VII Symposium

Portuguese GLIAL NETWORK

02·MAY

ALGARVE

ALBUFEIRA MUNICIPAL AUDITORIUM

satellite event of:

FRM 2023
FENS REGIONAL MEETING
3-5 MAY | ALGARVE, PORTUGAL

ABSTRACT BOOK

CHAIRS

Inês Araújo, ABC-RI, FMCB, University of Algarve

João Filipe Oliveira, ICVS - School of Medicine, University of Minho

SCIENTIFIC COMMITTEE

Adelaide Fernandes, iMed.ULisboa, Faculty of Pharmacy, University of Lisboa

Ana Raquel Santiago, iCBR, FMUC, University of Coimbra

Dora Brites, iMed.ULisboa, Faculty of Pharmacy, University of Lisboa

Federico N. Soria, Achucarro Basque Center for Neuroscience, Leioa, SP

Fernando de Castro, Cajal Institute, Madrid, SP

Francisco Ambrósio, iCBR, FMUC, University of Coimbra

Inês Araújo, ABC-RI, FMCB, University of Algarve

João Bettencourt Relvas, i3S, University of Porto

João Filipe Oliveira, ICVS - School of Medicine, University of Minho

Sandra Vaz, iMM, FMUL, University of Lisbon

Teresa Summavielle, i3S, University of Porto

ORGANIZING COMMITTEE

Ana Raquel Santiago, iCBR, FMUC, University of Coimbra

Clévio Nóbrega, ABC-RI, FMCB, University of Algarve

Daniela Santos, ABC-RI, FMCB, University of Algarve

David Brito, ABC-RI, FMCB, University of Algarve

Inês Araújo, ABC-RI, FMCB, University of Algarve

Isa Mestre, ABC-RI, FMCB, University of Algarve

João Filipe Oliveira, ICVS - School of Medicine, University of Minho

Marta Vitorino, ABC-RI, FMCB, University of Algarve

Sónia Simão, ABC-RI, FMCB, University of Algarve

WELCOME

The Portuguese Glial Network invites you to attend the VII Symposium of the Portuguese Glial Network, in collaboration with Red Glial Española. The Symposium will be held in Albufeira (Algarve) on May 2, 2023, as a satellite meeting of the FENS Regional Meeting 2023.

This year, we will have six invited speakers to bring us the latest on glial cell physiology, biology, signaling, and ultrastructure: Amanda Sierra (Achucarro Basque Center for Neuroscience and Department of Biochemistry and Molecular Biology, Spain), Ana Luísa Cardoso (University of Coimbra, Portugal), Christa Rhiner (Champalimaud Research Program, Portugal), Corrado Calì (Università degli Studi di Torino, Italy), Juliana Rosa (Hospital Nacional de Parapléjicos - IDISCAM, Spain) and Renzo Mancuso (Universiteit Antwerpen, Belgium). Six short talks will also be selected from the submitted abstracts by our Scientific Committee.

We are delighted to welcome you to our VII Symposium and gather again friends and colleagues of the Portuguese glial family for another great meeting. Registration is, as always, free (but mandatory), and the best poster and oral communication will receive awards!

Join us in the vibrant setting of the city of Albufeira and experience the sun and energy of the Algarve.

We are looking forward to seeing you all in May!

Inês Araújo and João Filipe Oliveira

Chairs

VENUE

Address:

Auditório Municipal de Albufeira

R. das Telecomunicações 2

8200-184 Albufeira, Portugal

GPS: 37° 5' 25.4544" N 8° 14' 46.302" W

Web: https://redeglial.weebly.com/vii-symposium.html

Twitter: https://twitter.com/GlialRede

Instagram: https://www.instagram.com/ptglianetwork

Facebook: https://www.facebook.com/RedeGlial

PROGRAMME

11h00 - 13h30 | REGISTRATION

13h30 | WELCOME

14h00 - 15h00 | KEYNOTE LECTURE

Chairs: Inês Araújo (PT) and Fernando de Castro (SP)

Microglial states and nomenclature: a field at its crossroads.

Speaker: Amanda Sierra (SP)

15h00 -16h45 | SESSION I

Chairs: Adelaide Fernandes (PT) and Federico N. Soria (SP)

The impact of early-life stress in neuroimmune interactions in the pre-frontal cortex: a tale of two sexes.

Speaker: Ana Luísa Cardoso (PT)

Neuro-glial clusters orchestrate regeneration in the injured fly brain.

Speaker: Christa Rhiner (PT)

The complex response of human microglia to neurodegeneration and the impact of genetics.

Speaker: Renzo Mancuso (BE)

Selected Talk 1: Increasing ascorbate transport in hippocampal microglia prevents disease progression in aß-depositing mice.

Speaker: Catarina Pacheco (i3S, Porto, Portugal)

Selected Talk 2: Characterization of microglial response to environmental enrichment: the role of the RhoGTPase RAC1.

Speaker: João Galvão (FMUP/i3s, Porto, Portugal)

16h45 - 18h00 | POSTER SESSION (includes coffee break)

18h00 - 18h30 | INAUGURATION OF FRM NEUROSCIENCE PHOTO EXHIBIT (Galeria Municipal João Bailote)

18h30 - 20h00 | SESSION II

Chairs: João Filipe Oliveira (PT) and Raquel Santiago (PT)

Cortical astrocytes increase efficiency of sensory processing by adjusting gain and sensitivity of neuronal circuitries.

Speaker: Juliana Rosa (SP)

Lactate derived from astrocytic glycogen is necessary for stabilization of synapses following learning.

Speaker: Corrado Calì (IT)

Selected Talk 3: Blood Brain Barrier Differences in the nucleus accumbens relate to natural variation in trait anxiety.

Speaker: Haissa de Castro (EPFL, Switzerland)

Selected Talk 4: Role of astrocytes on the abnormal synaptic transmission and plasticity in the motor cortex and hippocampus of sod1g93a mice.

Speaker: Sara Costa-Pinto (IMM/FMUL, Lisboa, Portugal)

Selected Talk 5: Increasing ascorbate transport in hippocampal microglia prevents disease progression in aß-depositing mice.

Speaker: Catarina Pacheco (i3S, Porto, Portugal)

Selected Talk 5: Live imaging of microglial behavior across the lifespan.

Speaker: Vanessa Coelho-Santos (CIBIT/ICNAS, Coimbra, Portugal)

Selected Talk 6: Changing paradigms on remyelination failure: the biomechanical point of view .

Speaker: Eva Carvalho (i3S, Porto, Portugal)

20h00 | AWARDS AND CLOSING SESSION

20h30 | DINNER (ALBUFEIRA CITY HALL)

KEYNOTE LECTURE

Amanda Sierra

Achucarro Basque Center for Neuroscience

Sponsored by Isaza

MICROGLIAL STATES AND NOMENCLATURE: A FIELD AT ITS CROSSROADS

In this talk, I will summarize the contents of a recent consensus papers of 94 authors (Neuron 2022) where we provide a conceptual framework and recommendations on the use of microglial nomenclature. Microglial research has advanced considerably in recent decades yet has been constrained by a rolling series of dichotomies such as resting versus activated and M1 versus M2. This dualistic classification of good or bad microglia is inconsistent with the wide repertoire of microglial states and functions in development, plasticity, aging, and diseases that were elucidated in recent years. New designations continuously arising in an attempt to describe thedifferent microglial states, notably defined using transcriptomics and proteomics, may easily lead to a misleading, although unintentional, coupling of categories and functions. In this talk, I will useexamples from research carried out in my lab to support the "DOs and DON´Ts" suggested in the paper, to guide researchers, reviewers and editors.

SESSION I

Ana Luísa Cardoso

Faculty for Science and Technology, University of Coimbra

THE IMPACT OF EARLY-LIFE STRESS IN NEUROIMMUNE INTERACTIONS IN THE PRE-FRONTAL CORTEX: A TALE OF TWO SEXES.

Exposure to early-life adversity (ELA) has been shown to profoundly influence brain development and to account for 50% of childhood and 30% of adult psychiatric disorders. Prospective studies in specific human populations, such as children raised in institutions, have clearly shown that early deprivation of social and nurturing interactions is among the most damaging adverse childhood experiences and can lead to long-lasting emotional and cognitive problems, which include deficits in sociability, working memory, visual recognition, associative learning and impulse control.

While human studies have provided causality between ELA and poor emotional and cognitive outcomes, studies in animal models have been instrumental to probe the molecular mechanisms behind these observations. Our most recent work, in an ELA model that mimics maternal separation and maternal unpredictable stress (MSUS), revealed significant morphological and functional alterations at the neuroimmune level, specifically in microglia cells from the prefrontal cortex. Importantly, these changes, which were mostly observed in male animals, conditioned early neuronal engulfment and the establishment of the inhibitory network in this region, resulting in changes in the intrinsic electrophysiological properties of PV interneurons and in a sex-specific hyperactive and impulsive behavioral phenotype.

Christa Rhiner

Champalimaud Research Program, Center for The Unknown

NEURO-GLIAL CLUSTERS ORCHESTRATE REGENERATION IN THE INJURED FLY BRAIN

Within the brain, highly coordinated cellular and molecular networks regulate brain function and ensure homeostasis. Brain injury acutely disrupts these cooperative networks and triggers fundamentally different cellular cross-talk. We use targeted stab lesion to the adult Drosophila brain to elicit potent repair programs. The genetic versatility of the fruit-fly model allows us to dissect the complex, multicellular injury responses with high cellular and temporal resolution and to screen for key factors promoting regeneration in the mature brain based on transcriptional signatures. To address the conserved nature of established mechanisms, we are collaborating with groups that work with rodent models of brain injury.

Taking advantage of the brain injury fly model, we recently discovered a mechanism by which neurons and glia cooperate in neuro-glial clusters to reactivate latent neural progenitor cells and promote regeneration (Simoes et al., 2022, Dev Cell). We found that glia secrete the lipocalin-like Swim/Lcn7 transporter in response to injury-induced tissue hypoxia. This leads to the spread of neural-derived Wg/Wnt ligands in the injured brain area, resulting in the activation of dormant progenitor cells near the injury site. Similarly, Lcn7 is strongly upregulated in astrocytes of the mouse hippocampus following brain injury, suggesting a conserved function of the HIF1-a/Swim/Wnt axis in connecting injury-sensing and regenerative outcomes.

Renzo Mancuso

Laboratory of Microglia and Inflammation in Neurological Disorders (MIND). VIB-Center for Molecular Neurology (CMN), VIB

THE COMPLEX RESPONSE OF HUMAN MICROGLIA TO NEURODEGENERATION AND THE IMPACT OF GENETICS

Microglial activation and neuroinflammation are initial steps in the pathogenesis of Alzheimer's disease (AD) and frontotemporal dementia (FTD). Studies in mouse models and human postmortem samples have yielded divergent results regarding microglia cell states relevant to AD. We have investigated 127,000 single cell expression profiles of human microglia isolated freshly from a xenotransplantation model for early AD to determine the range of cells states acquired by human microglia in disease. We observed that while they adopt a disease-associated (DAM) profile, they also display a much more pronounced HLA-cell state related to antigen presentation in response to amyloid plaques. In parallel, a distinctive pro-inflammatory cytokine and chemokine CRM response is mounted against soluble amyloid-beta aggregates. This wide landscape of cell states can be also captured in post-mortem studies. Genetic risk factors driving disease, such as TREM2, APOE or ABCA7, dramatically modulate certain aspects of the response of microglia to amyloid beta pathology. In light of our findings, it is clear that therapeutic strategies targeting microglia in AD need to carefully assess how they affect the different cell states, as the overall balance between distinct microglial profiles might determine a protective or damaging outcome.

SESSION II

Juliana Rosa

Hospital Nacional de Parapléjicos – IDISCAM

CORTICAL ASTROCYTES INCREASE EFFICIENCY OF SENSORY PROCESSING BY ADJUSTING GAIN AND SENSITIVITY OF NEURONAL CIRCUITRIES

Cortical astrocytes respond to peripheral sensory stimulation by mirroring the somatotopic neuronal arrangement. However, whether this astrocytic activity contributes to the computation of information to regulate behavior is less clear. We investigated this question in the context of sensory processing, focusing on distinct circuits across cortical layers and discrimination of sensory modalities. We demonstrate that by controlling the level of ongoing spontaneous activity and connectivity across layers, cortical astrocytes regulate the integration and processing of arriving inputs. The mechanism of such astrocytic control is mediated through a cell-circuit specialization with inhibitory neurons that adjust the gain and sensitivity of responding neuronal circuitries. Also, we show that astrocytic-modulation of sensory processing is layer-specific, impacting the discrimination of tactile and thermal inputs known to arrive at distinct layers of the cortical column. This study demonstrates that astrocytes control the background excitability of cortical circuitries to optimize neuronal computation of sensory inputs, thereby contributing to sensory processing and behavior output.

Corrado Cali

Neuroscience Institute Cavalieri Ottolenghi (NICO)Torino, Italy Department of Neuroscience, Università degli Studi di Torino

LACTATE DERIVED FROM ASTROCYTIC GLYCOGEN IS NECESSARY FOR STABILIZATION OF SYNAPSES FOLLOWING LEARNING

Long-term memory formation is an energy-expensive process, which is accompanied by structural changes at synapses, including, but not limited to an increase in spine density, for example. To directly investigate structural changes occuring during learning, and their dependance on brain energy metabolism, an in-depth 3D Electron Microscopy (EM) study was performed on adult mice brains subjected to a novel-object recognition (NOR) behavioral training, in presence of 1,4-Dideoxy-1,4-imino-D-arabinitol hydrochloride (DAB), a potent inhibitor of glycogenolysis. Memory consolidation and long-term potentiation impairments induced by the DAB treatment were reversed by intrahippocampal injection of lactate. The following 3D ultrastructural analysis on sparse reconstruction of spines and synaptic densities revealed that both density and size of spines increased significantly compared to naive animals, together with the appearance of glycogen clusters in astrocyte processes. The DAB treatment impaired the formation of new spines, and the application of L-Lactate together with the DAB rescued both memory formation and spine density, but failed to rescue the accumulation of glycogen clusters. Moreover, 3D analyses of dendritic mitochondria revealed an impaired fission, which was also rescued by intrahippocampal L-Lactate administration. Altogether, these evidences indicate that impairing lactate production from astrocytic glycogen results in the impairment of structural and biochemical synaptic plasticity features and memory consolidation.

SELECTED TALK 1

INCREASING ASCORBATE TRANSPORT IN HIPPOCAMPAL MICROGLIA PREVENTS DISEASE PROGRESSION IN Aß-DEPOSITING MICE

Monteiro-Pacheco A1,2, Santos E1,2, Almeida T1,4, Socodato R.1,3, Canedo T1, Tedim-Moreira J1,2, Relvas J1,2,3 and Portugal C1,3

1 University of Porto, Institute for Research and Innovation in Health (i3S), Porto, Portugal; 2 University of Porto, Faculty of Medicine (FMUP), Porto, Portugal; 3 University of Porto, Institute for Molecular and Cellular Biology (IBMC), Porto, Portugal; 4 University of Porto, School of Medicine and Biomedical Science (ICBAS), Porto, Portugal

Vitamin C is an important antioxidant present in the CNS. Ascorbate, the Vitamin C reduced form, enters CNS cells through the sodium vitamin C co-transporter 2 (SVCT2), which is expressed in neurons and microglia. Previously, we showed that ascorbate uptake through SVCT2 is critical for homeostatic microglia maintenance. The impairment of ascorbate uptake by diminishing SVCT2 expression is necessary and sufficient to induce microglial proinflammatory activation. Microglia play a significant role in Alzheimer's disease (AD) pathogenesis, characterized by progressive memory loss, synapse dysfunction, amyloid plaques, and neurofibrillary tangles. This work aims to investigate the role of SVCT2 overexpression in hippocampal microglia and establish the increase of SVCT2 expression in microglia as a strategy to halt pathology progression and cognitive rescue in AD mice. For that, we overexpressed SVCT2 in microglia using AAV injections into the hippocampus of 5xFAD mice. Compared to controls, our results show that SVCT2 overexpression in microglia attenuated microgliosis, reduced Aß content, avoided synapse loss, and prevented memory loss. We conclude that increasing microglial SVCT2 expression prevents AD pathology in mice. This work, therefore, highlights the importance of the SVCT2 transporter expression in microglia and its potential role in ameliorating some features in AD pathology.

