-of-the-art methodologies based on proximity biotinylation, new viral vectors targeting the central nervous system (CNS) and advanced mass spectrometry (MS) can resolve cell-type and subcellular-dependent proteomic alterations in animal disease models, providing the identification of new therapeutic targets in pathological/neurodegenerative contexts. Here, we have set up an in vivo proximity biotinylation assay to identify the early aSyn interactome in dopaminergic neurons. By expressing aSyn fused to a promiscuous biotinylase (TurboID) and adding biotin, proximal stable and transient interacting partners become biotinylated, are purified by affinity and identified by liquid chromatography-mass spectrometry (LC/MS). Adeno-associated viral (AAV9) vectors encoding TurboID and aSynTurboID were generated and injected by stereotaxia into the SN of mice. AAV9-SynTurboID mice showed increased aSyn levels in the SN and the striatum, nigrostriatal degeneration and motor deficits. Biotinylated substrates were pulled-down from dopaminergic striatal terminals and SN at early neurodegenerative stages. The analysis by semi-quantitative LC/MS has unveiled the identification of a previously unknown subcellular-specific aSyn interactome. A validation of the synaptic location of some of these substrates has been performed through fluorescence-activated synaptosome sorting and immunofluorescence. Moreover, we have identified aSyn-dependent alterations in non-anticipated biological process, such as RNA metabolism and translational regulation. The pathological significance of some of these proteins is currenly being evaluated in aSyn models and post-mortem brains from PD patients.

6. POSTER PRESENTATIONS

P1

Modulation of the synaptic translatome by astrocytic extracellular vesicles in AD mouse models

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Alzheimer's disease (AD) is the main cause of dementia and is almost twice as common in women than in men. Despite efforts to develop therapies to stop its progression, there is still no cure. Although the accumulation of Tau protein and beta-amyloid peptide (Abeta) are two of the main hallmarks of AD, synaptic dysfunction in early stages is the parameter that best correlates with cognitive impairment in patients (1). These changes are accompanied by alterations in the synaptic proteome, which can be shaped by proteins transported from the neuronal soma and/or by mRNAs that are delivered to synapses where they are locally translated. This mechanism is known as local translation. Local translation has been extensively studied in the nervous system in physiological contexts, but recent evidence indicates that this mechanism is deregulated in neurodegenerative diseases. In fact, diseases considered synaptopathies such as AD are accompanied by alterations in the localization of mRNAs and their localized translation in axons and synaptic terminals (2). Although the neuronal somatic compartment is considered the main origin of axonal, dendritic, and synaptic mRNAs there are evidence showing the existence of horizontal transport of mRNAs and even ribosomal subunits from Schwann cells to injured peripheral nerves, suggesting a glial contribution to local protein synthesis in neurons (3,4). Noteworthy, mRNAs and ribosomes can be transferred from glia to neurons in extracellular vesicles (EVs). EVs are membranous particles secreted by all types of cells involved in intercellular communication, since they transfer proteins, nucleic acids and lipids to the acceptor cells where these bioactive molecules perform their function (5). In our research group we have found evidence supporting that EVs secreted by astrocytes in the context of Abeta pathology modify the levels of newly synthesized proteins in axons in vitro. Based on these observations, our working hypothesis is that astrocytic EVs contribute to the synaptic local translatome in the Alzheimer mouse models. Therefore, EVs from control or Abeta-treated astrocytes were injected into the dentate gyrus of 5xFAD or APP/PS1 and wildtype female and male mice. The results show that EVs coming from astrocytes exposed to Abeta induce an increase in the relative levels of protein synthesis in the cholinergic pre-synaptic terminals of the dentate gyrus, suggesting an increase in cholinergic local translation. All these data describe an important role of astrocytic EVs in local translation.

P2

RNA binding protein IMP1/ZBP1 drives mRNA localization and local translation in microglial peripheral processes and mediates morphological changes, motility and phagocytosis in response to inflammation

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Central Nervous System cells exhibit significant diversity and specialization, not only between cell types and functions, but also regarding their morphology. Such morphological and functional polarization complexity is due to the uneven distribution of proteins at a subcellular level. This distribution can be obtained either by protein transport to a specific cell site, or by mRNA transport creating a site-specific transcriptome that can be translated in a stimulus dependent manner, allowing cells to react to environmental changes. This last mechanism is called local protein synthesis or local translation and has been long known to happen in neurons. However, evidence suggests that oligodendrocytes, astrocytes, and radial glia also rely on local translation to maintain protein homeostasis and functional integrity. Local translation in microglia has been observed, but its specific relationship to pathology remains unclear. Given the scant evidence, our study aims to investigate the existence of local translation in microglial peripheral processes (PeMPs) and unravel its functional significance in response to inflammation. Using primary microglia cultures and bacterial lipopolysaccharides (LPS), we have seen that Actb mRNA polarizes to PeMPs and is locally translated upon LPS exposure. Interestingly, downregulation of the Actb binding protein IMP1/ZBP1 impaires Actb mRNA polarization and its localized translation, and leads to defects in filopodia distribution, PeMP motility, directed migration and phagocytosis in microglia. In an in vivo study, we conducted immunohistochemical and in situ hybridization analyses following intraperitoneal LPS injections in 1mo mice. Results suggest that LPS enhances local protein synthesis in vivo, as phosphorylation levels of ribosomal protein Rsp6 increase in cortical PeMPs. We analyzed Actb mRNA levels in mice and observed an increase in cortical growth cone (GC)-like PeMPs and in phagocytic pouches in the dentate gyrus in response to LPS. Interestingly, IMP1 expression increases in primary PeMPs in the cortex and in phagocytic pouches in response to LPS. Indeed, we observed a significant positive correlation between Actb and IMP1 in phagocytic microglia suggesting IMP1 drives Actb localization in vivo. Altogether, our study confirms local translation in PeMPs and that it is enhanced in microglial inflammatory response with LPS suggesting a functional relevance of this molecular mechanism in response to inflammation. This adds to the evidence supporting mRNA

localization and localized translation in microglia, offering mechanistic insights into their crucial role in responding to inflammation.

P3

Vacuolar protein sorting-associated protein 4A validation as a Parkin substrate

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Parkinson's disease (PD) is a common, chronic, and incurable neurodegenerative disorder of central nervous system characterized by the progressive loss of dopaminergic neurons in the substantia nigra and formation of Lewy bodies, leading to motor and non-motor symptoms. The majority of the cases of PD are sporadic, suggesting a multifactorial etiology based on environmental factors, aging and genetic factors. Although it usually manifests after the age of 50 years, mutations in PARK2, among other genes, are responsible for the development of early-onset cases of PD. PARK2 encodes Parkin, an E3 ubiquitin ligase that post-translationally modifies proteins by covalently adding an ubiquitin moiety to lysine residues. Identifying Parkin substrates and unraveling the consequences of their reduced ubiquitination levels is critical to decipher the molecular mechanism underlying early-onset cases of PD. We previously identified a number of Parkin substrates in Drosophila melanogaster neurons, among which we validated VPS35 as being regulated by PARK2 in human cells. This was achieved using a GFP pulldown based ubiquitination assay developed in our lab, by comparing the ubiquitination levels of putative substrates when overexpressing the functional or a ligase dead version of Parkin. After testing another subset of putative Parkin substrates, we now conclude that Vacuolar protein sorting-associated protein 4A (VPS4A) is also a Parkin substrate in HEK293 cells, and that its ubiquitination leads to protein degradation. Further work is ongoing to identify the ubiquitination sites, and ubiquitin chain types that Parkin conjugates onto VPS4A.

P4

Increased surface expression of P2X4 receptors in microglia/macrophages ameliorates EAE symptoms in female mice

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Upon demyelinating injury in multiple sclerosis, microglia and peripheral macrophages clean up myelin debris and orchestrate a regenerative response that promotes myelin repair. We previously found that the P2X4 positive allosteric modulator ivermectin increases myelin phagocytosis, promotes remyelination and improves symptoms recovery in experimental autoimmune encephalomyelitis (EAE). To define accurately the selectivity of the pharmacological treatment and the role of microglia in the protective effect, we used P2X4mCherryIN knock-in mice. In these mice 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 were larger in non-internalized P2X4KI microglia, as expected, but also in oligodendrocytes. Constitutive P2X4KI mice and conditional CD11bCre-P2X4KI mice showed an amelioration of motor symptoms at all EAE stages in female mice, but not in males. P2X4-mediated inward currents did not differ between male and female P2X4KI microglia. However, lysosomes were larger in female than in male P2X4KI microglia, suggesting a sex-dependent increase in endolysosome fusion. In addition, acute application of progesterone enhances the amplitude of P2X4 inward and prolongs the deactivation kinetics, suggesting a direct impact of progesterone in channel gating. Our data demonstrate that increased surface P2X4 expression effectively ameliorates EAE and that the therapeutic potential of targeting microglial P2X4 has a clear gender dimorphism.

P5

Defining the role of APOE levels and isoforms in the developmental maturation of iPSC-derived human microglia

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Microglia are the specialized phagocytes of the brain, and they play a key role in maintaining its homeostasis. However, the molecular mechanisms by which microglia acquire their characteristic functionality are largely unknow. In contrast to what is usually assumed, our preliminary data show that these cells are not born-efficient phagocytes. Using confocal imaging, our laboratory found that microglia in mice hippocampus and cerebellum transition from a state of proliferative cells that colonize the brain parenchyma to postmitotic cells that begin to differentiate morphologically and become efficient phagocytes. With this data, we generated a mathematical model of microglial developmental maturation that predicts a switch at mice postnatal day 5 from early proliferative (P) cells to quiescent (Q) and functionally mature ones. We continued performing chromatin immunoprecipitation to identify potential epigenetic drivers of this P/Q switch and found a series of genes whose H3K4me3 methylation and therefore, its gene expression, decreases at this timepoint, among which stands out ApoE. In humans, but not in mice, APOE shows different isoforms, being APOE3 related to a normal Alzheimer's disease risk and APOE4 to a higher one. Here we will test the hypothesis that the levels and/or isoforms of APOE should impact the process of developmental maturation in microglia. For this purpose, we will compare two human cell lines of iPSCs bearing APOE3 or APOE4 and differentiate them into iMicroglia to study their development in terms of proliferation rate, morphology complexity and phagocytosis

efficiency. We will also perform these experiments in combination with GSK467, a demethylase inhibitor, to maintain the APOE expression levels constant during the whole process. Next, we will xenograft these iMicroglia into early postnatal mice to measure the same parameters in vivo. These experiments will give us insight on microglial developmental mechanisms, which is highly relevant as microglia are long-lived cells and early impairments on their maturation program may have an impact on the development of many neurological and neurodegenerative disorders in the long term.

P6

Phagocytosis-induced oxidative stress in brain macrophages

Cuesta, X; Márquez, M; Hambardzumyan, D; Sierra, A

Achucarro

Microglia are brain professional phagocytes mainly destined to the clearance of apoptotic or necrotic cells, synapses, axonal debris, and myelin, as well as combating central infections through direct phagocytosis of bacteria and viruses. However, phagocytosis is not a passive process of waste removal. Using an in vitro model, we have discovered that phagocytosis results in metabolic, mitochondrial, and transcriptional changes in phagocytes that leads to oxidative stress and cell death, suggesting that microglial functionality may be compromised following phagocytosis. Thus, to study whether the death of phagocytic microglia occurs as a consequence of oxidative stress induced by phagocytosis, we used an in vitro phagocytosis model in which microglial cells were fed with apoptotic SH-SY5Y neuronal cells and treated with different concentrations of the antioxidant N-acetylcysteine. Our preliminary results show that low concentrations of the antioxidant produce a reduction in the phagocytic microglia cell death, suggesting that it occurs as a consequence of oxidative stress. Additionally, we observed that low concentrations of the antioxidant did not have effect on microglial phagocytosis, suggesting that the lower percentage of microglial death observed was related to a protective effect of the antioxidant in the phagocytic microglia. Besides, we have studied the microglial response using a brain tumor model (glioblastoma), subjected to two consecutive doses of radiotherapy to induce apoptosis of tumor cells and we have determined that, despite continued apoptotic challenges, microglial cells were able to overcome the stress associated with phagocytosis, suggesting that the metabolic, mitochondrial, and transcriptional changes induced by phagocytosis were adaptive and intended to maintain phagocytic efficacy. Therefore, these results may lead to the use of antioxidant treatments as co-adjuvants in the treatment of glioblastoma (a high incidence glioma), to facilitate microglial survival and effectively eliminate tumoral cells, after treatment with radiotherapy/chemotherapy to induce tumor cell death.

P7

Microglial physiology changes in a stiffness-dependent manner in different 3D environments

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Biomaterials have been used in research for decades as a tridimensional microenvironment for cell culture, being the acrylamide-bisacrylamide-based hydrogels the most widely used material. Many of these biomaterials do not have a biologically mimetic composition for the cell grow, in particular when modelling the brain parenchyma. Microglia are resident macrophages of the brain that play an essential role in maintaining the homeostasis of the central nervous system (CNS) by sensing the environment. Here, we explore several biomaterials to achieve a more brain mimetic-like 3D-environment and studying whether matrix stiffness affect brain cell physiology in general and microglia in particular. For achieving these objectives, we created 3D scaffolds with different methacrylated polymers, such as alginate, gelatine, and hyaluronan or chitosan, and using Matrigel as control biomaterial. We evaluated cell viability, phagocytosis, motility and morphology in SV40 microglia cells. We have observed that these cells are more viable in methacrylate alginate, gelatine and Matrigel. Alginate-based scaffold allowed microglia to adopt the more brain-like morphology, while softer Matrigel scaffolds allowed for increased motility. Tridimensionality clearly affects microglial physiology and function, and it is in a 3D environment where brain cells can better mimic their in vivo counterparts. Whereas stiffness has a clear effect on movement and morphology, it is still not clear whether composition has a major effect on these parameters. However, composition and assembly protocol has a clear effect on cell viability. 3D scaffolds might prove a clear advantage over 2D cultures to better mimic the mechanical and chemical cues that cells find in the real brain, which might be useful for in vitro testing and screening.

P8

Microglial SIRT2 deficiency aggravates cognitive decline and brain pathology in Alzheimer's disease

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Several studies have highlighted the significant role of SIRT2 in neuroinflammation and Alzheimer's disease (AD). A previous study from our group has demonstrated that SIRT2 inhibition is beneficial in an AD mouse model. However, this treatment increased pro-inflammatory cytokines at the periphery. Consequently, we propose that targeting SIRT2 specifically within the CNS may offer the most effective therapeutic approach, maximizing benefits while minimizing peripheral adverse effects. Due to the central role of microglia in AD we aim to investigate the consequences of microglial SIRT2 deletion on AD progression. To remove SIRT2 exclusively from microglia, SIRT2flx/flx mice have been crossed with the Tmem119CreERT2 strain to generate the model SIRT2KO. Additionally, SIRT2KO was crossed with the AD model APP/PS1. A morphological and biochemical characterization of hippocampal microglia was performed using immunofluorescence, qPCR and flow cytometry.

Additionally, the consequences of microglial SIRT2 ablation were assessed analyzing the cognitive performance, amyloid pathology and neuroinflammation. SIRT2KO microglia show an increased gene expression of Cd68 and Trem2 and larger average branch length compared to WT. In the context of AD, microglial SIRT2 deficiency reduces the survival and worsens the cognitive capacities, the LTP and amyloid pathology. Our results indicate that SIRT2 deficiency impairs microglial function which aggravates learning and memory dysfunction, neurodegeneration, and AD-like pathology. Considering that several studies suggest SIRT2 inhibition as a potential therapeutic strategy for AD, further research is needed to understand the specific roles of SIRT2 in each cell type and the consequences of its genetic or pharmacological manipulation in neurodegenerative diseases.

P9

Molecular consequences of microglial SIRT2 deletion during postnatal development and adulthood

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Sirtuin 2 (SIRT2) is a deacetylase enzyme which has emerged as a potential pharmacological strategy in neurodegenerative diseases. However, its implication in early ages has not been studied. In order to deepen into the specific role of SIRT2 we decided to selectively delete the Sirt2 gene in microglia. Microglia have a key role during development and in the etiology of neurodegenerative diseases. Therefore, we aimed to assess the impact of SIRT2 deletion both in postnatal stage and in adulthood. To remove SIRT2 exclusively from microglia, SIRT2flx/flx mice have been crossed with the Tmem119CreERT2 strain to generate the model SIRT2KO. Tamoxifen was administered at different timepoints: P3-5 and P10-P12 for development study and weeks 6 and 10 for studies in adults. RNAseq was conducted in isolated microglia from adult mice. In addition, molecular characterization was carried out using immunohistochemistry in adults and quantification of mRNA and proteins at P25. RNAseq showed considerable alteration of genes involved in brain development and synaptic activity. Moreover, immunohistochemistry analysis revealed a reduction in cfos expression and an increased phagocytosis of synaptic proteins. An increase in Tnf-alpha and a significant decrease in synapse related proteins PSD95 and synapsin I was observed in development, suggesting an alteration in neuronal activity. Further studies will decipher the behavioral consequences of such alterations and the underlying molecular mechanisms. This study will widen the knowledge about the role of SIRT2 in physiological and pathological conditions.

P10

Myelin fatty acids boost oligodendroglia lineage progression and maturation

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Oligodendrocytes are the myelinating cells of the CNS In addition to building up the myelin sheath, oligodendrocytes provide energy substrates to axons to support action potential propagation Here we tested the idea of whether oligodendrocytes may use myelin components to support cell survival and differentiation To that end, we used Alexa 488 labelled myelin and fluorescent oleate along with time lapse imaging as well as light and electron microscopy immunohistochemistry We observed that cultured oligodendrocytes readily internalize labelled myelin and oleate that are viewed in the cytoplasm and proximal processes Notably, we also found that myelin and myelin derived fatty acids support oligodendrocyte survival and proliferation as well as differentiation in vitro while the number and size of mitochondria remained unaffected In turn, intracerebroventricular injections of fluorescent myelin and oleate into adult mice after fasting resulted in incorporation of that fatty acid into both oligodendrocyte progenitors and mature oligodendrocyte suggesting that oligodendroglia lineage can use myelin components when nutrients are scarce to sustain the energy demands needed to support their functions Together, these data provide evidence indicating that oligodendroglia internalize myelin components that help them fueling oligodendrocyte proliferation, differentiation and survival The conditions of energy demand in which myelin lipids are used by oligodendroglia remain to be elucidated.

P11

Boosting myelination as a therapeutic strategy in autism

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The neural basis of abnormal social behaviour in autism spectrum disorders (ASDs) is still not fully understood. Previous studies have shown that altered myelination might be involved in the pathophysiology of ASD. We have characterized the myelination process during development in the cntnap2-/- mouse model of ASD. We observed that oligodendrocyte differentiation and myelination is transiently impaired during cortical development in cntnap2-/- mice. Thus cntnap2-/- mice showed a reduction in myelinated fibres, in the nodes of Ranvier number and in the proportion of mature oligodendrocytes in the somatosensory cortex as well as in the corpus callosum. Moreover, we detected an increase in the number of Caspase-3+ oligodendrocytes, most of them in microglia phagocytic pouches, suggesting that oligodendroglial survival is also affected during development. Although myelination is nearly entirely restored in adult mice, a more detailed analysis of the column pattern of myelination revealed an altered orientation of myelinated fibres. Therefore, we hypothesized that restoring OL differentiation or promoting myelination during the brain plasticity window could partially reverse neurodevelopmental abnormalities as well as cognitive and social behaviour disturbances in ASD. Clemastine, a histamine H1 antagonist with anticholinergic properties against muscarinic M1 receptor, has promyelinating properties. Pranlukast, an antagonist of the orphan receptor GPR17, promotes oligodendrocyte differentiation and myelination. Treatment with the two drugs during development restored myelin deficits and rescued social deficits and behavioural inflexibility in cntnap2-/- mice. Our findings corroborate that myelin

deficits are associated with ASD and that promoting myelination could be a therapeutic target in this disorder.

P12

Nigrostriatal degeneration determines dynamics of glial inflammatory and phagocytic activity

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Glial cells are key players in the initiation of innate immunity in neurodegeneration. Upon damage, they switch their basal activation state and acquire new functions in a context and time-dependent manner. Since modulation of neuroinflammation is becoming an interesting approach for the treatment of neurodegenerative diseases, it is crucial to understand the specific contribution of these cells to the inflammatory reaction and to select experimental models that recapitulate what occurs in the human disease. In this study we hypothesized that glial activation states depend on time and intensity of dopaminergic neuronal death. Dopaminergic degeneration was induced with two MPTP intoxication patterns, subacute (sMPTP) and chronic (cMPTP). Our results show that in the sMPTP mouse model, the pro-inflammatory phenotype of striatal CD11b+ cells was counteracted by an anti-inflammatory astrocytic profile. In the midbrain the roles were inverted, CD11b+ cells exhibited an anti-inflammatory profile and astrocytes were pro-inflammatory. The overall response generated resulted in decreased CD4 T cell infiltration in both regions. Chronic MPTP exposure resulted in a mild and prolonged neuronal degeneration that generated a pro-inflammatory response and increased CD4 T cell infiltration. At the onset of the neurodegenerative process, microglia and astrocytes cooperated in the removal of dopaminergic terminals. With time, only microglia maintained the phagocytic activity. In the midbrain, astrocytes were the main phagocytic mediators at early stages of degeneration while microglia were the major phagocytic cells in the chronic state. In this scenario, we questioned which activation pattern recapitulates better the glial activation in PD. Glial activation in the cMPTP mouse model reflects many pathways of their corresponding counterparts in the human brain with advanced PD. Altogether, our results point toward a context-dependent cooperativity of microglia/myeloid cells and astrocytes in response to neuronal damage and the relevance of selecting the right experimental models for the study of neuroinflammation.

P13

Maturation-state dependent oligodendrocyte metabolic profile and lactate shuttle

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Oligodendrocyte precursor cells (OPCs) respond to neuronal signals and give rise to myelinating oligodendrocytes during development and adulthood. In turn, mature oligodendrocytes play a key role in glial-axonal signaling and metabolic support to neurons by providing the monocarboxylates lactate and pyruvate to the axonal compartment in an activity-dependent manner. Oligodendrocytes and OPCs lineage cells are involved in a growing number of neurological disorders and current evidence suggests that deficits in oligodendrocyte bioenergetic supply to neurons leads to axonal pathology. Nonetheless, the specific features of oligodendrocyte-axonal signaling during oligodendrocyte lineage progression remain poorly understood.Here, we have investigated the metabolic profiles of OPC and oligodendrocytes and their ability to release lactate in respond to neuronal signals using a combination of biochemical and imaging techniques applied to cell cultures. Differentiated oligodendrocytes displayed higher ability to release monocarboxylates than OPCs in basal conditions as determined by the analysis of extracellular lactate content. Consistently, mature oligodendrocytes showed increased basal glycolytic rates and reduced resting mitochondrial oxygen consumption as compared to precursor cells. This positive switch in ability of oligodendrocytes to release lactate was associated to an increased expression of monocarboxylate transporter 1 (MCT1) in differentiated cells. Further determinations of lactate concentrations in oligodendrocyte cultures and imaging of extracellular lactate using the newly generated biosensor eLACCO2.9 show that the ability of oligodendrocyte lineage cells to shuttled lactate in response to neuronal signals - such as glutamate, K+ and NH4+ - depends on their differentiation stage.These results suggest that oligodendrocyte maturation modulates the ability of these cells to release bioenergetic substrates in response to physiological stimuli associated with neuronal activity with potential implications for glio-axonal coupling in health and disease. Key words: oligodendrocytes, oligodendrocyte precursors, mitochondria, lactateAcknowledgements: Funded by FEDER and ISCIII (PI21/00629), the Basque Government (PIBA_2023_1_0046) and ARSEP Foundation.

P14

Searching for New Targets Involved in the Adaptive Response of Hypothalamic Astrocytes to Changing Glucose Concentrations

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Hypothalamic astrocytes play pivotal roles in both nutrient sensing and the modulation of synaptic plasticity within hypothalamic neuronal circuits, which are crucial for the control of feeding and systemic glucose metabolism. It has been shown that hypothalamic astrocytes rapidly respond to changes in circulating glucose levels by altering their activity, morphology, and electrical properties. Nevertheless, the differential cascade of events triggered by varying

glucose concentrations is poorly studied. Here, using -omics techniques, we investigate the main molecular components involved in the adaptive response of hypothalamic astrocytes to different energetic states. We have identified distinct signaling pathways modulated in hypo- and hyperglycemia.

P15

Circular RNA-associated eQTLs show stronger association with splicing-QTLs than to expression-QTLs

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Gene regulation is a widely studied process in neurological diseases. Its regulation has been characterized among other in multiple sclerosis (MS). In this context, we study the relation between loci associated with expression (eQTL) of circular RNAs (circRNAs) in people with MS. CircRNAs and their associated expression quantitative trait loci (circ_eQTLs) are essential to understand gene regulation mechanisms. However, their connection with traditional eQTLs and splicing quantitative trait loci (sQTLs) remains unclear. From our data, we hypothesized that circ_eQTLs have a stronger association with sQTLs than with eQTLs due to the close relationship between circRNA formation and RNA splicing.To test this hypothesis, we conducted circ_eQTL analysis by correlating genotypes of 4,824,059 imputed variants in autosomal chromosomes with the expression levels of 22,308 circRNAs. Only cis-QTLs within a maximum distance of 1 Mb were considered. A linear regression model was employed, controlling for group (MS or healthy controls) and sex. Significant interactions were identified with a corrected p-value (FDR) < 0.05, and a filtering threshold of minor allele frequency (MAF) > 0.1 was applied due to the size of our discovery cohort.We compared the identified circ_eQTLs to sQTLs and eQTLs from the GTEx database, which comprised 38,477 circ_eQTLs, 620,835 sQTLs, and 2,414,653 eQTLs. Given the differing sample sizes among these datasets, we employed a bootstrap method to compare their Jaccard indices. Our analysis revealed a significantly stronger relationship between circ_eQTLs and sQTLs compared to eQTLs ($p < 0.05$), suggesting that circRNA expression is more closely associated with splicing mechanisms. Additionally, genetic annotation analysis showed no significant enrichment of specific genetic elements in circ_eQTLs compared to eQTLs and sQTLs ($p > 0.05$). This suggests that the observed associations are broadly related to genetic influences on splicing rather than being driven by specific regulatory elements.In conclusion, our study provides evidence that circ_eQTLs are more strongly associated with sQTLs than with eQTLs, highlighting the importance of splicing in the regulation of circRNA expression. Future research should further explore the functional implications of these associations and the mechanisms by which genetic variants influence circRNA function.

P16

Nutritional-Culinary Intervention for the modulation of the microbiota in MS

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Multiple sclerosis (MS) is a chronic, immune-mediated and demyelinating disease of the CNS in which the immune system attacks the myelin sheath that covers the axons. It has been described that both the composition and function of the intestinal microbiota are altered in MS. The microbiota has a prominent role in the development and modulation of the immune system and it has a close relationship with the central nervous system. Therefore, we consider that the recovery of the microbiota and its function may play a key role in the evolution of MS, having the capacity of improving the quality of life of people with MS.Among the factors that can affect the composition of the microbiota, diet has been identified as one of the most influential in its acquisition and modulation. Therefore, this study proposes a 6 months nutritional-culinary intervention based on Mediterranean diet in a population with relapsing-remitting MS.The objective of the intervention is to evaluate the ability to modulate the intestinal microbiota and to observe its capacity to improve the evolution of the disease and the symptomatology of the patients as well as emphasizing nutritional education. Preliminary results show a reduction in physical fatigue levels as well as a lower probability of having anxiety at the end of the intervention. In terms of biochemical parameters, an increase in the concentration of vitamin D and a decrease in total cholesterol levels are observed. In addition, a reduction in BMI is reported due to a reduction in total fat mass. The analysis of the microbiota reveals a higher Shannon index as well as a significant reduction in the Proteobacteria phylum 6 months after starting the intervention.In general, positive feedback has been obtained from the participants, as well as an improvement in the quality of life in those who have most adhered to the proposed dietary guidelines.

