ABSTRACT BOOK

V SYMPOSIUM

PORTUGUESE GLIAL NETWORK 2021

Satellite Meeting of the XVII Meeting of the Portuguese Society for Neuroscience

Glial cells at the crossroad of health and disease

November 30 th

Auditório da Reitoria

Univ Coimbra (Polo I)

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WELCOME

Dear colleagues and students,

I am very pleased to welcome you all here in Coimbra, to attend the V Symposium of the Portuguese Glial Network, this year in Autumn, in the last day of November. As you certainly know, this Symposium is a satellite Meeting of the XVII Meeting of the Portuguese Society for Neuroscience, which will be held at Convento de São Francisco, December 1st-3rd.

Many of you are certainly aware this Symposium was supposed to take place last year, in May, here in Coimbra. However, due to the COVID-19 outbreak, we were forced to postpone it. Fortunately, due to massive vaccination, we now consider we have safety conditions to hold the Symposium. However, the Sars-Cov-2 virus is still there, and we must take precautions to prevent eventual contaminations.

We will have three invited foreign speakers, Andrew Greenhalgh (University of Manchester), Elena Galea (Universitat Autónoma de Barcelona) and Marta Navarrete (Instituto Cajal, Madrid), who have been developing excellent work in the field of glial cells. But we also have excellent science in Portugal in the glial arena, and so we will have three invited national speakers, Ana Falcão (ICVS, Braga), Raquel Santiago (iCBR-CIBB, Coimbra) and Teresa Summavielle (i3S, Porto). We are very grateful to all invited speakers for having accepted our invitation. There will be, as well, four selected talks. I can tell you it was very difficult to select the talks. The scientific work of those that applied for being selected for an oral communication is excellent.

In this Symposium, the role and heterogeneity of astrocytes, microglia and oligodendrocytes in the physiology of central nervous system, namely the brain and retina, will be covered, as well as the contribution of these cells for the pathophysiology of several diseases.

This (presential) Meeting will be a great opportunity to have the Portuguese glial family together again, after a two-year break. Here, we can foster interesting scientific discussions and set the ground for new collaborations between our members. This is an excellent opportunity for younger scientists to share and discuss their work. There will also be awards for best poster and best selected oral communication.

On behalf of the Organizing Committee, I thank you all for choosing to participate in this Meeting and share your research. Of notice, we had more than 170 registrations, a record, meaning that the Portuguese glial family is attracting new members, and glial cells are gaining more interest from the scientific community.

I wish you a pleasant and fruitful Symposium!

Next year we will meet again.

Francisco Ambrósio

Chair

VENUE

Address

Universidade de Coimbra - Polo I

Auditório da Reitoria

Rua Larga

3004-535 Coimbra

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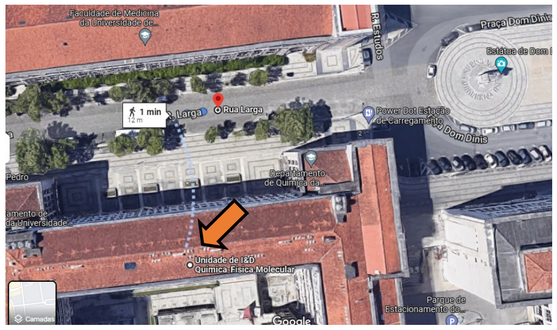
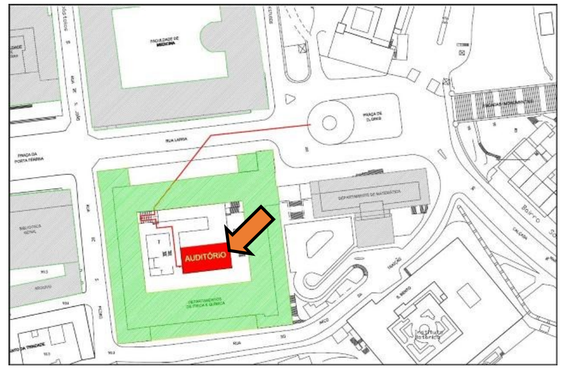


The “Auditório da Reitoria” is located in the building from the Faculty of Sciences and Technology, flanked by the departments of Chemistry and Physics.



Please be aware that Parking next to the University of Coimbra at Polo I is quite complicated on weekdays, and is even subject to constant policing by the competent authorities.

How to get here

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PROGRAMME

9:00-9:45 REGISTRATION

9:45 WELCOME

10:00 PLENARY LECTURE
Chair: *Francisco Ambrósio* (iCBR, Coimbra, Portugal)

10:00 ***The role of microglia and macrophages in CNS injury***
Andrew Greenhalgh (UM, Manchester, UK)

10:45 COFFEE BREAK

11:15-12:45 SESSION I
Chairs: *Paula Agostinho* (CNC, Coimbra, Portugal) and *João Oliveira* (ICVS, Braga, Portugal)

11:15 ***Astrocytic network heterogeneity within nucleus accumbens***
Marta Navarrete (CSIC, Madrid, Spain)

11:45 ***PsychoGlia: a crosstalk between glial cells under psychoactive drugs***
Teresa Summavielle (i3S, Porto, Portugal)