This work was supported by national funds from FCT- Fundação para a Ciência e a Tecnologia, IP, within the project EXPL/MED-NEU/0588/2021.

SELECTED TALK 2

CHARACTERIZATION OF MICROGLIAL RESPONSE TO ENVIRONMENTAL ENRICHMENT: THE ROLE OF THE RHOGTPASE RAC1

Galvão-Ferreira J[1, 3], O. Almeida T[1,2], C. Portugal C[1], C. S. Santos E[1], Tedim-Moreira J[1, 3], Canedo T[1], Magalhães A[1], Summavielle T[1, 4], B. Relvas J[1,3] and Socodato R[1]

[1] University of Porto, Institute of Research and Innovation in Health (i3S) and Institute for Molecular and Cell Biology (IBMC), Porto, Portugal; [2] University of Porto, School of Medicine and Biomedical Sciences (ICBAS), Porto, Portugal; [3] University of Porto, Faculty of Medicine (FMUP), Porto, Portugal; [4] Escola Superior de Saúde do Politécnico do Porto (ESS.PP), Porto, Portugal

Microglia, the quintessential innate immune cell of the CNS, have now been recognized to be crucial for a plethora of learning phenomena. Our way of learning consists of constant and complex stimulation from our surrounding environment. Yet, the microglial molecular drivers required for homeostatic learning related to cognitive performance remain largely elusive. To mimic this type of homeostatic and highly effective brain stimulation, we employed a protocol of environmental enrichment (EE) in adult mice. EE protocols have long been held to emulate a 'natural' learning process and are known to enhance cognitive performance, including in brain diseases.

Aims: To understand whether and how microglia are important for environmental enrichment dependent performance benefits, and what is the role of microglial Rac1 in that response.

Methods: We combined conditional cell-specific gene targeting, magnetic-activated cell sorting (MACS) isolation of microglia, high-throughput phosphoproteomics, bioinformatics, and animal behaviour.

Results: Phosphoproteomics profiling of microglia identified five main signalling modules triggered by EE, the biggest of which was RhoGTPase signalling. Inside this module we were able to rank the signalling pathways of the most important RhoGTPases, and single out Rac1, a master signal integrator, as the module's key regulator. We also found a plethora of cytoskeleton proteins with altered phosphorylation status, including many guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) along with coffilin1, whose activated form was more abundant in microglia from enriched mice. To understand how important Rac1 was for the response to EE, we ablated Rac1 specifically from adult microglia. We found a radically different phosphoproteomic response to EE in microglia from Rac1 cKO mice. Afterwards, we combined the phosphoproteomics datasets from control and cKO microglia to isolate the phospho-proteins that were dependent of either the absence or presence of Rac1 during the EE process. Results showed that Rac1 modulated more than 70% of microglial phosphoproteins during EE. Finally, we performed a battery of cognitive tests in control and cKO mice and found that removing Rac1 from microglia prevented them from having any EE-dependent improvement in cognitive performance.

Conclusions: Overall, our results show not only that microglia plays an essential role in EE, but also that EE-induced improvements in the cognitive performance occur in a microglial Rac1-dependent manner.

SELECTED TALK 3

BLOOD BRAIN BARRIER DIFFERENCES IN THE NUCLEUS ACCUMBENS RELATE TO NATURAL VARIATION IN TRAIT anxiety

De Castro-Abrantes H[1], Sandi C[1]

[1] Brain Mind Institute, EPFL, Lausanne, Switzerland

The nucleus accumbens (NAc), a brain region involved in reward and motivation, is implicated in the regulation of anxiety-related behaviors. Astrocytes are powerful modulators of neuronal activity, and their localization around blood vessels is important for brain metabolism. In this study, exploiting natural phenotypic variation in anxiety-like behaviors in outbred Wistar male rats, we aimed at investigating anxiety-related differences in astrocytic and endothelial features in the NAc. We classified animals as high (HA) or low anxious (LA) according to the time spent in the open arms of the elevated plus maze. Gene expression analyses indicated a significant decrease in the expression levels of Aquaporine 4 (Aqp4) and Claudine 5 in HA animals. Aqp4 is specifically expressed at the level of astrocytic endfeet around blood vessels. Therefore, we asked whether HA and LA animals show differences in blood brain barrier (BBB) permeability. Using Evan's Blue injections, we observed higher blood vessels' permeability in the NAc of HA animals. Using electron microscopy, we observed a lower number of endfeet processes around blood vessels in the NAc of HA animals. Furthermore, we observed a lower number of contacts between the mitochondria (mito) and the endoplasmic reticulum (ER) in those endfeets. Additionally, using RNAScope, we also found differences in mitofusin 2 (mfn2) expression in those endfeets. Therefore, we show differences in the coverage of blood vessels by astrocytic processes, mitochondria dynamic differences, and in the associated BBB permeability, as a function of individuals' anxiety. Our findings suggest that alterations in accumbal astrocytes may contribute to the vulnerability of high anxious individuals to develop depression phenotypes.

SELECTED TALK 4

ROLE OF ASTROCYTES ON THE ABNORMAL SYNAPTIC TRANSMISSION AND PLASTICITY IN THE MOTOR CORTEX AND HIPPOCAMPUS OF SOD1G93A MICE

Costa-Pinto S[1,2], Gonçalves-Ribeiro J[1,2], Moreira J[3], Socodato R[3], Relvas JB[3,4], Sebastião AM[1,2], Vaz SH[1,2]
[1] Instituto de Farmacologia e Neurociências, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal; [2] Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal; [3] Instituto de Investigação e Inovação em Saúde and Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Portugal; [4] Department of Biomedicine, Faculty of Medicine, University of Porto, Portugal

Aims: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease, affecting mainly motor function and, in some cases, cognitive function. The relevance of astrocytes in excitotoxicity and neurodegeneration has been highly recognized. Considering that increasing evidence suggests that ALS begins in the primary motor cortex (M1), we aimed to study alterations in synaptic function in the M1 of SOD1G93A and wild-type (wt) mice, as well as the astrocytic contribution. Since different studies on SOD1G93A mice have also shown synaptic alterations in hippocampus, we also assessed synaptic transmission and plasticity in this region.
Methods: Presymptomatic (4-6w) and symptomatic (14-18w) SOD1G93A mice, and age-matched wt mice, were used. Synaptic plasticity and transmission were assessed by eliciting long-term potentiation (LTP) protocols and recording input/output curves, respectively, in the CA1 area of hippocampal slices and layer II/III of M1 slices. Astrocytic metabolism was selectively reduced using fluorocitrate (FC). Whole-cell patch clamp recording was used to identify differences in M1 neurons intrinsic properties and action potential firing behaviour. Proteomic analysis was used to further investigate synaptic alterations of SOD1G93A mice.
Results: In the presymptomatic phase, SOD1G93A mice had a significant decrease in LTP magnitude in the hippocampus, while in the symptomatic phase there was an increase in LTP magnitude in these mice. When astrocytes were metabolically inhibited (200 µM), hippocampal synaptic plasticity was significantly impaired, as well as synaptic responses, in both wt and SOD1G93A mice. Regarding the motor cortex, presymptomatic SOD1G93A mice showed early impairments in LTP magnitude and synaptic transmission. Interestingly, these mice had lower protein levels of the NMDAR subunit 2B and AMPAR GluR1. Neurons from the M1 of presymptomatic mice also exhibited reduced firing frequency that progressed to increased frequency in the symptomatic phase. Moreover, the presence of FC (100 µM) led to an impairment of LTP and basal transmission only in wt mice, to similar levels of presymptomatic SOD1G93A mice. Finally, proteomic analysis highlighted major differences in neuronal transmission, metabolism, RNA processing and immune system.
Conclusions: Altogether, we further explored alterations in synaptic plasticity and transmission, as well as the role of astrocytes, in two affected regions of the SOD1G93A mice model. These findings suggest that, in the hippocampus, astrocytes are essential for the maintenance of LTP in healthy and ALS conditions. More importantly, SOD1G93A mice present early alterations in M1 synaptic function, plasticity and neuronal firing properties, and astrocytes seem to be impaired even before the onset of symptoms, which is supported by proteomic results. This opens new paths for the identification of novel therapeutic targets in ALS.

SELECTED TALK 5

LIVE IMAGING OF MICROGLIAL BEHAVIOR ACROSS THE LIFESPAN

Taryn Tieu[1], Anne-Jolene N. Cruz[1], Andy Y. Shih[1,2,3] and Vanessa Coelho-Santos[1,2, 4]
[1] Center for Developmental Biology and Regenerative Medicine, Seattle Children's Research Institute, Seattle, WA, USA; [2] Department of Pediatrics, University of Washington, Seattle, WA, USA; [3] Department of Bioengineering, University of Washington, Seattle, WA, USA; [4] University of Coimbra, Coimbra Institute for Biomedical Imaging and Translational Research (CIBIT), Portugal

Microglia are resident immune cells of the brain with key roles in development, neural plasticity and defense during brain injury. Their abnormal function has been associated with neurological diseases across the lifespan. While in vivo microglial dynamics have been thoroughly characterized in the adult mouse brain, little is known about their normal dynamics during early postnatal development and conversely during brain aging. Microglial responses to vascular injury at these early and late life stages is also unknown. To gain more insight, we used in vivo two-photon imaging to explore cortical microglial dynamics in neonatal (postnatal day 9), adult (3-5 months), and aged (21-23 months) microglial-labeled mice (CX3CR1-GFP+/-) under normal physiological conditions and after laser-induced capillary injury. Under basal conditions, microglia in both neonates and aged mice have less ramified processes compared to the adult mice. Microglia preferred contacting the vasculature with their processes rather than their somata, which is more common among microglia in the adult brain. Microglia in the neonate are higher in density, with more mobile somata and processes that extend/retract rapidly at baseline. While aged animals had similar microglia density as adults, their process were significantly less dynamic.

During vascular injury in the adult brain, microglia cells extended their processes in a concerted fashion to surround and contain sites of injury. The aging brain is still capable of reacting to microvascular injury, though the amount of process extension declined. In neonates, the response was uncoordinated with some cells extending rapidly toward the injury, while other cells presenting delayed responses. Curiously, only adults reacted to sham lesions that were made in the parenchyma, and not the capillary wall. Three days post-injury, microglial dynamics had largely recovered to baseline levels in the adult and aged brain, while neonates exhibited sustained microglial aggregation at the injury site and more broadly in surrounding brain regions.

Our findings support the idea that basal and injury-evoked responses of microglial cells differ based on life stage. In the neonatal period, microglia are highly dynamic but mount uncoordinated and enduring responses to vascular insult. In the aged brain, microglia exhibit reduced capacity to respond to focal insults. These findings reflect microglial immaturity during development, and a decline of microglial function with aging.

SELECTED TALK 6

CHANGING PARADIGMS ON REMYELINATION FAILURE: THE BIOMECHANICAL POINT OF VIEW

Carvalho ED[1,2,3], Morais MRG[1,2], Athanasopoulou G[1,2,4], Araújo M[1,2], Hubbe H[5], Mendes E[5], Barrias CC [1,2,4], Pêgo AP[1,2,4]

[1] i3S – Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal; [2] INEB – Instuto de Engneharia Biomédica, University of Porto, Porto, Portugal; [3] FEUP – Faculdade de Engenharia da Universidade do Porto, Porto, Portugal; [4] ICBAS – Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Portugal; [5] TU Delft – Delft University of Technology, Chemical Engineering Department, The Netherlands

INTRODUCTION & AIMS: In the central nervous system (CNS), myelination is initiated by oligodendrocyte precursor cells (OPCs), which differentiate into oligodendrocytes (OLs), enabling saltatory conduction of action potentials along axons. Destruction of myelin internodes, OL apoptosis and axonal degeneration are hallmarks of demyelinating diseases. Although there are OLs in the adult CNS which can partially regenerate myelin sheaths in denuded axons, this remyelination process fails with disease progression, leading to irreversible functional failure. Growing evidence suggests that the extracellular (ECM) composition and mechanical properties are changed in demyelinating conditions. The lack of platforms with biologically relevant features has hampered the study of these mechanobiology processes. Here we propose tissue-engineered models to study the impact of mechanical properties changes on OPC differentiation.

MATERIALS AND METHODS: A polydimethylsiloxane (PDMS) micropillar array with biological relevant diameters, low stiffness and amenable to be surface functionalized was designed to act as surrogate axons and uncover the role of axonal diameter and stiffness on OPC differentiation. Furthermore, OPCs were also embed within a modified alginate (ALG) matrix with tunable mechanical properties to fully recreate the 3D environment of the ECM.

RESULTS: A new formulation of PDMS was explored to fabricate axon surrogates, corresponding to a significant advance in the production of structures with a great aspect-ratio and reduced stiffness. OPCs were found to differentiate and wrap around the PDMS micropillars. OPC antigenic and morphological differentiation is accelerated in softer PDMS structures while the wrapping capability is increased for larger diameter micropillars. These events are mediated by epigenetic alterations (upregulation of Hdac1, 2 and 3).

ALG hydrogels were produced by combining ALG formulations containing the cell adhesive peptide RGD or the matrix metalloproteinase sensitive peptide PVGLIG. When cultured in these hydrogels OPCs are viable, metabolically active and can differentiate in OLs. By embedding OPCs in increased ALG content hydrogels we observed that softer matrices favored OPC differentiation in comparison with stiffer matrices (G*~ 100 Pa vs 350 Pa and 1300 Pa). We found that key mechanosensing genes (Yap1, Taz, Mapk14 and Ptk) are upregulated in OLs cultured in stiffer matrices.

CONCLUSIONS: We present evidence that supports that mechanical signals should be considered in the design of new therapies against demyelinating conditions. By embedding the micropillar array within the ALG we are expecting to recreate a 3D bioengineered platform containing axon surrogates allowing a deeper study on the role of biomechanical properties on OL biology.

POSTER 1

DECIPHERING THE ASTROCYTIC AND SYNAPTIC CHANGES UNDER CHRONIC ALCOHOL EXPOSURE USING A SELF-ADMINISTRATION PARADIGM

Ana Margarida Rodrigues[1,3], Teresa Canedo[1], Ana Filipa Terceiro[1,5], Joana Tedim-Moreira[3,4], Ana Isabel Silva[1,5], Ana Magalhães[1,5], João Relvas[3,4], Teresa Summavielle[1,2,6]

[1] Addiction Biology Group, Instituto de Investigação e Inovação em Saúde (i3S), Porto, Portugal; [2] Instituto de Biologia Molecular e Celular (IBMC), Porto, Portugal; [3] Faculdade de Medicina da Universidade do Porto (FMUP), Porto, Portugal; [4] Glial Cell Biology Group, Instituto de Investigação e Inovação em Saúde (i3S), Porto, Portugal; [5] Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Porto, Portugal; [6] Escola Superior de Saúde do Politécnico do Porto (ESS.PP), Porto, Portugal

Drug abuse is characterized by a compulsive and persistent drug-seeking behavior, despite the harmful emotional, physical and social consequences. Our laboratory has previously found that the neuronal-glial crosstalk is critical in relaying the changes caused by acute exposure to psychoactive drugs through neuroimmune mechanisms. We have also reported that microglia can engulf postsynaptic components in the prefrontal cortex (PFC) of mice after repeated alcohol exposure and this led to increased anxiety in mice. The adverse effects of alcohol on the central nervous system (CNS) are well described, with astrocytes becoming reactive and displaying changes in gene expression, activity and proliferation. However, the mechanisms involved are not yet fully understood. We are currently characterizing the astrocytic response under chronic alcohol consumption, taking into account the crucial interaction between neuronal and glial cells in the development and maintenance of addiction. Using a well-established voluntary alcohol drinking paradigm, we are evaluating alcohol-associated changes in PFC astrocytes, synapses and their behavioral correlates. Our preliminary results indicate similar alcohol consumption patterns between males and females, however, males, but not females, present altered weight gain and experience a significant increase in inhibitory synapse density after chronic exposure to ethanol when compared to the control group. Our work is contributing to a better understanding of the impact of chronic alcohol intake and may lead to the development of new strategies for pharmacological intervention in drug addiction, based on the targets identified as critical for the neuronal-glial crosstalk.