P17

A PROPOSED WORKFLOW TO ANALYZE BACTERIAL TRANSCRIPTS IN RNASEQ FROM BLOOD EXTRACELLULAR VESICLES OF PEOPLE WITH MULTIPLE SCLEROSIS

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The taxonomical characterisation of bacterial species derived from genetic material blood, including reads derived from bacterial extracellular vesicles (bEVs) poses certain challenges, such as the proper discrimination of true reads from contaminating reads. This is a common issue in taxa profiling and can lead to the false discovery of species that are present in the sample. To avoid such biases a careful approximation to taxa profiling is necessary. In this work we propose a workflow to analyze the presence of bacterial transcripts as indicative of putative bEVs circulating in the blood of people with MS (pwMS). In total, 4 RR-MS, 4 SP-MS and 4 HC samples are analyzed. The workflow includes several reference mapping steps against the host genome and a consensus selection of genera based on different taxa profilers. The consensus selection is performed with a flagging system that removes species with low abundance or with high variation across profilers. Additionally, the inclusion of biological samples from known cultured species as well as the generation of artificial reads constitute

two key aspects of this workflow. Finally, in silico samples with known species were created to benchmark the efficacy of the taxa profilers.Analysis of taxa profiler efficiency with in silico samples shows a balance between accurate assignation of reads to the genera, and the assignment of reads to not selected genera. Regarding the workflow, the inclusion of the 3-fold host mapping and flagging system show to be necessary since, even after the first two mapping steps, a vast range of reads are still being assigned to the host genome by the profilers. Additionally, the flagging system proves to be successful in discarding genera that are likely to be assigned spuriously by the profilers. The inclusion of bacterial controls also proves to be successful to discard species that are highly likely to be detected due to sample handling contamination. For instance, several genera, such as Cutibacterium or Dermacoccus, present in the skin and likely to be detected due to sample manipulation, are discarded thanks to the inclusion of bacterial control samples. Comparison of abundancy of genera between MS and HC samples show specific differentially abundant genera, such as Janibacter being more abundant in HC samples compared to RR-MS; 3 genera being more abundant in SP-MS compared to HC, and 5 genera being more abundant in SP-MS compared to RR-MS. No differentially abundant species were discovered after comparison between sexes. The low sample number highlights the necessity to include further samples to gain significance in the differential abundance comparisons.In this work we present a robust pipeline that shows the necessity of stringent filtering and sample-control matching to minimise the false-positive bias risk, common in metagenomic studies. Additionally, this study proves the existence of bEVs in blood plasma, both in MS and HC, with putative genera being differentially abundant between MS and control samples.

P18

Time-course of neuroinflammatory processes in alpha-synuclein-based models of Parkinson’s disease

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Parkinson's disease (PD) is characterised by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and the abnormal accumulation of the protein alpha-synuclein (alpha-syn). Previous studies in animal models of PD suggest that neuroinflammation and glial cell activation contribute to disease onset and progression. The aim of this study was to investigate the temporal expression of neuroinflammatory markers in an experimental mouse model of PD.Our experimental approach involved the unilateral or bilateral intranigral injection in seven-weeks-old C57BL/6J mice of adeno-associated viral vectors encoding human alpha-syn (AAV-halpha-syn) or empty vectors as control group. Animals were sacrificed at 15, 60, or 120 days after injection. To assess glial activation within

the striatum and substantia nigra (SN), immunofluorescence analysis was performed, and the percentage of area occupied by GFAP+ astrocytes and Iba-1+ microglia was quantified as indicators of neuroinflammatory responses. Our results revealed that alpha-syn overexpression peaked at 60 days post-injection in unilaterally lesioned animals. These animals exhibited an increase in astrocytic activation within the SN as early as day 15, although statistical significance compared to controls was not reached until day 60, and this effect was sustained at 120 days post-injection. Conversely, no differences were observed in the Iba1+ area within the SN or striatum. In bilaterally injected mice, the peak expression of alpha-syn was observed at 120 days post-injection. However, assessment of neuroinflammatory markers in both the SN and striatum showed no significant differences between alpha-syn-expressing animals and controls. In conclusion, these data indicate that unilateral intranigral injection of human alpha-syn promotes sustained reactive astrogliosis in the SN, and may help to understand the key role of neuroinflammation in the onset and progression of PD.Funding: Grant PID2 021-126434OB-I00 funded by MCIN/AEI/ 10.13039/501100011033 and ERDF A way of making Europe. It has also been funded by the Basque Government (IT1706-22, PUE21-03) and the UPV/EHU (COLAB20/07). This research was conducted in the scope of the Transborder Joint Laboratory (LTC) non-motor Comorbidities in Parkinson's Disease (CoMorPD).MZ is supported by UPV/EHU fellowship.

P19

Multimodal imaging evaluation of neurovascular inflammation following experimental cerebral arteriovenous malformation.

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IntroductionBrain arteriovenous malformations (bAVMs) are rare vasculature lesions characterized by abnormal connections between arteries and veins (1). Likewise, bAVMs trigger a cascade of pathological processes that induce blood brain barrier disruption (BBBd), inflammation and loss of patient functionality (2,3). However, the in vivo pathophysiology of vasculature, inflammation, and BBB integrity in bAVMs has been scarcely investigated to date. For this reason, the present study aims to decipher the pathophysiology of bAVM through the evaluation of vascular volume, inflammation, and BBBd.MethodsHai model of bAVM in rats by common carotid artery and external jugular vein fistula formation was used for imaging studies. The vascular volume and oedema were assessed with magnetic resonance angiography (MRA) and T2 weighted magnetic resonance imaging (T2W-MRI) at days 7, 14 and 21 after fistula formation and at 1 and 24 hours after bAVM resection (n=32). The assessment of inflammation was evaluated with positron emission tomography (PET) using [18F]DPA-714 (TSPO ligand) at days 7, 14 and 21 after fistula formation and at 24 hours after bAVM resection (n=10). BBBd was assessed with dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) (n=21). In addition, the in vivo findings were supported by fluorescence immunohistochemistry and Evans Blue at time points used for in vivo studies.Results/DiscussionbAVM resulted in changes in vascular volume at different days of

evolution, which were restored with the resection of the malformation, as determined by in vivo MRA and T2W-MRI. Moreover, bAVM rats did not show differences in [18F]DPA-714-PET uptake between different groups. However, ex vivo inflammation analyses of TSPO labelling showed mainly vascular inflammation in bAVM rats. Besides, a tendency of BBBd was observed along bAVM evolution by DCE-MRI and ex vivo Evans Blue labelling showed BBBd in bAVM rats. Furthermore, ex vivo vascular volume quantifications by fluorescence lectin are currently being conducted.ConclusionOur findings support that bAVM causes vascular damage followed by recovery after the malformation resection and suggest that bAMV causes brain vascular inflammation. Also, BBBd is observed in bAVM rats concluding the vascular alteration caused by this pathology. Therefore, these data shed light in the physiopathology of this cerebrovascular disease.AcknowledgementsThe authors would like to thank A. Lekuona, V. Salinas and Irati Aiestaran for technical support in radiosynthesis and technical assistance in the PET studies. This study was funded by Fundació La Marató de TV3 (248/C/2020). References1. Bazil, M. J., Shigematsu, T., Berenstein, A., Bederson, J., & Fifi, J. T. (2022). Modern Brain Arteriovenous Malformation Models: A Review. Stroke: Vascular and Interventional Neurology. https://doi.org/10.1161/svin.121.0003352. Hai, J., Ding, M., Guo, Z., & Wang, B. (2002). A new rat model of chronic cerebral hypoperfusion associated with arteriovenous malformations. Journal of Neurosurgery, 97(5), 1198-1202. https://doi.org/10.3171/jns.2002.97.5.11983. Revuelta, J. M., Zamarrón, Á., Fortes, J., Rodríguez-Boto, G., Vaquero, J., & Gutiérrez-González, R. (2020). Experimental rat model of chronic cerebral hypoperfusion-reperfusion mimicking normal perfusion pressure breakthrough phenomenon. Neurocirugía (English Edition), 31(5), 215. https://doi.org/10.1016/J.NEUCIE.2019.11.001Keywords: [18F]DPA-714; PET; bAVM; neurovascular-inflammation; MRI

P20

Temporal dynamics and vascular inflammatory response of blood-brain barrier disruption following ischemic stroke.

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The blood-brain barrier (BBB) is considered a key tissue in the regulation of substance transport, acting as a protective barrier of the brain against different harmful substances and molecules [1]. Dysfunction of the BBB after ischemic stroke affects endothelial cells located around brain capillary vessels, leading to an increase in BBB permeability [2]. This increased permeability is also associated with an inflammatory response in the cerebral parenchyma [3]. Therefore, the aim of the present study is to characterise the dynamics and inflammatory response associated with BBB dysfunction during the subacute (1, 3 and 7 days) and chronic (14, 21 and 28 days) phases on a rat model of ischemic stroke. For this purpose, dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) was performed to evaluate the in vivo BBB integrity (Ktrans) using gadolinium as the contrast agent. The results showed a progressive increase in the permeability during the first week peaking at day 7 after ischemia,

followed by a recovery of the BBB permeability values during the chronic phase. Additionally, a temporal assessment of ex vivo BBB disruption under ischemic conditions was conducted using Evans Blue dye extravasation. This study revealed a similar temporal BBB permeability profile to that observed with in vivo MRI. Moreover, these results were also supported by immunohistochemical evaluation of vascular cell adhesion molecule (VCAM) expression in brain blood vessels. These analyses confirm a significant vascular inflammatory response associated with BBB disruption under ischemic conditions. Overall, this study elucidates the dynamic temporal progression of BBB disruption following cerebral ischemia and highlights the critical role of vascular neuroinflammation. Therefore, we believe that this research is essential for understanding the role of vascular pathophysiology during the subacute and chronic phases of stroke, and highlights potential therapeutic targets for mitigating ischemic injury and preserving BBB integrity.References:1. Abdullahi, W., Tripathi, D., & Ronaldson, P. T. (2018). Blood-brain barrier dysfunction in ischemic stroke: Targeting tight junctions and transporters for vascular protection. American Journal of Physiology. Cell Physiology, 315(3), C343-C356. doi: 10.1152/ajpcell.00095.20182. Okada, T., Suzuki, H., Travis, Z. D., & Zhang, J. H. (2020). The Stroke-Induced Blood-Brain Barrier Disruption: Current Progress of Inspection Technique, Mechanism, and Therapeutic Target. Current neuropharmacology, 18(12), 1187,1212. doi: 10.2174/1570159X18666200528143301 3. Kawabori, M., & Yenari, M. A. (2015). Inflammatory responses in brain ischemia. Current medicinal chemistry, 22(10), 1258, 1277. doi: 10.2174/0929867322666150209154036

P21

Evaluation of neuroinflammation in a chronic unpredictable mild stress (CUMS) animal model

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Background: Neuroinflammatory hypothesis of depression states that the brain of patients with major depressive disorder (MDD) presents low-grade inflammation which may induce impairments in areas associated with mood and cognition. A dysregulated hypothalamic-pituitary-adrenal axis (HPA) could underlie this inflammatory response, triggered by environmental risk factors. Elevated levels of proinflammatory markers have been reported in peripheral blood of depressed patients. Chronic stress, in particular, has repeatedly been associated with increased risk to develop MDD. Chronic unpredictable mild stress (CUMS) model of MDD recapitulates broad symptomatic dimensions and neurochemical alterations related to the disease. However, studies report high heterogeneity in the neuroinflammatory state of chronically stressed rodents, as conflicting data have emerged.Aim: The aim of the current study was to evaluate the effect of chronic stress-induced HPA hyperactivity on the inflammatory status of CUMS animal model of MDD.Methods: Adult male C57BL/6J mice were randomly assigned to CUMS or control group (n=16 per group). CUMS animals were subjected to a stress protocol for 6 weeks, consisting of moderate stressful stimuli (confinement, stroboscopic lighting, hot/cold exposure, wet bedding, noise). Subsequent to behavioural evaluation of depressive/anxiety-like phenotype, mice were euthanized on week 8. Adrenal glands were harvested and weighed. Peripheral blood was collected and a subset of plasma

samples was used for cytokine evaluation by ELISA Luminex¬¨VÜ immunoassay (n=10 per group). The following markers were determined: interleukin (IL)-1alpha, IL-1≈í,â§, IL-2, IL-6, tumour necrosis factor (TNF)-alpha, interferon (IFN)-≈í,â• and IL-10. Brains were harvested and a subset of cortices were used for RNA extraction (n=8 per group). Quantitative real-time PCR was performed on single-stranded cDNA synthesised from DNase-treated RNA for Rela, Nfkbia, Il1b, Il6, Ifg, Tnf and Aif1, genes encoding for intracellular messenger NF≈í,à´B, its repressor I≈í,à´Balpha, inflammation markers IL-1≈í,â§, IL-6, TNFalpha and IFN-≈í,â•, and microglial marker Iba-1, respectively. All results were analysed using unpaired t test.Results: CUMS mice had increased adrenal gland weight compared to controls (t=11.53, p<0.0001). CUMS mice did not exhibit changes in peripheral IL-1alpha (t=0.65, p=0.53), IL-1≈í,â§ (t=0.80, p=0.44), IL-2 (t=0.93, p=0.37), IL-6 (t=1.27, p=0.22), IFN-≈í,â• (t=0.71, p=0.49) and IL-10 (t=1.09, p=0.29) compared to controls. Unexpectedly, TNF-alpha was found to be lower in CUMS compared to controls (t=2.52, p<0.05). Regarding cortical gene expression, no differences were found between control and CUMS mice for Rela (t=0.37, p=0.72), Nfkbia (t=0.31, p=0.76), Il1b (t=0.53, p=0.60), Il6 (t=1.61, p=0.13), Ifng (t=1.10, p=0.29), Tnf (t=1.01, p=0.33), and Aif1 (t=0.18, p=0.86).Conclusions: In spite of inducing behavioural impairments (including anhedonia, behavioural despair, apathy and anxious phenotype), HPA hyperactivity was not associated with the presence of hallmarks of inflammation in CUMS. The use of different animal models for the study of MDD, or a different time-point for tissue harvest subsequent to chronic stress should be considered for future studies on inflammatory processes associated to psychiatric illness.

P22

ROle of Kv channels in hearing loss and correlation with Alzheimer Disease

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IntroductionNeurodegeneration plays a crucial role in multiple sclerosis (MS), leading to progressive neuronal damage and functional decline. These detrimental changes are exacerbated by age-related immune alterations, collectively known as immunosenescence, which impair the immune system's capacity to trigger effective responses. One of the most characteristic phenomena of immune system aging is inflammaging, the chronic low-grade inflammation observed in the elderly. Marked by a sustained elevation of pro-inflammatory molecules, inflammaging has been shown to significantly contribute to the development and progression of various age-related diseases, including neurodegenerative disorders.Understanding the intricate interplay between aging-related immune dynamics and disease pathology is essential for optimizing therapeutic interventions and improving clinical outcomes.ObjectivesThe objective of our study was to analyze the inflammaging characteristic cytokines, addressing their age-associated patterns in both MS patients and healthy controls. Additionally, we aimed to analyze the levels of C-reactive protein (CRP) and neurofilament-light (NFL) as overall biomarkers of inflammation and neurodegeneration, respectively. MethodsPlasma samples were collected from 76 MS patients and 66 healthy controls (HCs) from several age ranges. The concentration of key cytokines (IL1-beta, IL-6, IL-8, IL-10 and TNF-alpha), CRP and NFL, were analyzed with ELLA instrument from ProteinSimple, an automated

immunoassay system.ResultsThe cytokine analysis exhibited elevated IL-6, IL-10, and TNF-alpha concentrations in MS patients. No differences were observed in CRP levels. Importantly, NFL levels were markedly elevated in MS patients compared to HCs.Regarding the age-correlations, IL-6, TNF-alpha, and NFL exhibited a significant age-related increase in both HCs and MS patients. It is noteworthy that for NFL, the correlation with age is stronger in the controls than in the patients. Conversely, the plasma concentration of IL-10 and CRP did not correlate with increasing age in HCs, whereas MS patients showed a significant increase of these proteins with age.ConclusionsOur findings showed relevant differences in several immune mediators in MS and with aging. As previously described, we reported increased levels of the neurodegeneration marker NFL in MS compared to HCs and we confirmed the elevation of this molecule in both groups with increasing age, with a stronger correlation in the case of HC. Regarding inflammation, IL-6 and TNF-alpha are increased in MS and they also correlated with aging in both groups, while for IL-10 and CRP the positive correlation is only significant in MS patients. Our results can help to gain deeper insights into the inflammatory and neurodegenerative processes in MS and their relation to aging. They highlight a marked inflammaging phenomenon in MS patients that should be further investigated.

P23

Transcriptional profiling of plasmacytoid dendritic cells reveals distinct signatures between relapsing remitting multiple sclerosis and healthy controls

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Multiple Sclerosis (MS) is an inflammatory disorder associated with immune abnormalities in the central nervous system (CNS) characterized by demyelination and nerve damage. Primary progressive (PP) MS is clinically defined as a disease in which neurological deficits accumulate in the absence of the exacerbations and remissions that are typical for the relapsing-remitting (RR) form of the disease. Evidence is available that the pathogenic mechanisms are partially different between both these major forms of the disease. Dendritic cells act as crucial actors in MS because of their function in presentation of antigen. Plasmacytoid dendritic cells (pDC) have been highlighted for their crucial role in either activating or suppressing effector T cells in MS. Cytokines released by pDCs, including type I interferon, IL-6, and TNF-alpha, have been linked to the development of MS. Furthermore, pDCs are present in the cerebrospinal fluid (CSF), leptomeninges and demyelinating lesions of patients with MS. Therefore, the purpose of this research is to analyze changes in the plasmacytoid dendritic cells transcriptome occurring in patients with MS (RR= 31, PP=24) compared to healthy controls (HC=33). We used RNA-Sequencing (RNA-Seq) to determine transcriptome profiles changes, to identify affected pathways and biomarkers with potential clinical use. We performed differential gene expression (DEG) analysis and pathway enrichment analysis by Gene Ontology Term

Enrichment (GO) and KEGG pathway map. In DEG analysis, we identified that the expression levels of 626 genes was significantly different between the two groups RRMS VS HC, with 40% of these upregulated and 60% downregulated.

P24

Uncovering novel biomarkers and therapeutic targets in multiple sclerosis through transcriptomics of conventional dendritic cells

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Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) characterized by inflammation and demyelination as well as axonal and neuronal degeneration. In the majority of patients, the disease begins with a relapsing remitting course (RRMS). However, a minority of patients (10%) show progressive disease from onset (PPMS). These patients have a shorter life expectancy and twice the risk of mortality than RRMS. There is no cure for this disease and unlike for RRMS, only a single treatment has been developed for PPMS. Although the exact causes of this disease are unknown, MS is considered to be predominantly a T-lymphocyte-mediated disease, with professional antigen-presenting cells, i.e., dendritic cells (DCs), playing an important role in the onset and course of the disease. Blood circulating conventional dendritic cells (cDC) display altered functionality and may express different levels of specific genes in MS compared to controls. In fact, studies have demonstrated that production of proinflammatory cytokines by cDC such as IL-12p70, tumor necrosis factor (TNF)-alpha and IL-23p19 is upregulated in RRMS patients, whereas cDC of patients with PPMS have a more immature phenotype compared with healthy controls, as indicated by reduced expression of the costimulatory molecules CD80 and CD86. For this reason, we propose that cDCs may be a source of biomarkers that may uncover novel therapeutic targets for treating MS. We have performed whole-genome gene expression profiling by means of RNAseq in conventional dendritic cells, purified from peripheral blood mononuclear cells (PBMCs) of PPMS and RRMS patients, and healthy controls. In our preliminary results, we were able to identify several genes that were differentially expressed between RRMS, PPMS and healthy controls. Functional enrichment analysis also showed that the pathways identified as most significant were different between the two mayor forms of the disease.

P25

Isolation of dendritic cells for omics approaches in multiple sclerosis research

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Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that promote immunogenic or tolerogenic response based on their subtypes and environmental signals. DCs are bone marrow-derived immune cells that are divided into two major categories: a) conventional DCs (cDC) and; b) plasmacytoid DCs (pDC). In MS, DCs are abundant in brain lesions, and display an altered function compared with healthy controls. This study aims to select the best isolation method for dendritic cDC and pDC from MS patients and healthy controls. For isolation of DCs, peripheral blood mononuclear cells (PBMCs) were extracted of whole blood from patients and controls by using density gradient centrifugation; after that, CD14+ were removed from the PBMCs by positive cell isolation using anti-CD14+ magnetic beads. Then, from CD14- PBMCs, pDC and CDC were enriched using two approaches: a) negative cell selection by labeling unwanted cell types for removal using magnetic beads; b) cell depletion by consecutive cell separation steps using magnetic beads. Finally, the enrichment of the DCs was evaluated by flow cytometry using an antibody panel for PBMC immunophenotyping. Here, we observed that the best approach for isolating cDC and pDC was the depletion method, with high percentages of purity for both cell types (>90%), whereas the negative selection approach presented low degrees of purity (<90%).

P26

In vivo multimodal imaging of purinergic P2X7 receptor in neuroinflammation after experimental stroke

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Introduction. Ischemic stroke leads to an increase of the concentration of adenosine 5'-triphospate (ATP). For this reason, purinergic receptors, including purinergic P2X7 receptor (P2X7R), have become of interest in the recent years as possible therapeutic target for ischemic stroke1. Nevertheless, the diagnostic and therapeutic potential of these receptors has been scarcely evaluated. Hence, the aim of this work is to monitor the in vivo expression of P2X7R in healthy and ischemic conditions and to study the role of P2X7R in brain inflammation by non-invasive imaging techniques.Methodology. The expression of P2X7R was evaluated with positron emission tomography (PET) ([18F]JNJ-64413739)2 before and at days

1, 3, 7, 14, 21 and 28 after a transient middle cerebral artery occlusion (tMCAO) in rats (n=18). Likewise, magnetic resonance imaging (MRI-T2W) studies were conducted at 24 hours to assess the extent of brain damage. PET signal uptake was quantified (%ID/cc) in different brain regions and radiotracer kinetic values were obtained with the Simplified Reference Tissue Model (SRTM)3 (n=7). Additionally, the possible role of P2X7R on brain infarction and inflammation after ischemic response was studied after the treatment with an antagonist of P2X7R during the following week after ischemia (vehicle, n=5; A 804597, 1mg/kg, n=6) by MRI and PET ([18F]DPA-714-inflammation). Currently, we are conducting ex vivo studies to validate the results obtained by in vivo imaging modalities. Results. [18F]JNJ-64413739-PET signal (%ID/cc) and kinetic studies showed that in healthy conditions P2X7R was barely expressed in the brain, with an almost negligible value of non-displaceable binding potential (BPnd) in different brain regions, including striatum, hypothalamus, thalamus, cortex, and hippocampus. At day 1 after stroke, MRI-T2W showed a cortico-striatal infarction triggering a progressive increase of the PET signal uptake (%ID/cc) until day 7 after stroke, followed by a gradual PET signal decline in both cortex and striatum. Additionally, the effect of the inhibition of P2XR7 was studied by MRI and PET, results showing non-significant differences of the ischemic volume between vehicle and treated groups. [18F]DPA-714-PET imaging demonstrated a lower uptake of [18F]DPA-714 in the treated group at day 7. Conclusions. The present study shows low BPnd values of [18F]JNJ-64413739 in the rat brain under healthy conditions that significantly increase after ischemic stroke. Likewise, this work also demonstrates a lower signal of [18F]DPA-714 after the inhibition of P2X7R, confirming the role of these receptors in the neuroinflammatory response underlying stroke. All these findings evidence that [18F]JNJ-64413739 is a valuable radiotracer to decipher inflammation after cerebral ischemia. References. [1] Burnstock, G. (2020). Purine and pyrimidine receptors. Cellular and Molecular Life Sciences, 64(12):1471-83.[2] Berdyyeva, T., et al. (2019). PET Imaging of the P2X7 Ion Channel with a Novel Tracer [18F]JNJ-64413739 in a Rat Model of Neuroinflammation. Molecular imaging and biology, 21, 871-878.[3] Lammertsma, AA, & Hume, SP (1996), Simplified Reference Tissue Model for PET Receptor Studies, NeuroImage, 4(3), 153-158.

P27

Peripheral and central nervous system cholesterol metabolism in people with multiple sclerosis

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Multiple Sclerosis (MS) is a chronic, immune-mediated, and heterogeneous disease characterized by demyelination, axonal damage, and physical and cognitive impairment. An increasing body of evidence suggests that alterations in cholesterol metabolism are associated with MS clinical progression. Cholesterol is essential for immune regulation and signaling and

is a crucial constituent of myelin. Non-cholesterol sterols (NCS) are widely used in biomedical research as surrogate markers for cholesterol absorption and synthesis. Oxysterols are oxygenated cholesterol metabolites, rapidly traverse the blood brain barrier and act as signaling mediators between the periphery and the CNS, amongst others helping maintain cholesterol homeostasis in the brain.In this study, besides cholesterol concentrations, also cholesterol-standardized levels of the non-cholesterol sterols (lathosterol, desmosterol, cholestanol, campesterol and sitosterol) and oxysterols (24-hydroxycholesterol, 25-hydroxycholesterol and 27-hydroxycholesterol) were determined in serum as-well-as cerebrospinal fluid (CSF) samples from 95 individuals with relapsing-remitting MS (RRMS), 22 individuals with clinically isolated syndrome (CIS), 10 individuals with primary progressive MS (PPMS) and 49 control subjects. Our results revealed alterations in cholesterol metabolism of MS patients, which were characterized by higher levels of cholesterol and cholesterol synthesis markers in the serum of individuals with MS. Conversely, an opposite trend, i.e lower cholesterol, lathosterol and desmosterol levels in MS patients versus controls, was observed in CSF samples, indicating a contrasting pattern between the periphery and the CNS. The serum-CFS correlation pattern was specific to the form of the disease. Based on our findings, we speculate that the lower endogenous synthesis in the brain of MS patients might underly their pathological developments. Additionally, some molecules were identified as potential biomarkers of diagnosis and disease progression. In summary, these advances in the field have the potential to improve our understanding of MS pathophysiology and aid in the identification of new biomarkers for diagnosis and monitoring as well as for treatment targets and strategies.