12:15 ***Contrasting patterns of morphologic remodeling of microglia and neurons and vulnerability to depression after short and long chronic mild stress exposure***
Rita Gaspar (iCBR, Coimbra, Portugal)

12:30 ***Astrocyte-driven changes in synaptic plasticity and memory function in Alzheimer' disease***
Daniela Sofia Abreu (iMM, Lisbon, Portugal)

12:45-14:00 LUNCH

14:00 POSTER SESSION

15:00 PLENARY LECTURE
Chair: *Dora Brites* (iMed.ULisboa, Lisbon, Portugal)

15:00 ***The phenotypic transformation of astrocytes in Alzheimer's disease unraveled with multi-transcriptomic and functional analyses***
Elena Galea (ICREA, Barcelona, Spain)

15:45 COFFEE BREAK

16:15-17:45 SESSION II
Chairs: *Rosa Fernandes* (iCBR, Coimbra, Portugal) and *João Relvas* (i3S, Porto, Portugal)

16:15 ***Oligodendroglial heterogeneity in health and disease***
Ana Mendanha Falcão (ICVS, Braga, Portugal)

16:45 ***Contribution of microglial exosomes to retinal neuroinflammation***
Ana Raquel Santiago (iCBR, Coimbra, Portugal)

PROGRAMME

17:15 ***Increasing Vitamin C uptake in microglia for halting Azheimer´s disease***
Camila Cabral Portugal (i3S-IBMC, Porto, Portugal)

17:30 ***Sacsin deletion promotes intermediate filaments aggregation in glial cells: potential implications for ARSACS***
Fernanda Murtinheira (ITQB-NOVA, Lisbon, Portugal)

17:45 SUMMARY AND AWARDS

BIOGRAPHY

Andrew Greenhalgh
Manchester, UK

Andrew Greenhalgh is a neuroimmunologist with a focus on acute CNS injury. After completing a PhD on the inflammatory mechanisms of stroke and subarachnoid heamorrhage with Prof. Dame Nancy Rothwell, in Manchester, UK, He moved to McGill University, Canada to work with Dr Sam David investigating the actions of microglia and macrophages in trauma. Following this he spent three years with Dr Sophie Layé in Bordeaux as a Marie Curie Fellow developing models of concussion before moving to back to Manchester, to his own lab on neuroimmunology of brain injury.

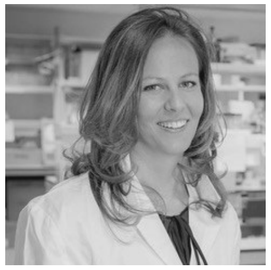
PLENARY LECTURE I

THE ROLE OF MICROGLIA AND MACROPHAGES IN CNS INJURY

Lydia Becker Institute of Immunology and Inflammation, The University of Manchester, Manchester, UK

Microglia are the resident immune cell in central nervous system and perform a multitude of functions in health, disease and response to injury. Microglia are themselves a type of tissue resident macrophage, though they are now distinguishable from other macrophages that reside in the borders of the CNS and those that are recruited from the circulation after injury. This talk will discuss the role of the various types of macrophage in the context of acute injury, including their potential interactions. We will examine new data sets investigating the immune cell response to mild traumatic brain injury (concussion) and future opportunities in the field of neuroimmunology in general.

BIOGRAPHY



Marta Navarrete
Madrid, Spain

Marta Navarrete is graduated in Chemistry-Physics and received her PhD in Neuroscience from UAM, Madrid. She is currently a leader of Synaptic Plasticity and Astrocyte-Neuron Iterations lab at the Cajal Institute, CSIC, Madrid. Her overall aim is to further study of the role of astroglial cells in the brain to understand how astrocytes participate in learning and memory, and how their alterations contribute to brain disorders, aiming to identify new cellular targets for potential therapeutic approaches. Her group use state-of-the-art techniques, that include optogenetics, chemogenetics, multiphoton microscopy, fiber photometry, combined calcium imaging and multiple electrophysiological recordings, in slices and in vivo, in transgenic and experimental animal models.

SESSION I

ASTROCYTIC NETWORK HETEROGENEITY WITHIN NUCLEUS ACCUMBENS

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

Unraveling the principles of information processing in complex cell circuits requires techniques capable of target and modulates specifically the activity of those elements involved.

Neuro-astrocyte networks display a surprising degree of complexity and state-of-the-art complementary tools are required to understand astrocyte involvement in circuit modulation and behavior. Although the evolution of genetic tools to study and control these circuits has focused mainly on neuronal activity, in this talk, I will show newly developed techniques in our laboratory to specifically dissect the active astrocyte circuits with spatio-temporal precision, i.e. CaMPARIGFAP (calcium-modulated photoactivatable ratiometric integrator under GFAP promoter) and Astro-Light (calcium- and light-gated switch to induce gene expression in activated astrocytes). Furthermore, I will discuss our recent data about mapping the functional astrocytic-circuitries in the Nucleus Accumbens (NAc) that reveal the existence of specific- astrocyte circuits in the NAC.

In short, I will present data, acquired using cutting-edge tools, which supports the idea that NAc astrocytic networks are critical players in the understanding of how the NAc integrates information.