POSTER 2

THE INVOLVEMENT OF ASTROCYTE CALCIUM-DEPENDENT SIGNALING IN FEAR MEMORY

Daniela Sofia Abreu[1,2], João Filipe Viana[1,2], Sónia Guerra-Gomes[1,2], João Luís Machado[1,2], Sara Barsanti[1,2], Diana Sofia Marques Nascimento[1,2], Luísa Pinto[1,2], Andreia Teixeira-Castro[1,2], João Filipe Oliveira[1,2,3]

[1] Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, 4710-057 Braga, Portugal; [2] ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal; [3] IPCA-EST-2Ai, Polytechnic Institute of Cávado and Ave, Applied Artificial Intelligence Laboratory, Campus of IPCA, Barcelos, Portugal

Astrocytes are critical players in the regulation of brain development and function. They sense and respond to neuronal activity by elevating intracellular calcium levels, which derive from different sources and display complex spatiotemporal properties. Calcium elevations appear spatially distributed in global (soma and main processes) and focal regions (microdomains). Such astrocytic calcium activity is expected to underlie the astrocyte involvement in synaptic transmission, metabolism, and brain homeostasis. In this work, we performed a multi-level analysis of the IP3 receptor type 2 knockout (IP3R2 KO) mouse model that lacks global calcium elevations in astrocytes to disclose its implications in behavior. Transcriptomic analysis of hippocampal tissue revealed that the lack of astrocytic global calcium causes the differential expression of hundreds of genes. Among these, 76 genes are regulated by the astrocyte-specific Foxo1 transcription factor, which is over-expressed in hippocampal astrocytes of this mouse model and regulates the expression of genes involved in spinogenesis and synaptic coverage. A detailed morphological analysis of hippocampal pyramidal neurons of this model revealed a shift to a more immature spine profile, that may underlie the previously described reduction of long-term depression and performance in a fear memory task. Indeed, we found that these mice lacking global astrocyte calcium display an enhancement of long-term fear memory. Overall, our results suggest a regulatory role of astrocytic calcium-dependent signaling through Foxo1 modulation of genes related to structural plasticity and fear memory performance. These findings broaden the scope of astrocytic modulation of brain circuits.

POSTER 3

CB1R DOWNREGULATES GLUTAMATE TRANSPORTERS ACTIVITY IN AN ASTROGLIAL CA2+-DEPENDENT MANNER IN THE RODENT MPFC

Gonçalves-Ribeiro J[1,2], Nanclares C[3], Costa-Pinto S[1,2], Neuparth-Sottomayor M[1,2], Rodrigues-Freitas L[1,2], Gualdino M[1,2], Sebastião AM[1,2], Araque A[3], Vaz SH[1,2]

[1] Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina da Universidade de Lisboa (FMUL), Lisboa, Portugal; [2] Instituto de Farmacologia e Neurociências, FMUL, Lisboa, Portugal; [3] Department of Neuroscience, University of Minnesota, Minneapolis, MN, USA

Glutamate transporters, which are expressed in astrocytes, are responsible to maintain extracellular glutamate levels at an optimal state in several brain areas, namely the medial prefrontal cortex (mPFC). Later studies have shown that glutamate uptake, mediated by specific glutamate transporters, is not a static process, evincing a possible correlation between this phenomenon and the efficiency of synaptic transmission, and suggesting that glutamate transporter activity can be modulated. The mechanism underpinning the modulatory effect of glutamate transporters over synaptic plasticity still remains unascertained. One possible mechanism is through changes in astroglial Ca2+ levels, possibly through astroglial CB1R activation which is known to elicit Ca2+ transients. Thus, this work aimed to unveil the astroglial CB1R role upon the modulation of glutamate transporters and ultimately its impact in mPFC synaptic function. Whole cell patch-clamp in layer V neurons and astrocytes of the mPFC was performed. IP3R2-knockout mice and age-matched WT received 0.2mg/kg ip. injections of WIN55,212-2 (a non-selective CB1R agonist) or vehicle. The open field test was used to assess locomotor activity and anxiety-like behavior. Working memory was assessed using Y-maze. FST explored the transition dynamics from active (swimming) to passive (immobility) modes of coping, testing depressive-like behavior. Synaptic transporters currents (STC) were induced by delivering a train of 75 Hz for 200 ms. In WT brain slices, a WIN 55-212 puff (100-300µM) significantly decreased the amplitude of STC being this effect lost in the presence of AM251 (1µM) - a CB1R antagonist – as well as in IP3R2KO slices. Dual patch recordings of layer V neurons and astrocytes showed that neuronal depolarization (ND), which is known to induce the release of endocannabinoids in neurons, elicited a decrease of the amplitude of STC in a nearby astrocyte, simultaneously increasing excitatory post synaptic current (EPSC) of the neuron that underwent ND. Regarding behavior, no drug-treated group showed significantly different locomotor activity or anxiety-like behavior from the vehicle-treated group. IP3R2-WT and IP3R2-KO did not differ in working memory, nor did WIN55,212-2 elicit differences in spontaneous alternation between the groups. When compared to vehicle, a low dose of CB1R agonist significantly decreased the total time spent in immobility for male IP3R2-WT; however, for female IP3R2-WT, no differences were found. Thus, it was shown that astroglial CB1R activation by exo- and endo-cannabinoids decreases glutamate transporters activity and that this is done in a Ca2+-dependent manner. Additionally, a low dose of CB1R agonist WIN-55,212 appears to be anti-depressant in IP3R2-WT males.

This work was funded by PD/BD/150342/2019 (JGR), PTDC/BTM-SAL/32147/2017, H2020-WDESPREAD (EpiEpinet) under grant agreement No. 952455 and 2021 ISN Career Development Grant. No conflict of interests.

POSTER 4

ASTROCYTE STRUCTURAL HETEROGENEITY IN THE MOUSE HIPPOCAMPUS

João Filipe Viana[1,2], João Luís Machado[1,2], Daniela Sofia Abreu[1,2], Alexandra Veiga[1,2], Sara Barsanti[1,2], Gabriela Tavares[1,2], Manuella Martins[1,2], Vanessa Morais Sardinha[1,2], Sónia Guerra-Gomes[1,2], Cátia Domingos[3], Alberto Pauletti[3], Jérôme Wahis[4], Chen Liu [4], Corrado Cali[5,6], Christian Henneberger[3,7], Matthew G. Holt[4,8], João Filipe Oliveira[1,2,9]

[1] Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, 4710-057 Braga, Portugal; [2] ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal; [3] Institute of Cellular Neurosciences, Medical Faculty, University of Bonn, Bonn, Germany; [4] Laboratory of Glia Biology, VIB-KU Leuven Center for Brain and Disease Research, Leuven, Belgium; [5] Department of Neuroscience, University of Torino, Torino, Italy; [6] NICO - Neuroscience Institute Cavalieri Ottolenghi, Orbassano, Italy; [7] German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany; [8] Synapse Biology Group, Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, 4200-135 Porto, Portugal; [9] IPCA-EST-2Ai, Polytechnic Institute of Cávado and Ave, Applied Artificial Intelligence Laboratory, Campus of IPCA, Barcelos, Portugal

Astrocytes are integral components of brain circuits, where they sense, process, and respond to surrounding activity, maintaining homeostasis and regulating synaptic transmission, the sum of which results in behavior modulation. These interactions are possible due to their complex morphology, composed of a tree-like structure of processes to cover defined territories ramifying in a mesh-like system of fine leaflets unresolved by conventional optic microscopy. While recent reports devoted more attention to leaflets and their dynamic interactions with synapses, our knowledge about the tree-like 'backbone' structure in physiological conditions is incomplete. Recent transcriptomic studies described astrocyte molecular diversity, suggesting structural heterogeneity in regions such as the hippocampus, which is crucial for cognitive and emotional behaviors. In this study, we carried out the structural analysis of astrocytes across the hippocampal subfields of Cornu Ammonis area 1 (CA1) and dentate gyrus (DG) in the dorsoventral axis. We found that astrocytes display heterogeneity across the hippocampal subfields, as astrocytes in the stratum radiatum and stratum moleculare possess more complex backbone arbors as compared to stratum oriens, stratum lacunosum moleculare and hilus. This pattern is conserved along the dorsoventral axis. We further found that astrocytes appear to contribute in an exocytosis-dependent manner to a signaling loop maintaining the backbone structure. These findings reveal astrocyte heterogeneity in the hippocampus, which appears to follow layer-specific cues and depend on the neuro-glial environment.

POSTER 5

ASTROCYTIC FOXO1 REGULATES HIPPOCAMPAL SPINOGENESIS AND SYNAPTIC PLASTICITY AND ENHANCES FEAR MEMORY

João Luís Machado[1,2], João Filipe Viana[1,2], Daniela Sofia Abreu[1,2], Sónia Guerra-Gomes[1,2], Cristina Martín-Monteagudo[3], Sara Barsanti[1,2], Diana Sofia Marques Nascimento[1,2], Marta Navarrete[3], Andreia Teixeira-Castro[1,2], João Filipe Oliveira[1,2,4]

[1] Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, 4710-057 Braga, Portugal; [2] ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal; [3] Instituto Cajal, CSIC, Madrid, Spain; [4] IPCA-EST-2Ai, Polytechnic Institute of Cávado and Ave, Applied Artificial Intelligence Laboratory, Campus of IPCA, Barcelos, Portugal

Astrocytes are active players in brain circuits, sensing and responding to neuronal activity with an impact in behavior production. Activation of astrocytes triggers intracellular calcium elevations displaying complex spatiotemporal properties. These intracellular calcium elevations were shown to underlie the modulation of synaptic transmission and brain homeostasis. Recently, we found a regulatory role of astrocytic calcium-dependent signaling through Foxo1 modulation of genes related to structural plasticity. We found that mice lacking global calcium elevations in astrocytes display a shift to an immature spine profile and enhancement of fear memory performance.

To confirm a causal relationship between these molecular, structural and behavioral observations, we used a viral approach to induce the overexpression of Foxo1 in hippocampal astrocytes in C57BL/6J mice. The overexpression of Foxo1 in hippocampal astrocytes replicated the structural, synaptic and behavioral effects, observed in mice lacking global calcium elevations in astrocytes. In summary, our data suggest that astrocytic Foxo1 modulates circuit structure and function involved in fear memory processing.

POSTER 6

THE RELEVANCE OF ASTROGLIAL CB1R IN THE RODENT mPFC SYNAPTIC PLASTICITY

Rodrigues-Freitas L[1,2], Gonçalves-Ribeiro J[1,2], Sebastião AM[1,2], Vaz SH[1,2]

[1] Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal; [2] Instituto de Farmacologia e Neurociências, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal

Major Depressive Disorder (MDD) is a neuropsychiatric disorder that may arise from electrophysiological alterations in specific regions of the brain, namely the medial prefrontal cortex (mPFC), a region associated with emotion and neuroplasticity. Moreover, the disruption of these processes, including Long-Term Depression (LTD), a form of neuroplasticity, is related to the onset of depressive-like symptoms. The endocannabinoid system (ECS) is a major modulator of emotion and neuroplasticity, and it acts in the brain mainly through the activation of the cannabinoid type one receptor (CB1R), found both in neurons and astrocytes. Astrocytes mediate cellular communication through intracellular Ca2+ transients, for which the astrocytic CB1R is a main contributor. Additionally, both astrocytes and the CB1R are implicated in MDD.

Thus, this work aims to elucidate the role of the astrocytic CB1R on endocannabinoid-mediated long-term depression (eCB-LTD) in the mPFC, and subsequently on depressive-like behavior. Ex-vivo field excitatory post-synaptic potentials (fEPSP) were recorded in the circuit II/III-V of the mPFC of IP3R2-WT and IP3R2-KO mice (lack astrocytic Ca2+ signals) while modulating the CB1R activity with AM251 (1 μM, CB1R inverse agonist) and WIN55,212-2 (5 μM, non-specific CB1R agonist). LTD was induced by Low Frequency Stimulation (LFS) and its magnitude evaluated. Depressive-like behaviour was accessed by Forced Swim Test (FST) prior to acute intraperitoneal injections of WIN55,212-2 (0.2 mg/kg). We observed that LTD was lost in the presence of AM251 and in the absence of astrocytic Ca2+ signaling, suggesting that mPFC LTD is eCB-mediated and astrocytic Ca2+-dependent. Additionally, mPFC LTD was enhanced in IP3R2-WT mice and attenuated in IP3R2-KO mice while superfusing the mPFC slices with WIN 55,212-2. Furthermore, the administration of WIN 55,212-2 seems to decrease the depressive-like phenotype only in IP3R2-WT males, while in females seems to increase the depressive-like phenotype only in the IP3R2-KO mice.

Altogether, these data suggest that astrocytic CB1R together with astrocytic Ca2+ signaling are essential for LTD maintenance in the mPFC and for WIN 55,212-2 antidepressant properties.

This work was funded by PD/BD/150342/2019 (JGR), PTDC/BTM-SAL/32147/2017, H2020-WDESPREAD (EpiEpinet) under grant agreement No. 952455 and 2021 ISN Career Development Grant. No conflict of interests

POSTER 7

INTERACTION BETWEEN CB1 RECEPTORS AND ADENOSINE A1 RECEPTORS ON ASTROCYTES: IMPLICATION ON HIPPOCAMPAL SYNAPTIC PLASTICITY

Gualdino M[1,2], Gonçalves-Ribeiro J[1,2], Sebastião AM[1,2], Vaz SH[1,2]

[1] University of Lisbon, Instituto de Medicina Molecular João Lobo Antunes (IMM), Faculty of Medicine, Lisbon, Portugal; [2] University of Lisbon, Instituto de Farmacologia e Neurociências, Faculty of Medicine, Lisbon, Portugal

Introduction: Astrocytes are glial cells with numerous physiological functions, among which the ability to detect and modulate neuronal activity, through the activation of specific receptors expressed on their plasmatic membrane. Both the type one cannabinoid receptors (CB1R) and the A1 adenosine receptors (A1R) are expressed in hippocampal astrocytes, whose activity is linked to glutamatergic signaling. Astrocytic CB1R activation leads to an elevation of astrocytic Ca2+ concentration with consequent release of glutamate, but nothing is known concerning the CB1R/A1R-crosstalk in astrocytes and its influence for synaptic function.
Aims: Thus, this work aims to study the role of astrocytic CB1R on the long-term potentiation (LTP) in the hippocampus of IP3R2-WT and IP3R2-KO mice (lack Ca2+ signaling in astrocytes) and its modulation by A1R.
Methods and results: LTP was induced by theta-burst stimulation, consisting of four trains composed by four stimuli each, separated by 200 ms, while recording field excitatory post-synaptic potentials (fEPSP) in hippocampus CA1 area. LTP magnitude was calculated as the percentage of change of fEPSP slope 50-60 min after LTP induction compared to baseline fEPSP (10 min before LTP induction). In IP3R2-WT mice, it was observed a decrease of the LTP magnitude in the presence of ACEA (1 μM), a selective CB1R agonist, compared to the control condition (absence of drugs), suggesting that activation of CB1R inhibits LTP. Furthermore, our results indicate that in both males and females, there are no differences between treatments with WIN55,212-2 (WIN, 0,2 mg/Kg), a non-specific CB1R agonist, and between mice (IP3R2-WT and IP3R2-KO) regarding working memory, that was accessed by the Y-maze test prior to acute intraperitoneal injections of WIN. We also observe no differences in hippocampal CB1R and NMDAR-NR2B protein levels between treatments (WIN and saline) and mice (IP3R2-WT and IP3R2-KO) that was accessed by Western Blot. Altogether, this suggests that WIN does not affect the working memory of mice and the protein levels of CB1R and NMDAR-NR2B.
Conclusions: Results from this work not only unveil a new astrocyte CB1R/A1R-crosstalk and its impact on glutamatergic signaling but also may contribute to the development of therapies for neuropsychiatric-related disorders since memory and learning are impaired in most of them. Besides, since these two receptors are modulated by two of the most heavily consumed psychoactive effect substances worldwide, caffeine and Δ9-Tetrahidrocanabinol (THC), this work will contribute for the identification of astrocytes role upon the consume of these drugs.
Acknowledgements: This work was funded by PD/BD/150342/2019 (JGR), 2021 ISN Career Development Grant and H2020-WIDESPREAD 05-2017-Twinning (EpiEpinet) under grant agreement No. 952455. No conflict of interests.