P28

Cerebral ischemia models optimised for two-photon imaging

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Brain ischemia is one of the most devastating diseases in the western world and causes long lasting disabilities in those suffering from it. Despite this, there is no effective treatment for cerebrovascular accidents due to the complex anatomy of the brain. Brain ischemia treatment is an ongoing research challenge. As a result, new methods are under development to study brain stroke from the onset to the chronic phases of the disease1.Here, we developed two stroke models to study brain ischemia using a two-photon microscopy. This microscopy allows us to analyse the progression of the disease in vivo being possible to examine blood-flow evolution after artery occlusion, clot formation, angiogenesis and even more, its relationship with the structural lesion1&2. In our work, the stroke model was induced via permanent ligation of one of the middle cerebral artery branches that irrigate the right somatosensory cortex of 2-month-old C57BL6 mice3. Our second model comprises the same steps but removing the not after 60 minutes of occlusion. Stroke models were characterized at 1, 3, 7, 15 and 28 days after the onset of the disease by two-photon imaging and immunohistochemistry as a control technique. Neuronal death, infarct volume, cellular reactivity and vascular architecture were measured. Our results indicate a new approach to study brain ischemia in live animals that allows real-time hemodynamic analysis following

brain stroke.The development of effective stroke models is essential to test promising therapeutic tools and achieve results with more accurate systems such as the two-photon microscope1. Only with this type of methodology that hard-to-reach ischemia events can be studied such as the blood-brain barrier breakdown.

P29

Mitochondrial Activity: A Biomarker for Motor Rehabilitation after FES treatment

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Strokes lead with motor disorders secondary to the initial injury. The oxygen deprivationtriggered by the lack of blood irrigation increases the oxidative stress at mitochondriallevel, leading to tissue damage, which correlate with motor function impairments.Functional electrical stimulation (FES) therapy can restore mitochondrial activity instroke patients and, in turn, improve muscular motor function.To study the impact of FES therapy on the mitochondrial electron transport chainactivity (ETC) and motor function, in vitro analyses were conducted using ultraviolet-visible spectrophotometry of the activity of complexes I, II, and IV, glycerol-3-phosphatedehydrogenase (GAPDH), and monoamino oxidase (MAO) in peripheral bloodmononuclear cells (PBMCs) from stroke patients. Complex II and MAO activity werealso determined in platelets. Motor function was assessed using various physiotherapyscales. Both variables were measured before and after 12 FES treatment sessions (2per week).The results revealed that the activity of ETC complexes, G3PDH, and MAO wassignificantly reduced in pre-treatment stroke patients, both in PBMCs and platelets,except for complex II in platelets and MAO in PBMCs. However, after FES treatment,mitochondrial activity of all complexes, G3PDH, and MAO was significantly increasedin stroke patients in both cell types. Additionally, significant correlations were detectedbetween enzymatic activity recovery and motor function in stroke patients.In conclusion, preliminary studies indicate that FES can restore motor function andmitochondrial activity, which are impaired in stroke patients. However, further studiesare needed to determine if changes in mitochondrial activity and motor function can beused as biomarkers of pathology and/or FES treatment efficacy.

P30

Early Mechanisms of Neurotoxicity associated with the intercellular Spreading of TDP-43 Pathology

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NA

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive motor neuron (MN) dysfunction and degeneration. The spread of neurodegeneration from an initial trigger to widespread MN loss suggests the involvement of pathogenic factors spreading between neurons and other cell types, including glial and muscle cells. TDP-43 proteinopathy, marked by cytoplasmic accumulation and nuclear depletion of TDP-43, is a major hallmark of ALS, likely leading to toxic gain of function.Rather than focusing solely on neurons bearing TDP-43 mutations, where compensatory mechanisms may obscure disease initiation, we investigate the impact of toxic factors from other pathological cells on healthy MNs to identify early degenerative mechanisms. We treated human iPSC-derived MNs with conditioned medium (CM) from various ALS-related cellular models with TDP-43 pathology. CM from muscle cells with mutant TDP-43 led to hyperexcitability followed by hypoexcitability and cell death in MNs. RNA-Seq analysis revealed upregulation of cell cycle and DNA metabolism pathways, along with an immature transcriptome, and downregulation of synaptic genes. Conversely, CM from muscle cells with loss of TDP-43 function induced hypoactivity in both control and mutant motor neurons.We anticipate comparing RNA-Seq results between the two CM treatments will provide insights into pathway dysregulations underlying ALS pathogenesis. Our findings suggest early degenerative mechanisms initiated by toxic inputs from pathological cells, highlighting potential targets for therapeutic intervention.

P31

Energy scarcity and impaired mitochondrial translation induce perinuclear stress granule clustering

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Many proteins linked to amyotrophic lateral sclerosis and fronto-temporal dementia (ALS-FTD) change their cellular location and coalesce in cytoplasmic inclusion bodies in the disease state; yet the factors that govern protein relocation and organization remain unclear. Here, we show that inhibition of glycolysis and mitochondrial protein synthesis causes many proteins involved in ALS-FTD to change location, and form a novel structure comprising a ring of stress granules

encircling the aggresome, a focal microtubule-based structure beside the nucleus. A perinuclear ring of stress granules also forms in activated microglia of mice exposed to the glycolytic inhibitor, 2-Deoxy-D-glucose. We propose that the new arrangement increases the risk of the stress granules merging and converting from the liquid phase to the insoluble inclusion characteristic of ALS-FTD. Thus, our findings suggest that that compromised nutrient and energy metabolism can precipitate a molecular cascade that ultimately leads to the pathological hallmark of ALS-FTD the perinuclear inclusion body.

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Biallelic variants in SNUPN cause a Limb Girdle Muscular Dystrohpy with myofibrillar-like features

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Alterations in RNA-splicing are a molecular hallmark of several neurological diseases, including muscular dystrophies with mutations in genes involved in RNA metabolism. Here, we present five patients from two unrelated families with a Limb-Girdle Muscular Dystrophy (LGMD) phenotype carrying a biallelic variant in the SNUPN gene. The protein encoded by SNUPN, Snurportin-1, plays a key role in the nuclear importation of small nuclear ribonucleoproteins (snRNPs), essential components of the spliceosome. However, it has never been related to human disease before.SNUPN patients showed a similar phenotype characterised by childhood-onset proximal weakness, restrictive respiratory dysfunction and prominent contractures, although interindividual variability wasfound. Muscle biopsies showed aggregates of p62 and myotilin, suggestive of myofibrillar-like features, and MRI showed predominant impairment of paravertebral, sartorius, gracilis, peroneal and medial gastrocnemius muscles. Conservation and structural analyses of the Snurportin-1 variant suggested a potential deleterious effect. In patient-derived fibroblasts, mislocalization of UsnRNP components was observed. Furthermore, RNA-splicing analysis in patients' muscle showed widespread splicing deregulation, specially in genes that are relevant for muscle development and splicing factors that participate in spliceosome assembly.We report that SNUPN variants are a new cause of LGMD according to its current definition, and it should be included in genetic testing of myopathies. These results further support the relevance of splicing-related proteins in muscle disorders.

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MP-001, a novel FKBP12 ligand, is a therapeutic candidate for Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a progressive and severe disease caused by mutations in dystrophin gene. Calcium (Ca2+) dysregulation and oxidative stress are two pathological mechanisms that underlie fiber death in DMD. Ryanodine receptors (RyRs) are Ca2+ channels responsible for sarcoplasmic reticulum Ca+2 release into the cytosol. Several molecules and post-translational modifications tightly regulate RyRs. In particular, FKBP12 proteins stabilize the close state of the channels. However, in DMD, oxidative stress induces modifications in RyRs that result in FKBP12 dissociation and Ca2+ leak into the cytosol. Within the group we have designed, synthetized and evaluated a novel family of FKBP12 ligands, named MP compounds that act as calcium modulators and chelators of reactive oxygen species. In this work, we have used high-throughput in vitro assays using microelectrode-array plates to identify a therapeutic candidate for DMD, based on the therapeutic index. Our compounds have shown efficacy as calcium modulators and protective agents against

oxidative stress. Among them, MP-001 presents favorable in vitro profile and extremely high therapeutic index. Also, MP-001 reaches heart and muscle tissues at therapeutic concentrations after oral administration in mice. Moreover, MP-001 can effectively protect against cardiac deficits in the mdx mouse model of DMD, reducing the long QTc syndrome and preventing cardiomyocyte death induced by isoproterenol challenge. Therefore, we propose MP-001 as a lead candidate for further development into the preclinical regulatory phase.

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Phenotyping novel RyR1 variants using in vitro human models

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Congenital myopathies are often associated with mutations in the ryanodine receptor type 1 (RyR1), the major calcium channel located in the sarcoplasmic reticulum of skeletal muscle fibers. RyR1 is essential for the transduction of the excitatory signal from the motor neuron, which involves the release of calcium stores from the sarcoplasmic reticulum in a process known as excitation-contraction coupling. Dysfunctional RyR1 channels can lead to abnormal muscle contraction by disrupting calcium homeostasis. In this study, we used patient-derived immortalised myotubes and HEK293 cell lines carrying RyR1 mutants to assess the functional impact of two novel RyR1 variants of uncertain pathogenicity: L2286V and G4638V, located in the central and C-terminal hotspots, respectively. Calcium handling studies in living cells revealed altered resting cytosolic and sarcoplasmic calcium levels associated with RyR1 mutations, together with abnormal spontaneous or electrically evoked calcium events. Impaired calcium signaling in HEK L2286V cells is consistent with atypical ryanodine binding and altered electrical activity at the single channel level. MP-001, a novel FKBP12 ligand that stabilizes RyR1 function under oxidative stress, partially rescues pathological phenotypes of L2286V and G4638V cellular models, opening the window to novel therapies for ryanodinopathies, such as central core disease or malignant hyperthermia.

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Feasibility of using multi-channel EMG signals for the calibration of a non-invasive multi-field FES system

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Functional Electrical Stimulation (FES) is a therapeutic technique widely used in rehabilitation, particularly for patients with neurological disorders. It consists on the application of electrical stimuli to the peripheral nerves in order to elicit muscle contractions of the paralyzed muscles and thus, allow the restoration of functional tasks. Within the non-invasive FES technology, multifield electrodes present a breakthrough due to a more selective stimulation and the ability to adjust stimulation parameters easily.When FES is aimed at the upper limb, the successful execution of different grasps and tasks depends on the precise calibration of stimulation sites and parameters, which is a challenging task due to the complex neuromuscular and biomechanical nature of the human hand and the high inter-patient variability. In this work, we show the feasibility of using multi-channel electromyography (EMG), and more concretely, stimuli-related M-wave characteristics, for performing an advanced automatic calibration of a multi-field FES system.A custom-built stimulator (Rehyb Stim 1.5) and a custom-designed multi-field electrode with 16 fields (15x25mm) were used. The electrode was placed covering distal/proximal and anterior/posterior sides of the forearm. The EMG recording was performed using the commercial Bittium NeurOne system, with 8 bipolar channels placed in the forearm in the form of a double ring in the midline between the wrist and the elbow.Stimuli of 5 seconds of duration with bursts of biphasic symmetric pulses of 250≈í¬∫s of duration were applied to a healthy volunteer with different stimulation parameters, such as frequency, amplitude and location. The results of the tests showed that the M-wave prominence varied depending on the distance from the active fields to the EMG electrodes. Its relative location also had an effect, as proximal stimuli generated a higher response on comparison with distal stimuli, which we assume that is caused by the physiological action potential transmission direction of efferent nerves. Finally, the M-wave prominence also showed an ascending trend within the same stimulus with high frequencies, which we assume it is an effect caused by the temporal summation of recruited fibres at frequencies above the tetanic muscle contraction threshold.In conclusion, based on these preliminary results, we believe that multi-channel stimuli-related M-wave characteristics, could be used for automatically calibrating non-invasive multi-field electrode FES systems.

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Mitochondrial activity in blood cells as a prodromic biomarker for Parkinsons disease

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Parkinson's Disease (PD) is the second neurodegenerative disorder with more prevalence. One of its pivot hallmarks is the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and this causes the classical motor symptoms that permit the diagnosis of the disease. Unfortunately, by the time these symptoms appear, SNc neuron loss is greater than 60%. Therefore, it is necessary to find biomarkers that allow the diagnosis in the asymptomatic phases, when treatment can be applied to slow the disease. Lewy bodies are one of the main features of idiopathic PD. These structures, formed in affected brain areas by the accumulation of alpha-synuclein (a-syn), are able to disrupt mitochondrial function, leading

to the formation of reactive oxygen species (ROS), oxidative stress and cell death. Thus, the aim of this study was to measure mitochondrial function in peripheral tissues from a prodromic model of PD. For this purpose, we established a rat model based on viral-vector induced overexpression of a-syn in the SNc. Four and Sixteen weeks after the surgery, we evaluated motor and non-motor behaviour in parkinsonian and control rats, confirming that the model was on a prodromal phase since no symptoms were found. However, animals showed mild dopaminergic degeneration in SNc and striatum (20% degeneration) at sixteen weeks. We also collected blood and brain samples to develop cell membrane microarrays (CMMAs) from platelets and PBMCs to analyze mitochondrial electron transport chain (ETC) activity. Mitochondrial activity was studied in CMMAs with platelets and PBMCs, cytochrome C oxidase activity showed significant changes in the a-syn group. By contrast, the level of serum inflammatory cytokines was similar in both groups (IL-1b, IL-2, IL-6, IL-10, IFNg and TNFa). Although further investigations are needed, this work shows that mitochondrial activity could serve as a potential biomarker in the prodromal phases of Parkinson¬¨¬•s Disease. Funding: Grant PID2 021-126434OB-I00 funded by MCIN/AEI/ 10.13039/501100011033 and ERDF A way of making Europe. It has also been funded by the Basque Government (IT1706-22, PUE21- 03, PIBA_2020_1_0048 and Bikaintek program: 007-B2/2020) and the UPV/EHU (COLAB20/07). This research was conducted in the scope of the Transborder Joint Laboratory (LTC) non-motor Comorbidities in Parkinson's Disease (CoMorPD).

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Insights from dysregulated lncRNAs in Parkinson's disease: characterization of LINC00520 as a putative biomarker of neuroinflammation in human in vitro models

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Parkinson's disease (PD) is a multifactorial disorder and represents the second major neurodegenerative pathology, after Alzheimer disease. Its global incidence rises with age, sex, environmental factors and genetic susceptibility (1).Over the last few years, long non-coding RNAs (lncRNAs) have emerged as principal regulators in numerous biological processes, specifically in the development of the Central Nervous System (CNS), neuronal proliferation, differentiation and synaptic plasticity, and in maintenance of proper homeostasis. Notably, even a slight alteration in their expression levels has been linked to neurological disorders, such as PD (2,3). We conducted a meta-analysis study to compare the transcriptome of post-mortem brain samples from individuals with Parkinson's disease (PD) and control patients and we were able to identify numerous differentially expressed gene loci. The data revealed a large number of lncRNAs that were never related to the disease. In particular, LINC00520 and LINC00641 showed marked up- and down-regulation, respectively, whereas LINC00674 expression remained stable in both conditions. The aim of this work is to confirm meta-analysis data in different human PD in vitro models, considering the most prevalent cell types in the CNS. Neuronal, microglial and astrocyte cell lines (SH-SY5Y, HMC3, and SV-40

immortalized astrocytes) were exposed to pro-oxidative and pro-inflammatory stimuli, such as 6-OHDA, Rotenone, IFN and TIC (TNFa, IL-1b, C1q) to mimic the typical cellular responses induced during neurodegeneration. Our results indicate a significant over-expression of LINC00520 after treatments in all cell types. Moreover, RNAi experiments using target specific siRNAs against the lncRNA led to an altered oxidative stress and inflammatory response in neurons and microglia. Lastly, we assessed the lncRNA gene expression profile in human induced pluripotent stem cells (hiPSCs), human fibroblasts, and dopaminergic neurons generated from hiPSCs. We found that LINC00520 and LINC00641 were expressed especially in neuronal cell types, indeed LINC00674 showed consistent gene expression levels in fibroblasts compared to the other cell types. Further investigations in cell lines obtained from PD patients are ongoing to better clarify the role of these lncRNAs in the pathogenetic mechanism. 1. Bloem BR, Okun MS, Klein C. Parkinson's disease. Lancet. Jun 12;397(10291):2284-2303 (2021).2. Lyu Y, Bai L, Qin C. Long noncoding RNAs in neurodevelopment and Parkinson's disease. Animal Model Exp Med. Dec 17;2(4):239-251 (2019).3. Lv Q, Wang Z, Zhong Z, Huang W. Role of Long Noncoding RNAs in Parkinson's Disease: Putative Biomarkers and Therapeutic Targets. Parkinsons Dis. Jun 12;2020:5374307 (2020).4. Mariani E, Frabetti F, Tarozzi A, Pelleri MC, Pizzetti F, Casadei R. Meta-Analysis of Parkinson's Disease Transcriptome Data Using TRAM Software: Whole Substantia Nigra Tissue and Single Dopamine Neuron Differential Gene Expression. PLoS One. Sep 9;11(9):e0161567 (2016).

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EFFECT OF ESKETAMINE ON MITOCHONDRIAL ACTIVITY OF BLOOD CELLS FROM PATIENTS DIAGNOSED WITH TREATMENT-RESISTANT DEPRESSION

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Treatment-resistant depression (TRD) presents a challenge due to a low percentage of patients experiencing symptom improvement, following pharmacological treatment. Esketamine nasal spray emerges as a rapid-acting antidepressant with an established safety and efficacy profile. Additionally, its effectiveness has been associated with various mechanisms of action, including its impact on mitochondrial energy metabolism.To study the impact of esketamine on mitochondrial activity, specifically on the mitochondrial electron transport chain (mETC), we developed microarrays containing peripheral blood mononuclear cells (PBMCs) from blood samples of 12 TRD patients and 6 healthy volunteers. We measured the activity of NADH dehydrogenase (complex I), succinate dehydrogenase (complex II), cytochrome c oxidase (complex IV), glycerol-3-phosphate dehydrogenase (mitochondrial shuttle), and monoamine oxidase (MAO) before and after esketamine treatment. Additionally, the activity of complex IV was determined spectrophotometrically in platelets.The results showed that intranasal esketamine treatment significantly reduced depressive symptoms, as demonstrated by scores obtained on the MADRS and BDI II scales. Moreover, the activity of complex IV, in PBMCs and platelets, was significantly reduced in TRD patients compared to healthy volunteers. However,

following esketamine administrations, the activity of this complex increased significantly in both cell types. It is important to note that there was a negative correlation between complex IV activity and BDI II scores, both before and after esketamine treatment, in PBMCs of TRD patients.The initial findings suggest that esketamine has the potential to restore the diminished mitochondrial activity of complex IV observed in TRD patiens, additionally there is a relationship between the BDI II clinical scale score and the activity of the complex IV. However, further research is required to determine whether these alterations in mitochondrial function could serve as biomarkers for TRD pathology and the effectiveness of esketamine treatment in TRD patients.

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Longitudinal characterization of the AD-like pathology in the TgF344-AD rat model: a multi-tracer PET study

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Introduction The transgenic TgF344-AD rat model stands out as one of the most promising and comprehensive Alzheimer's disease (AD) models today, exhibiting age-dependent AD features, such as amyloid beta (Abeta) plaques, neurofibrillary tangles, and neuroinflammation over time.1,2 In an effort to further enhance the in vivo characterization of AD-like pathology in this model, we present a longitudinal positron emission tomography (PET) study combining a second-generation Tau tracer (18F-Florzolotau), a clinically approved Abeta plaque tracer (18F-Florbetaben), and two radioligands that target neuroinflammation by binding to P2X7 and TSPO receptors. Methods Female TgF344-AD rats and age-matched wild-type (WT) F344 littermates (8-10 per group) underwent PET scans at different ages using [18F]Florzolotau (Tau), [18F]Florbetaben (Abeta), [18F]JNJ-64413739 (P2X7) and [18F]-DPA-714 (TSPO) (Fig.1A). PET images were reconstructed using OSEM3D, co-registered with a brain MRI atlas, and time-activity curves (TACs) were generated for different brain regions. Standardized Uptake Values (SUV) were calculated by averaging the last three frames of the scan. For 18F-Florzolotau, SUV ratios (SUVR) were used. Brain tissue samples (2 per group/time point) were collected at different ages for autoradiography and immunofluorescence assays with AT8, Iba1, P2X7, TSPO and Thioflavin. Results/discussion Tau PET with [18F]Florzolotau revealed significantly higher SUVR in the cortex, hippocampus, and cerebellum of AD rats in comparison to WT rats at 18 months and 22 months, indicating increasing levels of Tau over time (Fig. 1B). In vitro studies confirmed the presence of Tau aggregates in AD tissue supporting the in vivo results (Fig. 2). Amyloid plaques were visible in AD rats aged 23 months via Thioflavin staining (Fig. 2). However, despite the observed tracer's specificity for amyloid plaques in an autoradiography assay, our results suggested that [18F]Florbetaben failed to distinguish transgenic animals from healthy controls in vivo, due to excessive non-specific binding to white matter-rich areas (Fig. 1C). To this, an alternative application of the tracer was introduced detecting myelin loss in aged transgenic rats

compared to WT. Enhanced chronic neuroinflammation was detected in vivo in AD rats after 22 months, as evidenced by increased P2X7 and TSPO binding in [18F]JNJ-64413739 and [18F]DPA-714 PET studies, respectively (Fig. 1D-E). Conversely, control animals exhibited similar P2X7R expression levels at 15 months, followed by downregulation at 22 months, suggesting a reconfiguration of microglia in these animals, and with lower TSPO levels than AD. Both tracers exhibited specificity under blocking conditions in vitro, and immunofluorescence confirmed the overexpression of these markers in activated microglia around Abeta plaques in AD tissue (Fig. 2). Conclusions Our study has broadened the understanding of AD-like pathology in the TgF344-AD rat model, providing insights into disease progression using diverse techniques. This evaluation deepens our knowledge of the TgF344-AD model and its relevance to AD-related changes over time. It underscores the potential of various PET tracers, while also acknowledging the translational limitations between animal models and human conditions in certain cases.References1. Chaney, A. M., Lopez-Picon, F. R., Serrière, S., Wang, R., Bochicchio, D., Webb, S. D., Vandesquille, M., Harte, M. K., Georgiadou, C., Lawrence, C., Busson, J., Vercouillie, J., Tauber, C., Buron, F., Routier, S., Reekie, T., Snellman, A., Kassiou, M., Rokka, J., Boutin, H. (2021). Prodromal neuroinflammatory, cholinergic and metabolite dysfunction detected by PET and MRS in the TgF344-AD transgenic rat model of AD: A collaborative multi-modal study. Theranostics, 11(14), 6644, 6667. https://doi.org/10.7150/thno.560592. Cohen, R. M., Rezai-Zadeh, K., Weitz, T. M., Rentsendorj, A., Gate, D., Spivak, I., Bholat, Y., Vasilevko, V., Glabe, C. G., Breunig, J. J., Rakic, P., Davtyan, H., Agadjanyan, M. G., Kepe, V., Barrio, J. R., Bannykh, S., Szekely, C. A., Pechnick, R. N., & Town, T. (2013). A transgenic alzheimer rat with plaques, tau pathology, behavioral impairment, oligomeric Abeta, and frank neuronal loss. Journal of Neuroscience, 33(15), 6245, 6256. https://doi.org/10.1523/JNEUROSCI.3672-12.2013

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A COMPUTER VISION FRAMEWORK FOR 3D HUMAN HAND MOVEMENT EVALUATION: POTENTIAL APPLICABILITY IN MOVEMENT DISORDERS.

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A portion of tests involved in the diagnosis and follow-up of Parkinson's Disease (PD) frequently rely on the visual exploration of a patient performing a specific movement or motor act. Although this approach provides very important information about the state of the patient, it is prone to subjectivity as it depends on the interpretation of the specialized neurologists guiding the test. There is a rising need in developing objective, automatable, quantifiable and robust procedures to properly characterize the motor state of the PD patients.In this work we developed a workflow based on computer vision and deep learning software to semi-automatically annotate behavioral patterns in human models. In particular, we adopted some tests included in the Unified Parkinson's Disease Rating Scale (UPDRS). Namely, Finger Taps, Hand Movements and Rapid Alternating Movements of Hands. To assess the validity of our approach, it was compared to the use of accelerometers, a state-of-the-art approach also used to provide objective and quantifiable evaluation.In this project we used 2 cameras to triangulate key-points in human hands in 3D space. This way we extract more representative information about human movements than using only 2D approaches used in other animal models.To extract the key-points of interest in human hands the use of the deep

learning application Mediapipe was used. With this software we can achieve even real-time key-point extraction. As opposed to the use of an accelerometer, this procedure does not require markers nor wiring that may interfere with the patient. The main hypothesis considered on this project is that this software, combined with an accurate triangulation, should be able to produce as much and even greater amounts of useful information regarding spatial movements as current state-of-the-art approaches. This translates into the possibility of developing better and more precise models. The hardware needed is easily obtainable and affordable without being overly complex, facilitating periodic follow-ups and even at home implementations.In conclusion, the workflow presented here provides an unbiased and quantitative method to extract hand movements with results comparable to other state-of-the-art techniques. It could therefore represent an opportunity to implement for the evaluation and follow-up of the motor disturbances in PD patients.

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Exploring central nervous system-derived extracellular vesicles as biomarkers in multiple sclerosis: insights from a single vesicle analysis

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Multiple sclerosis (MS) is a chronic and demyelinating disease of the central nervous system (CNS). 85% of patients present the relapsing-remitting form (RRMS) of the disease, and 10-15% present a worsening of symptoms from the onset, known as primary progressive form (PPMS). Extracellular vesicles (EVs) are lipid bilayer membrane-delimited nanoparticles whose bioactive cargo reflect the molecular state of their cells of origin. L1CAM and GLAST were reported biomarkers to identify putative neuron- and astrocytes-derived EVs respectively. The aim of this study was to unravel the L1CAM and GLAST EV levels in CSF from MS patients using the ExoView R200+ platform. For this purpose, CD63, CD81, CD9, L1CAM, and GLAST-captured EVs were analysed in unprocessed CSF (diluted 1/10) of MS and non-MS patients using L1CAM, GLAST, and CD9 as detection antibodies. This single EV imaging platform enable us to carry out phenotyping and colocalization analysis in unprocessed samples, avoiding the EV isolation method, and with a clinically feasible volume. Negative controls represented by disrupted EV samples with Triton X-100 were included into the analysis.After analysing L1CAM+ and GLAST+ EVs, neither tetraspanin CD63-, CD81-, nor CD9-captured spots exhibited a significant difference between intact and disrupted EV samples in CSF. Similarly, analysing CD9+ EVs in L1CAM- and GLAST-captured spots, neither L1CAM- nor GLAST-captured spots revealed a significant difference between intact and disrupted EV samples in CSF. These results suggest that putative L1CAM and GLAST CNS-derived EVs showed a very low presence of tetraspanin proteins. Next, GLAST-captured spots showed a significant increase in the number of L1CAM+ and GLAST+ EVs compared to L1CAM-captured spots. So, a lower presence of L1CAM+ EVs compared to GLAST+ EVs in CSF were observed. Finally, the L1CAM+ and GLAST+ signals in L1CAM- and GLAST-captured spots were contrasted among RRMS, PPMS, and non-MS samples. Only L1CAM+ EVs in L1CAM-captured spots and GLAST+ EVs in GLAST- captured spots showed a significant increase when intact RRMS were compared with disrupted EV samples

in CSF. Surprisingly, a significant decrease in the particle count of GLAST+ EVs was detected in CSF from PPMS subjects compared to RRMS and non-MS samples. To conclude, ExoView technology is a sensitive and innovative platform to directly trace a CNS-derived EV biomarker that is altered in MS, helping to overcome the barrier between research and clinical applications of EVs.