SESSION I

Teresa Summavielle is a Principal Investigator and Research Coordinator at Instituto de Investigação e Inovação em Saúde (I3S), University of Porto, heading the Addiction Biology research group. She coordinates courses on Neurobiology of Addiction at Doctoral and Master programs at FMUP. Presently, her research is focused on the role of glial cells under exposure to psychoactive substances and their crosstalk with neuronal cells.

Teresa Summavielle

Porto, Portugal

SESSION I

PSYCHOGLIA: A CROSSTALK BETWEEN GLIAL CELLS UNDER PSYCHOACTIVE DRUGS

Addiction Biology Group, i3S-Instituto de Investigação e Inovação em Saúde and IBMC -Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal.

Methamphetamine (Meth) is a powerful and highly neurotoxic psychostimulant. Upon disrupting the monoaminergic system and promoting oxidative brain damage, Meth also causes neuroinflammation, contributing to synaptic dysfunction and behavioral deficits. Here, we aimed at unraveling how Meth activates microglia and how this contributes to the addictive behaviour. To do so, we used a binge Meth administration and complementary in vivo and in vitro approaches, which included RNAse, advanced morphology analysis, cell sorting, FRET, knockout mouse models and behavior. We found that binge Meth exposure caused microgliosis and disrupted risk assessment behavior, and these required astrocyte-to-microglia crosstalk. Mechanistically, Meth triggered a critical increase of glutamate release from astrocytes (in a process dependent on TNF production and calcium mobilization), promoting microglial expansion and reactivity. Suppressing TNF production, or astrocytic calcium mobilization, prevented Meth-elicited microglia reactivity and re-established risk assessment behavior as tested by elevated plus maze (EPM). These data indicate that glial crosstalk is critical to relay alterations caused by acute Meth exposure and that interfering with glial signaling is sufficient to prevent Meth induced behavior.

SESSION I—SELECTED 1

CONTRASTING PATTERNS OF MORPHOLOGIC REMODELING OF MICROGLIA AND NEURONS AND VULNERABILITY TO DEPRESSION AFTER SHORT AND LONG CHRONIC MILD STRESS EXPOSURE

Rita Gaspar (1,2,3), Carina Soares-Cunha (4,5), Ana Verónica Domingues (4,5), Bárbara Coimbra (4,5), Filipa I. Baptista (1,2,3), Luísa Pinto (4,5), António F. Ambrósio (1,2,3), Ana João Rodrigues (4,5), Catarina A. Gomes (1,2,3,6)

(1) Univ Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, Portugal; (2) Univ Coimbra, Centre for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Portugal; (3) Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal; (4) Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal; (5) ICVS/3B's –PT Government Associate Laboratory, Braga/Guimarães, Portugal; (6) Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal

In a daily basis, individuals are exposed to stress stimuli that can imprint changes in the brain cells (neurons and microglia) and ultimately lead to disease, including anxiety and depression. The duration of stress exposure is determinant for the clinical presentation of stress-related disorders, but eventual correlations between stress duration and cellular morphologic adaptations are not known. The aim of this study was to clarify whether short or long unpredictable chronic mild stress (uCMS) trigger similar or different patterns of morphologic remodeling of neurons and microglia in the nucleus accumbens and, in parallel, to characterize anxiety- and depression-like behavior.

The results show that stress duration influences behavior in a sex-specific manner: short-term stress is sufficient to induce anxiety-like behavior, but only males presented depressive-like behavior after long-term uCMS. Globally, short and long uCMS triggered transient, sex-specific (opposite) changes in microglia morphology. In contrast, the morphology of neurons was not affected by stress in females and, in the case of males, stress was paralleled by the hypertrophy of medium spiny neurons.

These findings raise important questions: may the manifestation of depression-like symptoms be causally associated with neuronal morphology changes, in turn paralleled by a particular pattern of microglial remodeling, as observed in males exposed to stress? These issues deserve further investigation and would be valuable in clarifying sex specificities of anxiety and depression clinical presentation.

Funding:

Foundation for Science and Technology (FCT): PD/BD/114116/2015; PTDC/MED-NEU/29071/2017 (REWSTRESS), UID/NEU/04539/2013; UID/NEU/04539/2019; UIDB/04539/2020; UIDP/04539/2020; UIDB/50026/2020; UIDP/50026/2020; and COMPETE-FEDER POCI-01-0145-FEDER-007440; POCI-01-0145-FEDER-016428 (MEDPERSYST); Centro 2020 Portugal Regional Operational Programme (CENTRO-01-0145-FEDER-000008; Brain Health 2020); Norte 2020 Portugal Regional Operational Programme NORTE-01-0145-FEDER-000013 and NORTE-01-0145-FEDER-000023 and by Bial Foundation 30/2016.