POSTER 8

DIFFERENCES IN DEVELOPMENTAL MILESTONES AND ASTROCYTIC COMPLEXITY OF GENETIC ABSENCE EPILEPSY RAT FROM STRASBOURG (GAERS)

Neuparth-Sottomayor M[1,2], Morais TP[3,4], Gonçalves-Ribeiro J[1,2], Costa-Pinto S[1,2], Sebastião AM[1,2], Crunelli V[3,4], Vaz SH[1,2]

[1] Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Portugal; [2] Instituto de Farmacologia e Neurociências, Faculdade de Medicina, Universidade de Lisboa, Portugal; [3] Neuroscience Division, School of Bioscience, Cardiff University, Cardiff, United Kingdom; [4] Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta, Msida, Malta

Absence seizures (ASs) are genetic generalized seizures characterized by sudden and brief lapses of consciousness accompanied by 2.5-4Hz spike-and-wave discharges in the EEG. In ASs animal models, dysfunction of thalamic GABA transporter-1 (GAT-1), exclusively expressed in thalamic astrocytes enhances tonic inhibition at postnatal day 17 (P17), being this enhancement essential and sufficient for the generation of ASs (P30). Additionally, it has been shown in childhood absence epilepsy (CAE) that 60% of patients show psychiatric comorbidities. So, a better understanding of ASs animal models' behaviour is needed. Using the Genetic Absence Epilepsy Rat from Strasbourg (GAERS), and respective non-epileptic control (NEC), by performing behaviour tests, we aimed to assess reflex, motor, and learning developmental milestones from PND10-28, as well as to study astrocytic morphological differences between epileptic and non-epileptic animals on crucial developmental milestones: P8-10 (no behavioural and EEG abnormalities are present in GAERS); P14-15 (just before the thalamic tonic GABA-A current is increased); P18-19 (just after the thalamic tonic GABA-A current is increased), and P90. Immunohistochemistry and Western blot assays were used to assess molecular and cellular morphological differences between epileptic and non-epileptic animals. Our data suggests that, according to physical parameters, eye-opening first occurs in GAERS when compared to NEC (P10). A significant decrease in body weight was observed for GAERS compared to NEC (P10). Concerning sensory-motor maturation, GAERS climbed angled platforms (positive geotaxis) faster and with higher success rates on P10. GAERS also demonstrated a reduction in upward turn latency for negative geotaxis. In terms of locomotion, GAERS show increased mean velocity and distance traveled on P17 when tonic current increases. On the Barnes Maze, spatial leaning is visible at P18, and both groups show maturation of recognition memory at P21. Although exploratory drive was decreased, working memory was not affected in GAERS at P28. At P90, GAERS displayed an increase in astrocytic morphological complexity, mainly in the hippocampus, thalamus, and motor cortex, and increased GFAP expression levels compared to Wistar and NEC.

In conclusion, this study suggests changes in the physical and sensorimotor development of GAERS animals. Memory deficits in GAERS do not exist before seizures are completely developed (P30), although changes in behaviour are observed when the tonic current is enhanced (P17). Since GAT-1 is expressed in astrocytes and there is an increase in morphological complexity and GFAP expression in GAERS (P90), the involvement of astrocytes in the pathology of ASs is reinforced.

Acknowledgements: This work was funded by iMM/BI/36/2023 (MNS), H2020-WDESPREAD (EpiEpinet) under grant agreement No. 952455. Authors declare no conflict of interests. This project was funded by FCT (PTDC/BTM-SAL/32147/2017).

POSTER 9

MODULATION OF CB1R IN SYNAPTIC PLASTICITY THROUGH ALS PROGRESSION

Pinto-Coelho M[1,2], Costa-Pinto S[1,2], Sebastião A[1,2], Vaz S[1,2]

[1] Instituto de Farmacologia e Neurociências, Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal; [2] Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal

Aims: ALS is a neurodegenerative disease with progressive loss of motor neurons in the spinal cord, brainstem and motor cortex. Studies on ALS SOD1 animal model have also shown alterations in hippocampal synaptic transmission and plasticity. Additionally, astrocyte dysfunction has been associated with ALS progression. Other studies also suggest a relationship between the endocannabinoid system and neurodegenerative diseases, regarding the fact that endocannabinoids have a neuromodulatory role in synaptic transmission, through the activation of CB1 and CB2 receptors. Thus, we aimed to characterize the endocannabinoid system in the SOD1(G93A) mouse model throughout disease progression, by determining the protein expression of CB1 and CB2 receptors in the hippocampus, as well as, its cellular location. We will also modulate these receptors and assess their impact on hippocampal synaptic function.

Methods: Congenic SOD1(G93A) mice are being used in a pre-symptomatic phase (4-6weeks) and symptomatic phase (14-18weeks). Both sexes were used during the studies. Protein levels were assessed by Western blot. Modulation of CB1R was achieved pharmacologically by using a specific agonist and antagonist, and synaptic plasticity was then assessed by eliciting long-term potentiation (LTP) through a theta-burst stimulation of the Schaffer collaterals fibers of the hippocampus CA1 region.

Results: In the hippocampus, CB1R level is increased in the pre-symptomatic phase, when compared with the age-matched wt mice ($p<0.05$, $n=12$). When looking at synaptic plasticity, in the pre-symptomatic phase, SOD1(G93A) mice had a significant decrease in LTP magnitude ($p<0.05$, $n=10$-13), while in the symptomatic phase there was an increase in LTP magnitude in these mice ($p<0.05$, $n=10$-13). In the presence of the selective CB1R agonist ACEA (1μm), in the pre-symptomatic phase of wt mice, it was observed a significant inhibition of synaptic plasticity ($p<0.01$, $n=4$), but in the SOD1(G93A) mice is not possible see any significant difference, so far.

Conclusions: Altogether, the before mentioned results show that there are alterations in the protein levels of CB1R in ALS, and changes in the location of CB1R in astrocytes will be evaluated next. Moreover, SOD1(G93A) mice present changes in hippocampal synaptic plasticity and, as of now, even though no conclusions can be withdrawn from the modulation of endocannabinoid receptors, these seem to influence synaptic plasticity.

POSTER 10

BRINGING ASTROCYTES INTO THE SPOTLIGHT OF ELECTRICAL BRAIN STIMULATION THERAPY

Aroso M[1,2], Silva S[1,2,3], Castro D[1,2,4], Aguiar P[1,2]

[1] INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Portugal; [2] i3S - Instituto de Investigação e Inovação em Saúde da Universidade do Porto, Portugal; [3] Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Porto, Portugal; [4] Faculdade de Engenharia, Universidade do Porto, Portugal

The development of electrical brain stimulation therapies opened a new window for the treatment of neurological diseases. An excellent example is deep brain stimulation, a surgical technique used to modulate brain function with proven efficacy in movement disorders (e.g. Parkinson Disease). However, the mechanism of action of electrical brain stimulation is still far from being understood. Neurons have been on the spotlight so far, but new findings point to the involvement of astrocytes as well. Astrocytes, primarily viewed only as supportive units, are now emerging as active players in the information processing of the brain. Here, we assessed if astrocytes may have a role on electrical brain therapies by being able to respond to the same electrical stimulus used to modulate neuronal activity. To do so, we took advantage of microelectrode arrays (MEAs) capability to simultaneously record and deliver extracellular electrical signals. Additionally, we synchronized the recording of electrophysiological data with the recording of calcium activity, a hallmark of astrocytic activity. Although astrocytes are thought to be electrically silent, we reveal that they respond to electrical stimulation with the generation of high frequency oscillations (small membrane potential deflections) and the simultaneous production of calcium waves, demonstrating, unequivocally, that astrocytes respond to electrical stimulation on the same range as neurons do. Importantly, these responses are dependent on the stimuli amplitude. Therefore, a clear characterization of the electrical stimulation effect on astrocytic activity and, subsequently, on neuronal modulation is urgently needed to improve the development and implementation of electrical brain stimulation therapies.

Acknowledgments: This work was partially financed by Prémio Mantero Belard from Santa Casa da Misericórdia de Lisboa in the framework of project smartDBS (Ref: MB12-2022). MAroso, DCastro and SSilva are funded by the Fundação para a Ciência e a Tecnologia (FCT) (CEECIND/03415/2017, SFRH/BD/143956/2019 and 2020.05463.BD respectively).

POSTER 11

GLIA TO THE RESCUE: ASTROCYTES AND MICROGLIA AS THE BRAIN'S MECHANICS CREW

Morais MRG[1,2,3], Carvalho ED[1,2,4], Athanasopoulou G[1,2,3], Araújo M[1,2], Barrias CC[1,2,3], Pêgo AP[1,2,3]
[1] University of Porto, Institute for Research & Innovation in Health (i3S), Porto, Portugal; [2] University of Porto, National Institute of Biomedical Engineering (INEB), Porto, Portugal; [3] University of Porto, Institute of Biomedical Sciences Abel Salazar (ICBAS), Porto, Portugal; [4] University of Porto, Faculty of Engineering (FEUP), Porto, Portugal

Neurodegenerative disorders of the central nervous system (CNS) have a tremendous socioeconomic impact, which is expected to escalate with the increasing life expectancy. Astrogliosis and neuroinflammation are a common hallmark in many neurodegenerative disorders. The two main players in this process are astrocytes and microglia. Microglia has been shown to induce neurotoxic reactive astrocytes by secreting a cocktail of cytokines, while astrocytes are also capable of producing immunoregulatory molecules that influence microglia response to stimuli. Additionally, extensive alterations in ECM composition and, consequently, in tissue mechanical properties occur during neuroinflammation. Here we propose a 3D astroglial tissue engineered model that recreates key features of neuroinflammation. Primary rat astrocytes were embedded within alginate hydrogels containing the cell adhesion (RGD) and matrix metalloproteinase-sensitive (PVGLIG) peptides in order to recreate the ECM environment. A combination of LPS/IFN was used to mimic a pro-inflammatory environment, in which astrocytes acquired an astrogliosis-like phenotype with increased expression of Lcn2, IL-6 and production of nitrite oxide. Mechanical properties were dynamically tuned using an external chelator (Ba2+) that led to a six-fold increase in the stiffness of the hydrogels, without remarkable alterations on cell metabolic activity, viability and functionality (assessed by calcium imaging). Matrix stiffening was accompanied by astrocytic morphology changing and an up-regulation of the mechanotransducer Piezo1. When astrocytes were stimulated via combination of biochemical and mechanical stimuli, both pro-inflammatory and mechanosensing markers were up-regulated. Primary rat microglia cells were also embedded within modified alginate matrices and cellular response to the 3D environment was dependent on the cellular density as well as matrix properties, including the mechanical properties.

The crosstalk between astrocytes and microglia has not been explored as a function of mechanical properties in a 3D environment. To further understand this, both cell types will be co-cultured within the 3D alginate model. This model will ultimately be used to explore novel mechanotransduction molecular targets that can later lead to the development of new therapeutic approaches to overcome neuroinflammation in neurodegenerative disorders.

Acknowledgments: The authors acknowledge the funding from the FCT-UT Austin Portugal Program (project UTAPEXPL/NTec/0057/2017) and the Air Force Defense Research Sciences Program, USA (Award FA9550-20-1-0417). M. R. G. Morais and E. D. Carvalho acknowledge FCT for their Ph.D. fellowships (grants 2022.13353.BD and SFRH/BD/140363/2018, respectively). G. Athanasopoulou acknowledges ASTROTECH Project for her Ph.D. fellowship (Grant Agreement number: 956325 — ASTROTECH — H2020-MSCA-ITN-2020).

POSTER 12

ASTROGLIAL RESPONSIVENESS IN OVERT HEPATIC ENCEPHALOPATHY: AN IMMUNOHISTOCHEMICAL APPROACH IN THE HEPATIC DEVASCULARIZATION MODEL

Nahla Ouard[1] Assmaâ Tali[2] Samir Ahboucha[3]

[1] Sultan Moulay Sliman University, Polydisciplinary Faculty Khouribga, Multidisciplinary Laboratory of Research and Innovation (MLRI); Research Team: Technological Applications, Environmental Resources and Health; [2] Sultan Moulay Sliman University, Polydisciplinary Faculty Khouribga, Multidisciplinary Laboratory of Research and Innovation (MLRI); Research Team: Technological Applications, Environmental Resources and Health; [3] Sultan Moulay Sliman University, Polydisciplinary Faculty Khouribga, Multidisciplinary Laboratory of Research and Innovation (MLRI); Research Team: Technological Applications, Environmental Resources and Health

Hepatic encephalopathy is a serious complication of hepatic cirrhosis with multiple neuropsychiatric manifestations, protean and variable degrees of severity. While its pathophysiology remains elusive, many studies have demonstrated a primary gliopathy, mainly in astrocytes, in patients with hepatic encephalopathy as well as in animal models of acute liver failure. Here, we have investigated in an animal model of acute liver failure (ALF): Portocaval anastomosis (PCA), followed by a hepatic arterial ligation (HAL) in Wistar rats before and after coma, the expression of the key marker of mature astrocytes: the glial fibrillary acidic protein GFAP in different brain areas. The Immunohistochemical study showed, a decrease in the number of astrocytes demonstrated by a loss in GFAP expression in ALF compared to the operated controls (shams) and a difference of expression from one region to another especially in cerebral cortex and Thalamic regions. We suggest that these differential astroglia responses could reflect the specificity of each region and its particular neuronal neighboring environment leading to the diverse neuronal impairments during the different episodes of hepatic encephalopathy.

POSTER 13

STRUCTURAL ANALYSIS OF ASTROCYTES IN DIFFERENT EXPERIMENTAL CONDITIONS

Barsanti S[1,2], Machado J L[1,2], Viana J F[1,2], Veiga A[1,2], Abreu D[1,2], Wahis J[3], Liu C[3], Holt M[3,4], Oliveira J F[1,2,5]

[1] University of Minho, Life and Helath Sciences Research Institute (ICVS), School of Medicine, Braga, Portugal; [2] ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal; [3] Laboratory of Glia Biology, VIB-KU Leuven Center for Brain and Disease Research, Leuven, Belgium; [4] University of Porto, Synapse Biology Group, Instituto de Investigação e Inovação em Saúde (i3S), Porto, Portugal; [5] IPCA-EST-2Ai, Polytechnic Institute of Cávado and Ave, Applied Artificial Intelligence Laboratory, Campus of IPCA, Barcelos, Portugal.