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Generation and characterization of midbrain and striatal organoids from hIPSCs.

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Brain organoids are tridimensional cultures that imitate the spatial and cellular organization of a particular brain region. Organoids can be generated from induced pluripotent stem cells (iPSC) derived from human skin fibroblasts, which makes them useful tools for studying the development, functioning and even pathology of human brain. By combining specific small molecules, iPSCs can be differentiated into any type of neural cell with region-specific properties. During the last decade midbrain-like organoids enriched in dopaminergic neurons have emerged as a promising tool to model Parkinson's Disease (PD). PD is a progressive neurodegenerative disorder which results from the destruction of dopaminergic neurons of the nigrostriatal pathway. This dopamine pathway projects from the substantia nigra pars compacta of the midbrain to the dorsal striatum of the forebrain. However, in the absence of a target to innervate, dopaminergic neurons in midbrain organoids often lack specific directionality or orientation, and their activity is far from being considered physiological. For that reason, the fusion between midbrain and striatum organoids to generate nigrostriatal assembloids could lead to a substantial improvement in the spatial organization, functionality and physiological relevance of these three-dimensional models. To this end, midbrain organoids were generated using a protocol based on Shh and Wnt signaling pathways, which are key in midbrain development and differentiation of dopaminergic neurons. The peculiarity of our protocol lies in a gradual transition between the different media, which makes the exposure of the cells to guiding small molecules more physiological. The organoids generated by this protocol showed dopaminergic markers (such as FOXA2 or TH) from very early stages of development. On the other hand, striatal organoids were generated based on the protocols published by Miura et al. (2022) and Chen et al. (2022), although in both cases slight modifications, such as progressive media changes, were also applied. Organoids were generated from hiPSCs, both control and (LRRK2(G2019S)) lines. In the near future we plan to generate functional nigrostriatal assembloids to better investigate the dopaminergic neurodegeneration in PD. Once both types of organoids reach their maturation stage, they will be co-cultured in a 1:1 ratio to promote their fusion and obtain assembloids.

P43

Predicting Multiple Sclerosis Disease Status and Progression Using Machine Learning Techniques

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Naru Intelligence

Multiple Sclerosis (MS) results from a complex interplay between host genetic factors and environmental components, with growing attention on the role of the gut microbiota (GM). Recent studies have aimed to predict MS disease progression using Machine Learning (ML) methods in various biological data, including GM. The aim of this study was to assess the viability of using ML methods to support early diagnosis and predict MS disease status (people with MS (pwMS) and healthy controls (HC)) and its progression stages. For that, a workflow was developed for evaluating the best combination of several variables and ML techniques. GM data, along with other demographic and clinical data was used, and feature selection and data transformation techniques were evaluated. Regarding ML classifiers, 6 different were trained. In order to conduct a comprehensive assessment for selecting the optimal combination, several performance metrics were considered: balanced accuracy, recall, precision, f1-score, confusion matrix and ROC curve (AUC score). The workflow revealed the best variable and technique combinations for classifying both disease status and progression, with really satisfactory scores, higher than or around 0.80 across all performance metrics. This demonstrates the potential and viability of predicting MS, based, mostly, on GM data, as well as, that many factors must be considered for achieving the best performance of those predictions.

P44

APOE3 and APOE4 hiPSC-derived astrocytes differentially contribute to Alzheimer's pathology in chimeric mice

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Increasing evidence for a direct contribution of astrocytes as well as different APOE variants to Alzheimer's disease (AD) main pathological hallmarks comes from cellular and molecular studies in rodent models. Yet these models cannot fully mimic how human astrocytes affect AD pathology 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 APOE3 and APOE4 isoforms within the live brain by

transplanting human induced pluripotent stem cell (hiPSC)-derived astrocyte progenitors into neonatal brains of suitable AD model mice. APOE3 and APOE4 transplanted cells similarly differentiate into astrocytes within the mouse host brain and very often integrate in upper layers of one cortical hemisphere. Interestingly, in AD chimeric mice, APOE3 and APOE4 astrocytes differentially affect amyloid-beta load, microglial responses, Tau pathology and neuritic dystrophy. We reveal here a fundamental contribution of human astrocytes to Alzheimer¬¨¬•s pathology in AD chimeric mice and show that human astrocytes with different APOE variants differentially affect main pathological hallmarks of AD. Now to determine the molecular differences between APOE3 and APOE4 astrocytes in AD and wild type chimeric mice, we are planning to do both bulk and single nuclei RNA sequencing of the human astrocytes.

P45

Impact of the LRRK2 G2019S mutation on microglial activity and alpha-synuclein oligomer accumulation in Parkinson's Disease.

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The G2019S mutation in the leucine-rich repeat kinase (LRRK2) is a major cause of familial Parkinson's disease (PD), a complex neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc). This mutation enhances LRRK2 activity, leading to increased aggregation of alpha-synuclein (alpha-syn), potentially accelerating PD pathology. Recent studies in PD animal models have highlighted the role of neuroinflammation in disease progression, with evidence of heightened brain inflammation mediated by microglia in both animal and human carriers of G2019S LRRK2. This research is focused on understanding how the G2019S LRRK2 influences the accumulation of alpha-syn oligomers, as well as microglial activity and response in the SNc, striatum, hippocampus, motor cortex, anterior olfactory nucleus, and medulla oblongata of male and female LRRK2 wildtype and G2019S LRRK2 knock-in mice, aged 12 or 48 weeks. Results have revealed a complex interplay between sex, LRRK2 genotype, and age leading to changes, on the one hand, in the number and distribution of alpha-syn oligomers in the brain, and, on the other hand, in the microglial phenotype, where parameters such as microglia cell number, the phagocytized number of oligomers and cell morphology happen to be altered.

P46

Elevated cholesterol in ATAD3 mutants is a compensatory mechanism that leads to membrane cholesterol aggregation

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Dysregulation of cholesterol metabolism as a consequence of mitochondrial disease occurs in Harel-Yoon syndrome, which have been traced to mutations in the ATAD3 gene cluster. In this study, we report a novel missense mutation in ATAD3A in a family that display a neurological syndrome, and we show that cells carrying this mutation feature cholesterol and mitochondrial abnormalities similar to the other variants. Moreover, ATAD3 mutant cells have an expanded lysosome pool, most of which contain unprocessed membranes - whorls that are a hallmark of lysosomal storage disorders. The use of Drosophila melanogaster with the equivalent pathological point mutation in ATAD3 confirms the severity of the variant and displays a similar expansion in lysosome number as the huamn cells. A new tool to label the membrane-associated cholesterol in vivo shows that cells expressing mutant ATAD3 have aggregates of membrane-bound cholesterol, many of which co-localize with lysosomes. The patient-derived human cells also have increased membrane-bound cholesterol in vitro. Viability is enhanced in the dATAD3 mutant flies raised on cholesterol supplemented diets, indicating a beneficial effect of cholesterol for these mutants. Collectively, these findings strengthen the view that mitochondrial and cholesterol metabolism are interdependent and strongly suggest the elevated cholesterol mitigates the pathological effects of mutant ATAD3. However, the excess cholesterol leads to its aggregation in membranes, which the lysosomes struggle to clear, and thereby creating a lysosomal storage disease that likely contributes to the neurological features of the disease.

P47

Impact of epileptic conditions on GABAergic (hub) neurons in hippocampal organotypic cultures: a full optical dissection of circuits' connectivity and dynamics

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NA

Epilepsy is a neurological disorder characterized by abnormal neuronal activity (hyperexcitability). Mesial temporal lobe epilepsy (mTLE) represents the most common form of epilepsy that generally affects the hippocampus. One of the main causes of hyperexcitability points towards a disequilibrium between neuronal excitation and inhibition processes. GABAergic neurons shape neural networks and participate in network synchrony. Thus, the correct maturation, excitability and connectivity of GABAergic neurons is essential for the correct functioning of neural circuits and network synchrony. Interestingly, GABA hub neurons present a high structural and functional connectivity, capable of modifying the synchronous activity of the developing hippocampus as shown by P.Bonifazi et al. The relationship between microcircuits' connectivity, GABAergic hub neurons and epilepsy has not been established yet. In order to address this question, we induce by pharmacological disinhibition (i.e. blocking GABAA transmission using PTX) epileptogenic conditions in hippocampal organotypic slice cultures (hOTC). Circuits' spontaneous dynamics and dissection of functional connectivity of GABAergic vs non-GABAergic neurons with single cell resolution are reconstructed using wide field imaging via genetically encoded calcium sensors (virally expressed via AAV1-hSyn-Gcamp8f), and RFP markers (virally expressed via AVV1-Dlx-mRuby). This work provides deep insights into impaired microcircuits' dynamics and connectivity patterns following and induced by epileptogenic conditions. Simultaneous calcium imaging and optogenetics of single neurons, with special focus on GABAergic neurons, will next complement the full characterization and impact of epilepsy on GABAergic hubs in relation to their role in orchestrating spontaneous circuits' synchronizations both in physiological and pathological conditions.

P48

Open Hardware Implementation of Real-Time Phase and Amplitude Estimation for Neurophysiologic Signals

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Adaptive deep brain stimulation (aDBS) is a promising concept in the field of DBS that consists of delivering electrical stimulation in response to specific markers. Novel brain adaptivity arises when stimulation targets dynamically changing states, such as oscillatory phase or amplitude of certain frequencies. In this case, it is necessary to deliver stimulation in close temporal proximity to the maker, which calls for a reliable and fast causal estimation of the phase and amplitude of the signals of interest.Here we present an open hardware implementation for phase and amplitude computation of neurophysiological signals in real-time. Our approach exploits the concepts of resonators and Hilbert filters embedded in an open-hardware electronic prototyping platform to obtain a real-time, causal estimator of the

phase and amplitude of neurophysiological signals.To emulate real-world scenarios, we built a setup that included a system to replay neurophysiological signals, a real-time processor, an ADC converter to record the raw signal and the TTL pulses delivered by the processor. By doing so, we can assess the accuracy of the phase and amplitude estimation.We illustrate system performance by computing the phase and amplitude for several scenarios such as epileptic seizures from a preclinical mice model, accelerometer tremor, and STN activity (including beta, gamma, and HFO activity) from Parkinsonian patient's. Results show that the system can track in real-time the phase and amplitude of neurophysiological signals with a minimum (and fixed) delay. To summarize, here we describe a real-world, easily reconfigurable hardware implementation for real-time estimation of phase and amplitude that can deal with very high frequencies of neurophysiological signals. Based on an open hardware platform this framework may be adopted in adaptive neuromodulation studies to quickly test biomarkers in clinical and preclinical settings, supporting the advancement of aDBS.

P49

Advanced Methods for Neuronal Activity Detection: Quantum Dot-Based Membrane Voltage Sensing

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The quest to unravel the brain's complex neural networks has been a primary focus in neuroscience. To understand the dynamic interactions within the nervous system, it is essential to develop tools for accurate mapping of neuronal circuits with high spatial and temporal resolution. Inorganic voltage nanosensors, particularly quantum dots (QDs), show great potential for non-invasive recording of electrical activity. These nanosensors offer fast temporal response, simultaneous monitoring of spiking activity across multiple neurons, and exceptional photostability. Their tunable emission and ability to conjugate with various molecules make them superior to commonly used organic dyes for bioimaging applications.Transmembrane electric fields, characteristic of neuronal spiking, can modulate the electronic structure of QDs, leading to changes in the intensity and position of emission wavelength (Quantum Confinement Stark Effect). This modulation enables the reporting of voltage dynamics with millisecond precision. However, the effectiveness of QDs as voltage sensors depends on their precise localization within the cellular membrane, a challenge due to the exponential decay of electric fields outside the lipid bilayer caused by Debye screening. Successfully integrating QDs into neuronal membranes could revolutionize our ability to visualize and understand neural network dynamics.In this presentation, we share our research on the use of small QDs for measuring changes in membrane potential of self-spiking HEK cells. We focus on the phase transfer of nanocrystals from non-aqueous to aqueous mediums through their encapsulation within micelles and liposomes. This approach aims to enhance their integration into the lipid bilayer without compromising their optical properties. Our ongoing work seeks to establish the viability of QDs as effective tools for neurobiological research.

P50

Extreme low frequency magnetic field protects neuronal and microglia cells from oxygen-glucose deprivation

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Ischemic stroke involves the rapid death of neurons due to the blockage of a brain vessel, leading to damage in the surrounding area known as the ischemic penumbra. The brain tissue at the core of the lesion becomes permanently damaged, but the ischemic penumbra can potentially be saved during the early stages after the stroke. Therefore, new therapeutic strategies are urgently needed to reduce ischemic damage and prevent it from spreading to the penumbral region. Consequently, we investigated the effect of Extreme Low Frequency Electromagnetic Stimulation (ELF-EMS) on primary neuronal and microglial cultures under oxygen-glucose deprivation (OGD) conditions in vitro. ELF-EMS significantly reduced neuronal cell death under OGD conditions and decreased ischemia-induced Ca2+ overload. Additionally, ELF-EMS influenced microglial activation and reduced OGD-induced microglial cell death. Thus, this study suggests that ELF-EMS may offer potential benefits in limiting irreversible ischemic damage under in vitro stroke conditions, and supports further in vivo preclinical validation of ELF-EMS as a potential therapeutic strategy for ischemic stroke.

P51

Virus delivered, brain circuit targetable, tightly-controlled inducible gene expression system

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Pathological protein levels are altered in many neurological diseases, leading to brain dysfunctions. Stringent control of gene expression is crucial for basic research and clinical applications. In most cases, to establish a relationship or causality between gene expression and cellular function, behavior, disease, or brain circuits, it is imperative to turn-off and turn-

on gene expression during the different stages of biological processes, which the use of inducible gene expression systems can accomplish, with the tetracycline (Tet)-dependent regulatory systems being the most widely used. With the Tet-On system, an activator (rtTA) switches gene expression ON only in the presence of an inducer, doxycycline (Dox). However, in the OFF state, the Tet-responsive promoter shows a leaky, undesired basal activity. We have characterized an advanced Tet-On system to overcome this limitation, where a Tet-controlled transcriptional silencer (tTS) is deployed along with the activator. The system was delivered by recombinant adeno-associated viruses (rAAVs) into cultured primary neurons, hippocampal organotypic slices, and in vivo mice brains, where the reduction in basal activity and the activation-inactivation kinetics were assessed. The use of rAAVs and cell-type specific promoters gives high versatility and flexibility to our system (capacity to target multiple brain regions and different cell types). In all three models tested, the basal activity of the promoter was significantly reduced. Reduced background levels increase the system's dynamic range, and the silencer's strength provides additional safety. Therefore, our advanced rAAV-delivered Tet-On system is highly suitable and versatile for neuroscientific research and gene therapy.

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Bioresorbable polymeric scaffolds coated with graphene oxide promote the selection of neuroectodermal commitment of human embryonic and pluripotent stem cells.

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Advances in neural tissue engineering have shown that biocompatible materials can be used to improve cell therapy. In this context, our research team has created bioresorbable nanostructured scaffolds functionalized with polydopamine (PDA) and graphene oxide (GO), demonstrating their properties to facilitate the differentiation of murine neural stem cells (NSCs) into neural and glial lineages, highlighting their potential in regenerative medicine [1] and are biocompatible with human stem cells [2].Given the significant applicability of human embryonic stem cells (hESCs; ES-2) and induced pluripotent stem cells (hiPSCs; KiPS-4F-1), our objective is to use these cell sources to differentiate towards neural tissue on PDA- and GO-functionalized scaffolds. Actin immunofluorescence, scanning electron microscopy (SEM), and cell culture monitoring systems revealed that the materials' nanotopography supported aligned orientation and reduced cell migration, resulting in orderly distribution of ES-2 and KiPS-4F-1 cell lines. In addition, apart from guiding their alignment and migration processes, RT-qPCR analysis showed an increase of their pluripotent ability. Interestingly, cell viability and proliferation assays using flow cytometry, XTT, KI67/CASP3 immunofluorescence and RT-qPCR

showed a selection and direct specification of both human cells towards ectoderm germ layer. The resultant cell populations enhanced their differentiation capacity into neural and supportive glial cells from central nervous system (CNS). Indeed, GO scaffolds accelerate the differentiation process into Schwann cells, the main glial cells from peripheral nervous system (PNS).These findings demonstrate that the use of bioengineered nanostructurated scaffolds of PDA and GO supports in a first phase, the attachment and alignment of ES-2 and KiPS-4F-1 cell lines, and at long term GO forces a selection towards neuroectodermal fate committing ES-2 and KiPS-4F-1 human cells for neural and glial differentiation. Overall, this innovative approach could be used as a technical alternative for future cell therapy.This work has been financed by the University of the Basque Country (UPV/EHU) (COLAB19/03, COLAB22/07 & PPGA20/22), the Basque Government (Grants 2021333012, 2023333035 & IT1751-22). YP has a Bikaintek Postdoctoral grant (010-B1/2023), IMR has a Ph.D. fellowship from University of the Basque Country (UPV/EHU) (PIFBUR21/05) and BPR has a Ph.D. fellowship from Basque Government (PRE_2023_2_0038). [1] Polo Y et al. (2021). Nanostructured scaffolds based on bioresorbable polymers and graphene oxide induce the aligned migration and accelerate the neuronal differentiation of neural stem cells. Nanomedicine: nanotechnology, biology, and medicine. 31:102314. https://doi.org/10.1016/j.nano.2020.102314 [2] Pineda JR et al. (2022). In vitro preparation of human Dental Pulp Stem Cell grafts with biodegradable polymer scaffolds for nerve tissue engineering. Methods Cell Biol.170:147-167. https://doi.org/10.1016/bs.mcb.2022.02.012

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Bioresorbable polymeric scaffolds coated with graphene oxide for human dental pulp stem cell delivery in a model of intracerebral stab wound injury

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Most therapies aiming to regenerate the neural tissue rely on anchoring scaffolds to promote the self-regeneration or on direct implantation of stem cells. However, both approaches encounter challenges that hinder their effective translation, thus presenting an unresolved clinical obstacle. In this study, we propose the combination of nanostructured bioresorbable polymeric scaffolds [1], loaded with human dental pulp stem cells (hDPSCs) [1,2] to improve the recovery of the injured rostral migratory stream (RMS) in a immunosuppressed murine model. A copolymer based on DL-lactide and ≈í¬µ-caprolactone (PLCL) was synthetized following the established procedures [3] through ring opening polymerization (ROP). Subsequently, PLCL underwent characterization via proton nuclear magnetic resonance (NMR), gel permeation chromatography (GPC) and differential scanning calorimetry (DSC). Next, PLCL films were nanopatterned with a 350 nm linewidth grating and functionalized with polydopamine (NanoPDA) to facilitate the subsequent immobilization of graphene oxide (GO)

(NanoGO). Degradation studies were conducted to address the suitability of the scaffolds to implant into central nervous system (CNS). For neural commitment studies, hDPSCs were seeded on the scaffolds and immunostained against stem (Nestin, GFAP), neuronal (DCX, NeuN) and glial (S100≈í,â§, p75) markers after 7 or 14 days. Mobility patterns were also examined for up to 16 h. Finally, NanoPDA and NanoGO, either alone or combined with hDPSCs, were implanted into a stab-wound injured RMS to evaluate their restorative potential. Our NanoPDA and NanoGO scaffolds based on a completely amorphous PLCL facilitated attachment, elongation, migration, and differentiation of hDPSCs into neurons and glial cells in vitro, while still preserving some stem cell characteristics to support the survival of the transplanted cells. In comparison with the hDPSCs grafted alone, the support of the NanoPDA scaffold promoted the survival of grafted hDPSCs in the brain, thereby promoting the restoration of the injury after 3 months. Our results underscore the advantages of combining this alternative source of stem cells with bioresorbable polymeric scaffolds for repairing damaged CNS tissue.This work has been financed by the University of the Basque Country (UPV/EHU) (COLAB19/03, COLAB22/07 & PPGA20/22), the Basque Government (Grants 2021333012, 2023333035 & IT1751-22). YP has a Bikaintek Postdococtoral grant (010-B1/2023), IMR has a Ph.D. fellowship from University of the Basque Country (UPV/EHU) (PIFBUR21/05) and BPR has a Ph.D. fellowship from Basque Government (PRE_2023_2_0038).
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P55

3D neuronal monitoring platforms for electrochemical sensing of neurotransmitters

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Neurotransmitter dysfunctions are known to be the root cause of various neurological diseases including Alzheimer's and Parkinson’s disease. The understanding of neurotransmission dynamics is crucial for the development and assessment of new drugs for neuroprotection.This project aims to develop an electrochemical biosensor that quantifies neurotransmitter concentration in 3D neuronal cell cultures. The biosensor comprises a 3D hybrid hydrogel based on extracellular matrix (ECM) for mimicking the biological environment and functionalized carbon nanotubes (f-CNTs) as transducer material between the biological signal and the metal collector.As first step, this study presents a comprehensive investigation

into the development of neuronal cultures with distinct neurotransmission phenotypes, which constitutes an integral component of the monitoring platform and will be use as the validation of the system. Models were developed from iPSC-derived neurons resulting in mature dopaminergic and glutamatergic neurons with observable morphological traits and functional properties. Additionally, the impact of carbon nanotubes (CNTs) on neuronal differentiation was examined, revealing improved cell adhesion and gene expression in the presence of CNTs. In particular, CNTs were functionalized with gold nanoclusters (AuNCs) or cobalt phthalocyanine (CoPC) as redox moieties followed by the immobilization of glutamate oxidase for dopamine and glutamate detection, respectively. The CNT-Au biosensor exhibited excellent performance and limit of detection, as well as the ability to detect dopamine release from the iPSC-derived dopaminergic neurons in vitro. Overall, this study lays the foundation for a 3D neuronal culture monitoring platform coupled to the iPSC-derived neurons with potential applications in neuroscience research and drug discovery.

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Evaluating the neuromodulatory effectiveness of non-invasive brain-spine interfaces

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Brain-spine interfaces (BSI) have emerged as a potential tool for interacting with the nervous system and repairing neural damage in patients with motor disorders, such as stroke or spinal cord injury. BSIs rely on a timely-linked activation between brain and spinal networks via spinal cord stimulation and have demonstrated efficacy in recovering lower-limb functionality. Recently, we engineered the first non-invasive BSI for continuous control of trans-spinal magnetic stimulation (ts-MS) using electroencephalographic activity. However, the mechanisms by which this BSI can induce changes in neural excitability in the human nervous system remain to be elucidated. With the aim of evaluating and characterizing the neurophysiological effectiveness of a BSI-based intervention, we conducted a single-session experiment with twenty-one healthy participants, who underwent a comprehensive set of assessments before and after the intervention. The evaluation of both central and peripheral neural networks was considered in these assessments, which encompassed motor evoked potentials (MEP), trans-spinal motor evoked potentials (ts-MEP), and H-reflexes. Our results showed for the first time that an intervention based on a non-invasive BSI significantly increased the excitability of corticomuscular pathways, quantified as the peak-to-peak amplitude of MEP, and that this increase was sustained for 30 minutes afterwards. Furthermore, the enhancement of corticomuscular synaptic efficacy was positively correlated with the BSI performance. No significant changes were observed in either ts-MEP or H-reflexes. Overall, our findings evidenced the capacity of the non-invasive BSI to effectively neuromodulate the excitability of the nervous system and could contribute to the development and design of BSI technology as a therapeutic tool for lower-limb paralysis.

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ELECTROHAIR: Design of wearable system for 24x7 EEG monitoring

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Electrical activity can be measured across the skin surface of mammals, including humans. This electrical activity is associated with various underlying processes, such as muscle activation, nerve signals, and neural activity. Current systems for detecting this electrical activity fall into two categories: inexpensive, non-invasive but noisy solutions, and costly, high-quality but invasive solutions. This dichotomy is evident in EEG recordings at the head level. Despite the recent growth in companies offering semi-dry or dry EEG products (which do not require gel to improve impedance), these technologies still lack adequate signal quality due to instability from movement or electrode/skin contact. The time-intensive application of gel-based EEGs, in terms of technician hours, and the poor ergonomics of these products have led to the development of peripheral devices (such as wristbands) that attempt to infer brain activity indirectly with limited success (e.g., sleep cycles). Consequently, existing dry or semi-dry technology devices are primarily used in applications like neuromarketing and video games, reflecting their limited adoption in scenarios requiring a minimum level of stability and reliability.In this work, it is presented a novel system for continuous EEG monitoring in ambulatory settings using an hair-like electrodes. These electrodes are partially implanted into the skin at a dermal or subdermal depth. The electrode support is primarily longitudinal and includes conductive pathways that connect the registration component at the implanted end to the external end of the support, where a recording device register and process the EEG signals. Solution is designed in the form of a cap, bandana or a band allowing 24x7 signal recording with high quality, opening the door for a new range of applications in sleep monitoring, epilepsy seizures monitoring and prediction and novel brain computer interfaces.

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Combining High and Low Frequency Signals for Stable Classification of Hand Movements in Post-Stroke Rehabilitation

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Introduction: Rehabilitation of motor function in post-stroke patients often leverages Brain Machine Interfaces (BMI) for robotic exoskeleton control to enhance functional neuroplasticity through contingent and repetitive movement training over several sessions. BMI decoders based on intracortical spiking activity often are less stable across sessions due

to high-frequency signal degradation over time and discrete nature of the spiking signal. Conversely, low-frequency signals like local field potentials (LFPs) are known to be more stable but less specific to movement types. This study aims to develop a kinematic decoder from intracortical continuous brain recordings that maintains a stable performance across multiple sessions by combining various continuous signal features, extracted by discrete wavelet transform (DWT). Methods: Intracortical recordings were obtained during initial pre-training familiarization/calibration sessions from a post-stroke patient implanted with a 96-channel Utah array in M1, where the exoskeleton fully assisted the grasping movement of the paretic hand. Using the methods in [1], discrete wavelet transform (DWT) was applied in data windows of 100 ms from raw recordings acquired at 30kHz. Recorded robot kinematics were categorized as classes -1 (hand opening), 0 (rest), and 1 (hand closing). In the process of optimal frequency band selection as decoding feature, an initial search identified low-frequency DWT scales optimizing the class separation of rest phase from movement. After that, rest periods were excluded, and a secondary search was performed to find scales with best separation between hand opening and closing movements. Two 1D Convolutional Neural Networks (CNN) classifiers were trained on data from one session and tested on seven additional sessions spanning over two weeks. Classifiers were hierarchically combined: the low-frequency decoder predicting movement intervals and the high-frequency decoder differentiated between hand closing and opening within predicted intervals. Results: Low-frequency scales (7.3-29.3 Hz) were most effective for distinguishing movement phases from rest, overlapping with the beta power band decreased activity during motor tasks. High-frequency scales (3750-15000 Hz) best differentiated hand opening from closing, correlating with spiking activity. The hierarchically combined decoders maintained acceptable accuracy across multiple sessions, suggesting the feasibility of long-term stable decoders to enhance rehabilitation outcomes for post-stroke patients by providing stable and contingent feedback about their neural strategy for motor control. Future work will focus on decoding actual velocity values and evaluating the time resolution of decoders to prove the possibility of using such decoders in real-time BMI applications. [1] Zhang, M., Schwemmer, M. A., Ting, J. E., Majstorovic, C. E., Friedenberg, D. A., Bockbrader, M. A., ... & Sharma, G. (2018). Extracting wavelet based neural features from human intracortical recordings for neuroprosthetics applications. Bioelectronic Medicine, 4, 1-14.