SESSION I—SELECTED 2

ASTROCYTE-DRIVEN CHANGES IN SYNAPTIC PLASTICITY AND MEMORY FUNCTION IN ALZHEIMER' DISEASE

Daniela S. Abreu (1,2), Joana I. Gomes (1,2), Mariana N. Sottomayor (1,2), Filipa F. Ribeiro (1,2), Hugo Vicente-Miranda (3), Marta C. Marques (1), Teresa Summavielle (4,5), Renato Socodato (4,5), João B. Relvas (4,5), Ana Maria Sebastião (1,2), Nicole Déglon (6,7) and Sandra H. Vaz (1,2)

(1) Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal; (2) Instituto de Farmacologia e Neurociências, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal; (3) Chronic Diseases Research Center, NOVA Medical School, Lisboa, Portugal; (4) i3S-Instituto de Investigação e Inovação em Saúde and IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; (5) Faculdade de Medicina da Universidade do Porto, Porto, Portugal; (6) Neuroscience Research Center, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland; (7) Department of Clinical Neuroscience, Laboratory of Neurotherapies and Neuromodulation, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland

Although new insight has been gained about the neurotoxic mechanisms and the clinical aspects of Alzheimer's disease (AD), the actual neuropathogenic processes remain unclear. Amyloid-β (Aβ), an AD hallmark, induces a Ca^{2+}-signaling dysregulation in astrocytes with consequent abnormal gliotransmission, which in turn activates NMDA receptors (NMDARs), that play an important role in synaptic plasticity (eg: long-term potentiation (LTP)) and memory function. NMDAR requires the binding of an agonist (glutamate) and a co-agonist (D-serine), and can be neuroprotective (synaptic, sNMDAR) or initiate cell death pathways (extrasynaptic, eNMDAR). Thus, we hypothesize that Aβ: (1) exacerbates astrocytic glutamate release, excessively activating eNMDARs, which increases neuronal excitability, (2) and reduces D-serine release, poorly activating sNMDAR, which overall leads to the disruption of synaptic plasticity with subsequent memory impairment. Therefore, we aim to investigate how, in AD, changes in astrocyte-neuron signaling suppress synaptic plasticity mechanisms and contribute to memory deficits. To clarify this, we used dnSNARE transgenic mice, where SNARE-dependent gliotransmission is selectively disrupted in astrocytes. To study synaptic plasticity, we incubated hippocampal slices from dnSNARE and control animals, with Aβ1-42 oligomers (200nM), memantine (1μM, eNMDAR blocker) and D-serine (10μM). In parallel, we injected in the hippocampus of dnSNARE and Wt mice AAV vectors carrying the mutated form of human amyloid precursor protein (APP) and presenilin 1 (PS1), to mimic in vivo the pattern of APP expression observed in the hippocampus of human AD patients. Then, the animals were submitted to the open field test (OFT) (general locomotor activity, anxiety, and willingness to explore), Y-maze spontaneous alternation (spatial working memory), and Morris water maze (MWM) (spatial and long-term memory). Additionally, primary astrocyte cultures were transfected with a FRET-glutamate biosensor, to evaluate the impact of Aβ oligomers on glutamate release by astrocytes. Our work shows that the etiology and treatment of AD can be mechanistically linked, via astrocytes, since: (1) impairing gliotransmission restores LTP inhibition mediated by Aβ; (2) astrocytes release more glutamate in the presence of Aβ; (3) memantine prevented Aβ-induced LTP inhibition; and (4) D-serine enhanced sNMDAR activation and potentiated LTP induction, even in the presence of Aβ. Besides, behavioral preliminary results suggest that AAV-APP/PS1 injection does not induce an alteration in OFT nor Y-maze test. However, in MWM, gliotransmission impairment rescues memory deficits caused by AD-like APP processing, suggesting a critical role of astrocytes in memory decline in the early stage of AD. Together, our results place astrocyte at the central hub for synaptic plasticity and memory dysfunction in AD, opening the search for innovative approaches and targets for the treatment of AD.

BIOGRAPHY

Elena Galea

Barcelona, Spain

Neurobiologist with a passion for all things cerebral, although I mostly study astrocytes. The unique anatomical relationship between neurons and astrocytes has always intrigued me; I suspect that it might be key to the understanding of higher brain functions and some CNS diseases. Current projects of my lab include, on the basic side, the theoretical exploration of astrocytes as CNS microcircuits and, on the translational side, the development of astrocyte targeted therapeutics for Alzheimer's disease and neurotrauma using systems biology tools and artificial intelligence.

PLENARY LECTURE II

THE PHENOTYPIC TRANSFORMATION OF ASTROCYTES IN ALZHEIMER'S DISEASE UNRAVELED WITH MULTI-TRANSCRIPTOMIC AND FUNCTIONAL ANALYSES

What happens to astrocytes in Alzheimer's disease, and what is their contribution to the disease progression is not well understood. In this talk, I will describe our recent search for astrocytic signatures in transcriptomic data of about 800 patients, as well as a study on changes of functional of Ca2+ based astrocyte excitability caused by ApoE4, the most important genetic risk factor in Alzheimer's disease.Taken together, the data support a model of astrocyte malfunction in Alzheimer's disease driven by impairment of the endolysosome and mitochondrial systems. Pathological impact and therapeutic implications will be discussed.

BIOGRAPHY

Ana Falcão

Braga, Portugal

Ana Falcão has recently started her research team at ICVS - University of Minho in Portugal. She were awarded my PhD in ICVS and then moved to Karolinska Institutet in Sweden for her postdoc. There, she has developed research projects focused on oligodendrocyte cell biology and multiple sclerosis. Currently, she combining the expertise she acquired so far to answer basic questions on the role choroid plexus in myelination and in multiple sclerosis.