Astrocytes are the most abundant glial cells in the brain, displaying a ramified star-shaped morphology. This extended feature, which is heterogeneous and dependent on the physiological state, is pivotal to understanding their dynamic interactions with neighboring neurons and glia. Therefore, assessing astrocytic structures is crucial to understanding their regional distribution and role in brain networks. The immunostaining of the Glial Fibrillary Acidic Protein (GFAP), the astrocyte-specific cytoskeletal protein, is among the most used procedures to visualize astrocytic structure in the absence of genetically encoded fluorescent labels. The staining procedure could influence the visualization and reconstruction of the detailed astrocytic structure. Thus, in this work, we evaluate the experimental parameters that could affect the morphological reconstruction of the astrocytic backbone in the mouse hippocampus: antigen retrieval, slice thickness, slicing method, and cell tagging method. Here, we performed the 3D reconstruction of the backbone of hippocampal astrocytes in brain slices of C57BL6/J mice using GFAP immunostaining, manipulating these different experimental parameters. Furthermore, we also compared GFAP immunostaining with the AstroTRAP labeling, which results from expressing GFP specifically in astrocytic ribosomes. For this, we used the open-source and semi-automatic FIJI tool SNT to quickly screen different morphometric features of the main astrocytic structure: number of processes, total process length, and arbor complexity (Sholl analysis). Our results describe to which extent the different experimental procedures influence the final 3D reconstruction.

The authors acknowledge the Foundation for Science and Technology (FCT) fellowships to JFV, JLM and DSA; projects PTDC/MED-NEU/31417/2017 and POCI-01-0145-FEDER-016818; grants from Bial Foundation (037/18) and "la Caixa" Foundation (LCF/PR/HR21/52410024) to JFO; ICVS Scientific Microscopy Platform, member of the national infrastructure PPBI - Portuguese Platform of Bioimaging (PPBI-POCI-01-0145-FEDER-022122; by National funds, through the Foundation for Science and Technology (FCT) - project UIDB/50026/2020 and UIDP/50026/2020.

POSTER 14

A TRIPARTITE MICROFLUIDIC DEVICE WITH INTEGRATED MICROELECTRODES TO STUDY THE NEURON-ASTROCYTE CROSSTALK

Silva SC[1,2], Castro D[1,3], Mateus JC[1], Aroso M[1,4]*, Aguiar P[1]*

[1] Neuroengineering and Computational Neuroscience Lab, i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto 4200-135, Portugal; [2] Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto; [3] Faculdade de Engenharia da Universidade do Porto; [4] Instituto Nacional de Engenharia Biomédica da Universidade do Porto.

*Equal contribution

Astrocytes have been shown to be an active element at the tripartite synapse, being able to sense and respond to neuronal activity. Upon neuronal synaptic transmission, astrocytic microdomains increase their intracellular calcium levels, which can lead to a calcium wave propagating throughout the astrocyte and the astroglial syncytium. Increased calcium levels can result in the release of gliotransmitters by astrocytes, which in turn are capable of regulating neuronal activity. Although significant progress has been made to study the role of astrocytes in shaping the synaptic transmission, there are still a lot of aspects of the astrocytic calcium dynamics and their interplay with neuronal synapses left to uncover, particularly at the electrophysiological domain.

With this in mind, we designed an in vitro platform combining the advantages of microElectrode arrays and microFluidics. This platform, the tripartite µEF, allowed us to compartmentalize cells to mimic the tripartite synapse, while performing both electrophysiological recordings and stimulation, combined with calcium imaging. In the synaptic compartment, besides purified astrocytes, we observed both axons, which extended from the pre-synaptic compartment through the microchannels, and neurites coming from the post-synaptic compartment. By using AAVs, it was possible to specifically target astrocytes and neurons to express GCaMP and RCaMP respectively, allowing the screening of their calcium dynamics while monitoring the cellular electrophysiological profile of each cell population. These validation results demonstrate that our tripartite µEF platform is suitable to study the astrocyte-neuron interaction and its underlying calcium activity, while reducing the complexity of the surrounding microenvironment of astrocytes.

Sara Costa Silva acknowledges the support from FCT (2020.05463.BD) and the Doctoral Programme on Cellular and Molecular Biotechnology Applied to Health Sciences (ICBAS). The authors declare no conflicts of interest.

POSTER 15

EXPLORING THE ADVERSE EFFECTS OF OBESITY ON CLINICAL PROGRESSION AND CNS PATHOGENESIS OF MULTIPLE SCLEROSIS: AN IN VIVO MODEL STUDY

Neto A[1], Alberro A[2], Barros C[1], Ribeiro R[1], Fernandes A[1,3], Barateiro A[1,3]

[1] University of Lisbon, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Lisbon, Portugal; [2] Biodonostia Health Research Institute, San Sebastian, Spain; [3] University of Lisbon, Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Lisbon, Portugal

Introduction/Aims: Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease affecting the CNS. Obesity is a known risk factor for MS that could contribute to onset/progression, however, the mechanisms underlying that are not clear. We aimed to investigate how obesity affects the onset/progression of MS in an animal model of the disease (experimental autoimmune encephalomyelitis, EAE) to identify new targets for innovative therapeutic strategies.

Methods: 4-week-old C57BL/6 mice were fed with standard or high-fat diet (HFD) for 20-weeks (animals in HFD reach 40 g of weight, being considered diet-induced obese, DIO, mice) followed by EAE induction. Mouse weight, clinical score and EAE-associated frailty index were monitored. Immunohistochemistry revealed changes in myelination and microglia proportion in spinal cord samples at 18 (disease peak) and 30 (chronic disease) days post-induction.

Results: DIO mice showed more aggressive clinical symptoms, with higher weight loss, clinical scores, and frailty index, that persisted over time, while those on standard diet had lower scores with better recovery from chronic EAE pathogenesis. DIO mice spinal cords exhibited wider areas of white matter lesions, with a pronounced degree of demyelination in the surrounding regions, as well as increased density of microglia, not only in the peak of the disease but also in the chronic phase, which could explain at least in part the more aggressive and non-recoverable phenotype.

Conclusion: These findings suggest that obesity exacerbates MS disease severity and progression, emphasizing the importance of addressing obesity in MS management to potentially improve patient outcomes.

Conflicts of interest: The authors declare no competing interests. Funded by EXPL/MED-NEU/1033/2021 to AB from Fundação para a Ciência e Tecnologia, Portugal (FCT); and in part by UIDB/04138/2020 and UIDP/04138/2020 - from FCT to iMed.ULisboa.

POSTER 16

METHAMPHETAMINE ACTIVATES RAC1 IN STRIATAL MICROGLIA

Terceiro AF[1,2], Canedo T[1], Silva AI[1,2], Magalhães A[1,2], Relvas J[3,4], Summavielle T[1,5]

[1] Addiction Biology Group, i3S – Instituto de Investigação e Inovação em Saúde, Universidade o Porto; [2] Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto; [3] Glial Cell Biology, i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto; [4] Faculdade de Medicina da Universidade do Porto (FMUP); [5] Escola Superior de Saúde do Politécnico do Porto (ESS.PP)

Methamphetamine (Meth), a powerful psychostimulant, induces profound synaptic and morphological alterations alongside with detrimental neuroinflammatory responses, in the brain reward system. Yet, the mechanisms regulating these processes in microglial cells are not clear. We have previously shown that exposing WT mice to Meth (4x5 mg/kg, 2h intervals) induces microgliosis concomitant with decreased microglia cell volume and ramification1. Furthermore, psychostimulants are known to induce structural plasticity mechanisms in neurons, and Rho GTPases, important regulators of the actin cytoskeleton, are involved in these responses. Here, we evaluate if Rho GTPases, specifically rhoA, rac1 and cdc42, are critical in the response to Meth in microglia. Exposing WT mice to the same pattern of Meth administration, we found an increase in the activation of rac1 in the striatum, 15 min following the last administration of Meth. To further explore these results, we then used a conditional mice model for ablation of rac1 in adult microglia (Rac1fl/fl:Cx3cr1CreER+) and exposed these mice to the same pattern of Meth administration. Rac1 ablation was sufficient to prevent Meth-induced morphological alterations in the striatum. Currently, we are assessing whether ablation of rac1 is also sufficient to prevent the neuroinflammatory response induced by Meth. Overall, we identified rac1 as a novel target of Meth in microglial cells. With these results, we expect to clarify if targeting Rho GTPases may contribute to improving the treatment of addictive disorders.

POSTER 17

PRIMED MICROGLIA AFTER ACUTE NEUROINFLAMMATION MAY DRIVE AN ENHANCED RESPONSE

León-Rodríguez A[1,3], Grondona JM[1,3], Fernández-Arjona MM[3], Pedraza C[2,3], López-Ávalos MD[1,3]

[1] Universidad de Málaga, Departamento de Biología Celular, Genética y Fisiología (Área de Fisiología Animal), Málaga, Spain; [2] Universidad de Málaga, Departamento de Psicobiología y Metodología en las Ciencias del Comportamiento, Málaga, Spain; [3] Instituto de Investigación Biomédica de Málaga y Plataforma en Nanomedicina-IBIMA Plataforma Bionand, Málaga, Spain.

AIMS: Microglial cells become activated during acute neuroinflammation, and usually they return to their basal surveillant state in a few days. However, sometimes microglia evolve towards a primed state characterized by an exacerbated response to new stimuli, which may jeopardize brain functions. Here we aimed to explore microglial priming in the hypothalamus and its consequences on the neuroendocrine regulation of the stress response.

METHODS: To induce priming we used a model of acute ventricular neuroinflammation by intracerebroventricular (ICV) injection of the enzyme neuraminidase (NA). Three months later, an acute stressor (consisting in forced swimming) was applied to investigate the activation of the hypothalamic-pituitary-adrenal axis and the stress response elicited, as well as the inflammatory activation of hypothalamic microglial cells. Afterwards, blood samples were retrieved to determine corticosterone levels by ELISA, and the animal subjected to an open field test in order to study their locomotor and exploratory behavior. Lastly, the brains were extracted to analyze microglial cells in histological sections by immunohistochemistry with IBA1 and inflammatory markers by qPCR.

RESULTS: Stressed rats previously injected with NA had increased plasma levels of corticosterone compared to control rats that were equally stressed but had been ICV injected with saline. Also, qPCR studies revealed that NA-treated rats presented an increased expression of the microglial marker IBA1 and of the inflammasome protein NLRP3. Concomitantly, the morphological analysis of hypothalamic microglial cells showed a morphological bias towards a slightly activated state in microglia of NA injected rats compared to those of saline injected controls. Furthermore, in the open field test NA-treated rats displayed increased locomotor activity.

CONCLUSIONS: These results suggest that prior neuroinflammatory episodes might result in subtle but persistent changes in microglial cells that could determine the response to future challenges such as stressful events.

This work was carried out with funding from: FEDER/Ministerio de Ciencia, Innovación y Universidades – Agencia Estatal de Investigación/_Proyectos SAF2017-83645 y PID2020-117464RB-I00.

POSTER 18

PROMISE OF A PROTEIN CK-1Δ INHIBITOR FOR RESHAPING INFLAMMATORY MIRNAS IN THE TDP-43 (A315T) TRANSGENIC MICE

Matos AT[1], Almería MG [2,3,4], Martín Baquero R[2,3,4], Martínez A[4,6], Rodríguez-Cueto C[2,3,4], de Lago E[2,3,4], Vaz AR[1,5], Brites D[1,5]

[1] Neuroinflammation, Signaling and Neuroregeneration Lab, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; [2] Instituto de Investigación en Neuroquímica, Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad Complutense de Madrid, 28040 Madrid, Spain; [3] Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain; [4] Centro de Investigación Biomédica en Red Sobre Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, 28031 Madrid, Spain; [5] Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; [6] Centro de Investigaciones Biológicas Margarita Salas, CSIC, Calle Ramiro Maétzu 9, 28040 Madrid, Spain.

Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease with no effective therapy, compromising both motor neuron (MNs) and glial cells. ALS can be sporadic, the most common form, or familial involving at least 31 genes, including SOD1 and TARDBP encoding the RNA/DNA-binding protein TDP-43. Both models develop CNS inflammation, axonal pathology, and motor impairment. The main difference is the cognitive impairment that is characteristic of ALS associated to frontotemporal dementia (FTD) in the last model. So far, the therapy mostly used in ALS (and the only approved in Portugal) is the Riluzole by its antiglutamatergic properties, though only slightly increasing patient survival. Therefore, identification of novel therapeutic strategies is urgently needed. Our previous data in the SOD1G93A (mSOD1) mouse model identified the presence of upregulated inflammatory-miRNAs in the symptomatic stage of the disease, such as miRNA(miR)-124, miR-146a and miR-155 (doi: 10.1007/s12035-017-0631-2) and upregulated miR-124 in MNs has a key role in ALS pathology (doi: 10.3390/ijms22116128). Moreover, we showed that its modulation by anti-miRNA-124 engineering in cells/secretome prevented ALS disease progression in the mSOD1 mouse model (doi: 10.3390/biomedicines10092120). Only recently, some studies highlighted the association of dysregulated miRNAs in the TDP-43 pathology, where insoluble inclusions of phosphorylated TDP-43 have been identified. Interestingly, we have lately demonstrated that the protein CK-1δ kinase inhibitor treatment preserved MNs and decreased in vivo TDP-43 phosphorylation (doi: 10.1038/s41598-020-61265-y). Here, we propose to assess whether the inflammatory miRNAs are dysregulated in the lumbar section of the SC tissue from Prp-hTDP-43A315T (mTDP-43) mice vs. their WT counterparts, and if so, whether injection of IGS-2.7, Riluzole or IGS-2.7+Riluzole could rescue their profile. mTDP-43 animals were injected intraperitoneally starting at the age of 65 days old (early symptomatic stage), with IGS-2.7 (0.5 mg/Kg of body weight) and/or Riluzole (5 mg/kg of body weight) daily, until 95 days old (symptomatic stage), and were sacrificed 24 h later. Non-treated control animals received vehicle injections. Assessment of hit miRNAs included miR-124, miR-125b, miR-146a, miR-155 and miR-21. Our preliminary data identified alterations in miR-124, miR-146a and miR-21 in the mTDP-43 spinal cord samples. From the tested therapeutic strategies, the combined action of IGS-2.7+Riluzole showed to be the most promising in reshaping the miRNA profile in the mTDP-43 animals. In the future these data will be consolidated, and miRNAs specifically associated to TDP-43 activity will be additionally evaluated.

Funding: FCT-PTDC/MED-NEU/2382/2021 and LISBOA-01-0145-FEDER-031395 to DB, UIDB/04138/2020 and UIDP/04138/2020 to iMed.ULisboa, La Caixa Foundation-Luzón Foundation (HR21-00931) to DB, and PhD fellowship from FCT (2022.11128.BD) to ATM.

POSTER 19

INSIGHTS INTO THE ROLE OF A NOVEL AUTISM-ASSOCIATED GENE IN MICROGLIA DEVELOPMENT

Tavares AT[1], Seco P[1,2], Matiz L[1], Madureira T[1], Monteiro JF[2], Certal AC[2], Jacinto A[1]
[1] NOVA Medical School | Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisbon, Portugal;
[2] Champalimaud Foundation, Champalimaud Research, Lisbon, Portugal

Background: Microglia, the resident immune cells of the central nervous system (CNS), play essential functions during prenatal development by regulating neurogenesis, neuronal apoptosis and synaptic pruning. In the last decade, increasing evidence supports a key role for microglia in the pathogenesis of neurodevelopmental disorders such as autism spectrum disorders (ASD). During embryonic development, microglial cells arise from yolk-sac hematopoietic progenitors (or hemangioblasts) that colonize the CNS before the blood-brain barrier is established. However, little is known about the molecular regulators of microglia ontogeny that, when mutated, may contribute to the pathogenesis of neurodevelopmental disorders. In our previous work, the transcriptome analysis of chick hemangioblasts led to the identification of DIPK2B (Divergent Protein Kinase 2B; also known as DIA1R, Deleted in Autism 1 Related) as a novel X-linked gene highly expressed in hematopoietic progenitors that was recently associated with primary immunodeficiencies, X-linked intellectual disability (XLID) and ASD. Sequence and phylogenetic analyses revealed that DIPK2B genes are found exclusively in vertebrates, but their functions remain largely unknown.