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Neurophysiological assessment of a stroke patient throughout a novel multimodal motor rehabilitation therapy

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Stroke patients often undergo clinical assessment using standard scales that evaluate motor function based on behavioral observation. However, individuals with similar behavioral

performance can present heterogeneous neuromotor pathophysiology that might not be effectively captured by standard clinical evaluation. As a complementary measure, electromyography (EMG) recordings provide a direct measurement of the recruitment patterns of muscles, offering a valuable signal for the quantitative assessment of the combined central and peripheral neurophysiology after stroke and throughout a recovery process. In this study, we analyzed the EMG activity of a severely affected chronic stroke patient undergoing neurorehabilitation. The patient participated in a multimodal therapy combining novel hybrid neural-interfacing and motor control relearning paradigms where the intervention was progressively tailored based on the patient's behavioral and neurophysiological condition. In addition to clinical motor scales, surface EMG activity of upper arm, forearm, and hand muscles in both the paretic and healthy (functional reference) limbs were recorded during diverse dynamic motor tasks at different time points throughout a longitudinal intervention. We investigated different EMG-based features: muscle activity patterns, significant muscle contraction levels and antagonistic muscle pairs reciprocal inhibition level.The clinical scores demonstrated an overall significant improvement of motor function over the course of the intervention. More specifically, EMG analysis revealed a significant gain from negligible muscle recruitment capacity at enrollment to increased voluntary cortico-muscular descending volley evoking a general muscle coactivation first, followed by specific agonist-antagonistic reciprocal inhibition in later phases. EMG monitorization over the longitudinal intervention shed light on the adopted neuromuscular strategies by the patient in response to different feedback-based training and permitted an optimization of the therapy to the neurophysiological capacities of the patient. We concluded that EMG-based assessment holds promise as a valuable tool for understanding neuromotor changes related to motor recovery with higher specificity and tailoring personalized interventions in stroke patients.

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Metabolic changes in stroke alter nanoparticles biodistribution and clearance

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Introduction:Stroke is the second leading cause of death worldwide(1), however, there is still a lack of effective treatments(2). This may be due to the obstacles of accessing the brain, as the blood-brain barrier (BBB) prevents most drugs from entering(3). To bypass this, there are several strategies, one of the most innovative is targeting nanoparticles (NPs), decorating them to facilitate entry to the BBB3. We evaluated four glyco-conjugated NPs on their ability to enter the brain and described changes in metabolism that appear in stroke conditions affecting the biodistribution and elimination of these NPs.Methods:Male Sprague Dawley rats (n=31) were subjected to temporal middle cerebral artery occlusion (tMCAO) and then Magnetic Resonance Imaging (MRI) at 24 h after ischemia onset to evaluate the extent of the infarct; the neurological deficit was assessed by the Neuroscore test. Age-matched rats (n=14)

were used as controls. Seven days after ischemia, rats underwent Positron Emission Tomography (PET) imaging with 18F-labelled glyco-conjugated NPs. Four different NPs were used: Glucose coated NPs (Glc-NPs), mannose coated NPs (Mann-NPs), and the same NPs with additional fluorinated glucose (Glc/GlcF-NPs and Mann/GlcF-NPs) to increase hydrophobicity of NPs surface and attempt to enhance their brain uptake. After scans rats were sacrificed and radioactivity was determined by gamma counter.Results/Discussion:We observed NP uptake in the brain in all four NP types, meaning all the NPs were able to reach the brain in both stroke and control conditions. However, there were differences among the NPs. All Glucose-conjugated NPs showed higher radiotracer uptake than the mannose ones. In addition, Glc/GlcF-NP had higher uptake than the not fluorinated NPs, while Mann/GlcF-NP showed less uptake than the not fluorinated counterpart.When we analysed the rest of the organs, differences in the distribution of the NPs between stroke and control conditions were detected. Stroke caused the liver uptake of the NP to double in all four NP types and also increased spleen NP uptake slightly.PET studies showed that NPs were mainly excreted to the intestines via the liver in both conditions, so this uptake increase in the liver in stroke conditions could mean that the clearance pathway is not working properly or is saturated and therefore the NPs are accumulating in the liver.Conclusion:Stroke causes metabolic changes in the organism which affect the NPs biodistribution and clearance, therefore altering their brain access. This means that when treating stroke, the metabolic changes it causes have to be taken into account. These changes need to be studied longitudinally and better described, so we can have a full picture that helps produce more effective probes and drugs to better treat it.References1. Saini V, Guada L, Yavagal DR. Global Epidemiology of Stroke and Access to Acute Ischemic Stroke Interventions. Neurology. 2021 Nov 16;97(20_Supplement_2). 2. Yang C, Hawkins KE, Doré S, Candelario-Jalil E. Neuroinflammatory mechanisms of blood-brain barrier damage in ischemic stroke. American Journal of Physiology-Cell Physiology. 2019 Feb 1;316(2):C135,53. 3. Sethi B, Kumar V, Mahato K, Coulter DW, Mahato RI. Recent advances in drug delivery and targeting to the brain. Journal of Controlled Release. 2022 Oct;350:668-87.

P61

Role of Kv channels in hearing loss and correlation with Alzheimer Disease

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Ion channels are essential for life as they play a fundamental role in neuronal signaling, muscle contraction or even nutrient transport.Among all ion channels, the key regulators of neuronal excitability are the voltage-gated potassium channels (KV), the largest family of K+ channels. These KV channels are divided into twelve subfamilies, named as KV1- KV12. During Alzheimer disease (AD), the amyloid beta-peptide (Abeta) deposition causes synaptic dysfunction and consequently neuronal loss. There have been identified several KV channels that can regulate the firing rate (KV1, KV4 or KV7) or the duration of the action potential (KV2 or KV3). Some of this channels have been associate to hearing loss as they participate in the acoustic signaling transmission.Moreover recent studies correlate Alzheimer Disease and hearing loss but the mechanism in which both diseases are related is still unknown. In this work we identify several

ion channels in the auditory cortex and inferior colliculus of AD mice and demonstrate that there is a channel dysfunction in AD mice compared to control ones in this areas, confirming the role of this channels in both Diseases.

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Mutation Profile of Inherited Retinal Dystrophies in a Basque Country Cohort (Spain)

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Inherited retinal dystrophies (IRD) are a heterogeneous group of diseases that mainly affect the retina, causing a progressive and severe vision loss, with more than 250 genes involved in their pathogenesis. The clinical and genetic heterogeneity complicates the identification of causal mutations.In order to advance the molecular genetic characterization of patients with IRD, a retrospective study has been done on 353 affected individuals from 289 unrelated families, using different molecular approaches, including gene panels, whole-exome sequencing (WES), whole-genome sequencing (WGS), Multiplex Ligation dependent Probe Amplification (MLPA), CGH arrays and midigene splice assays.In total, 57% (166/289) of the studied families have been solved and 12% (34/289) have been likely solved. We have found 214 different variants of which the majority, 45%, were missense; 21% frameshift; 14% nonsense; 6% CNV; 6% splice site; 4% splicing; 2% in frame; 1% stoploss and a 1% rearrangements. These variants were identified in 80 different genes, but over 50% of the families where carriers of mutations in 8 genes. The genes with more mutations were USH2A, CERKL and SNRNP200, in 18, 10 and 8 families, respectively. The most frequent variants were c.2276G>T (p.Cys759Phe) in USH2A, c.847C>T (p.Arg283Ter) in CERKL and c.3260C>T (p.Ser1087Leu) in SNRNP200.Our study enabled us to solve nearly 70% of the families in our cohort. This has significant implications for the diagnosis of inherited retinal diseases (IRDs). With the implementation of complementary approaches, such as long-read whole genome sequencing (WGS), we hope to increase our diagnostic yield significantly.

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Exploring the therapeutic potential of MP compounds in diabetic retinopathy: insights from an ex vivo model

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Our research group has been developing a novel approach to treat inherited retinal dystrophies through the ocular instillation of a small molecule, MP-004, using the rd10 mouse model. MP-004 operates through a dual mechanism: it modulates oxidative stress (OS) and maintains calcium (Ca2+) homeostasis. Dysregulation of OS and intracellular Ca2+ represents common pathogenic mechanisms shared by various retinal disorders, including diabetic retinopathy (DR), the leading cause of visual loss among working-age adults in the Western population. In DR, neuroinflammation triggered by diabetes plays a pivotal role in exacerbating photoreceptor degeneration, predominantly mediated by glial and microglial cells. Proper regulation of cytosolic calcium levels is crucial for glial cell function, thus ensuring adequate calcium regulation in the context of inflammatory processes in DR holds significant therapeutic potential. Moreover, neuroinflammation associated with glial activation is implicated in most cases of retinal degeneration. Therefore, we used GFAP expression levels as a readout of the effect of MP compounds over glial activation. The aim of this study was to evaluate the efficacy of MP compounds in an ex vivo model of DR. Retinal explants from young WT (C57BL/6) mice were isolated and cultured in standard DMEM medium or in a diabetic-like medium containing high glucose (55mM), H2O2 (300 mM), and IL1≈í,â§ (20ng/ml). Time of incubation ranged from 2 to 14 days. GFAP expression levels were measured by immunohistochemistry in fixed whole mounted retinas. Our results show that compounds MP-001 and MP-004 effectively reduce GFAP levels, suggesting their potential candidacy for the treatment of DR.

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Hippocampal-dependent memory in a model of epileptiform activity

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Medial Temporal Lobe Epilepsy (MTLE) is a neurological disorder characterized by neuronal hyperactivity and unpredictable seizures, often accompanied with memory impairments. During the early stages of this condition, and between seizures, patients begin to develop abnormal brain activity known as epileptiform activity (EA), mainly presented in the hippocampus. Moreover, EA is not exclusively related to MTLE, but to other neuropathologies such as Alzheimer, Parkinson's disease, and traumatic brain injury. Taking advantage from a new model of EA, our aim is to evaluate whether this model presents hippocampal-related cognitive deficits due to EA. To achieve this, we assessed the innate emotional behaviour of male and female mice using open field, elevated plus maze and light-dark box tests. In general, we did not find significant differences between groups, which suggests this model does not present emotional disturbances. Furthermore, we have assessed spatial memory using Barnes maze. EA mice showed worse performance along all trials, spending greater time, longer distances, and more perseverance (i.e. visiting previous explored holes). Then, we established clusters depending on the cognitive strategy used and we found specific cognitive deficits in random (exploratory) and spatial (cognitive), but not in serial strategies. Lastly, our current research focus on in vivo electrophysiology to correlate with the hippocampal-dependent

memory deficits found before. Thus, we have implemented new behavioural protocols for spatial and recognition memory, allowing for recording in freely moving mice with tetrode implants. In parallel, we are carrying out histological analysis in CA1 and Dentate Gyrus to study layer dispersion, cell differentiation and astrocytes activation during EA.

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Mild Hyper-Excitation Induces Functional and Morphological Changes in Dendritic Spines in Mouse Brain Slices

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Epileptogenesis is often a very slow gradual process emerging spontaneously or in response to a given event such as trauma. Most experimental efforts have explored epileptogenesis in response to an initial trauma or seizure event, whereas the mechanisms underlying idiopathic epilepsy remain obscure. It appears likely to involve a very slowly developing shift in the excitation/inhibition balance that reaches a threshold. Here, to identify the earliest functional 'footprint' of hyperexcitability on the hippocampal circuit, we induced modest hyperexcitation in mouse hippocampal slices using the GABAA receptor blocker picrotoxin (PTX). After 30 min PTX treatment we investigated intrinsic membrane and dendritic spine structure in CA1 pyramidal neurons. Modest hyperexcitation, without sustained discharging, did not induce changes in intrinsic neuronal properties, though we found subtle changes in dendritic spines shape, that may impact signaling independently of synaptic release and post-synaptic receptor mechanisms. Our further efforts will explore whether these changes may contribute to a slow shift in the excitation/inhibition balance and epileptogenesis, or whether they reflect a beneficial homeostatic response to maintain the balance.

P66

CELLULAR SPECIFICITY AND SEXUAL DIMORPHISM OF HIPPOCAMPAL CB1RECEPTORS ON BEHAVIORAL PROCESSES

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In this work, we want to dissect the specific and sex-dependent role of CB1 signalling in different cell types and its impact on hippocampalfunctions such as navigation or memory.A specific Cre-mediated CB1 deletion on neurons (excitatory and inhibitory), astrocytes, or interneurons in CB1-flox males and females was performed. Viralvectors (AAV-Cre) were injected bilaterally into the hippocampus of these mice. The Barnes maze was used for learning, memory, navigation and the NovelObject Recognition Test (NORT) for recognition memory. Anxiety-like behavioural protocols were also performed before cognitive evaluation to establish anemotional baseline. We then analysed the inhibitory cell subpopulations by immunofluorescence image analysis. CB1-flox mice carrying a hippocampal CB1deletion in

different cell types presented different changes in cognitive processes compared to control littermates. CB1 deletion from all neurons produceda specific decrease in the spatial strategy in the Barnes Maze, and an impairment in the recognition memory in males. Specific CB1 deletion fromhippocampal interneurons affected the innate emotional response in females. Hippocampal CB1 deletion in astrocytes presented an impairment in learningand memory performance. Field excitatory postsynaptic potentials (fEPSPs) were evoked in CA1 stratum radiatum by stimulating Schaffer collaterals (SCs)with a tetanic stimulation (4 trains at 100 Hz for 1 s; 30 s intervals) to induce long term potentiation (LTP). This plasticity was absent neuronal or astrocyticCB1 deleted males. Finally, different expression patterns of hippocampal cells were observed and quantified in the immunohistochemistry assays.

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Widespread reversible reduction in brain myelin content upon marathon running

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Recent evidence suggests that myelin lipids may act as glial energy reserves when glucose is lacking, a hypothesis yet to be solidly proven. Hereby, we carried out an observational study, for which no pre-registration was required, to examine the effects of running a marathon on myelin content by MRI. Our findings show that marathon runners undergo widespread myelin decrease at completion of the effort. This reduction involves white and gray matter and includes primary motor and sensory cortical areas and pathways, as well as the corpus callosum and internal capsule. Notably, myelin levels recover within two months after the marathon. These results reveal that myelin use and replenishment is an unprecedented form of metabolic plasticity aimed at maintaining brain function during extreme conditions.Supported by Spanish Ministry of Science and Innovation and Universities, Basque Government, CIBERNED Network-Instituto Carlos III, and Ikerbasque Foundation.

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Transcriptomic study in the hippocampus of centenarians reveal high levels of metallothioneins associated with successful aging

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Aging is a systemic, multifactorial and degenerative process characterized by the deterioration and loss of physical and mental capacities. Different individuals display diverse aging

trajectories and some individuals can give clues about how to age in a healthy way. Centenarians exhibit extreme longevity and this is commonly accompanied by fewer comorbidities, better cognitive function and greater quality of life. Different approaches have been used to decipher the biology and genetics of centenarians, however, these studies have been performed mostly in blood samples and the mechanisms responsible for promoting the maintenance of cognition and long-lived aging that characterize centenarians are largely unknown. We addressed this question by performing transcriptomic studies on human hippocampus samples from individuals of different ages (centenarians, septuagenarians and young). We identified a differential gene expression pattern in the brain of centenarians compared to the other two groups. In particular, members of the metallothioneins (MTs) family of genes were highly expressed in the hippocampus of centenarian individuals. MTs are low molecular weight proteins with the ability to bind heavy metals such as Zinc or Copper that play an important role in their detoxification when they reach high concentrations. Our omic findings were validated in two independent cohorts at mRNA and protein level. At cellular level, we revealed that MTs were mainly expressed by astrocytes. Functional studies in primary astrocyte culture revealed the role of MTs on their viability and activity. Indeed, silencing of MT1 or MT3 resulted in decreased proliferation, increased apoptosis and senescence together with increased expression of inflammatory genes. Overall, our results show that the hippocampus of centenarians display high levels of MTs, which are mainly expressed by astrocytes becoming a defensive mechanism that could provide neuroprotection in the brain during aging.

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Evaluation of potential cognitive effects of psilocybin in naive mice

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Background: Psilocybin is a natural hallucinogenic alkaloid that has demonstrated promising therapeutic potential for a variety of disorders, such as depression or anxiety. Nevertheless, the mechanism underlying its efficacy remains unclear. Psilocybin has also been proposed to affect specific cognition-associated domains such as cognitive flexibility, which is frequently associated with cognitive impairments observed in various psychiatric disorders.Aim: We aimed to evaluate the long-term cognitive effects of psilocybin on learning and cognitive flexibility.Methods: To assess the effects of psilocybin on different cognitive domains, two cohorts of adult C57BL/6J male mice were used. Cohort 1 underwent Pairwise Visual Discrimination and Reversal Learning (PVD/RL) behavioral test in the automated Bussey-Saksida Touchscreen Operant Chamber for mice. This cohort was used to assess visual learning (discrimination between two different stimuli) along with cognitive flexibility. Cohort 2 was assigned to Barnes Maze (BM) test, with an acquisition phase (to assess spatial learning) and a reversal phase (to assess cognitive flexibility). Mice were randomly assigned to psilocybin (n(PVD/RL)=14, n(BM)=15) or saline (n(PVD/RL)=13, n(BM)=12) groups. Two doses of psilocybin (1 mg/kg, i.p.) or saline were administered. This dose was selected on the basis of its maximal psychedelic-like effect elicited in mice. All results were analysed using unpaired t

test or two-way repeated measures ANOVA, followed by Bonferroni post hoc test. Results: In cohort 1, control mice successfully completed the 5-phase pre-training of the PVD/RL tasks (habituation, initial touch training, must touch stimuli, must initiate and punish incorrect). No significant differences were found between control and psilocybin group during any of the pre-training stages. During task, all mice demonstrated learning evidence by improving the percentage of correct choices along time in both acquisition (F trial(3.391, 77.32)=12.52, p<0.0001) and reversal learning (F trial(3.336, 76.72)=26.89, p<0.0001) phases. No effect of psilocybin was found when evaluating the percentage of correct choices along sessions, in either acquisition or reversal learning phases. During Barnes Maze, cohort 2 mice exhibited task learning, evidenced by progressive reduction in primary latency to target, as revealed by significant effect of trial in acquisition (F trial(7, 198) = 4.837, p<0.0001) and reversal (F trial(5,259)=38.68, p<0.0001) learning phases. No effect of psilocybin was found in primary latency in either acquisition or reversal phases.Conclusions: Administration of two systemic doses of psilocybin had no effect on spatial or non-spatial associative learning, nor in cognitive adaptation to new environmental contingencies. Other experimental approaches, such as the use of animal models for the assessment of cognitive deficiencies, or a broader test battery should be considered for future studies. Conflicts of interest: JJM is supported by an unrestricted grant from Janssen. The rest of the authors declare no conflict of interest.Funding: This work was supported by MCIN/AEI/10.13039/501100011033 (PID2021-123508OB-I00) and the Department of Education (IT-1512-22) of Basque Government. E.H.-H holds grant FJC2022-048338-I, funded by MCIN/AEI/10.13039/501100011033 and by the European Union NextGeneration EU/PRTR. I.E-S and N.M-A received a predoctoral fellowship from UPV/EHU (PIF19/308) and Basque Government (PRE_2022_1_0256), respectively.

P70

Quantification of reading circuits in the ventral occipitotemporal cortex

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Substantial evidence shows that one of the circuits supporting reading is located in the ventral occipito-temporal cortex (VOTC), a region that was first functionally identified and denominated visual word form area (VWFA). Recent evidence combining functional, structural, and quantitative MRI suggests that the VOTC can be segregated into at least two sub-regions: one involved in visual feature extraction in the posterior occipito-temporal sulcus (pOTS), and one implicated in integrating with the language network in the middle occipito-temporal sulcus (mOTS). Due to the heterogeneity of functional localizers, it is usually difficult to know if the same cortical region is being located across studies and if the results of the analyses are comparable. The aim of the present work was to develop a single-subject multimodal VOTC word recognition localizer, replicable across different labs and studies. Our experiment followed a dense-sampling (5 repetitions) strategy with 5 participants. We acquired fMRI reading region localizers, and structural MRI scans (diffusion MRI, T1w images). Our results revealed that by combining multimodal MRI measurements we can segregate three different reading regions at the individual subject level and that with 5 repetitions, we can measure the variability of the localization. Furthermore, we developed and tested a

shorter localizer capable of capturing similar individual variability, that will be made available to the scientific community. In sum, here we propose a new protocol to harmonize inter-lab and inter-study visual word recognition, which will be critical to advance our understanding of the role of the VOTC in neurobiology of reading.

P71

Fatty acid dysregulation in astrocytes: A driver of ALS neurodegeneration

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive motor neuron disease lacking effective treatment. Lipids serve crucial roles in the central nervous system as membrane components, signaling molecules, and energy stores. During periods of high neuronal activity, astrocytes support energy demands by utilizing energy from lipid acyl groups through oxidative phosphorylation, which they can handle better than neurons due to enhanced anti-oxidative stress mechanisms. Given the emerging evidence implicating glial cells in ALS pathogenesis and the recognized dysregulation of lipid metabolism in ALS, we investigated how ALS-associated factors, focusing on TDP-43 loss-of-function, affect astrocyte metabolism and its impact on lipid processing and neuronal health. Using astrocyte models from rat primary cultures and human iPSCs, we found impaired energy production pathways in TDP-43 knockdown astrocytes, potentially linked to disrupted fatty acid oxidation and accumulation of saturated species. Pharmacological interventions targeting saturated fatty acid production rescued these effects in Drosophila melanogaster models of TDP-43 dysfunction. These findings highlight potential therapeutic targets and strategies for ALS treatment.

P72

GCN2 inhibition reduces mutant SOD1 clustering and toxicity and delays disease progression in an Amyotrophic Lateral Sclerosis mouse model.

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Amyotrophic lateral sclerosis (ALS) is a fatal disease caused by the death of motoneurons that, unfortunately, has no cure. Although mostly sporadic, about 10% of ALS patients suffer familial, inheritable forms of the disease (fALS). fALS-causing gene mutations have uncovered the diverse etiology of fALS and sporadic ALS, but also common molecular hallmarks such as protein misfolding and activation of the Integrated Stress Response (ISR). The ISR is an intracellular homeostatic mechanism initiated by four stress-sensing kinases (PKR, PERK, GCN2, and HRI) which, upon activation, reprogram translation to promote a coping gene expression program; however, under chronic stress, the ISR can signal apoptosis.Mutations in the SOD1 gene cause fALS leading to abnormal protein aggregation in patients and models. We previously developed an ALS neuronal model based on the mutant allele SOD1 G93A expression that recapitulates mutant ISR activation and neuronal death, and found that a downstream inhibitor of the ISR (ISRIB) improved neuronal survival. Still, the stress-sensing kinase accounting for ISR activation and the ISRIB neuroprotective mechanism remains unknown. Here, the effect of pharmacological and genetic inhibition of specific ISR components was analyzed in ALS SOD1-based experimental models. Changes in SOD1-dependent clustering rates and the risk of neuronal survival (by longitudinal survival analysis) were analyzed in HEK293 cells and rat primary neurons. ALS disease progression was evaluated in the SOD1G93A transgenic mouse; symptomatology with a clinical score, muscle denervation with longitudinal electromyography analysis and strength with the hanging-wire test. We found that ISRIB reduces SOD1 clustering. Interestingly, both CRISPR/Cas9-dependent GCN2 protein level reduction and its pharmacological activity inhibition robustly prevented mutant SOD1 foci formation and improved neuronal survival. Moreover, GCN2 pharmacological inhibition in fALS SOD1G93A transgenic mice delayed muscle denervation, strength loss, weight loss, and the appearance of ALS symptoms. This work strongly suggests a previously undescribed key role of GCN2 in the pathology of SOD1-based ALS models. Therefore, we propose GCN2 as potential therapeutic target for ALS.

P73

Retromer-mediated recruitment of the WASH complex involves discrete interactions between VPS35, VPS29 and FAM21

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Endosomal trafficking ensures the proper distribution of lipids and proteins to various cellular compartments, facilitating intracellular communication, nutrient transport, waste disposal, and the maintenance of cell structure. Retromer, a peripheral membrane protein complex, plays an important role in this process by recruiting the associated actin-polymerizing WASH complex to establish distinct sorting domains. The WASH complex is recruited through the interaction of the VPS35 subunit of retromer with the WASH complex subunit FAM21. Here, we report the identification of two separate fragments of FAM21 that interact with VPS35, along with a third fragment that binds to the VPS29 subunit of retromer. The crystal structure

of VPS29 bound to a peptide derived from FAM21 shows a distinctive sharp bend that inserts into a conserved hydrophobic pocket with a binding mode similar to that adopted by other VPS29 effectors. Interestingly, despite the network of interactions between FAM21 and retromer occurring near the Parkinson's disease-linked mutation (D620N) in VPS35, this mutation does not significantly impair the direct association with FAM21 in vitro.

P74

Alpha-synuclein overexpression in the Locus Coeruleus as a presymptomatic rodent model of Parkinson's Disease

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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. Interestingly, the locus coeruleus (LC) that contains the highest density of noradrenergic neurons in the brain, is among the brain stem areas first displaying Lewy bodies in the disease, and is associated with prodromal non-motor symptoms. Despite its small size, it projects to almost all brain areas and exerts a key influence on the homeostasis of dopaminergic networks. The aim of this study is to examine the noradrenergic dysfunction in Parkinson Disease. For that, we studied the impact of alpha-synuclein overexpression in the LC at molecular, functional, and behavioural levels in a pre-symptomatic rodent model of PD. Through these data, we can gain a better understanding of how noradrenergic dysregulation occurs at the earliest stages of PD, and identify potential therapeutic strategies to dampen PD progression.Funding: Grant PID2 021-126434OB-I00 funded by MCIN/AEI/ 10.13039/501100011033 and ERDF A way of making Europe. It has also been funded by the Basque Government (IT1706-22, PUE21- 03) and the UPV/EHU (COLAB20/07). This research was conducted in the scope of the Transborder Joint Laboratory (LTC) non-motor Comorbidities in Parkinson's Disease (CoMorPD). JR holds a PhD grant from the University of the Basque Country.

P75

Alpha-synuclein overexpression induces axonal pathology preceding dopaminergic cell loss in a mouse model of Parkinson's disease

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Parkinson's disease (PD) is the most common chronic neurodegenerative movement disorder. Its pathophysiology is mainly associated with the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and the presence of intracytoplasmic inclusions called Lewy bodies. The alpha-synuclein (alpha-syn) protein is the primary component of Lewy bodies and plays an essential role in the development and progression of the disease. Axonal pathology is a prominent feature in PD preclinical models characterised by swelling and fragmentation of affected axons as the disease progresses. The main objective of this study was to assess axonal pathology induced by the overexpression of human alpha-syn, specifically in projection sites of SNc dopaminergic neurons, and examine whether these alterations precede the loss of SNc cells.Mice received uni- or bilateral intranigral injection of adeno-associated viral vectors encoding human alpha-syn (AAV-halpha-syn) and were sacrificed at 60 or 120 days post-surgery (n=4 in each group). At these time points, using immunohistochemical techniques for tyrosine hydroxylase (TH) and alpha-syn immunolabelling, axonal morphological changes were analysed at the level of the striatum by quantifying the density of axonal varicosities, also known as axonal swellings, with two methods: stereological quantification and ImageJ macro-based workflow. While the density of dopaminergic cells in the SNc was not significantly reduced at 60 or 120 days, axonal swellings were already evident in the striatum. Indeed, loss of striatal TH reactivity was observed as early as 60 days and continued to decrease at 120 days in bilaterally injected mice. Notably, striatal TH+ optic density showed a significant correlation with the amount of axonal swellings using both stereological quantification with Spaceballs and the mean positive area calculated in Fiji ($r=0.53$, $p=0.041$ and $r=0.82$, $p<0.001$, respectively). The intraclass correlation coefficient of both methods was 0.624 ($p=0.004$). However, the amount of axonal swellings did not correlate with alpha-syn expression. Our results suggest that alpha-syn induces axonal pathology, and that axonal swellings precede dopaminergic axonal and cell loss.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 a UPV/EHU fellowship.