SESSION II

OLIGODENDROGLIAL HETEROGENEITY IN HEALTH AND DISEASE

Oligodendrocytes (OL) are more heterogeneous than previously thought. By means of single-cell RNA-sequencing 12 distinct OL lineage populations were identified. In Multiple Sclerosis (MS) myelin-forming OLs are lost during an immune system attack to the myelin. Nevertheless, it remains unexplored if there is preferential OL population targeted in MS and how are living OLs responding to this autoimmune challenge. New myelin can be formed through the differentiation of OL precursor cells (OPCs) but only at initial stages of the disease. It is also poorly investigated how are OPCs responding to demyelination, the reasons why they fail to remyelinate in chronic stages of the disease and the mechanisms underlying it. We have investigated these unsolved questions by performing single-cell RNA-seq in OL lineage cells of a model of MS and identified populations uniquely found in disease. Currently, we are exploring the role of the choroid plexus, the major producer of the cerebrospinal fluid, in the remyelination processes.

SESSION II

Ana Raquel Santiago
Coimbra, Portugal

Ana Raquel Santiago has been interested in the contribution of glial cells and neuroinflammation to retinal neurodegeneration, and she has been focused on the identification of therapeutic strategies for retinal diseases targeting neuroprotection and neuroinflammation control.

SESSION II

CONTRIBUTION OF MICROGLIAL EXOSOMES TO RETINAL NEUROINFLAMMATION

Univ Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, Portugal; Univ Coimbra, Centre for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Portugal; Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal; Association for Innovation and Biomedical Research on Light and Image (AIBILI), 3000-548 Coimbra, Portugal.

Glaucoma refers to a group of optic neuropathies characterized by progressive degeneration of retinal ganglion cells and damage to the optic nerve (retinal ganglion cell axons). It is a leading cause of blindness worldwide, and elevated intraocular pressure and ageing are important risk factors for disease onset and progression. Chronic neuroinflammation plays an important role in glaucoma pathogenesis. Microglia become reactive when challenged with elevated pressure, releasing cytotoxic factors that contribute to retinal ganglion cell death. The control of microglia-mediated neuroinflammation in animal models of glaucoma is sufficient to protect retinal ganglion cells from damage, highlighting the crucial role of microglia to this disease.

Exosomes are nanovesicles constitutively released by most cells to the extracellular space and are important vehicles of intercellular communication, conveying lipids, proteins and genetic material.

Exosomes derived from microglia exposed to elevated hydrostatic pressure, to mimic elevated intraocular pressure, trigger a pro-inflammatory response in naïve microglia that is reflected by the increased production of proinflammatory cytokines, microglia motility, phagocytosis, and proliferation. These exosomes also increase cell death and impact the survival of retinal ganglion cells. The depletion of retinal microglia with a selective colony-stimulating factor 1 receptor kinase inhibitor, halted neuroinflammation induced by exosomes isolated from microglia at elevated pressure, suggesting that microglial exosomes preferentially interact with microglia.

Exosomes isolated from microglia challenged with elevated pressure are enriched in proteins associated with inflammatory signaling and RNA processing and in miRNAs that modulate the NFKB pathway.

Our results show that exosomes derived from retinal microglia have an autocrine function and propagate the inflammatory signal in conditions of elevated pressure, contributing to retinal degeneration in glaucomatous conditions. Exosomes from elevated pressure-induced reactive microglia can be foreseen as a new route of communication in pathological conditions, being able to propagate and amplify inflammation in the retina. The elucidation of the signaling mechanisms triggered by exosomes will certainly open new possibilities to address how intercellular communication occurs in retinal degenerative diseases with an inflammatory component, as it may also reveal new targets for treatment.

Funding: Fundação para a Ciência e a Tecnologia (FCT), Portugal (UID/NEU/04539/2019; UIDB/04539/2020; UIDP/04539/2020), COMPETE-FEDER (FCOMP-01-0124-FEDER-028417; POCI-01-0145-FEDER-007440), Centro 2020 Regional Operational Programme (CENTRO-01-0145-FEDER-000008: BRAINHEALTH2020).

SESSION II—SELECTED 3

INCREASING VITAMIN C UPTAKE IN MICROGLIA FOR HALTING ALZHEIMER´S DISEASE

Camila C. Portugal (1), Evelyn S. Santos (1), Teresa Canedo (2), Fabiana Oliveira (1), Tiago O. Almeida (1), Joana Tedim-Moreira (1), Renato Socodato (1), Teresa Summavielle (2) and João B. Relvas (1)

(1) Glial Cell Biology Lab and (2) Addiction Biology Lab, both from Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Porto, Portugal

Vitamin C is an antioxidant highly concentrated in the brain playing an essential role in its development and functioning. The isoform 2 of the Sodium Vitamin C co-transporter (SVCT2) is responsible for Vitamin C uptake by CNS, being expressed by neurons and microglia. Reduced ascorbate levels in the brain can lead to neuropsychiatric scurvy (characterized by depression, changes in mood, and behavior) and seem to be involved in neurodegenerative disorders, including Alzheimer's disease (AD).