Aims: The major goal of our research is to uncover the role of DIPK2B in microglia ontogeny and its impact on neurodevelopment and behaviour. Here we present our preliminary results on the expression pattern and potential function of dipk2b in microglia development using the zebrafish as a model.

Methods: Zebrafish dipk2b expression was analyzed at different developmental stages by whole mount RNA in situ hybridization. To investigate the potential role of dipk2b in zebrafish microglia development, we evaluated the number of microglia progenitors and differentiated cells in dipk2b morphant and mutant zebrafish larvae. dipk2b knockdown in morphant embryos was achieved by injecting one-cell-stage embryos with morpholino antisense oligonucleotides, whereas dipk2b loss-of-function mutants were generated by CRISPR/Cas9 genome editing.

Results: We have shown that zebrafish dipk2b is expressed in regions where the hematopoietic progenitors of microglia are located, such as the rostral blood island and the dorsal aorta. In addition, we observed that the number of microglia in the brains of DIA1R morphant and mutant zebrafish larvae is significantly lower when compared to the respective controls.

Conclusions: Our results suggest that dipk2b promotes the differentiation of hematopoietic progenitors into microglial cells. Since microglia dysfunction can contribute to neurodevelopmental disorders, we hypothesize that the role of DIPK2B in microglia ontogeny may underlie its implication in XLID and ASD.

Funding Support: This work was supported by iNOVA4Health (UIDB/04462/2020; UIDP/04462/2020) and R&D project grants (PTDC/BTM-SAL/29377/2017; 2022.02611.PTDC) from Fundação para a Ciência e Tecnologia.

POSTER 20

DEVELOPMENT OF A HUMAN TRICULTURE MODEL OF NEURON-MICROGLIA-ASTROCYTE CROSS-TALK TO INVESTIGATE AD AND TEST ENGINEERED MIR-124-3P EXOSOMES

Évora A[1], Rubi A[1], De Vitis E[2], Podobnik E[1], Matos AT[1], Vaz AR[1,3], Gervaso F[2], Polini A[2], Garcia G[1], Brites D[1,3]

[1] Neuroninflammation Signaling and Neuroregeneration, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; [2] CNR NANOTEC—Institute of Nanotechnology, c/o Campus Ecotekne, Via Monteroni, Lecce, Italy; [3]Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder, still without effective therapy. Accumulation of amyloid-beta peptide (Aβ), and hyperphosphorylated tau are among the hallmarks of AD. Lately, the dysregulation of miRNAs was associated with AD pathology. Our data showed that miR-124 regulation was key for neuron-glia homeostasis in AD (doi: 10.3390/cells10092424; doi: 10.3389/fphar.2022.833066). miR-124 dissemination mainly occurs by its inclusion in exosomes (30-200 nm). Regulation of miR-124 showed to be key in sustaining brain homeostatic balance.

Thus, we first aimed to produce the most effective miR-124 enriched exosomes to be delivered as a therapeutic strategy. For that, we compared the efficacy of transfecting pre-miR-124-3p into SH-SY5Y cells (CF124) and collecting their exosomes with that by direct transfection in exosomes (EF124) by Exo-Fect® and using microglia as the recipient cell. Our second goal was to develop a microglia-neuron-astrocyte triculture in a microfluidic device able to recapitulate AD features and test the regenerative ability of the engineered exosomes. Primary cortical microglia were prepared from 2-day-old wild-type mouse pups. Exosomes were isolated by differential ultracentrifugation and their number and size were obtained by Nanoparticle tracking analysis. Exosomes were labeled with the PKH67 dye to track their internalization by microglia using confocal microscopy. Gene/miRNA expression was performed by RT-qPCR. Tricultures were established in microfabricated multi-compartment devices (doi: 10.1038/s41598-021-86300-4) with neurons (SH-SY5Y or SH-SY5Y Swedish cells), astrocytes (P10251-IM) and microglia (CHME3). Tricultures were incubated overnight with fluorescent Hylite-Aβ1-42, washed, and labeled with S100B, β-III-tubulin, and P2RY12, and visualized by confocal microscopy.

CF124 and EF124 exosomes were captured and upregulated microglial miR-124-3p, though EF124 was 20-fold more efficient. Only EF124 decreased IBA1 immunofluorescence, while upregulated TREM2 and arginase-1 gene expression levels. Interestingly, EF124 did not increase iNOS gene expression, in contrast with CF124. The best cell ratio in tricultures was 50% neurons, 30% astrocytes, and 20% microglia. Data showed immunostaining specificity of β-III tubulin (neuronal), S100b (astrocyte-enriched), and P2RY12 (microglial). Hylite-Aβ1-42 addition led to its internalization mostly by microglia and neurons (no differences were noticed between SH-SY5Y and SH-SY5Y Swedish cells).

Our study identified direct exosomal transfection with pre-miR-124 as being more efficient to target microglia and optimized a triculture model to efficiently test miR-engineered exosomes in reshaping neuro-glia dynamics in AD.

POSTER 21

ANALYSIS OF DOPAMINERGIC DYSFUNCTIONS, SOCIAL DEFICITS AND TEMPORAL PATTERN OF MICROGLIA DIFFERENTIATION IN A MODEL OF SCHIZOPHRENIA

Ana C. Rodrigues-Neves[1,2,3,4], Bárbara Coimbra[5], Rita Gaspar[1,2,3], Verónica Domingues[5], Carina Soares-Cunha[5], Patrícia Patrício[5], Luísa Pinto[5], João Bessa[5], Ana J Rodrigues[5], António F Ambrósio[1,2,3], Catarina A. Gomes[1,2,3,4]

[1] University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, Coimbra, Portugal; [2] University of Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal; [3] Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal; [4] Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal; [5]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal

Dopaminergic transmission alterations are a hallmark of schizophrenia. Mesocortical hypofunction (targeting prefrontal cortex - PFC), associated to negative symptoms (e.g. social withdrawal), has been explained by abnormal synaptic pruning during neurodevelopment. Synapse pruning is physiologic, operated by microglia, from early development and particularly active during adolescence, when schizophrenia onset is common. Interactions environment-genome seem to dictate schizophrenia emergence and models of prenatal exposure to xenobiotics (e.g. methylazoxymethanol acetate, MAM) have been used to identify pharmacologic targets. We used the MAM model to confirm if a hypodopaminergic tonus of the mesocortical pathway coexists with social deficits in adulthood and, as a main purpose, if a defect in the morphological differentiation of microglia in the PFC between birth and adolescence, could be identified as a hallmark and potential target in disease modeling, accompanied by behavioral changes. Males exposed to MAM (maternal injection during pregnancy) present social deficits and decreased PFC activity. In line with our hypothesis, postnatally, a negative impact in neurodevelopmental milestones related with emotionality was observed (e.g. maternal odor recognition), persisting until adolescence (social deficits). We also observed postnatal alterations in microglia morphology, that are transient and absent in adolescence.

There is a gap in the identification of putative causes for the hyperfunction of mesolimbic pathways linked to positive symptoms (e.g. hallucinations). Interestingly, we previously observed a different pattern of temporal morphological differentiation of microglia in these pathways. Our results strongly suggest that microglia morphology may be a readout of dopaminergic dysfunction, a topic deserving further investigation.

FCT (UID/NEU/04539/2019, UIDB/04539/2020, UIDP/04539/2020, EXPL/MED-NEU/1002/2021 and individual PhD fellowship SFRH/BD/141754/2018); COMPETEFEDER (POCI-01-0145-FEDER-007440); Centro 2020 Regional Operational Programme (CENTRO-01-0145-FEDER-000008: BrainHealth 2020); and Faculty of Medicine, Coimbra University and Santander Totta Bank (FMUC-BST-2019).

POSTER 22

UNRAVEL THE PUTATIVE ROLE OF GLIAL CELLS IN AUTOSOMAL RECESSIVE SPASTIC ATAXIA OF CHARLEVOIX-SAGUENAY (ARSACS)

Farsetti E[1,2], Spirito E[3], Maltecca F[3], Fernandes A[1,4]

[1] Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal; [2] Department of Biology and Biotechnology ""Lazzaro Spallanzani"", University of Pavia, 27100 Pavia, Italy; [3] Mitochondrial Dysfunctions in Neurodegeneration Unit, Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy; [4] Department of Pharmaceutical Sciences and Medicines, Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a distinctive form of hereditary early-onset neurodegenerative disease, now considered one of the most common recessive ataxias worldwide. ARSACS is clinically characterized by loss of Purkinje cells in the cerebellum (progressive cerebellar ataxia), peripheral neuropathy and spasticity. The disease is due to mutation of the SACS gene with more than 200 pathogenic mutations reported. The SACS gene encodes a protein called Sacsin, which is expressed in several different tissues, namely in the CNS and skin. In the brain, Sacsin is highly expressed in the motor system, including the cerebellum, especially in Purkinje cells, but a recent study also found high levels of this protein in astrocytes and microglia. Sacsin seems to be involved in the regulation of Intermediate Filaments - Neurofilaments assembly and disassembly, chaperon activity, autophagosomes and lysosomes activity and also it seems to be important for mitochondrial activity and localization. Although it is known that glia cells play a key role in developmental and neurodegenerative disorders, in ARSACS studies no reference has been made to glial cells and their potential inflammatory role in neurodegeneration or demyelination, both occurring in ARSACS patients. So, here we aim to describe the phenotype and function of glial cells in the SACS Knock Out (KO) mice brain. We evaluated the brains of Wild Type (WT) and SACS KO mice at 8 months, focusing on the areas of the cortex, hippocampus and cerebellum. To study Neuroinflammation and Gliosis, we first analysed the transcript levels of different markers for glial cells and mediators of inflammation using RealTime-qPCR. Preliminary results show a particular increase in the transcript levels of GFAP (astrocytes), MBP (oligodendrocytes), CD11b (microglia) and PDGFα (oligodendrocyte progenitor) in the cortex area of the SACS KO mice compared to the WT ones, indicative of glial cell proliferation/reactivity. Also, high transcript levels of CSFR1 and P2RY12, a sign of microgliosis, were found in the cortex and cerebellum of the KO mice. Furthermore, the transcript levels of IL-17, TNFα and IFNγ were higher in the KO animals when compared to WT mice, indicating a possible immune cell infiltration, particularly in the area of the cortex and cerebellum. In ongoing experiments, we are confirming these alterations by immunohistochemistry and analysis of protein content by Western Blot. Overall, our results firstly demonstrate that animals mimicking ARSACS pathology have alterations in glial cell markers in distinct brain regions that may account for the phenotype of this disease. Further studies are needed to identify new potential targets for therapeutic intervention for this debilitating disorder.Funded by ARSACS grant 2022 to AF, ERASMUS+ grant to EF; in part by UIDB/04138/2020 and UIDP/04138/2020—from Fundação para a Ciência e Tecnologia, Portugal (FCT) to iMed.ULisboa.

POSTER 23

HOW ISOFLURANE INTERACT WITH MICROGLIA TO PROTECT BRAIN PARENCHYMA FROM NEUROINFLAMMATION

Dhaya I[1], Konsman JP[2]

[1] University of Tunis El Manar, Tunisia; [2] University of Bordeaux, CNRS UMR 5164- ImmunoConcEpT, France

We have recently demonstrated that general isoflurane-induced anesthesia of rats for MRI examination affects the morphology of non-neuronal cells in the brain. Isoflurane, an inhalated general anesthetic agent, has been widely used in human surgery and animal experimentation. It is now evident, from clinical and experimental approaches, that isoflurane can interact with immunocompetent cells, which for the nervous system include brain-resident (microglia) and brain border-associated macrophages (BAMs). Such effects may have consequences for central nervous system (CNS) pathologies in which neuroinflammation has been proposed to play a role. The binding sites of isoflurane have not been extensively determined, but its interactions with ion channels, such as those associated with gamma-aminobutyric acid (GABA) receptors, have been studied most.

The aim of this work was therefore to identify potential binding sites contributing to the effects of isoflurane on microglia. Based on molecular docking and biodistribution analyses of isoflurane, we propose a microglia membrane-mediated mechanism, consisting to an action on Toll-like receptors (TLR) which, in turn, could interfere with Piezo in order to mitigate the effects of neuroinflammation in the cerebral parenchyma.

POSTER 24

DEVELOPMENT OF A HIGH-THROUGHPUT PIPELINE TO CHARACTERIZE MICROGLIA MORPHOLOGICAL STATES AT A SINGLE-CELL RESOLUTION

Jennifer Kim[1], Dr. Paul Pavlidis[1,3], Dr. Annie Ciernia[1,2]

[1] Graduate Program in Neuroscience, University of British Columbia, Vancouver, British Columbia, Canada; [2] Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada; [3] Department of Psychiatry, University of British Columbia, Vancouver, British Columbia, Canada

As rapid responders to their environments, microglia engage in functions that are mirrored in morphology, where microglia are classically thought to exhibit a ramified morphology in homeostatic conditions which switches to an ameboid form in immune-active conditions. However, microglia display a wide spectrum of morphologies outside of this dichotomy, including rod-like, hyper-ramified, and dystrophic states, which have been observed across brain regions, neurodevelopmental timepoints, and various pathological contexts. We developed a dimensionality reduction and clustering approach that considers contributions of multiple morphology measures together to define a spectrum of various microglial morphological states. Using ImageJ tools, we first developed an automated approach to characterize 27 morphology features from hundreds to thousands of individual microglial cells in a brain subregion-specific manner. Within this pool of morphology measures, we defined distinct sets of highly correlated features that describe different aspects of morphology, including branch length, branching complexity, territory span, and cell circularity. When considered together, these sets of features drove different morphological state-relevant clusters. Furthermore, our analysis toolset was able to characterize morphological states similarly and robustly when applied to independent datasets collected in different labs, from different species, and using different immunofluorescent markers for microglia. We have compiled the morphology pipeline into an accessible and open-source ImageJ plugin and R package that the neuroscience community will hopefully find use for in their own analyses. Outcomes from this work will supply the field with new tools to systematically evaluate the heterogeneity of microglia morphological states across various experimental models and research questions.

POSTER 25

MICROGLIA-NEURON CROSSTALK IN CHRONIC METHAMPHETAMINE EXPOSURE

Rodrigues, João[1,2,9]*; Bravo, Joana[1,2,3,4]*; Andrade, B. Elva[2,3,4,5], Vieira; Ricardo[6]; Rodrigues, João[1,2], Canedo, Teresa[1,2]; Lorga, Inês[2,3,5]; Azevedo, Maria[7]; Magalhães, Ana[1,2,3], Relvas, João[8,9], Summavielle, Teresa[1,2,4]

[1] Addiction Biology, i3S-Instituto de Investigação e Inovação em Saúde, Universidade do Porto (UP); [2] IBMC - Instituto de Biologia Molecular e Celular, UP; [3] ICBAS - Instituto de Ciências Biomédicas de Abel Salazar, UP; [4] Escola Superior de Saúde, Politécnico do Porto; [5] Immunobiology, i3SInstituto de Investigação e Inovação em Saúde, Universidade do Porto (UP); [6] European Center of Angiosciences, University of Heidelberg; [7] Advanced Light Microscopy Scientific Platform, i3S-Instituto de Investigação e Inovação em Saúde, Universidade do Porto (UP); [8] Glial Cell Biology, i3S-Instituto de Investigação e Inovação em Saúde, Universidade do Porto (UP); [9] FMUP - Faculdade de Medicina da Universidade do Porto.
*Equal contribuition

Exposure to methamphetamine (Meth), a highly addictive widely used psychostimulant, is classically associated with damage to neuronal terminals, but its neurotoxicity can also be mediated via activation of the neuroinflammatory response. We have recently shown that acute methamphetamine (Meth) causes microgliosis and increases microglia activation through astrocytic-TNF release, and we are now focused on the effects of chronic Meth exposure. To explore this, we performed a proteomic analysis in the hippocampus, in different phases of the addictive process. We found a proteome profile that varied substantially with exposure to Meth for ten days (Chronic).and after a short- (2d WD) and long-term withdrawal (10d WD) periods. We identified significant differences in Wnt signaling, which was linked to regulation of microglia reactivity. As such, we evaluated the microglia profile and we found an increase in microglia number at Chronic and at 2d WD. Microglia presented a more ameboid-like shape at 10d Meth, but its ramified morphology was recovered at 2d WD. Importantly, during Meth withdrawal, several microglial receptors were downregulated, suggesting that microglia was in a "primed" state. Interestingly, we found that the signaling of CD47-SIRPα is disrupted after chronic Meth administration and during withdrawal and this may be related with microglia homeostasis dysregulation. CD47-SIRPα signaling is an important "don't eat me signal" that has been shown to protect synapses from excessive microglia-mediated pruning. Furthermore, in cancer cells CD47 expression is modulated by IFN-γ. Consistently, after chronic Meth, we observed synaptic dysregulation, accompanied by a significant decrease of meningeal T cells and a reduction in their production of IFN- γ. Currently, we are aiming to clarify if IFN-γ is regulating CD47 in the brain, after chronic Meth administration, and consequently regulating synaptic pruning.