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Unravelling the role of PLC≈í,â§1 splice variants and the impact of their subcellular localisation in determining the cell fate of the human neural lineage Ntera-2

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Ntera-2 pluripotent human embryonic carcinoma stem cells (NT2 cells) offer a valuable platform for exploring the mechanisms underlying neural cell differentiation due to their ability to differentiate into various neural cell types, including neurons and astrocytes and have been even proposed as a tool for regenerative medicine in brain lesions. The role of PLC≈í,â§1 in cell cycle regulation and differentiation has been extensively documented across various cell lines, including NT2 cells [1]. Indeed, gene silencing of PLC≈í,â§1 prevents AraC-induced neuronal differentiation, whereas transient overexpression of PLC≈í,â§1a or PLC≈í,â§1b in the absence of differentiation inducers drives differentiation towards neurons and astrocyte-like cells, underlining the essential and sufficient role of PLC≈í,â§1 in the differentiation of NT2 cells [2]. Here, we sought to investigate the role of the two splicing variants PLC≈í,â§1a and PLC≈í,â§1b and their localisation or not in the cell nucleus in the final cell fate decision. To this end, we overexpressed PLC≈í,â§1a and PLC≈í,â§1b and their corresponding nuclear localization mutants PLC≈í,â§1a-M2b and PLC≈í,â§1b-M2b, which are reported to localize entirely in the cytoplasm, and conducted combined neurochemical and morphometric analyses. Cell phenotypes, classified on the basis of NF200 and GFAP immunostaining intensity, were morphometrically analysed according to González-Burguera [3]. Results demonstrated that cells with a NF200High/GFAPLow phenotype, indicative of a neuronal identity, exhibited significantly larger Ferret's diameter and higher aspect ratio values compared to NF200Low/GFAPHigh cells, which displayed markedly higher circularity and solidity scores typical of astroglial cells. Using this approach to classify cells into neuronal and non-neuronal phenotypes, we showed that overexpression of PLC≈í,â§1a promoted a higher proportion of neuronal cells compared to PLC≈í,â§1b, whereas nuclear localization mutants PLC≈í,â§1a-M2b and PLC≈í,â§1b-M2b led to a significant reduction in neuronal cell counts and a corresponding increase in glial cell numbers compared to the native isoforms. Collectively, our findings highlight the critical interplay between PLC≈í,â§1 splicing variants and their subcellular localization in governing neural differentiation and providing valuable insights into the complex regulatory mechanisms underlying neural development.Key words: NT2 cells, differentiation, PLC≈í,â§1, splicing variants, nuclear localization. REFERENCES[1] González-Burguera I, Ricobaraza A, Aretxabala X, Barrondo S, García del Caño G, López de Jesús M, Sallés J. Highly efficient generation of glutamatergic/cholinergic NT2-derived postmitotic human neurons by short-term treatment with the nucleoside analogue cytosine beta-D-arabinofuranoside. Stem Cell Res. 2016 Mar;16(2):541-51. doi: 10.1016/j.scr.2016.02.038. Epub 2016 Mar 3. PMID: 26985738.[2] Suzanne Scarlata, Imanol González-Burguera, Guanyu Lin et al. PLC≈í,â§1 By-Passes Early Growth Response -1 to Induce and Maintain the Differentiation of Neuronal Cells, 16 January 2024, PREPRINT (Version 1) available at Research Square [https://doi.org/10.21203/rs.3.rs-3717468/v1].[3] González-Burguera I, Ricobaraza A, Aretxabala X, Barrondo S, García Del Caño G, López de Jesús M, Sallés J. Data for the morphometric characterization of NT2-derived postmitotic neurons. Data Brief. 2016 Apr 13;7:1349-54. doi: 10.1016/j.dib.2016.04.021. PMID: 27158648; PMCID:

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P77

Modelling Parkinson's Disease (PD) and Frontotemporal Dementia (FTD) using fused region-specific brain organoids derived from human pluripotent stem cells

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Cerebral organoids represent a promising cell culture model for investigating human neurodegenerative diseases given their three-dimensional architecture, cellular diversity and intercellular interactions, offering significant advantages over other stem cell-derived cultures. Recently, a novel cell culture model, termed assembloid, has advanced in vitro research. These assembloids consist of fused organoids differentiated into distinct brain regions where cells can migrate and establish connections, thus mimicking the interactions observed in the living nervous system.In this project, we are developing an in vitro model to study Parkinson's disease (PD) and Frontotemporal Dementia (FTD) by fusing three regionalized brain organoids: cortex (CX), striatum (ST), and midbrain (MB). Using this assembloid model, our aim is to analyze the neurotoxic effects resulting from exposure to alpha-synuclein (alpha-syn) preformed fibrils (PFF) and TDP-43 protein in the substantia nigra -specifically the nigrostriatal pathway- in PD and the cortex in FTD.Preliminary results show that our brain organoids express region-specific markers and point out a significant reduction in tyrosine hydroxylase (TH) levels in assembloids exposed to alpha-syn PFF. Future perspectives include assessing the effects of TDP-43 exposure and conducting a more comprehensive characterization of the molecular response to both alpha-syn and TDP-43 pathological proteins.

P78

Pigmentation of Midbrain Catecholaminergic Neurons in Mice Following the Systemic Delivery of a BBB-Penetrant AAV9 Capsid Variant

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Throughout the past few years, the field of CNS gene therapy has witnessed acontinuous race for the development of novel AAV capsid variants that can bypass theBBB upon systemic delivery. Among existing possibilities, Voyager Therapeutics(Cambridge, MA) recently developed several AAV9 capsid variants by taking advantageof a RNA-driven screen platform

known as TRACER (see PMID: 33553485). Here we took advantage of the AAV9-P31 capsid variant for modeling Parkinson's disease in naive, non-transgenic mice. To this aim, AAV9-P31 encoding the human tyrosinasegene (the enzymatic precursor of neuromelanin) was delivered in the retro-ocular plexusof C57BL/6 mice. One cohort of animals was euthanized four weeks post-AAV delivery,whereas another cohort was sacrificed with a follow-up time of 16 weeks. In all animals,the conducted histological processing revealed a specific, time-dependent pigmentationof dopaminergic neurons located in the substantia nigra pars compacta and the ventraltegmental area, together with a weaker pigmentation of noradrenergic neurons in thelocus coeruleus. Moreover, preliminary results suggested the presence ofintracytoplasmic inclusions positive for traditional markers of Lewy body pathology suchas P62 and alpha-synuclein. Therefore, the conducted approach enabled thedevelopment of a novel preclinical mice model of Parkinson's disease mimicking theknown neuropathological hallmark of this disorder, including Lewy bodies. By going thisway, it is also worth noting that intraparenchymal delivery of AAVs will no longer berequired, an issue obviously broadening the gene therapy field by allowing theimplementation of a whole range of novel therapeutic strategies.

P79

Characterization of a 3D Cellular Model for Parkinson's Disease Using iPSC with the E46K Alpha-Synuclein Mutation

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Parkinson's disease (PD) is a complex neurodegenerative disorder characterized by the selective death of dopaminergic neurons in the substantia nigra (SN). While most cases are sporadic, certain types of Parkinson's have been associated with specific genetic mutations. To advance our understanding of the disease, we have characterized a three-dimensional (3D) cellular model based on a mutation associated with the disease, the E46K mutation in the alpha-synuclein gene, identified in a Basque family with a history of parkinsonism and Lewy body dementia.We first focused on optimizing midbrain differentiation in 3D using induced pluripotent stem cell (iPSC) lines from The Jackson Laboratory carrying the E46K mutation, as well as the isogenic control line in which the mutation has been reversed. We compared the effect of different culture matrices -porcine brain extracellular matrix (ECM) and/or synthetic ECM (Geltrex) and conditions (embedding or not)- on the induction of midbrain dopamine neurons in a 3D organoid model by analyzing the presence of midbrain progenitors and neurons by immunofluorescence at 30 and 45 days. Quantification of FOXA2, a transcription factor necessary for midbrain dopamine neuron specification and Tyrosine Hydroxylase (TH) the rate-limiting enzyme for dopamine biosynthesis showed that addition of liquid matrices, such as liquid brain ECM, alone or in combination with Geltrex were optimal for the timed induction of dopamine neurons. For reference we also mapped these markers in post-mortem human tissue samples to have an accurate physiological model control. In addition, we have

started to analyze whether there are differences in the expression of alpha-synuclein in TH positive neurons in the midbrain organoids with and without the E46K mutation.

P80

EXPLORING THE ROLE OF NEDDYLATION IN GLIAL CELLS: IMPLICATIONS FOR BRAIN DEVELOPMENT AND TRAUMATIC BRAIN INJURY

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Glial cells, abundant in the central nervous system (CNS), are essential for brain development, function, and homeostasis. Oligodendrocytes produce and maintain myelin sheaths, facilitating rapid signal transmission. Astrocytes provide structural support, regulate the blood-brain barrier permeability, and maintain the extracellular environment. They also contribute to the response to injuries like Traumatic Brain Injury (TBI), which induces CNS damage through various mechanisms including synaptic dysfunction, protein aggregation, and neuroinflammation. Following TBI, oligodendrocytes undergo apoptosis, leading to demyelination, while, astrocytes activate, proliferate, and show hypertrophy, among others. Neddylation, a post-translational modification process involving the covalent attachment of the ubiquitin-like protein NEDD8 to substrate proteins, regulates myriad cellular processes, including protein degradation, signal transduction, and cell cycle progression. Its dysregulation has been associated with some pathological contexts such as neurodegenerative diseases. This mechanism can be modulated using MLN4924 (MLN; Pevonedistat), a small-molecule inhibitor of the initiating enzyme of the neddylation cascade (NAE1). Despite existing data on neddylation in the CNS, primarily focused on neurons, its role in glial cells and TBI is not well understood. Previous results from our group showed that the pharmacological inhibition of neddylation by MLN compromised the viability of oligodendrocyte precursor cells under proliferation conditions and was also crucial for their differentiation. Following this line of research, this study aimed to investigate the role of neddylation in astrocytes in vitro and in the context of TBI. We demonstrate that, upon the inhibition of neddylation with MLN, astrocytes exhibited reduced viability and drastic morphological changes. Additionally, in a preliminary approach where we induced focal TBI in mice, we observed increased NEDD8 and NAE1 expression in the ipsilateral brain 24 hours post-injury. Interestingly, MLN-treated mice demonstrated improved neurological scores 24 hours after injury. These preliminary results suggest that while neddylation inhibition is detrimental under physiological and developmental conditions, it may ameliorate the effects of TBI by counteracting the over-activation of neddylation observed during this process. This

study highlights the significant role of neddylation in glial cells and suggests further research into its mechanisms and therapeutic potential in injury contexts like TBI.

P82

Characterization of AAV8-mediated MBP overexpression in oligodendrocytes in vitro and in vivo

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Oligodendrocytes generate myelin around axons, which allows for rapid and efficient nerve conduction and is essential for proper cognitive function of the central nervous system (CNS). In Alzheimer's disease (AD), alterations in myelinated axons have been described, suggesting that myelin dysfunction may also be a relevant pathophysiological feature of this disease. Myelin Basic Protein (MBP) is the major component of the myelin and it is overexpressed in AD models. Therefore, we hypothesize that MBP overexpression may promote oligodendrocyte dysregulation and myelin alterations in AD.Based on these findings, we investigated the adeno-associated viral (AAV)-mediated MBP and green fluorescent protein (GFP) expression driven by the MBP promoter in cultured oligodendrocytes in vitro. Our data showed that MBP mRNA and protein levels were increased in AAV8-MBP promoter-MBP-IRES-GFP (AAV-MBP-GFP) infected oligodendrocytes compared to AAV8-MBP promoter-GFP (AAV-GFP) infected control cells. Additionally, we stereotactically injected adeno-associated viral (AAV) vectors in the dentate gyrus of hippocampus of 12-month old 129/Sv mice. AAV8 led to a rapid onset of transgene expression (within 2 weeks), with expression persisting up to three months. Evaluation of cell types using confocal microscopy and glia and neuron-specific antibodies, revealed that AAV-MBP-GFP and AAV-GFP preferentially transduced oligodendrocytes. Furthermore, mice injected with AAV8-MBP-GFP showed significant changes on search strategies in Barnes maze compared to AAV8-GFP infected mouse controls.In conclusion, we have a novel tool to further study the role of MBP overexpression. Importantly, our preliminary results support that overexpression of MBP modify the behavior of wild type mice towards a similar strategy used by AD mice, highlighting the detrimental role of MBP overexpression. Further research is needed to clarify the mechanism behind myelin-associated alterations in AD. Funded by MICIU, EITB-Maratoia and Basque Government

P83

Human biomodels to study glial bioenergetic in Parkinson's disease

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Human biomodels, which include 2D cell models and 3D organ-like structures derived from human iPSCs, have become an essential tool to deeply understand and to model the biological, biochemical and molecular hallmarks of brain physiopathology, as in

Parkinson¬¨¬•s Disease (PD).The principal hallmark of PD is the selective neurodegeneration of dopaminergic neurons. Mounting evidences, confirmed by our previous studies in hiPSC-derived astrocytes and human brain tissues, suggested that astrocytes contribute to dopaminergic neurodegeneration through decreased homoeostatic support and loss of neuroprotection. In order to characterize the metabolic hallmarks of astrocytes in PD, we analyzed the mitochondrial activity and Ca2+ homeostasis in hiPSC-derived astrocytes from PD patients (LRRK2(G2019S)) and age pared control donors. It has recently been demonstrated that the distance between endoplasmic reticulum (ER) and mitochondria contact sites (MERCS) is a critical parameter for Ca2+ flux between organelles. Using a novel family of genetic probes (SPLICS), we found that PD astrocytes manifested impairment of mitochondrial bioenergetics and the alteration of the normal ER-mitochondrial distance (40-50 nm long).Taken together, our data indicate that astrocytic asthenia observed in patient-derived iPSC cultures with LRRK2(G2019S) mutation may contribute to neuronal death through decreased homoeostatic support, elevated oxidative stress and failed neuroprotection.Finally, now we have generated and characterized human midbrain organoids in order to corroborate these results in a more complex and complete model that better preserves tissue architecture and cell heterogeneity and interactions.Funded by Heath Department of Basque Country Government (2022333055), BIOEF (BIO21/COV/012) and EMBO Fellowship program.

P84

CO-CULTURE OF a-SYNUCLEIN-TREATED IPSC-DERIVED DOPAMINERGIC NEURONS AND MICROGLIA AS AN IN VITRO MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is a progressive neurodegenerative disorder associated with a loss of dopaminergic neurons (DAN) of the substantia nigra pars compacta. Multiple cellular and molecular mechanisms contributing to neuronal cell death include the abnormal accumulation of alpha-synuclein (alphaS) aggregates, mitochondrial dysfunction, oxidative stress, excitotoxicity, proteasomal dysfunction and neuroinflammation. Due to the complexity of neurodegenerative diseases, it is not surprising that non-neuronal cells such as microglia, are also involved in the pathological progression of PD.Microglia is the most abundant cell type involved in the immune response and homeostasis of the central nervous system. They continuously scan their microenvironment, monitor ongoing synaptic activity, phagocytose apoptotic cells, and provide trophic support for neurons. In the context of PD, previous studies in pluripotent stem cell-derived macrophages, demonstrate that the excess of alphaS can compromise alphaS clearance, and probably contributes to its accumulation in DAN. Therefore, it is crucial to examine the role of microglia in this process and, conversely, to understand whether microglial function is affected by the presence of an excess of the alphaS.

In our previous work, we have established protocols for the differentiation of iPSC-derived dopaminergic neurons and microglia. Here, we combine both cell lineages and establish a new co-culture model which is subsequently insulted with alphaS aggregates, thus establishing an in vitro model that better resembles PD.Our aim is to study the role of microglia in PD and to screen for different antiaggregant drug candidates with the objective of developing disease-modifying drugs that slow or halt the underlying neurodegenerative process. Future studies will not only focus on the validation of the phagocytic capacity of microglia, but also on the immune response to damage, the study of its morphology, the profile of pro- and anti-inflammatory cytokines, as well as changes in the expression of different receptors.

P85

Molecular and Morphological Changes in Oligodendrocyte Lineage Cells in Parkinson's Disease In Vivo

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NA

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the gradual loss of dopamine-producing neurons in the substantia nigra pars compacta (SNc). The cause of PD is unknown, but both genetic and environmental factors are thought to play a role in the development of the disease. Emerging studies have shown that targeting glial cells in Parkinson's disease may be a potential therapeutic strategy. Oligodendrocyte progenitor cells (OPCs) are found in the central nervous system (CNS) that can differentiate into oligodendrocytes, the cells responsible for myelinating axons in the CNS. Recent studies have suggested that OPCs may have a broader range of functions beyond myelination, including roles in inflammation, neuroprotection, and synaptic plasticity. The role of OPCs in PD is still unknown, however studies have shown that human single-cell transcriptome analysis from both SN and cortex have a large population of OPCs in PD subjects, and that PD genetic risk correlates better with oligodendrocyte lineage cells (OLs) than other cell types in SNc. In our study we used human SNCA-overexpressing mice (SNCA-OVX) as an in vivo PD model and analyzed OPC gene expression in wild-type and SNCA-OVX mice by RNAseq. Immunohistochemical analysis confirmed the dysregulation observed by RNAseq, namely that the OPC population increases in SNc and cortex of SNCA-OVX aged mice, and in addition, that the morphology of OPCs is also significantly in these animals. Although it is known that dopaminergic neurons are rather sparsely myelinated, OPCs could be involved in PD pathology given their plasticity and genetic correlation with PD. Further studies will be conducted in order to establish the functional relevance of these findings for the pathophysiology of PD.

P86

Noradrenergic disfunction in prodromal Parkinson’s disease: the Locus Coeruleus case.

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Parkinson's Disease (PD) is the second most prevalent age-related neurodegenerative disorder, in developed societies, after Alzheimer's Disease. Despite its prevalence, PD diagnosis is still based on the appearance of the classical bradykinesia, rigidity, and resting tremor. Patients may, however, present nonmotor symptoms as hyposmia, sleep disorders or cognitive/mood issues years prior to the onset of the motor signs. The Locus Coeruleus (LC) is one of the first areas to exhibit Lewy bodies and neurodegeneration. Its dysfunction is associated to the appearance of nonmotor symptoms due to its noradrenergic (NA) projections and influence on the homeostasis of dopaminergic (DA) networks. We have set up a prodromal mouse model of human alpha-syn overexpression at the LC by viral vector stereotaxic injections. Our goal is to characterize cognitive and molecular/histological changes driven by dysregulation of the LC-NA system - locally and in projecting hippocampal CA1 and CA3 regions. Thus, we have started to study behavioural phenotypes as locomotion and innate emotional responses, as well as spatial, recognition, and emotional memory. Moreover, we have analysed structural changes in local inflammatory markers, as astrocytes and microglia, together with molecular alterations both in glia and neuronal populations. These data, along with neurodegeneration histological studies, provide an insight into how LC-NA dysregulation occurs at very early phases of PD and will lead to the development of further functional research. Funding: Grant PID2 021-126434OB-I00 funded by MCIN/AEI/ 10.13039/501100011033 and ERDF A way of making Europe. It has also been funded by the Basque Government (IT1706-22, PUE21- 03, PIBA_2020_1_0048) and the UPV/EHU (COLAB20/07). This research was conducted in the scope of the Transborder Joint Laboratory (LTC) Non-motor Comorbidities in Parkinson's Disease (CoMorPD). LDlHG holds a PhD grant from the UPV/EHU.

P87

Automated detection and quantification of subtle motor changes in an early Parkinson's disease mouse model in the balance beam test

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Parkinson's disease (PD) is the most common motor neurodegenerative disease and to this day, remains incurable. PD is characterised by aggregated alpha-synuclein (alpha-syn) forming Lewy bodies, the pathological hallmark of PD. Here, we aimed to investigate temporal changes in motor impairments in a mouse model of early PD induced by alpha-syn overexpression, by combining conventional manual analysis of the balance beam test with a novel approach using machine learning algorithms to automate behavioural analysis. We used markerless pose estimation in DeepLabCut together with automated behavioural classification in Simple Behavior Analysis for automated video-based animal tracking.Our automated procedure was able to accurately detect and quantify subtle motor deficits in mouse performances in the balance beam test that would have been difficult to score manually. Our novel approach provided in-depth information on classifiers such as walking and falls, by generating the duration and frequencies of uninterrupted, continuous behavioural episodes (bouts) constituting the classifiers. The automated model revealed significant temporal differences for the walking behaviour in the mean interval between each behavioural bout, the median event bout duration and the classifier probability of occurrence in male PD mice, even though no statistically significant loss of tyrosine hydroxylase in the nigrostriatal system was found in either sex. These findings are valuable for early detection of motor impairments in animal models of earlier stages of PD. Here, we provide a user-friendly, step-by-step guide for automating video-based quantification of mouse motor behaviour in the balance beam test, with a reproducible and flexible protocol that does not require any significant computational or programming skills.This study was funded by Grants from the Basque Government (IT1706-22) and the Spanish Ministry of Science and Innovation (MCIN/AEI/10.13039/501100011033; PID2 021-126434OB-I00). This research was conducted in the scope of the Transborder Joint Laboratory (LTC) non-motor Comorbidities in Parkinson's Disease (CoMorPD). M.Z has a fellowship from the University of the Basque Country.

P88

Galectin-1 O-GlcNAcylation controls migration and phagocytosis responses to Amyloid ≈í‚â§ peptide

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Protein O-GlcNAcylations are dynamic post-translational modifications (PTM) that consists of the attachment of O-linked N-acetylglucosamine (O-GlcNAc) moieties to cytoplasmic, nuclear and mitochondrial proteins in serine and threonine residues. This PTM regulates many biological responses such as stress and nutrient responses and its controlled by a single pair of enzymes, O -GlcNAc transferase (OGT) and O-GlcNAcase (OGA). 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). In our laboratory, we have identified by LC-MS/MS 60 O-GlcNAcylated proteins that were exclusively present in the amyloid beta 1-42 (Abeta1-42) oligomers-stimulated lysates. Galectin 1 (Gal-1) was one of them, this is a key proten in the regulation of cell responses, controlling different intracellular and extracellular responses. 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 O-GlcNAcylated Gal-1 in cell biology, we studied the migration pattern in immortalized human microglia and astrocytes, mouse embryonic stem cells (mESCs) and differentiate astrocytes from mESCs. For that, we conducted Wound Healing and Transwell assays and the results showed different patterns in migration and different response to the Abeta1-42 oligomers stimulation. Furthermore, the absence of O-GlcNAcylation in Gal-1 promotes an uncontrolled migration, accentuated in the presence of Abeta1-42. In addition, the presence of Abeta1-42 oligomers also promoted uncontrolled migration in Gal-1WT and Gal-1S8C. Based on this results, we suggest that O-GlcNAcylation of Galectin-1 controls cell migration and its necessary for a correct function. On top of that, Abeta1-42 also contributes to uncontrolled cell responses.On the other hand, we investigated whether the phagocytosis pattern was altered by of O-GlcNAcylation in Gal-1 in human immortalized microglia. The results obtained showed that the absence of O-GlcNAcylation in Gal-1 promotes an uncontrolled phagocytosis, accentuated in the presence of Abeta1-42. These pattern adds up with the migration pattern. Taken together, we conclude that O-GlcNAcylation in Gal-1 is essential for a proper migration and phagocytosis. These results could be a new target in order to characterize and control the neurodegeneration in Alzheimer disease.

P89

Nigrostriatal organotypic cultures to study neuromelanin dynamics in dopaminergic circuits

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It has been recently demonstrated that overexpression of human tyrosinase in the mouse substantia nigra causes neuromelanin accumulation and dopaminergic cell death in vivo. However, little is known about the dynamic interaction between extracellular neuromelanin and the surrounding microenvironment, including cells and the extracellular matrix. Brain slice cultures provide the opportunity to monitor pathological brain circuits and follow cell dynamics over time. Here we have developed a protocol for the preparation and maintenance of rat organotypic cultures from parasagittal brain slices that preserve the nigrostriatal circuit throughout the culture process. We use a 13¬¨,à´ slicing angle and a customized cocktail of small molecules and growth factors, to maximize the integrity of the nigrostriatal pathway and ensure the survival of dopaminergic neurons, respectively. The slices maintain the basic cytoarchitecture of the brain, including glia and the extracellular matrix, but not including vessels nor immune system. The cultures can be used to study dopaminergic degeneration, cell-cell and cell-matrix interactions and are particularly suitable for AAV-mediated overexpression of transgenes, brightfield or fluorescence time-lapse live imaging and longitudinal studies. Specifically, they allowed us to keep track of dopaminergic cell death in the substantia nigra and neuromelanin accumulation 20 days after AAV-hTyr infection. Long-term real-time monitoring of the pathological process within the brain parenchyma will enable to study the dynamics (accumulation and clearance) of neuromelanin and possibly other aggregates.

P90

Neuroendocrinal Sex Related Differences After Acute Social Defeat Stress in Pubertal CD1 Mice

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Although social stress-related psychiatric disorders affect mainly to women, most preclinical research has been conducted in male mice due to the lack of reliable female social stress animal models. Due to the differing social dynamics among sexes, proposed models for one sex has resulted inefficient to the other. In our study, a defeat stress protocol, based on the appliance of adult male urine, was used. In this context, the objectives of the current study were twofold: (1) to validate the defeat stress protocol under an acute paradigm in pubertal mice; (2) to analyze the possible neuroendocrinal sex discrepancies after the defeat stress protocol. To this end, male and female pubertal subjects were exposed to the female social defeat protocol over a 24-hour period. Behavioral results indicated the correct functioning of the model in the laboratory. Neuroendocrinal results indicate that stressed females showed lower progesterone and testosterone compared to their non-stressed counterparts. Overall, the current findings remark the need to conduct preclinical research considering always both sexes.

P91

LRRK2 mutations induced energy metabolism alterations as triggers of neurodegeneration in models of Parkinson¬¨¬•s Disease

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Parkinson's disease (PD) represents the second most common neurodegenerative disorder, and its occurrence is on the rise within our aging population. The loss of dopamine-producing neurons, which is at the core of PD, has long been associated with compromised energy production within the mitochondria. Meanwhile, ongoing research suggests that irregularities in lysosomal as well as mitochondrial metabolism play crucial roles in the development of this disease. Recent investigations have revealed deviations in mitochondrial function and nucleotide metabolism linked to PD in human neurons. Mitochondria, housing DNA (mtDNA) vital for energy production and overseeing autophagy, form a functional loop that we propose includes defects in mitochondrial respiration and DNA maintenance. Any alterations in these pathways, particularly in highly susceptible cell types as higly energy demanding dopaminergic neurons, can potentially lead to neuronal cell death and the onset of PD. Our project aims to explore the relevance of Parkinson's disease-causing mutations, specifically LRRK2 mutations, in relation to mitochondrial function, autophagy, and DNA metabolism. We will apply fibroblasts extracted from skin biopsies of PD patients carrying LRRK2 mutations and Drosophila melanogaster models. Through the analysis of functional and molecular outcomes derived from our models, we demonstrate that hyperactivation of LRRK2, causing alterations

in autophagy, resulting in energy source crucial switch that affects mitochondrial oxidation and mtDNA metabolism defects, triggering neuronal survival and locomotor function. These findings could result in the identification of new disease modifying therapeutic targets against LRRK2 mutations-derived and non-derived PD.