AD is characterized by progressive memory loss, behavioral changes, synapse dysfunction, amyloid plaques, and neurofibrillary tangles. Oxidative stress occurs very early in AD, supporting its role in disease pathology. Microglia, the resident immune cells of the CNS, play a significant role in AD pathogenesis. Their exposure to amyloid induces a signature called Damage-Associated Microglia (DAM), which can cause neuronal damage (via increased production of inflammatory mediators and ROS). Previously, we showed that ascorbate uptake through SVCT2 is critical for homeostatic microglia maintenance. We described that impairment of ascorbate uptake by diminishing SVCT2 expression is necessary and sufficient to induce microglial proinflammatory activation. We also demonstrated that SVCT2 overexpression abrogates LPS-induced microglial activation. So, we decided to explore the SVCT2 expression in microglia from an AD mice model and establish the increase of SVCT2 expression in microglia as a strategy to halt pathology progression and rescue cognitive performance in AD mice.

Our data show a decrease in SVCT2 expression in microglia from pre-plaque 4-month-old female APP/PS1 mice, suggesting that disruption of microglial SVCT2 expression is an early event during APP/PS1 pathology progression. To investigate whether preventing SVCT2 decrease in microglia ameliorates APP/PS1 pathology progression, we performed stereotaxic injections of AAV9 particles to deliver a transgene expressing both SVCT2 and mCherry (SVCT2 virus) or only mCherry (empty virus), under the control of the CD68 promoter, to hippocampal microglia of 4 months-old APP/PS1 mice. After confirming the SVCT2 overexpression in hippocampal microglia, we firstly observed that SVCT2 overexpression attenuated microgliosis. Regarding Aß load, we observed that SVCT2 overexpression in microglia reduced the Aß content in the hippocampus by three different techniques (ELISA for Aß1-42 quantification, BAM10, and methoxy-X04 staining). We also evaluated the excitatory synapse content in the hippocampus and observed that the SVCT2 overexpression in microglia prevented the synapse loss observed in APP/PS1 mice. Regarding cognition, we observed that the SVCT2 overexpression in hippocampal microglia improved memory in AD mice. Altogether, we conclude that increasing microglial SVCT2 expression delays AD pathology progression in the AD mice.

SESSION II—SELECTED 4

SACSIN DELETION PROMOTES INTERMEDIATE FILAMENTS AGGREGATION IN GLIAL CELLS: POTENTIAL IMPLICATIONS FOR ARSACS

F. Murtinheira (1,2,3), M. Miguéis (1,2), R. Letra-Vilela (1,2,3), V. Martin (4), C. Rodriguez (4), C. Valente (5), C. Duarte-Olivenza (4) and F. Herrera (1,2)

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Aims:

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS), a neurodevelopmental disorder with onset in childhood, is one of the most prevalent recessive ataxias. ARSACS is caused by different mutations in the SACS gene that encodes the 520 kDa multidomain protein sacsin. ARSACS phenotype includes progressive cerebellar ataxia, spasticity, axonal demyelination and Purkinje cell loss. The enormous size of sacsin has complicated the research into its function, but evidence suggests that it is linked to molecular chaperones and protein quality control. In neuronal cells, sacsin modulates mitochondrial network architecture, as well as the proper polymerization of the neuronal intermediate filaments vimentin and neurofilaments. So far, the contribution of glial cells to ARSACS pathology has not been investigated. We are interested in the consequences of sacsin loss in glial cells, as well as the role of glial cells in ARSACS dysfunctions.

Methods:

We generated a glial-like C6sacs$^{-/-}$ cell line employing a CRISPR/Cas9 approach and characterized it by means of western blot, immunocytochemistry, and flow cytometry.

Results:

Sacsin was highly expressed in astrocytes and C6 rat glioblastoma cells. In C6 cells, sacsin depletion resulted in the removal of mitochondria from the juxtanuclear area, where abnormal aggregation of the intermediate filaments glial fibrillary acidic protein (GFAP), nestin, and vimentin was observed. Sacsin-negative cells were more vulnerable to toxic challenges and produced more reactive oxygen species.

Conclusion:

Taken together, our results indicate that the glial-like C6sacs$^{-/-}$ cell line is a suitable model for gaining a better understanding of sacsin functions, with a particular emphasis on intermediate filaments. Our findings could be useful not only for studying the consequences of sacsin deficiency in ARSACS, but also for a wide range of human pathologies caused by disturbance of the intermediate filament networks, such as Alexander disease and Giant Axonal Neuropathy. Our results shed light on a core function for sacsin in glial cells and suggests that astroglia may be involved in ARSACS pathology.

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POSTER 1

CALCIUM SIGNALING INVOLVING P2X7 RECEPTOR ACTIVATION IN ASTROCYTES OF APP/PS1 ALZHEIMER´S DISEASE MICE

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Aims:

Alzheimer´s disease (AD) is the most common form of dementia. AD is characterized by the accumulation of Aβ in senile plaques and hyperphosphorylated tau in neurofibrillary tangles, as well as a neuroinflammation response. Damaged cells can release ATP, which acts as a danger signal and activates purinergic receptors, such as P2X7 receptor (P2X7R). The P2X7R is an ionotropic receptor that allows the entry of Ca^{2+} after its activation. Considering the well-described neuropathological implications of calcium dyshomeostasis in AD, which elicits mitochondrial damage, the aim of this work is to study the role of the purinergic P2X7R in calcium signaling involving the mitochondria in cultured astrocytes, and possible alterations in P2X7R activity in APP/PS1 AD transgenic mice.