POSTER 26

THE USE OF PBMCS-DERIVED MICROGLIA TO STUDY DEMYELINATION-ASSOCIATED MECHANISMS IN MULTIPLE SCLEROSIS

Pinto MV[1]*, Barros C[1]*, Costa I[1], Santos FMF[1], Ravasco J[1], Gois PMP[1,2] Fernandes A[1,2]

[1] Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal; [2] Department of Pharmaceutical Sciences and Medicines, Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal

*Equal contribution

Microglia are the resident macrophages of the central nervous system. Under pathological situations like in Multiple Sclerosis (MS), microglia have been shown to impact on a variety of processes associated with MS progression and recovery. In our Lab, using a new boronic acid-based fluorescent probe (BASHY) to stain myelin debris (MD), we recently showed the colocalization of microglia with MD in demyelinating lesions of the MS in vivo model, the Experimental Autoimmune Encephalomyelitis. These results go in line with previous data evidencing the presence of highly phagocytic microglia, in active MS lesions, playing the critical role of clearing the accumulated MD to promote effective recovery. However, it is also described that upon MD intracellular processing, myelin lipids can induce critical physiological changes in microglia towards a more pro-inflammatory signature, that may contribute to propagation of a maladaptive inflammatory response and further demyelination. Hence, novel human-derived models are fundamental to better understand the microglial role in MS disease course.

Here, we aimed to implement and characterize microglia derived from human peripheral blood mononuclear cells (PBMCs) to further expose them to external stimuli mimicking demyelination. We first combined different published protocols to improve our method of isolation and differentiation of human PBMCs from healthy donors. For 14 days, adherent cells were cultured in differentiating media containing IL-34 (50ug/ml) and GM-CSF (20ug/ml) and microglial differentiation was followed-up by cell imaging using EVOS microscope. At the end of differentiation, microglia were incubated with MD previously labeled with BASHY (MD-BASHY, 10mg/mL), for 1h. Then, cells were characterized, and the different microglial phenotypes over myelin internalization were evaluated by immunohistochemistry. We observed that 3 days post-differentiation PBMCs-derived cells still have a monocyte-resembling shape. At the fifth day in vitro, cells changed from an elongated form to a more microglial-like and ramified morphology. Additionally, microglia were positive for both specific markers, P2Ry12 and TMEM 119, confirming a successful differentiation. After myelin incubation, MD-BASHY were observed inside microglial cells presenting a less ramified conformation, confirming an effective clearing process and efficient morphologic alterations into a more phagocytic-associated shape. Ongoing studies aim to further characterize our PBMCs-derived microglia and their responsiveness to MD stimuli by flow cytometry and RT-qPCR.

Overall, with this model, we intent to accurately understand how myelin-rich microglia might be fundamental for proper remyelination and possibly use it to assay different therapeutic approaches for MS.

POSTER 27

INTRATHECAL INJECTION OF mir-124-ENGINEERED SECRETOME FROM ALS MOTOR NEURONS MAY DIFFERENTLY IMPACT ON SEPARATE BRAIN REGIONS

Corraliza-Gomez M[1,2,3], Barbosa M[1], Ianni M[1,4], de Sousa N[5,6], Vaz AR[1,7], Salgado AJ[5,6], Brites D[1,7]
[1] Neuroinflammation, Signaling and Neuroregeneration Lab, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; [2] Division of Physiology, School of Medicine, Universidad de Cadiz, Cadiz, Spain.; [3] Instituto de Investigacion e Innovacion en Ciencias Biomedicas de la Provincia de Cadiz (INiBICA), Cadiz, Spain; [4] Università degli Studi di Trieste, Trieste, Italy; [5] School of Medicine, Life and Health Sciences Research Institute (ICVS), University of Minho, Portugal; [6] CVS/3B's Associate Lab, PT Government Associated Lab, Guimarães, Portugal; [7] Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Amyotrophic lateral sclerosis (ALS) is a rare, progressive, and fatal neurodegenerative disorder resulting from the degeneration of the motor neurons (MNs) and activation of glial cells. The disease affects the motor cortex, the brainstem and the spinal cord (SC) and typically occurs in one of two main ways: limb onset ALS or bulbar onset ALS. We have previously shown that overexpression of miR-124 has a key role in ALS pathology (doi: 10.3390/ijms22116128) and that it circulates as part of the exosome cargo (doi: 10.3389/fnins.2017.00273). Intrathecal injection of the secretome from ALS MNs regulated for miR-124 showed to prevent motor impairment and muscle atrophy, and to halt astrocyte, microglia, and oligodendrocyte reactivity/dysfunction in the symptomatic SOD1G93A (mSOD1) mice (doi: 10.3390/biomedicines10092120). Data indicated that such preconditioned secretome may be a promising therapeutic for the limb onset ALS patients. Lately, some studies highlighted that SC injury can modify the brain structure and alter the brain function. Therefore, we wondered whether the intrathecal administration of the secretome from anti-miR-124-treated mSOD1 MNs can reconfigure the brain expression of genes and miRNAs associated to brain inflammation and neurodegeneration. The preconditioned secretome (concentrated 100x) was injected (2 µg/1 µL/g of animal weight; mouse weight 30-35 g) between L4 and L5 in WT and mSOD1 mice at the early symptomatic stage of the disease (12-week-old animals). The animals were sacrificed at 15-week-old and the brain and the hippocampus used for the evaluation of miRNAs and genes associate to neuro-immune imbalance. First data indicate that miR-124 ($p<0.05$) and miR-155 (n.s.) are increased in the brain of the mSOD1 mice. Injected preconditioned secretome only caused a slight decrease of miR-124 and miR-155 in the brain. No changes were observed for the miR-124 in the hippocampus of the transgenic mice, but a mild (n.s.) decrease was observed after the treatment with the secretome, while it was significant for miR-155 ($p<0.05$). Other modifications included a decrease of the arginase-1 in the mSOD1 mice that was prevented by the preconditioned secretome. Interestingly, the decrease of the NeuN in the ALS mice appears to be counteracted by the preconditioned secretome, thus representing a promising beneficial effect at far distance of the administration. No changes were observed for MBP or GFAP in the cortex or the hippocampus. We are now specifically evaluating changes in the brain cortex and assessing miR-146a that we previously found to be decreased in this region. With additional data we anticipate to demonstrate that the intrathecal injection holds promise to regulate miR-124 and to recover homeostatic balance in the CNS of ALS patients.

Funding: FCT-PTDC/MED-NEU/2382/2021 and LISBOA-01-0145-FEDER-031395 to DB, UIDB/04138/2020 and UIDP/04138/2020 to iMed.ULisboa and La Caixa Foundation-Luzón Foundation (HR21-00931) to DB.

POSTER 28

IMMUNOCOMPETENT BRAIN ORGANOIDS AND INDUCED-MICROGLIA-LIKE CELLS TO STUDY THE PATHOLOGICAL MECHANISMS OF FRONTOTEMPORAL DEMENTIA

Solange Martins[1,2,4], Mariana Martins[1,2,5], Mafalda Neves[2,5], Ana Maria Cardoso[1,2,3] ,Maria Rosário Almeida[1,2], Isabel Santana[2,6,7], João Peça[1,2,8] and Ana Luísa Cardoso[1,2,3]

[1] CNC—Center for Neuroscience and Cell Biology, University of Coimbra, 3004-504 Coimbra, Portugal; [2] Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, 3004-504 Coimbra, Portugal; [3] Institute for Interdisciplinary Research (IIIUC), University of Coimbra, 3030-789 Coimbra, Portugal; [4] Doctoral Programme in Experimental Biology and Biomedicine (PDBEB), CNC—Center for Neuroscience and Cell Biology, University of Coimbra, 3004-504 Coimbra, Portugal; [5] Faculty of Pharmacy, University of Coimbra, 3000-548 Coimbra, Portugal; [6] Neurology Department, Centro Hospitalar e Universitário de Coimbra, 3004-531 Coimbra, Portugal; [7] Faculty of Medicine, University of Coimbra, 3004-504 Coimbra, Portugal; [8] Department of Life Sciences, Faculty of Science and Technology, University of Coimbra, 3000-456 Coimbra, Portugal

In the last two decades, heterozygous loss-of-function progranulin mutations have been associated with the development of frontotemporal dementia (FTD), a devastating neurodegenerative disorder characterized by distinct pathological signatures. Several different molecular hallmarks have already been reported, but a clear relation between them is still to be unravelled. Such hallmarks include lysosomal dysfunction, increased neuroinflammation, the presence of TDP-43 aggregates in the cytoplasm, as well as selective pruning of thalamic inhibitory synapses. In this work, we generate both brain organoids, macrophage precursors and induced microglia-like cells from iPSCs lines obtained from fibroblasts isolated from skin biopsies derived from progranulin mutation carrying FTD-patients. Macrophage precursors (preMacs) can be generated by first aggregating iPSCs into embryoid bodies (EBs). After 4 days of induction using VEGF, SCF and BMP4 to induce mesodermal fate, EBs are plated in T75 flasks, where they will be induced to form yolk-sac-like structures, from where macrophage precursors will arise and be released into the medium. Here, we show that after 4 weeks in culture, a pure population of macrophage precursors can be obtained. We also show that before that point, more than one population is present and the cells cannot yet be used for further purposes. This is evidenced by the expression of mature myeloid markers by 100% of cells at 4 weeks but only a few cells expressing them prior to that. At this stage, preMacs are ready to be used either in 2D cultures, where they will be further differentiated into induced-microglia-like cells (iMGs) or for organoid incubation. We demonstrated that iMGs can be obtained after 14 days in culture with IL-34 and GM-CSF supplementation, as evidenced by the expression of microglia-related genes. iMGs were demonstrated to express IBA-1, TMEM119, P2RY12, as well as the specific-lysosomal marker CD68, by immunocytochemistry. Furthermore, these cells are able to perform engulfment and respond to LPS stimuli by altering their morphology and transcriptome. In addition, we show that, after 1 month inside organoids, preMacs differentiate into induced microglia-like cells. We also report that these cells become more ramified with time of incubation. In fact, wild type iMGs inside brain organoids heterozygotic for PGRN become ramified more rapidly and show an inferior LAMP-1 content, suggesting lysosomal impairment. In summary, in this work we dissect cellular and molecular differences between wild-type controls and heterozygotic mutation carrying lines, both in induced in 2D cultures of iMGs and in immunocompetent brain organoids.

POSTER 29

MICROGLIAL RESPONSE TO EXTERNAL STIMULI IS MODULATED BY RHO GTPASE RAC1

Almeida TO[1,2], Socodato R[1], Portugal CC[1], Santos ECS[1,3], Tedim-Moreira J[1,3], Canedo T[1], Magalhães A[1], Summavielle T[1,4], Relvas JB[1,3]

[1] Institute of Research and Innovation in Health (i3s) and Institute for Molecular and Cell Biology (IBMC), University of Porto, Portugal; [2] ICBAS – School of Medicine and Biomedical Sciences, University of Porto, Portugal; [3] Faculty of Medicine, University of Porto, Portugal; [4] ESS.PP, Escola Superior de Saúde, Instituto Politécnico do Porto, Portugal

Within the brain, microglia are the primary active immune cells by excellence. By extending and retracting their several processes, these cells are constantly surveilling their surrounding environment. When triggered, microglia dramatically change their morphology and their transcriptomic profile. Microglial response is also characterized by reactive oxygen species production and NF-κB pathway activation. Prolonged or exacerbated responses can thus be detrimental to the brain.

Rho GTPases are critical regulators of the actin cytoskeleton. Rac1 is the most well-known and studied element of this family and is classically associated with lamellipodia formation. Besides, Rac1 is part of the NADPH oxidase complex, it is a key protein for phagocytic cup formation, and it coordinates Nrf2 and NF-κB pathways in other immune cells. Since Rac1 studies in microglia are almost inexistent, we hypothesized that Rac1 could be essential for microglia homeostasis and for its response capacity.

Thus, combining microglia-specific conditional gene ablation, flow cytometry, RNA sequencing, phosphoproteomics and Förster resonance energy transfer (FRET) live cell imaging we aimed at characterizing the roles of Rac1 for microglia in homeostasis and in a paradigm of neuroinflammation.

RNAseq analysis showed that Rac1 ablation in microglia did not impact microglia's homeostatic engulfment capacity nor its oxidative state. RNAseq and phosphoproteomics unveiled a subset of altered pathways associated with immune signaling and Rho GTPase signaling. To functionally validate these observations, we exposed microglia in vitro to three different stimuli: lipopolysaccharide (LPS), phosphatidylcholine, and ATP. We observed that there was an attenuation or absence of response to these stimuli when Rac1 was knocked down. To mimic a neuroinflammatory paradigm, we injected mice with LPS intraperitoneally. Rac1 ablation prevented microgliosis and blocked the increase in CD68 expression induced by LPS.

Overall, our data show that Rac1 ablation lessens microglia's capacity to respond to external stimuli. These findings place Rac1 as an essential protein for microglia to respond to inflammatory cues and to control their inflammatory response.

POSTER 30

GO THE EXTRA MILE: IN VITRO AND IN VIVO MODULATION OF ADULT OLIGODENDROGENESIS BY BDNF

Mateus JM[1,2], Moreira JB[1,2], Lourenço DM[1,2], Sebastião AM[1,2], Dawson N[3], Xapelli S[1,2]

[1] Instituto de Farmacologia e Neurociências, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal; [2] Instituto de Medicina Molecular João Lobo Antunes (iMM), Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal; [3] Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster, UK

In multiple sclerosis (MS), the myelin sheath that insulates the nerve fibers, enabling an efficient conduction of nervous impulses, is attacked and lost, as well are the oligodendrocytes (OLs), the cells responsible for its production in the central nervous system. Although in patients with MS, and in mouse models of the disease, parenchymal oligodendrocyte precursor cells (OPCs) and subventricular zone-derived neural stem cells (SVZ-NSCs) can differentiate into OLs and partly repopulate the lesioned regions, remyelination from both cell types can be limited. Our group has previously shown that adenosine A2A receptors (A2ARs) can enhance hippocampal-derived neurogenesis, and that this effect is mediated by brain-derived neurotrophic factor (BDNF). However, their role on adult oligodendrogenesis from SVZ-NSCs remains to be described. Hence, we aimed at studying how these modulators and the putative crosstalk between them can influence OL differentiation from postnatal SVZ-NSCs. Results obtained using SVZ-NSCs cultures show that treatment with BDNF tends to increase OPC formation (NG2/PDGFRα-positive cells) after 4 days in vitro (DIV) (n=3; CTRL set to 100%, BDNF 203.8±27.59; $p=0.0548$), whilst significantly increasing the number of OPCs at DIV7 (n=7-8; CTRL set to 100%, BDNF 210.2±21.87; $p<0.0001$) without affecting OL maturation (MBP-positive cells). Importantly, BDNF effects on OPC formation at DIV7 were partially abrogated by the A2AR antagonist (n=4-8; CTRL set to 100%, BDNF+ZM241385 174.0±8.951; $p<0.01$), while the antagonist by itself had no effect when comparing with control (ZM241385 117.6±15.47; $p>0.05$). No changes were observed after treatment with the A2AR agonist at these timepoints in both OPC formation and OL maturation. This work outlined the role of BDNF in promoting the formation of OPCs from SVZ-NSCs. Then we explored how physical exercise (PE), which is known to upregulate the expression of neurotrophic factors, could potentiate adult oligodendrogenesis in vivo using the cuprizone (CPZ) mouse model of demyelination. For this, CPZ-fed mice were subjected to a PE protocol and tested for cognitive and motor performance. Cellular and molecular analysis to assess the changes in OL population and shifts in brain connectivity are currently being evaluated. With this work we expect to identify PE as a potential inducer of adult oligodendrogenesis and remyelination, restoring brain connectivity and cognition in MS, through neurotrophic factor signaling.