P92

Astrocytic insulin receptor ablation precipitates Alzheimer's disease onset

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Objectives: Insulin is an important regulator of glucose homeostasis and metabolism. However, its role in the central nervous system is not yet fully understood. The role of insulin and its receptor (i.e. the insulin receptor, IR) in neurons has been widely studied. However, the role of insulin action in astrocytes remains unknown. Astrocytes provide structural, energetic and metabolic support to neurons, and also form the blood-brain barrier, being indispensable for adequate brain homeostasis. Interruption of astrocytic insulin signalling could change those astrocytic functions and thus, alter neuronal activity leading to cognitive deficiencies. In this line, this study aims to evaluate the impact of astrocytic IR deletion on Alzheimer's disease (AD).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. Once IR deletion is obtained those mice were crossed with APP/PS1 AD mouse model. Brain glucose metabolism was evaluated by 18F-FDG PET. Peripheral metabolic characterization was performed by glucose tolerance and insulin tolerance tests. Memory was assessed using the Fear conditioning and Morris Water Maze tasks. Beta-amyloid accumulation was assessed by immunohistochemistry and ELISA. Results: IR-ablated astrocytes featured reduced glucose uptake and glycolysis, upon amyloid-beta treatment. In our hands astrocytic IR deletion precipitates AD pathology in APP/PS1 mice inducing cognitive deficiencies. This cognitive perturbances are accompanied by lower brain glucose uptake. Interestingly, IR deletion in astrocytes induced an increase in amyloid-beta burden in AD mice. Conclusions: Astrocytic IR ablation drives brain and systemic metabolism towards a less efficient glucose-handling phenotype and accelerates AD features inducing cognitive deficiencies and elevated amyloid burden.Key words: Astrocytes, Alzheimer's Disease, insulin receptor, glucose, brain metabolism.

P93

Comparison of tectal mesencephalic development in vertebrates neurogenesis and cell types

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Visual information is processed by equivalent structures in all vertebrates. A primary brain center in the visual pathway is the tectal mesencephalon. It is thought that this tectal mesencephalon -optic tectum for the non-mammals and superior colliculus for mammals- is a conserved structure among species due to its layer organization and shared neuronal inputs and outputs. However, sauropsids process major retinal information through the optic tectum, whereas mammals do it mainly through the thalamus. In addition, there is a clear difference in the relative size and architecture of the tectal mesencephalon between mammals and sauropsids. This important difference may be due to differences on the developmental trajectories of both structures: the neurogenesis of different layers, cell type diversity and higher transcriptomic heterogeneity. In the present work, we aim to compare and find the key differences in the formation of the several tectal mesencephali among amniotes. We are performing comparative immunohistochemistry, cell birthdating, retrograde tracing and bioinformatic Seurat integration of transcriptomic cell profiles in developing and adult samples of key vertebrate species. We are focusing on the neurons of the retino-tecto-thalamic visual pathway, and the transcriptomic expression of the optic tectum and superior colliculus. These tectal mesencephali display differences that indicate that even though they are conserved at the circuit level, and share an equivalent layer organization pattern, its diversity resides on the transcriptomic level. This means that the tectal mesencephalon acts as an intermediate point in the divergence of the visual pathway between the retina, a highly conserved structure among different species, and the thalamus, a highly divergent structure among different species.

P94

MP-004 as a promising candidate targeting calcium dysregulation and oxidative stress in retinitis pigmentosa

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Calcium dysregulation and oxidative stress are crucial mechanisms leading to photoreceptor death in several retinopathies, such as retinitis pigmentosa. Ryanodine receptor type 2 (RyR2), endoplasmic reticulum calcium channels, are essential for photoreceptor calcium homeostasis and RyR2 dysfunction has been involved in photoreceptor degeneration. Our group has developed a group of FKBP12 ligands (MP compounds) aiming to restore FKBP12/RyR interaction and rescue abnormal RyR function under nitro-oxidative stress. Our compounds have demonstrated antioxidant capacity and are able to normalize intracellular calcium levels in cardiac and muscle cellular models. Our purpose is to study the therapeutic potential of MP-004 in retinitis pigmentosa and unravel its mechanism of action. To this end, we have evaluated its efficacy in rescuing H2O2-induced cell toxicity and calcium dysregulation in the photoreceptor cell line 661W. In addition, we also evaluated its efficacy in the rd10 mouse model of retinitis pigmentosa, which carries a mutation in the PDE6B gene that triggers photoreceptor death from postnatal day 16. Our results support MP-004 as an interesting candidate for retinitis pigmentosa by targeting calcium dysregulation and oxidative stress, and ultimately preventing photoreceptor cell death.

P95

Amyloid oligomers dysregulate oligodendrocyte differentiation and myelination via PKC in the spinal cord of zebrafish larvae

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Amyloid oligomers (Abetao) have been proposed as candidates to induce oligodendrocyte (OL) and myelin dysfunctions, which are early events in Alzheimer's disease (AD) pathology. However, little is known about how Abetao affect OL differentiation and myelination in vivo, and the underlying molecular mechanisms. In this study, we investigated the effects of a brain intraventricular injection of Abetao on OLs and myelin in the developing spinal cord of zebrafish larvae. Using quantitative fluorescent in situ RNA hybridization assays, we demonstrated that Abetao alter myrf and mbp mRNA levels and the regional distribution of mbp during larval development, suggesting an early differentiation of OLs. Through live imaging of Tg(myrf:mScarlet) and Tg(mbp:tagRFP) zebrafish lines, both crossed with Tg(olig2:EGFP), we found that Abetao increased the number of myrf+ and mbp+ OLs in the dorsal spinal cord at 72 hpf and 5 dpf, respectively, without affecting total cell numbers. Furthermore, Abetao also increased the number of myelin sheaths per OL and the number of myelinated axons in the dorsal spinal cord compared to vehicle-injected controls. Interestingly, the treatment of Abetao-injected zebrafish with the pan-PKC inhibitor G6983 restored the aforementioned alterations in OLs and myelin to control levels. Altogether, not only do we demonstrated that Abetao induce precocious oligodendroglial differentiation leading to dysregulated myelination, but we also identified PKC as a key player in Abetao-induced pathology.

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Intracarotid grafting of human Dental Pulp Stem Cells contributes to neovascularization and cell migration to affected area in ischemic stroke

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Ischemic stroke results from decreased cerebral blood flow, leading to cellular death due to oxygen and nutrient deprivation. Additionally, subsequent recanalization also influences the course of the ischemic event. A key challenge in treating ischemic stroke is developing neuroprotective strategies to protect the brain before and during recanalization [1]. Due to

their versatile properties, human Dental Pulp Stem Cells (hDPSCs) are promising candidates in tissue regeneration therapy. Known for their immunomodulatory and neurotrophic capabilities, hDPSCs are particularly interesting because of their potential to differentiate into various cell lineages, including vascular cells. Previous studies have demonstrated that hDPSCs can differentiate into both endothelial cells and pericytes, and even give rise to complete blood vessels in the brain of nude mice after cell inoculation [2]. This study aimed to investigate whether intracarotidally grafted hDPSCs could integrate and promote neovasculogenesis in the brain of ischemic rats. Furthermore, we evaluated the impact of hDPSC grafting on the recovery of ischemic animals and stimulation of neurogenesis processes. For this purpose, hDPSCs isolated from the third molars of healthy donors were initially cultured in standard media for amplification and then transferred to serum-free media for ten days to promote vascular differentiation. Subsequently, hDPSCs were inoculated via right carotid artery into Sprague-Dawley male rats previously induced to ischemic conditions induced by 75 minutes of middle cerebral artery occlusion (MCAO). The neurological state of ischemic animals was periodically assessed to compare the recovery of cell-grafted and control rats. Fourteen days after the ischemic events, animals were injected with labelled lectin for imaging vasculature and sacrificed with 4% PFA perfusion. Post-fixed brain, liver, and spleen tissues were dissected and processed for immunohistochemical analysis. hDPSCs were labelled with Stem121 human marker and our intra-arterial administration allowed grafted cells not only to reach the infarct core but to contribute to in situ neovascularization, particularly in the periphery of the brain lesion. Interestingly, hCD31+ hDPSCs were found in functional blood vessels surrounded by astrocytes, as confirmed by lectin and GFAP labelling. Some isolated hDPSCs were also engrafted in liver and spleen sections with no signs of tumour generation. Neurological assessment showed a tendency for hDPSC-grafted animals to have better recovery. Finally, DCX staining did not reveal differences in neurogenesis between the two groups, but indicated a higher migration of neural progenitors towards the affected zone after cell grafting. Overall, the use of hDPSCs for brain vasculature regeneration appears highly promising as a strategy for treating brain ischemic stroke and other neurovascular disorders.[1] Paul, S., & Candelario-Jalil, E. (2021). Emerging neuroprotective strategies for the treatment of ischemic stroke: An overview of clinical and preclinical studies. Experimental neurology, 335, 113518. [2] Luzuriaga, J., Pastor-Alonso, O., Encinas, J.M., Unda, F., Ibarretxe, G., & Pineda, J.R. (2019). Human Dental Pulp Stem Cells Grown in Neurogenic Media Differentiate Into Endothelial Cells and Promote Neovasculogenesis in the Mouse Brain. Frontiers in physiology, 10, 347. This work has been financed by the University of the Basque Country (UPV/EHU) (PPGA20/22), the Basque Government (Grants 2021333012, IT1751-22 and PIBA_2023_1_0013), Fundació La Marató-TV3 248/C/2020), ERA-NETNEURONJTC2022(CV_043IMatrix) and the Spanish Ministry of Science and Innovation MICINN/AEI/10.13039/50110001103 (PID2019-104766RB-C21). JSM and BPR obtained a Ph.D. fellowship from Basque Government (PRE_2023_2_0112 & PRE_2023_2_0038).

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Autophagic regulation of oligodendrocyte physiology in cerebrovascular disease

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Autophagy regulated lysosomal digestion determines the physiology and survival of brain cells during metabolic shortage imposed by cerebrovascular disease. In turn, lysosome-related Scarb2 mutant zebrafish exhibit impaired oligodendrogenesis and a pathological vascular phenotype, suggesting a reciprocal connection between autophagy, oligodendrocytes and vascular disease. The objective of this work is to study the bidirectional relationship between autophagic (dys)function in oligodendrocytes and cerebrovascular disease. To this end, we are using in vitro and in vivo approaches to analyze the effects of genetic (SCARB2, ATG4B) and environmental (hypoxia) cerebrovascular disease factors in oligodendrocyte autophagy and physiology. Our preliminary results show that SCARB2 mainly localizes to degradative vesicles (late endosomes/lysosomes) in oligodendrocytes, although non-degradative vesicles (early endosomes/autophagosomes) also contain SCARB2, suggesting broad roles for SCARB2 on autophagy regulation. Acute hypoxia does not alter neither the subcellular distribution nor the expression levels of SCARB2 in oligodendrocytes, although it does enhance autophagic turnover in a time-dependent manner. It will be interesting to test if chronic hypoxia differentially affects oligodendrocyte autophagy and SCARB2 dynamics with complementary in vivo analysis of the cortex and corpus callosum of cerebrovascular disease mouse models, which are underway. Interestingly, preliminary data using autophagy deficient ATG4B KO mice exhibit increased numbers of OLIG2+ oligodendrocytes in the cortex and corpus callosum. We are now performing siRNA based Scarb2 and Atg4b silencing experiments to decipher the underlying mechanisms. The understanding of the mechanistic connections between oligodendrocyte autophagy and the vasculature may eventually aid on identifying new therapeutic targets for the treatment of cerebrovascular disease.

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Evaluation of the deleterious effect of hyperglycemia in experimental stroke: role of the hypoxia-inducible factor (HIF).

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Ischemic stroke is a current important health problem due to its high incidence and theworsening quality of life of patients. Hyperglycemia during stroke has been related to a worse prognosis, however, the mechanisms involved are still unknown. The imbalance between O-glycosylations/phosphorylations of different proteins as consequence of hyperglycemia and, in particular, HIF glycosylations following ischemia could be key.In order to study alterations in HIF, adult male Sprague-Dawley rats were subjected to 75-minmiddle cerebral artery occlusion (MCAO). Hyperglycemic and normoglycemic rats receivedeither dextrose 15 minutes before MCAO (351±42 mg/dL at the occlusion; n=85) or vehicle(water; 217±38 mg/dL; n=68). Neurofunctional impartment was evaluated at 0, 1, 3, 6, 12 and 24 hours after MCAO and post-mortem brains were subjected to TTC and Fluoro-

Jade(neurodegeneration) stainings. In vitro model of ischemia-reperfusion injury was carried outusing 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 was determined by Trypan-Blue as well as glycogen labeling. Glycogen storage was also visualized in rat astrocytes by immunofluorescence and HIF-1 alpha was measured by Western-Blot.Hyperglycemic rats showed a significant worsening in neurofunctional recovery after ischemia along with an increase in infarct volume and a decrease in HIF-1 alpha protein expression starting at 3 hours following reperfusion. At 24 hours after ischemia, hyperglycemia increased degenerative neurons using Fluoro-Jade and hypoxia promoted a decrease in nuclear glycogen stores in the astrocyte but not in combination with hyperglycemia. Besides, immortalized human astrocyte cultures showed that hypoxia followed by hyperglycemia induced a reduction in cellular proliferation and HIF-1 alpha expression. Similar to in vivo conditions, hypoxia induced a decrease in the nuclear glycogen stores during the first 24 hours that was increased in combination with hyperglycemia.In conclusion, hyperglycemia worsened the progression of ischemic injury after MCAO in rats and the viability of astrocytes in vitro which was related to a decreased in HIF-1 alpha protein expression. Finally, nuclear glycogen stores in the astrocyte increased after hypoxia with hyperglycemia in both in vitro and in vivo cerebral ischemia.ReferencesDavis, C. K., et al. Frontiers in cell and developmental biology 2018, 6, 175.Dengler, V. L., et al. Critical reviews in biochemistry and molecular biology 2014, 49 (1), 1-15.Kruyt, N. D., et al. Nature reviews. Neurology 2010, 6 (3), 145- 55.Martin, A., et al. Stroke 2006, 37 (5), 1288-95.Scott, J. F. et al. Lancet (London, England) 1999, 353 (9150), 376-7.

P99

Behavioural effects of Cplx1/2 knockdown at GABAergic or glutamatergic hippocampal neurons in the PS19 murine model of tauopathy.

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Tauopathies, including Alzheimer's disease (AD), are currently understood as prion-like diseases in which altered excitatory/inhibitory (E/I) balance could play an important role in tau spread and early symptom manifestation. E/I imbalance may arise from specific GABAergic deficits, including the loss of complexin-I (Cplx1) in early stages of AD. Here, we evaluated the effects of complexin-I/II (Cplx1/2) knockdown (KD) in GABAergic or glutamatergic hippocampal neurons on the behavioural phenotype of adult PS19 transgenic mice overexpressing pathogenic P301S-tau species. To this end, adeno-associated viral vectors (AAV) encoding five micro-RNAs (miR) silencing both Cplx1 and Cplx2 gene expression under selective GABAergic (Dlx; n=9) or glutamatergic (Camk2a; n=8) promoters were unilaterally infused into the dentate gyrus of the right hippocampus of 8-week-old PS19 mice. Control mice (n=10) received similar vectors carrying an empty miR cassette. A complete behavioural evaluation was performed from week 11 through 14, including assessment of anxiety-like behaviours (open field test, OFT; and elevated plus maze, EPM), cognitive performance (Barnes maze), and motor coordination (rotarod test, RRT). At the end of the behavioural phenotyping, mice were perfused transcardially, and brains were vibratome-sliced into 40-

thick sections. Immunohistochemical experiments (IHC) were performed to (1) confirm the selectivity of AAVs for GABAergic or glutamatergic neurons using specific cell-type markers, and (2) test miRs capacity for inhibiting Cplx1/2 expression. Datasets were analysed with one- or two-way repeated measures (TW-RM) ANOVA followed by Tuckey's post hoc test. IHC assays confirmed both the selective expression of AAVs at their target neuronal populations and a significant inhibition of Cplx1/2 protein expression in the ipsilateral (but not contralateral) hippocampus 21 days after AAV infusion. Cplx1/2-KD in either neuronal type was not associated with abnormally elevated anxiety-like behaviours measured in the OFT and EPM. Cplx1/2-KD and control mice showed similar learning and spatial memory abilities in the Barnes maze, as evidenced by a gradual improvement in test performance across trials. No differences in motor coordination were found longitudinally in the latency to fall in RRT. However, Cplx1/2-KDDlx mice exhibited significantly greater gait performance in the last two trials (trial 5: +119 ¬¨¬± 24.5%, $p < 0.01$; trial 6: +55 ¬¨¬± 10.8%, $p < 0.05$) compared to the control group. In conclusion, the present study showed that Cplx1/2-KD at GABAergic or glutamatergic hippocampal neurons has no significant impact on the behavioural phenotype in an AD mouse model other than a marginal improvement in motor coordination following repeated trials in Cplx1/2-KDDlx mice. The generated models will be useful to further test the role of GABAergic and glutamatergic functionality in the pathological spread of tau.Supported by BIO22/ALZ/002 (BIOEF/Maratoia-EiTB) and IT1512/22 (Basque Government) grants. EH-H (FJC2022-048338-I) and AR-M (CNS2023-143768) hold grants funded by MCIN/AEI/10.13039/501100011033 and the European Union NextGenerationEU/PRTR.

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Modelling Parkinson's Disease Using iPSC-Derived Organoids Focusing on the Locus Coeruleus

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder. It is classically defined by motor symptoms such as bradykinesia, tremor, and gait disturbances, but also presents non-motor symptoms including cognitive impairment, depression, and sleep disorders. While the motor symptoms are primarily due to the loss of dopamine neurons in the substantia nigra pars compacta, the degeneration of the locus coeruleus (LC) is possibly responsible for non-motor symptoms which often precede the motor ones. While the majority of Parkinson's cases are sporadic, some of them result from genetic mutations, such as the E46K mutation in the alpha-synuclein gene (SNCA) that causes parkinsonism and Lewy body dementia, which we are using to model the disease.In our quest to develop innovative and predictive models of PD, we are using organoids, three-dimensional cellular models derived from human induced pluripotent stem cells (iPSCs)- to recreate brain regions differentially affected in PD . The modelling of locus coeruleus was challenging due to the lack of existing protocols and the existing differences between rodent and human locus development. Finally, we have adapted a recently published protocol from the laboratory of Yunlong Tao, for the induction of noradrenergic neurons from iPSCs in 2D, to generate a 3D-organoid model of the

human LC.We previously generated iPSC cells from dermal fibroblasts of several patients from the SNCA E46K family. For this study we used a pair of isogenic lines which also carry a tdTomato fluorescent reporter under the TH promoter. This novel approach provides a promising model for studying the non-motor symptoms of PD and the role of the LC in the initiation and progression.

P101

Molecular and functional characterization of human pluripotent stem cell-derived oligodendrocytes to model Alzheimer's disease

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Oligodendrocytes (OLs) are the glial cells that produce myelin within the central nervous system, crucial for a proper conduction of electrical signals. It is known that OL dysfunction and myelin degeneration occur in Alzheimer's disease (AD) but the specific mechanisms are not well understood. 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 disease. In this sense, the human pluripotent stem cell hPSC technology constitutes an essential tool to model AD.We generated OLs from three hPSC lines: a parental line from a non-affected individual and isogenic APOE3/3 and APOE4/4 lines derived from the parental line. Cells were characterized by immunocytochemistry at different developmental stages, and from DIV 75 on, molecular and functional analysis were performed. Oligodendroglial lineage was characterized at DIV 95 by staining with O4, CNPase and MBP markers, showing different levels of maturation of OLs. Calcium recordings revealed spontaneous calcium transients in O4+ cells, showing signal coordination with MAP2+ cells. O4+ OLs also showed AMPA calcium responses mediated by functional glutamatergic receptors. We are currently studying the dysregulation of calcium dynamics in AD conditions after stimulation of AMPA receptors. Ongoing and future experiments are intended to understand how different APOE variants affect cholesterol homeostasis and myelination in OLs in control and AD conditions.

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CROSS-TALK BETWEEN NEUROMELANIN AND SYNUCLEIN IN PARKINSON'S DISEASE

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The main characteristic features of Parkinson¬¨¬•s disease are the accumulation of alpha-synuclein (alpha-syn) into Lewy body (LB) inclusions and the selective loss of melanizeddopaminergic neurons in the substantia nigra pars compacta (SNpc). The

pigmentneuromelanin (NM), which accumulates in dopaminergic neurons during normal agingin humans, seems to play a protective role during dopamine synthesis. However, itseems evident that, when the accumulation of NM exceeds a pathological threshold, itcan trigger the degeneration of dopaminergic neurons, characteristic of PD. It has beenalso suggested that NM can modulate neuronal vulnerability in PD by inducing alpha-synexpression and aggregation. In contrast to humans, rodents, commonly used as PDanimal models, lack NM. In order to mimic an age-dependent accumulation ofneuromelanin within nigral dopaminergic neurons, Carballo-Carvajal et al. (2019)successfully injected an adeno-associated viral vectors carrying the gene that codes forhuman tyrosinase (AAV-hTyr) in the rat substantia nigra. Here, in order to study thepathological mechanism underlying neuronal loss in PD, we used the approach ofCarballo-Carvajal et al. (2019) and we injected an AAV-hTyr into the SNpc of a knock-inmice for human alpha-syn. Our results showed that AAV-hTyr injected mice showed a clearmotor phenotype characteristic of the disease and the corresponding striataldopaminergic denervation due to the loss of dopaminergic neurons in the SN. Weperformed RNA-seq analyses on midbrain and the Gene ontology (GO) analysis showedmany significantly enriched genes associated with microglial activation, confirmingthat and excessive activation of microglia may exacerbate the neuronal death. Theidentification of specific genes that can modulate this neuroinflammation-mediatedneurotoxicity will open new avenues for the treatment of PD.

P103

Potential therapeutic effect and mechanism of action of PLA2G4E for Parkinson's disease

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Neurodegenerative diseases are emerging as prominent illness in the 21st century due to extended life expectancy. Parkinson's disease (PD) is one of the main neurodegenerative disorders, and although extensive research has been conducted to understand the pathogenesis of this complex disease, efficient treatments remain elusive. This study aimed to evaluate whether PLA2G4E, a cytosolic phospholipase A2 group IV, has a neuroprotective role in a PD neuromelanin-producing animal model. The model involved the administration of an adeno associated viral vector (AAV-Tyr) to induce human tyrosinase overexpression in the substantia nigra pars compacta (SNpc) of mice. AAV-PLA2G4E and AAV-Tyr were co-injected in the SNpc with additional groups of animals receiving equivalent amounts of either AAV-Tyr or AAV-PLA2G4E separately. A successful PLA2G4E overexpression covering the whole nigro-striatal pathway was confirmed by immunohistochemistry. Behavioral studies demonstrated that the therapeutic virus prevented the motor deficits induced by Tyr overexpression. Stereological quantification of tyrosine hydroxylase (TH+) neurons revealed statistically significant changes on neuronal count in the treated group compared to the untreated one, indicating a neuroprotective effect. Also, immunohistochemistry stains indicated less reactivity in astrocytes and higher prevalence of healthy microglia in the treated group. This led to conclude that PLA2G4E overexpression has neuroprotective effects by preventing dopaminergic neuronal loss and mitigating immune response, inherent to PD. This work contributes to the ongoing pursuance of effective treatments for neurodegenerative diseases.

P104

Uncovering the contribution of RNA-misprocessing in Glioblastoma for the development of novel therapeutic strategies

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NA

IntroductionGlioblastoma (GBM) is the most frequent and most malignant brain tumor, with a devastating prognosis of a median overall survival of 15 months. Its aggressiveness is closely associated with a strong therapy resistance which arises from a small population of dormant but highly tumorigenic self-renewing glioma stem cells (GSCs). RNA-processing is a tightly regulated molecular mechanism that includes 5'mRNA capping, splicing and 3'end processing. This process ensures a correct gene expression pattern and expands transcriptome and proteome diversity. Emerging evidence indicates that misregulation of RNA-processing is a hallmark of cancer, that contributes to tumor initiation and evolution. However, it is still poorly studied in GBM. The main objective of this project is to uncover RNA-misprocessing events in the context of GBM and specific of GSCs that can be exploited to develop new therapeutic approaches for GBM.Material and methodsOur group has previously established patient-derived GSCs from tumor samples of a panel of GBM patients from Donostia University Hospital, which have been phenotypically characterized in vitro and in vivo. We performed high-throughput mRNA sequencing in GSCs (n=17) and GBM tumor samples (n=26), together with samples from normal brain tissue (n=3), astrocytes (n=5) and human neural stem cells (hNSCs) (n=6) as controls. In parallel, transcriptomic data of high-grade glioma GBM (n=49) and low-grade gliomas astrocytoma (n=34) and oligodendroglioma (n=39) primary tumor samples was obtained from Chinese Glioma Genome atlas (CGGA) public dataset. Gene expression and alternative splicing (AS) data were analysed using Salmon and Vast-tools pipelines, respectively.Results and discussionRNA-seq data from the CGGA demonstrated that GBM not only separates from low-grade gliomas (LGG) by gene expression, but also by splicing profile. We detected many dysregulated AS events (mostly exons) comparing GBM versus Astrocytoma (WHO grade 2) or GBM vs Oligodendroglioma (WHO grade 2). However, there were very low number of dysregulated AS events detected when comparing LGG between them, confirming that RNA-processing signature is specific of the tumor grade. Importantly, we have identified over 100 AS events that were commonly dysregulated in GBM vs LGG that might be associated with GBM aggressiveness. Analysis of mRNAseq data from our own GBM cohort, demonstrates that the GSCs established in our lab recapitulate the features of GBM tumors by gene expression. Based on GBM molecular classification, we have established cultures of different GSC types including Proneural (PN), Mesenchymal (MES) and Classical (CL), that showed different phenotypic features in vitro. Moreover, preliminary analysis shows specific AS events dysregulated in GSCs compared to hNSCs and astrocytes, opening the window to a new source of disease-relevant targets which can be exploited for disease diagnosis, response to therapies or to develop novel therapeutic approaches.