Methods:

Primary cultures of astrocytes retaining cell purity around 85-90% were obtained from WT and APP/PS1 postnatal mice. Calcium signaling experiments were performed using Fura2-AM and Rhod2-AM fluorescent probes. Modulation of the P2X7R was assessed by using the well-known receptor agonist Bz-ATP, and the antagonist JNJ. The levels of proteins involved in ER-mitochondria crosstalk and in mitochondrial dynamics were also analyzed by western blotting.

Results:

APP/PS1 astrocytes exhibited an increase in Ca^{2+} flux through P2X7R, when compared to WT astrocytes, which was significantly inhibited by JNJ, the P2X7R antagonist. Selective stimulation of P2X7R also produced an increase in mitochondrial calcium levels, which was similar in APP/PS1 and WT mouse astrocytes. Moreover, we observed an increase in Mitofusin2 and OPA1 protein levels in APP/PS1 mouse astrocytes, suggesting higher mitochondrial fusion events in astrocytes derived from APP/PS1 mice.

Conclusions:

APP/PS1 astrocytes show an alteration in calcium entry through P2X7R, suggesting that these receptors are a potential molecular target to this neurodegenerative disease.

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POSTER 2

ASSESSING THE ROLE OF THE SECRETOME OF BRAIN METASTATIC BREAST CANCER CELLS IN THE PHENOTYPIC SHIFT AND OXIDATIVE RESPONSE OF MICROGLIA

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Introduction:

Introduction: The MDA-MB-231 (TNBC) and JIMT-1 (HER2+) breast cancer subtypes have a higher incidence of brain metastasis. It is well recognized that primary tumors prepare the microenvironment of distant organs, the so-called premetastatic niche (PMN) before metastasis onset. Microglia (MG), as the major innate immune cells of the brain, are likely to take part in the metastatic process, but the underlying mechanisms are poorly understood.

Aims:

We intend to investigate the microglial response when exposed to the secretome of brain metastatic breast cancer cells (BCC), with a focus on oxidative stress, and the role of extracellular zinc on microglia response. We also aim to evaluate whether BCC's-induced MC activation may lead to endothelial cell dysfunction.

Materials and Methods:

Conditioned mediums (CMs) were collected from TNBC and HER2+ cells and their corresponding brain-tropic variants. Human microglia cells (HMC3) were exposed to these CMs and NO production was evaluated by the Griess assay, expression of inflammatory mediators and oxidative stress markers by RT-qPCR, production of intracellular reactive oxygen species (iROS) with the H2DCFDA probe, proliferation rate with WST-1 reagent, and motility by a scratch assay. Activation of stress-activated protein kinases (SAPKs) was evaluated by Western blot. Extracellular zinc depletion experiments were carried out using the chelating agent DTPA. The hCMEC/D3 monolayer integrity was assessed by measuring the transendothelial flux of the 4kDa-FITC probe and the Transendothelial Electric Resistance (TEER).

Results: The exposure of MG to the CMs of BC cells leads to a significant increase in NO and iROS generation, as well as higher proliferation and reduced motility, being the most pronounced achieved with the CM from TN brain-tropic cells. Gene expression of inflammatory mediators and SAPKs signaling also points to an M1-like phenotype under the effect of BCC's secretome. Increased ROS generation appears to be of mitochondrial origin as indicated by the upregulation of the uncoupling protein 2 (UCP2). The depletion of extracellular Zn^{2+} impaired the NO and iROS generation by MCs and their proliferation. Conversely, inflammatory signaling was markedly upregulated and the NRF2 antioxidant gene appears to denote an oxidative stress state. The barrier properties of ECs were disrupted when incubated with the secretome of activated MCs.

Conclusion: MG reacts to the secretome of metastatic BCCs by acquiring an M1-like phenotype, as indicated by the increased production of NO, iROS, and the predominance of pro-inflammatory cytokines. Zinc chelation with DTPA reduced the production of iROS and NO generation by MG while exacerbating the inflammatory environment by upregulating either anti- and pro-inflammatory mediators. These findings highlight the contribution of Zn^{2+} in eliciting a microglial response. Further studies are required to understand how these findings can be related to the PMN formation"

POSTER 3

UNDERSTANDING GLIAL-IMMUNE INTERPLAY ALONG AGE IN AN IN VIVO MODEL OF MULTIPLE SCLEROSIS

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Introduction:

Multiple Sclerosis (MS) is a chronic autoimmune, inflammatory and neurodegenerative demyelinating disorder of the Central Nervous System (CNS). As people get older, the ability to initiate an effective immune response is lost due to a strong accumulation of health deficits leading to the development of progressive forms of MS. Over the past years, some studies have focused on the role of aging in MS progression and in the deregulation of the immune system. We recently describe that in an in vivo model of MS, age is positively correlated with worse disease phenotype. However, the exact cellular mechanisms behind the interplay between the immune system and glial cells response in MS and how it may correlate with age and disease progression/severity is poorly understood.

Aim:

We aimed to evaluate glial and immune cells response that may impact on disease worsening in the in vivo model of MS, the Experimental Autoimmune Encephalomyelitis (EAE).

Methods:

EAE was induced in 3, 6 and 12-month-old female C57BL6 mice and both Clinical Score and Frailty Index (FI) were assessed. On the day of sacrifice, spinal cords were collected for immunohistochemistry (IBA1 for microglia/macrophages, and GFAP for astrocytes) and for RT-qPCR gene expression.