Authors declare no conflicts of interests. Authors received funding from European Union's Horizon 2020 research and innovation programme under the grant agreement No. 952455, ISN (career development award), IBRO (early career award), the fellowships PD/BD/150343/2019 (JMM), DFA/BD/4492/2020 (JBM) PD/BD/141784/2018 (DML) from Fundação para Ciência e Tecnologia, the EMBO Scientific Exchange Grant - PNG 10018 (JMM) and the GAPIC projects 20210009 and 20220011.

POSTER 31

POLYOXOMETALATES IMPACT IN ALZHEIMER DISEASE

Mateus J[1,2] and Aureliano, M[1,2]

[1] FCT, University of Algarve, Faro, Portugal; [2] CCMar, University of Algarve, Faro, Portugal

Polyoxometalates (POMs) are clusters of units of oxoanions of transition metals such as Mo, W, V and Nb that like transformers showed a broad diversity of structures. On these structures it can be included others elements or even one of the unites could be lack and/or substituted by other metals such as Co, Ni or As thus exhibiting fantastic physical, chemical and biological properties. Thanks to these properties, these inorganic clusters have been studied in environmental, chemical and industrial fields, having applications in catalysis, macromolecular crystallography, as well as in biomedicine such as cancer, bacterial and viral infections, and neurological diseases such as Alzheimer's disease (AD) [1-4]. AD is a neurodegenerative disease characterized by loss of memory. Although no single cause of Alzheimer's has been discovered, there is evidence that metals and oxidative stress can play a role in disease progression. The disease is associated with the accumulation of β-amyloid plaques in the brain that have the capability of interacting with redox-active metals, such as copper, zinc and iron. Although POMs's studies with Alzheimer effects are increasing, their mechanisms of action are still distant to be clarified. There are only a limited number of studies characterizing POMs effects on the development of Alzheimer's disease or its progression. Herein, we described recent studies of POMs in AD. Some studies found that POMs was able to ameliorate AD symptoms through a number of mechanisms including inhibition of Aβ aggregate formation. These results should encourage studies on the use of POMs in the treatment of neurodegenerative diseases such as AD.

This study received Portuguese national funds from Foundation for Science and Technology (FCT) through projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020 (M.A.).

1. Amante, C.; De Sousa-Coelho, A.L.; Aureliano, M. Vanadium and Melanoma: A Systematic Review. Metals 2021, 11, 828. https://doi.org/10.3390/met11050828.

2. De Sousa-Coelho, A.L.; Aureliano, M.; Fraqueza, G.; Serrão, G.; Gonçalves, J.; Sánchez-Lombardo, I.; Link, W.; Ferreira, B.I. Decavanadate and metformin-decavanadate effects in human melanoma cells. J. Inorg. Biochem. 2022, 235, 111915. https://doi.org/10.1016/J.JINORGBIO.2022.111915.

3. Aureliano, M.; Gumerova, N.I.; Sciortino, G.; Garribba, E.; Rompel, A.; Crans, D.C. Polyoxovanadates with emerging biomedical activities. Coord. Chem. Rev. 2021, 447, 214143. https://doi.org/10.1016/j.ccr.2021.214143.

4. Aureliano, M.; Gumerova, N.I.; Sciortino, G.; Garribba, E.; McLauchlan, C.C.; Rompel, A.; Crans, D.C. Polyoxidovanadates' interactions with proteins: An overview. Coord. Chem. Rev. 2022, 454, 214344. https://doi.org/10.1016/j.ccr.2021.214344.

POSTER 32

NEW MODEL OF BRAIN DISORDERS IN ACOMYS CAHIRINUS: FROM BRAIN MAPPING TO ACCURATE STEREOTATIC COORDINATES

Marta Vitorino[1,2,3], Sónia Simão[1,3,4], João B. Moreira[1,3], Joana Rodrigues[1,3], Joana Nogueira-Rodrigues[4,5], Joana Silva[1], Ana Sofia Lourenço[1], Vítor Fernandes[1,3], Monica M. Sousa[4], Gustavo Tiscornia[2,6] and Inês M. Araújo[1,3,4,7]

[1] Faculty of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal [2] Center for Marine Sciences (CCMAR), University of Algarve, Faro, Portugal; [3] Algarve Biomedical Center Research Institute (ABC-RI), University of Algarve, Faro, Portugal; [4] Nerve Regeneration Group, Instituto de Biologia Molecular e Celular (IBMC), Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Porto, Portugal; [5] Graduate Program in Molecular and Cell Biology, Instituto de Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, Porto, Portugal; [6] Clínica Eugin, Barcelona, Spain; [7] Champalimaud Research Program, Lisbon, Portugal

The African spiny mouse (Acomys cahirinus) is an emerging model of mammalian epimorphic regeneration that has aroused the interest of the scientific community in the last decade. This animal is able to regenerate several organs and tissues such as skin, ear, muscle and most impressive spinal cord. To date, studies on brain regeneration have been hindered by the lack of knowledge on the neuroanatomy of this species. Here, we present a coronal brain atlas in stereotaxic coordinates, which allows for three-dimensional identification and localization of the brain structures of this species.

The brain of 12-week-old spiny mice was mapped in stereotaxic coordinates using cresyl violet-stained brain sections obtained from coronal cryosectioning of the brain after transcardial perfusion with fixative. The atlas is presented in 42 plates representing sections spaced 240 μm apart.

The Atlas stereotaxic coordinates were validated using a model of Parkinsonian lesion of the striatum with 6-hydroxydopamine, labeling of the corticospinal tract in the spiny mouse spinal cord using AAV1/2-GFP intracortical injections and labeling of newborn neurons with AAV5.hsyn-FLEX-EGFP in pAAV.GFAP-Cre cells in dentate gyrus. This work presents a new tool in A. cahirinus to study the regenerative ability of A. cahirinus in several models of brain disorders.

POSTER 33

METALS OXIDATIVE STRESS AND NEURODEGENERATIVE DISEASES

Santos R[1] and Aureliano M[2,3]

[1] FMCB, University of Algarve, Faro, Portugal; [2] FCT, University of Algarve, Faro, Portugal; [3] CCMar, University of Algarve, Faro, Portugal

Metals are known to have neurotoxic effects and contribute to a number of neurodegenerative diseases presumably through the introduction of oxidative stress. Neurons are surrounded by a myelin sheath which is important for the development of the electric potential and the ability to transmit electrical impulses in the form of action potentials quickly. Vanadium exposure has been reported to cause damage to the myelin sheath and, as a result neuronal death. Vanadium accumulates in the brain after exposure indicating that the toxic effects of vanadium relating to membrane destruction may play a role in the reported neurodegenerative diseases such as Parkinson's and Alzheimer's. Parkinson's disease (PD) is a neurodegenerative disease that has been associated with several failures in the brain. A decrease in the neurotransmitter dopamine has been correlated with the onset of Parkinson's which, with disease progression, leads to a failure in the dopaminergic system. Some basis of knowledge around metals, specifically manganese (Mn), and the onset of Parkinson's or the onset of similar symptoms called Parkinsonism exists. Manganese, like vanadium, undergoes redox cycling and is known to have many neurotoxic effects. The ability of manganese to produce ROS has been well characterized and shown to cause effects on mitochondrial function similar to those observed with vanadium treatment, including the loss of the mitochondrial membrane potential. With high doses of manganese, symptoms of Parkinson's disease are seen and correlated with the onset of Parkinson's. Given the similarities between vanadium and manganese, the effects of vanadium on the onset of Parkinson's are likely to be similar. Some studies have reported a link between vanadium neurotoxicity and its effect on the dopaminergic system and its function in cell signaling mechanisms. Vanadium increased ROS and decreased motor function in Melanogaster drosophila, both wild-type and PD models, and that these effects were alleviated with chelators or the administration of L-DOPA [1-3].

1. Harischandra, D.S.; Ghaisas, S.; Zenitsky, G.; Jin, H.; Kanthasamy, A.; Anantharam, V.; Kanthasamy, A.G. Manganese-Induced Neurotoxicity: . Front. Neurosci. 2019, 13, 654. https://doi.org/10.3389/fnins.2019.00654.

2. Afeseh Ngwa, H.; Kanthasamy, A.; Anantharam, V.; Song, C.; Witte, T.; Houk, R.; Kanthasamy, A.G. Toxicol. Appl. Pharmacol. 2009, 240, 273–285. https://doi.org/10.1016/j.taap.2009.07.025.

3. Ohiomokhare, S.; Olaolorun, F.; Ladagu, A.; Olopade, F.; Howes, M.-J.R.; Okello, E.; Olopade, J.; Chazot, P.L. . Int. J. Mol. Sci. 2020, 21, 6719. https://doi.org/10.3390/ijms21186719.

POSTER 34

ROLE OF HDACS IN THE REGENERATION OF AGEING PERIPHERAL NERVES

Patel N[1,2], Bertolini A[2], Velasco-Aviles S[1,2], Casillas-Bajo A[1,2], Armengol-Gomis A[1] Suelves M[3], Cabedo H[1,2] and Gomez-Sanchez JA[1,2]

[1] ISABIAL, Hospital General Universitario de Alicante Dr Balmis, Alicante 03010, Spain; [2] Instituto de Neurociencias de Alicante CSIC-UMH, San Juan de Alicante 03550, Spain; [3] Germans Trias i Pujol Research Institute (IGTP), Badalona 08916, Spain

Nerve regenerative capacity is impaired in aged animals by a poorly understood mechanism. Repair Schwann cells are necessary for peripheral nerve regeneration and remyelination after nerve damage. It is known that after injury there is an upregulation of c-Jun, a transcription factor pivotal for the switch to the repair Schwann cell phenotype. It has been recently reported that c-Jun induction is lower in the aged nerves, which causes defects in the activation of the repair Schwann cell phenotype delaying axonal growth. Class I Histone Deacetylases (HDACs) play a central role in myelin development and maintenance [1]. Among them, HDAC3 has been shown to be important for maintaining myelin homeostasis in aged nerves.

The aim of this work is to study if class IIa and class V HDACs are also involved in the myelination of aged nerves. To this aim, we explore if there was any difference in myelin homeostasis and remyelination after injury in aged mice lacking HDAC4, HDAC5, HDAC7 or HDAC11 by electron microscopy images of developmental, and young and aged uninjured or injured nerves. With these images, we have calculated the number of myelin sheaths, g-ratio, Schwann cell number and nerve areas.

The results show that class IIa HDACs play a more relevant role in myelination and remyelination than class V HDACs. HDAC11 does not play a major role in Schwann cells. However, a similar compensatory mechanism seen in young animals is observed in aged mutants of class IIa HDACs [2], and the triple mutant HDAC4, HDAC5 and HDAC7 show a large delay in remyelination following nerve damage in ageing.

In conclusion, class IIa HDACs play a fundamental role in Schwann cell biology, whereas class V HDACs do not appear to play a role in peripheral nerve homeostasis and regeneration.

[1] Gomez-Sanchez JA. et al., International Journal of Molecular Sciences 2022

[2] Velasco-Aviles S at al., eLife 2022

Jose A. Gomez-Sanchez was funded by a Miguel Servet Fellowship (CP22/00078) from the Instituto de Salud Carlos III (Spain)

POSTER 35

ROLE OF MECHANOSENSITIVE ION CHANNEL PIEZO1 IN MICROGLIA RESPONSIVENESS AND REACTIVITY TRIGGERED BY ELEVATED PRESSURE

Ângela Soares[1,2,3], Catarina Gonçalves Carvalho[1,2,3], Ricardo Machado-Ribeiro[1,2,3], António Francisco Ambrósio[1,2,3,4], Raquel Boia[1,2,3], Ana Raquel Santiago[1,2,3,4,5]

[1] University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, Coimbra, Portugal; [2] University of Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal; [3] Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal; [4] Association for Innovation and Biomedical Research on Light and Image, Coimbra, Portugal; [5] University of Coimbra, Institute of Immunology, University of Coimbra, Faculty of Medicine, Coimbra, Portugal.

Aims: Glaucoma is a chronic degenerative disease characterized by retinal ganglion cell loss. Elevated intraocular pressure is a major risk factor for disease onset and progression. Chronic neuroinflammation plays an important role in glaucoma, with microglial cells contributing to the neurodegenerative process. However, the sensor for elevated pressure in microglia was not identified yet. Recently, Piezo1 was described as a mechanosensitive ion channel that senses pressure and shearing stress. We hypothesized that Piezo1 plays a role in microglia responsiveness and reactivity triggered by elevated pressure.

Methods: BV-2 microglial cells were incubated with 1, 5, 10 and 20 μM Yoda1 (Piezo1 agonist) for 4, 6, 8, 12, and 24 h or with 0.1, 1 and 10 μM GsMTx4 (Piezo1 inhibitor) for 4, 8, and 24 h. Cell viability was determined using the resazurin reduction assay. BV-2 cells were exposed to elevated hydrostatic pressure (EHP; 70 mmHg above normal pressure) for 4 and 24 h, in the presence or absence of Yoda-1 or GsMTx4. Piezo1 protein levels were quantified by Western blot. Single cell calcium imaging was performed after loading cells with Fluo4-AM. Phagocytosis was assessed with fluorescent microbeads. NO production was determined with the NO-sensitive probe DAF-FM.

Results: The incubation of BV-2 cells with Yoda-1 or with GsMTx4 did not significantly affect cell viability for the concentration and time points analyzed, as assessed by the resazurin reduction assay. Piezo1 protein was present in BV-2 cells and the incubation with Yoda1 caused alterations in the intracellular calcium concentration. Yoda1 (1 and 10 μM) slightly increased phagocytic efficiency from 50.9±6.2%, in the control condition, to 69.6±8.6% and 84.8±6.4%, respectively. The exposure to EHP increased the NO production, and the presence of GsMTx4 did not cause significant alterations. The production of NO slightly increased after the incubation with Yoda1.

Conclusions: These preliminary results indicate that BV-2 cells express functional Piezo1. The activation of Piezo1 in BV-2 cells might cause microglia reactivity. More experiments need to be performed to evaluate the role of Piezo1 activation in microglia and its contribution to EHP-induced microglia reactivity.

Acknowledgements: This work was funded by: FCT-Portugal, 2022.01354.PTDC, PEst UIDB/04539/2020 and UIDP/04539/2020.

VII Symposium

Portuguese GLIAL NETWORK

02·MAY

Sponsors:

SPN SOCIEDADE PORTUGUESA DE NEUROCIÊNCIAS

ABC ALGARVE BIOMEDICAL CENTER

UAlg FMCB UNIVERSIDADE DO ALGARVE FACULDADE DE MEDICINA E CIÊNCIAS BIOMÉDICAS

izasa scientific a werfen company

Albufeira CÂMARA MUNICIPAL

satellite event of:

FRM 2023 FENS REGIONAL MEETING 3-5 MAY | ALGARVE, PORTUGAL