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A Rapid and Efficient Human Neural Model from iPS Cells to Study Lysosomal Disruption and TDP-43 Pathology in the Context of FTD Neurodegeneration

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Biogipuzkoa HRI, CiberNED, UPV/EHU, Ikerbasque Basque Foundation for science, Miaker Developments

Frontotemporal Dementia (FTD) is recognized as the second most common early-onset dementia, generally affecting patients under 65 years of age. Mutations in GRN gene, which codes for progranulin (PGRN), lead to haploinsufficiency of PGRN, causing autosomal-dominant FTD with TDP-43 pathology (FTLD-GRN). Although its role is unclear, PGRN is widely associated with lysosomal function. Moreover, TDP-43 depletion has also been related to autophagolysosomal degradation defects, linking PGRN, TDP-43 and lysosome in neurodegeneration.Methods to differentiate human induced pluripotent stem cells (hiPSCs) into neurons have recently provided experimentally tractable cell models. In this context, we developed a novel neuronal model for investigating FTD-PGRN by inducing the differentiation of hiPSCs carrying the GRN mutation (Q125X) and wild-type (WT) hiPSCs into human cortical neurons using protocols mediated by inducible transcription factors. These neurons exhibited the expression of both ≈í,â§3-Tubulin, a marker specific to neuronal populations, and VGLUT, a marker specific to glutamatergic neurons, and displayed indications of mature network activity as early as 20 days post-differentiation. As expected, GRN-Q125X neurons display GRN haploinsufficiency, as indicated by lower levels of GRN. In addition, GRN-Q125X neurons show a significant reduction in lysosomal acidification with respect to WT neurons. Despite the absence of cytoplasmic TDP-43 inclusions at 20 days of differentiation, there is a trend of lower nuclear TDP-43 expression in GRN-Q125X neurons compared to WT. The preliminary characterization of this neuronal culture offers a legitimate prospect for studying neuropathological mechanisms associated with lysosome defects in the context of FTD neurodegeneration.

P106

Effect of Iododiflunisal on Amyloid-beta and Neuroinflammation in the 5xFAD Alzheimer's Disease Mouse Model: A Longitudinal PET Study

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NA

Alzheimer disease (AD) is characterized by the accumulation of amyloid-≈í,â§ (Abeta) plaques. Transthyretin (TTR) is a potential therapeutic target due to its neuroprotective ability to bind and clear Abeta. However, the altered tetramer form of TTR in AD hinders optimal Abeta binding. Iododiflunisal (IDIF) enhances TTR-Abeta interaction, reduces Abeta plaques and improves cognition early on in AD animal models. In this work, we assess IDIF effect on early-stage plaque deposition using PET imaging.Fifty 2-months-old female 5xFAD mice were divided into 5 groups (n=10 per group) and treated with different doses of IDIF (0, 10, 30, 100 and 300 mg/kg/day) administered in the drinking water for 6 months. Treatment efficacy assessment was based on changes in longitudinal deposition of Abeta in the hippocampus

(HIP) and the cortex (CTX), as determined using PET-[18F]-Florbetaben. Neuroinflammation was also evaluated thought PET-[18F]-DPA-714 at the end of the treatment. Complementary studies were carried out to evaluate anatomical brain alterations and spatial learning and memory using MRI and the Barnes Maze behavioral test, respectively. For behavioral tests, 11-month-old wild type mice were used as control. Post-sacrifice, ex-vivo studies were carried out to confirm in vivo studies.Standard uptake values relative to the cerebellum (SUVR) of [18F]-Florbetaben in CTX and HIP of non-treated animals progressively increased from 2 to 9 months of age. However, SUVR for animals treated with IDIF 300 mg/kg/day remained constant both in CTX and HIPP during the early stages of the treatment, suggesting that IDIF treatment can delay Abeta plaque formation. The Barnes maze test revealed spatial learning deficits in all AD mice, except in the group of animals treated with IDIF 100 and 300 mg/kg/day. PET-[18F]-DPA-714 after 6 months of treatment shows higher levels of neuroinflammation in the animals treated with 100 and 300 mg/kg/day compared with the non-treated animals. MRI shows no anatomical differences between groups. Our study strongly indicates that IDIF treatment at doses of 100 and 300 mg/Kg/day effectively delays amyloid-≈í,â§ plaque deposition in the 5xFAD mouse model during the early-stage of AD. This is the first longitudinal study of IDIF in the 5xFAD model, highlighting the potential of TTR stabilizers as disease-modifying drugs for AD. However, further investigation is needed to fully understand the mechanisms underlying these effects.

P107

Cyclin-dependent kinase 5 activator p25 is associated with synaptic preservation in the prefrontal cortex of participants in a large ageing study

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The role of the CDK5 activator cofactors, p25 and p35, in Alzheimer's disease (AD) remains controversial. Human postmortem brain studies addressing p25/p35 expression levels in AD have shown contrasting results. Using postmortem dorsolateral prefrontal cortex (DLPFC) samples from a well-characterized cohort of participants of the Rush Memory and Aging Project (MAP), we reported lower p25 density in AD samples, and observed that higher p25 brain levels were associated with better cognitive outcomes. Additionally, preservation of intact SNARE complex functionality significantly contributed to improve cognitive reserve in the same cohort. Against this background, the aim of the present study was to assess the potential associations of CDK5/p35/p25 protein levels with available estimates of synaptic degeneration and/or dysfunction, and with the burden of AD-related neuropathologies. To this end, CDK5, p35 and p25 protein levels were quantified by Western blotting in DLPFC samples from 150 MAP participants. Multivariate pairwise correlation analyses surveyed putative associations between the immunodensities of CDK5/p35/p25 and the levels of multiple presynaptic proteins (CPLX1/2, STXBP1, SNAP25, STX1, and VAMP), their protein-

protein interactions (PPIs), and the burden of AD-related neuropathology (Ab plaques and tangles) in the DLPFC. Furthermore, regression models adjusted for demographics and neuropathologies were used to estimate the effect of p25 accumulation on cortical indices of synaptic degeneration (i.e., SNARE proteins and STXBP1) and dysfunction (i.e., SNARE PPIs). Results of the multivariate analyses showed that greater p25 density correlated with both higher amounts of all synaptic proteins examined ($r = 0.201, 0.589$, $p < 0.001$) and better indices of synaptic function, including SNARE PPIs ($r = 0.402, 0.543$, $p < 0.001$). In contrast, p25 levels were inversely correlated with Ab accumulation ($r = -0.169$, $p < 0.05$) and neuritic and diffuse plaque counts ($r = -0.174, -0.261$, $p < 0.05$). Similar results were obtained using p35 but not CDK5 immunodensities. In regression models, loss of p25 density was strongly associated with greater indices of synaptic degeneration, predicting 16% and 28% of between-subject variance in SNARE PPIs and 41% of STXBP1 expression. In conclusion, the present findings support a potentially important role for p25 in safeguarding both synaptic density and functionality against lifelong accumulation of AD neuropathology. Future studies will determine whether these associations underlie a potential contribution of p25 to cognitive reserve.Supported by grants BIO22/ALZ/002 (BIOEF/Maratoia-EiTB), IT1512/22 (Basque Government), CNS2023-143768 (NextGenerationEU/PRTR/MCIN/AEI), and R01AG17917 (NIH). EH-H holds grant FJC2022-048338-I, funded by MCIN/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRTR.

P108

CEPsh cell-specific degradation of DAF-2 shortens lifespan in C. elegans

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Insulin/IGF-1 signaling pathway is involved in longevity, stress resistance and healthy aging.While traditionally attributed to insulin's influence on neurons, recent research reveals thepresence of insulin receptors in astrocytes. Astrocytes are essential for maintaining cerebralhomeostasis, providing an appropriate microenvironment for meeting neuronal energydemands and functions.In C. elegans, whole-body mutations in DAF-2 receptor, an insulin/IGF-1 receptor ortholog, can double lifespan. However, the specific impact of DAF-2 ablation in CEPsh cells (similar to astrocytes) remains unexplored. Therefore, the present study aims to evaluate how astrocyte-specific DAF-2 deletion using AID (Auxin-inducible-degron) system in adulthood affects lifespan, aging, development, entry into dauer-diapause and oxidative stress.Our findings demonstrate that specific DAF-2 ablation in astrocyte-like cells (CEPsh::DAF-2strain) in C. elegans not only reduces lifespan and accelerates the aging process, but alsodiminishes resistance to oxidative stress compared to the whole-body DAF-2 deletion strain.In a scenario in which decreasing insulin signaling is assumed to improve lifespan in nematodes,we provide a proof-of-concept that silencing astrocytic DAF-2 is detrimental. Therefore, this result opens a new horizon for the development of novel treatment opportunities for age-related diseases focused on astrocytic insulin receptor improvement.

P109

GABAB receptor signaling in oligodendrocytes and its therapeutic potential against demyelinating diseases

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Myelination in the central nervous system and myelin repair in demyelinating conditions, such as multiple sclerosis, are driven by oligodendrocyte progenitor cell (OPC) differentiation into mature oligodendrocytes. Our recent research demonstrates that GABAB receptor (GABABR) promotes OPC maturation in vitro, and that GABABR selective agonist baclofen accelerates remyelination after lysolecithin-induced primary demyelination in the mouse spinal cord. In this study, we investigate the impact of baclofen administration on the experimental autoimmune encephalomyelitis (EAE), a more complex model of multiple sclerosis that mimics both acute and chronic features of the disease. With that aim, we injected baclofen intraperitoneally into adult mice following EAE induction to test the prophylactic and therapeutic potential of the drug, starting the injections either at the onset of the model (day 0) or at the onset of the symptoms (day 12). Interestingly, baclofen treatment from day 0 delayed disease progression, while treatment from day 12 notably attenuated model severity. To further explore the constitutive role of GABABR in oligodendrocytes, we evaluated the impact of GABAB1 ablation in NG2-positive cells, using conditional knockout mice. We employed two-photon microscopy to study oligodendroglial calcium activity in the mouse corpus callosum. Our results revealed a significant increase in the frequency of calcium events in OPCs from knockout mice, probably in compensation for the reduced amplitude of these signals following GABAB1 deletion. Additionally, we assessed the effect of baclofen, used as an antispasticity drug, in patients with MS. Simoa analysis showed significant alterations in the plasma levels of several cytokines, suggesting that baclofen may influence the inflammation associated with MS. These findings underscore the significance of GABABR in the course of demyelinating diseases and identify its agonist baclofen as a promising candidate for therapeutic strategies against MS.This research was funded by Basque Government (2021333019, 2022333021, IT1203-19, IT1551-22), MICINN (PID2019-108465RB-I00), CIBERNED (CB06/05/0076), Basque Government grant to L. BC (PRE-2019-1-0132) and MICINN grant to BI.OB (FPU20/06365).

P110

IS HSP70 A THERAPEUTIC TARGET FOR PARKINSON'S DISEASE?

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Protein folding and its quality control is key in cell homeostasis. Under stress conditions, protein folding can be altered resulting in aberrant-structure proteins which display toxic functions for the cell. To counteract the toxic effect of these aberrant proteins, cells have different protective mechanisms such as ubiquitin-proteasome system, autophagy-lysosome pathway, the translational machinery, and molecular chaperones. The latter, molecular chaperones, display several important functions related to the protection of the proteasome from stress, folding of new proteins, and unfolding and degradation of misfolded proteins. The human Hsc70 disaggregase, a chaperone complex formed by Hsp70 and its cochaperones Dnajb1 and Hsp110, plays a pivotal role in protein disaggregation and it has been recently linked to Parkinson's disease (PD) because its preferential ability to disaggregate toxic oligomers and short fibrils of alpha-synuclein.Here we present data that indicate the protective role of Hsp70 against alpha-synuclein toxicity in PD, reducing the levels of oligomers and rescuing the mitochondrial and autophagy-lysosome phenotype. Moreover, a Gene Set Enrichment Analysis (GSEA) revealed a dysregulation of Hsp70-related genes (Hspa8, cochaperones-encoding genes) in a synuclein-overexpressing mice model.This study aims to confirm or discard Hsp70 as a target for PD in vitro through genetic and chemical modulation, by using two different cell models: SH-S5Y5 and iPSC-derived dopaminergic neurons. To recapitulate the pathogenesis of PD, SH-S5Y5 will be transfected with alpha-synuclein plasmid while dopaminergic neurons will be treated with preformed fibrils (PFF). Hsp70 overexpression will be achieved through plasmid transfection, whereas its activation will be performed using the chemical activator YM-1. We will investigate the functional effects of both overexpression and activation of Hsp70 on alpha-synuclein aggregation, as well as the mitochondrial and autophagosome-lysosome pathway phenotype in health and pathology. Furthermore, we plan to study the relation between Hsp70 and the glia (astrocytes and microglia) in PD.

P111

Monocarboxylate transporter 2 in oligodendroglia contributes to myelin maintenance and regeneration and is downregulated in multiple sclerosis

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NA

Monocarboxylates (MCs) (lactate, pyruvate, and ketone bodies) are increasingly recognized as important metabolic fuels for the brain, and their trafficking is mediated by monocarboxylate transporters (MCTs). MCT2 has the highest affinity and supplies neurons with astrocyte-

derived lactate, fueling synaptic activity. Here, we first showed that MCT2 is expressed by neonatal and adult oligodendroglial cells, and by remyelinating oligodendrocyte progenitors (OPCs) in mice, as well as on oligodendrocytes in the human cerebellum. We investigated potential changes in MCT2 expression in multiple sclerosis (MS). In silico analyses of publicly available databases and post-mortem immunohistochemical analyses showed decreased MCT2 expression by oligodendrocytes in MS. We then performed loss of function experiments to investigate the role of MCT2 in myelin generation, maintenance, and regeneration. To delete MCT2 in adult oligodendrocytes, we used AAV-mediated Cre expression in MCT2flox/flox mice, which resulted in myelin loss and mild increase in inflammation, without death of transduced oligodendrocytes. In turn, MCT2-KO oligodendrocytes showed reduced expression of enzymes associated with lipid synthesis. Importantly, neighboring axons showed upregulation of lactate dehydrogenase A, swelling, and damage.We then generated a mouse line with inducible deletion of MCT2 in OPCs/newly generated oligodendrocytes. Postnatal MCT2 deletion resulted in decreased numbers of oligodendroglia and myelin sheaths in defined spinal cord areas. MCT2 deletion during adult myelin regeneration decreased OPC differentiation.Our findings suggest that MCT2 regulates myelin (re)generation and maintenance, likely by enabling oligodendroglia to use monocarboxylates as sources of ATP and/or carbons to sustain myelin synthesis and axonal support, which becomes dysregulated in MS.

P112

AGE-RELATED CHANGES IN METABOTROPIC GLUTAMATE RECEPTOR DENSITIES IN THE PREFRONTAL CORTEX OF SUBJECTS WITH SCHIZOPHRENIA

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Patients with schizophrenia (SZ) may experience accelerated aging: age-related brain changes associated with SZ could evolve abnormally faster in subjects with SZ in comparison to healthy individuals(1). Among the possible molecular indices of brain aging, loss of metabotropic glutamate receptors (mGluRs) has been postulated as probable underpinnings of SZ-related glutamatergic miswiring. mGluRs have also been proposed as promising targets for novel antipsychotic drug development(2). Thus, we evaluated whether subjects with SZ display abnormal age-related changes of mGluR densities, compared to control (C) subjects. Postmortem samples from the dorsolateral prefrontal cortex (DLPFC; aiming Brodmann area 9) of SZ and C subjects were obtained at autopsies in the Basque Institute of Legal Medicine. Twenty-one pairs of SZ and C subjects were matched by age (range 28-60 years old), sex and postmortem delay. Group I mGluR1 and mGluR5, group II mGluR2 and mGluR3, and group III mGluR4 and mGluR7 protein densities were quantified by Western blot (WB) using selective antibodies. The effect of diagnosis (SZ vs C) on mGluR protein densities was evaluated by paired t-test. Overall age effects on mGluR protein density were evaluated by Pearson's

correlation. Linear regression models (LRM) were built to estimate the effect of age on mGluR densities across groups (SZ vs C) of subjects by adding the AgeXDiagnosis interaction term to the model.WB experiments detected specific immunoreactive bands for mGluR1a (150 kDa), mGluR5 150 kDa), mGluR2 (95 kDa), mGluR3 (110 kDa), mGluR4 (100 kDa), and mGluR7 (90 and 100 kDa). Compared to C, SZ samples had significantly lower immunodensities of mGluR2 (-33%, $p<0.05$), mGluR4 (-28%, $p<0.05$), and mGluR7 00 and 100 kDa (-64%, $p<0.001$; -31%, $p<0.05$; respectively). Aging was associated with lower mGluR3 densities ($r=-0.4$, $p<0.01$). However, aging affected mGluR expression similarly across groups, as AgeXDiagnosis interaction terms did not reach statistical significance in any of the LRMs generated. In conclusion, group II mGluR2(3) and group III mGluR4 and mGluR7 were downregulated in SZ DLPFC. Only mGluR3 protein density was affected by age in the overall sample. However, the results do not support our hypothesis, as similar age-related changes of mGluR densities were observed in both SZ and C samples. The sample size may be insufficient to test this hypothesis. This work was supported by grants PID2022-137848OB-I00 (MCIU/AEI/FEDER) and IT1512/22 (Basque Government), PRE_2022_1_0075 (Basque Government to JAS), PIF19/306 (UPV/EHU to OMP) and PIF19/14 (UPV/EHU to JDlCB). The authors report no conflict of interest. 1Kochunov, P.V. & Hong, L.E. (2024). Biol Psychiatry. 95(11):988-989.2Dogra, S. & Conn, P.J. (2022). Mol Pharmacol. 101(5):275-285.3DelaCuesta-Barrutia J, et al. (2024). Transl Psychiatry. 14(1):113

P113

Mitochondrial DNA replication depends on the lipid-raft protein ERLIN2 and calcium transfer levels in the endoplasmic reticulum-mitochondrial contact sites

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Mitochondrial DNA replication requires a complex apparatus and strict regulation to respond to energy demands. The contacts between the endoplasmic reticulum (ER) and mitochondria (ERMCs) support replication, and may play an important role in this regulation. However, little is known about the ER factors that support mtDNA replication, nor how the process is regulated. Here, we show that repression of ER Lipid Raft Associated protein 2, ERLIN2, which is concentrated at ERMCs, inhibits mtDNA replication in human cells. Moreover, repression of the tubular ER protein Reticulon 4 and the ERMC bridging protein GRP75 have the same effects. A major function of the ERMCs is calcium transfer from the ER to the mitochondria. MFN2 KO cells transfer less calcium than control cells and the depletion of ER calcium stores with thapsigargin markedly inhibits mtDNA synthesis. However, those conditions where ERMCs were disrupted with ERLIN2 or RTN4 silencing show a higher release of mitochondrial calcium by uncoupling with FCCP and therefore, mtDNA synthesis was not inhibited owing to a shortage of calcium in the mitochondria. Hence, calcium transfer via ERMCs appears to be required for mitochondrial DNA synthesis and could be a regulatory of the process, although synthesis is not always directly proportional to calcium levels in mitochondria.

P114

Unraveling dysregulated pathways in glial cells during Parkinson's disease.

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NA

Parkinson's disease (PD) is a progressive neurodegenerative disorder primarily characterized by the loss of dopaminergic neurons in the substantia nigra. Recent research has highlighted the crucial role of glial cells -including astrocytes, microglia and oligodendrocytes- in the pathogenesis of PD. However, little is known about which are the key molecular alterations in glial cells that take place during PD. This study aims to unravel the dysregulated pathways in glial cells that contribute to PD pathology in both mouse and human samples in order to identify new potential therapeutic targets.We have performed gene set enrichment analysis (GSEA) on human PD single nuclei RNA-Seq data previously obtained in the laboratory. Our preliminary results reveal an upregulation of pathways involved in cellular stress; such as HSF1 activation pathways on astrocytes, microglia and oligodendrocytes. Additionally, we have observed a downregulation of multiple pathways on glial cells of PD patients, being some related to the establishment of synapses (on microglia), sterol biosynthesis (astrocytes) or cellular signaling (oligodendrocytes). These results agree with those obtained on the GSEA performed with bulk RNA-Seq and spatial transcriptomics data from mouse PD model (SNCA-OVX) samples. In the future, by integrating data from human and mouse models, we will identify conserved dysregulated pathways that may serve as potential therapeutic targets. Our study underscores the importance of glial cell dysfunction in PD pathology and highlights the need for further investigation into glial-specific interventions. This comprehensive analysis provides a foundation for developing novel strategies aimed at modulating glial cell function to mitigate neurodegeneration in Parkinson's disease.

P115

PHARMACOLOGICAL CHARACTERIZATION OF THE CS1P 1 COMPOUND AS A S1P 1RECEPTOR ANTAGONIST

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The sphingosine 1-phosphate (S1P) system is a lipid-based neuro-signaling mechanism that is present throughout the organism. The endogenous ligand, sphingosine 1-phosphate (S1P), exhibits anti-apoptotic, proliferative, and cell-trafficking effects, which confer neuroprotective properties against several central nervous system (CNS) diseases associated with neuroinflammation and neuronal loss, such as Alzheimer's disease (AD) and multiple sclerosis

(MS). In this study, we characterize the selective S1P1 radioligand, [3H]CS1P1, for use in binding and autoradiographic assays as a novel pharmacological tool to delineate the localization and density of S1P receptors in the CNS.[3H]CS1P1 was evaluated in binding affinity assays in the presence of various S1P1 agonists and antagonists in cell membranes overexpressing S1P1 receptors, as well as in cell membrane homogenates of the rat cerebral cortex. Concurrently, the agonist or antagonist properties of the CS1P1 compound were assessed in cell membrane homogenates of the rat cerebral cortex using the [35S]GTP≈í,â•S binding assay. Well-known S1P1 agonists (CYM5442) and antagonists (W146 and NIBR-0213) were able to inhibit [3H]CS1P1 binding in both cell membranes overexpressing S1P1 receptors and cell membrane homogenates of the rat cerebral cortex (CYM5442 99.16%; W146 100%; NIBR-0213 100%). CS1P1 reduced CYM5442-stimulated [35S]GTP≈í,â•S binding by 60%, with a logKi of 11.83, indicating an antagonist-like effect on S1P1 receptors. Therefore, CS1P1 is an antagonist with high affinity for S1P1 receptors, making it a valuable drug for S1P1 pharmacological analysis, including binding and autoradiographic assays for new selective S1P1 molecules.In conclusion, CS1P1 represents a novel pharmacological tool for studying the S1P system, thereby facilitating the further development of S1P-based therapies for AD, MS, and other neurodegenerative disorders.

P116

Baclofen Protects Oligodendrocytes from Metabolic Impairment and Dysfunctional Myelination in Alzheimer's Disease Models

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Oligodendrocyte (OL) differentiation from oligodendrocyte progenitor cells (OPCs) is vital for myelin sheath formation in the central nervous system (CNS), enabling fast and energy-efficient axonal impulse propagation. Alzheimer¬¨¬•s disease (AD) is an irreversible neurodegenerative disorder characterized by the accumulation of amyloid beta-peptide oligomers (Abetao) which alters, among others, OLs and myelin function. As OLs maintain neuronal health and metabolism, impaired OL function and myelin integrity could be an upstream risk factor for neuronal disability in AD. In this line, recent works of our group show that GABA receptors (GABARs) activation in OPCs promotes their survival and differentiation to mature myelinating OLs and previous data point to alterations in mitochondrial genes in cultured rat OLs treated with Abetao. Considering that, in this study we explored the protective role of GABARs activation against Abetao-derived mitochondrial dysfunction in OLs. Baclofen, specific GABABRs agonist, mitigated Abetao-induced mitochondrial membrane hyperpolarization and abnormal mitochondrial-Ca2+ efflux in cultured OLs. Baclofen also preserved OL metabolic functions altered by Abetao, like proton efflux (PER), oxygen consumption (OCR) and ATP production rates. In addition, we examined Abetao and baclofen's effect on the progression of the oligodendroglial lineage in vitro. First, OPC proliferation rate remained unaltered at 1 or 2 days in vitro (DIV) after Abeta or baclofen treatment. Nonetheless, Abetao reduced the OPC pool at 2DIV, identified by PDGFRalpha expression, but the administration of baclofen together with Abetao reversed this effect. Furthermore, Abetao increased myelin basic protein (MBP) expression at 3DIV, both alone and in

combination with baclofen. Finally, in order to assess the impact of in vivo baclofen application, we compared OL status from 15-months old wild-type (WT) and 3xTgAD mice, treated or not with baclofen during 30 days. 3xTgAD mice exhibited fewer OPCs and mature OLs in the hippocampus compared to WT mice. However, chronic baclofen treatment increased these populations in both the hippocampus and corpus callosum of 3xTgAD, as well as the expression of MBP in these areas. Overall, these findings suggest a protective role of GABABR activation by baclofen against Abetao-induced mitochondrial and myelin disturbances in cultured OLs and in the 3xTgAD model, thus evidencing the potential of GABA system as an oligodendroglial protectant in CNS disorders.

P117

Evolution of the Basal Ganglia in Amniotes (Research Plan)

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In recent years, the basal ganglia have received substantial attention due to their medical relevance, given their relationship with neurodegenerative diseases such as Parkinson's. Therefore, a deep understanding of their development and the evolutionary history that has led to its current structure and function can be very useful. So far, the idea that it is a highly conserved structure in vertebrates has been reinforced, both at the level of structures, connections, cell types, and neurotransmitters. The aim of this project is to apply the perspective of EvoDevo, a multidisciplinary field that maintains that variations in development can explain phenotypic variability and allow the evolutionary selection. Through this lens, our purpose is to compare at different levels the development of the basal ganglia in three key vertebrate species: gecko, chicken, and mouse. At the level of neurogenesis, to describe the neurogenic formation of the different neurons in the ganglia and thus be able to detail the moments of embryonic development in which each structure is formed along with its location and function. At the molecular level, to describe the transcriptional profiles of the different populations during their differentiation and maturation. At the functional level, to study the existing connections between the structures that compose the basal ganglia. In this way, we can obtain a broader and more integrated view of the development and functioning of these structures. Moreover, the integration of results will reveal the small modifications within such a conservative evolutionary history that led the specific modifications we observe in each vertebrate taxa.

P118

Exploring a neuroprotective role for the transcription factorREST in Parkinson’s Disease in vivo

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The expression of the Repressor Element 1-Silencing Transcription (REST) factor is induced in the human aging brain, and it has been shown to repress cell death and promote cellular stress response, leading to neuroprotection in healthy aging. On the other hand, REST deletion was shown to lead to age-related neuronal loss in a conditional mouse knock-out model. In neurodegenerative pathologies, such as Dementia with Lewy Bodies (DLB), REST is lost from the nucleus, and it is recruited to autophagosomes together with pathological misfolded proteins. A recent study suggests that REST is retained in Lewy Bodies preventing its neuroprotective functions in Parkinson's Disease (PD) patient samples. In agreement with these findings, we have previously shown that REST and its target genes are dysregulated in PD models. This evidence suggests REST as an interesting target for PD treatment, promoting neuroprotective effects such as the repression of cell death, the reduction of oxidative stress and the increase of mitochondrial health, which are dysfunctional in PD. Our aim is to confirm or discard REST as a target for PD in vivo, by using two mouse models: the human SNCA-overexpressing (SNCA-OVX) mice and the intracranial PFF (alpha-synuclein Pre-Formed Fibrils) injection model. SNCA-OVX mice recapitulate several aspects of the early pathogenesis of PD, including age-dependent neuronal loss, alpha-synuclein oligomers and glial response, whereas the PFF injection model presents more advanced Lewy-body-like lesions. REST overexpression will be achieved through viral injection. We will investigate the functional effects of REST upregulation on dopaminergic cell counts, alpha-synuclein aggregation, as well as mitochondrial function. In addition, we plan to evaluate motor and non-motor pathology in REST-injected animals versus controls in vivo.Here we present data obtained from immunohistochemistry and behaviour tests on injected animals.

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