Results:

In the overall health, 12-month-old mice showed an atypical EAE clinical course reaching the highest FI score at the peak of disease (0.36±0.04 for 3-month-old vs 0.54±0.04 for 12-month-old, $p<0.05$) with no sign of spontaneous recovery at the end of experiment. Regarding glial reactivity, 12-month-old EAE mice showed a significantly increase in the percentage of IBA1+ and GFAP+ staining in spinal cord infiltrated areas when compared to 3- and 6-month-old EAE mice ($p<0.05$). Concerning inflammation-associated genes EAE induction resulted in a reduction of chemokine CCL5 and CXCL10 gene expression but enhanced levels of IL-1β and IL-17 mRNA in the 12-month-old EAE mice when compared to 3- EAE mice ($p<0.05$). Interestingly, when looking for microglia enriched genes, we observed that EAE induction significantly decreased the expression of genes associated with the complement (C1qA, B and C), phagocytosis (TREM2) and microglia survival (CSF1R) in the older mice when compared with the 3- and 6-month EAE mice ($p<0.05$).

Conclusion:

In sum, EAE course changes throughout aging and is accompanied with increase gliosis with variable expression of inflammatory molecules. Moreover, EAE induction and aging alter crucial functions of a regenerative microglia phenotype thus leading to disease progression.

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POSTER 4

MICROGLIA SPEFICIFIC BEHAVIOR ACROSS CENTRAL NERVOUS SYSTEM

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Microglia are the resident immune cells of the Central Nervous System (CNS) representing the first line of immune response to neurodegenerative or trauma diseases. Cortex microglia are widely used for in vitro studies of microglia function and properties independently of the research focus. However, recent evidences suggest that subpopulations of microglia, with different characteristics and functional profiles, can be found across different CNS areas. In fact, the first description on the existence of microglia subtypes can be traced back to 1919 by Rio-Hortega in his original description of microglia. These facts lead us to clarify how at the in vitro level microglia isolated from two central nervous system distinct areas, cortex and spinal cord, are different on morphology, function and response to activation. Isolating microglia from cortex and spinal cord and using the same protocol, we demonstrate a region-specific behavior of microglia, presenting several physiological differences. Indeed, we observed that although cortex microglia present the same cell area as spinal cord microglia, they are much different on their complexity. Also, when evaluating phagocytosis function, spinal cord microglia presented less phagocytic activity on both resting and activated state. Therefore, it is important to note that even for in vitro studies, the origin of the microglial cells in study should be carefully selected having in consideration the research focus, since microglial cells behave differently accordingly to their CNS source.

POSTER 5

ASTROCYTIC GPR55 RECEPTORS FUEL GLYCOLYSIS

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Introduction:

Astrocytes support the energy expenditure of neuronal activity via the lactate shuttle. We previously showed that the CB1 and the CB2 endocannabinoid receptors can modulate cerebral glucose turnover (10.1016/j.neuint.2011.10.019; 10.1016/j.neuropharm.2016.03.015). The novel cannabinoid-like receptor GPR55 has also been implicated in systemic metabolic regulation. Furthermore, the L-α-lysophosphatidylinositol-(LPI)-GPR55 signaling cassette is a marker of cancer aggressiveness and promotes glycolysis in breast cancer. We asked now if GPR55 is also present in astrocytes to stimulate glycolysis.

Subjects and Methods:

Acute hippocampal and cortical slices of male young adult C56bl/6 mice and Wistar rats as well as rat primary astrocytic and neuronal cultures were used for quantitative polymerase chain reaction (qPCR), [3H]deoxyglucose/[14C]-U-glucose uptake measurement, and proton nuclear magnetic resonance assays (1H-NMR).

Results and Discussion:

qPCR revealed that the CB1R mRNA was largely neuronal while GPR55 was predominantly astrocytic in primary rat cortical cultures. The GPR55 agonist Δ9-tetrahydrocannabinol (EC50=49 nM), the endogenous GPR55 agonists, LPI (EC50=29 nM) and oleoylethanolamide (10 µM) all significantly stimulated [3H]deoxyglucose uptake in acute rat hippocampal and frontocortical slices by ~20%. The endogenous agonists, LPI (100 nM) and palmytoylethanolamide (100 nM), as well as the synthetic, GPR55-selective agonist, O-1602 (100 nM) all significantly stimulated by ~20% the uptake of [3H]deoxyglucose and the metabolism of [14C]-U-glucose in primary astrocytic culture. 1H-NMR analysis of the culture medium revealed a significant ~20% increase in astrocytic glucose consumption, lactate release and glycolytic rates upon LPI (100 nM) administration.

Taken together, GPR55 activation ramps up glycolysis in astrocytes, with possible implications for glioma research.

POSTER 6

MICROVESICLES RELEASED BY MÜLLER GLIAL CELLS PROMOTE THE SURVIVAL OF RETINAL GANGLION CELLS

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Glaucoma, a leading cause of blindness, is an optic neuropathy characterized by retinal ganglion cell (RGC) loss and optic nerve degeneration. Current therapeutics focus in lowering intraocular pressure (IOP), the main risk factor, but the disease progresses in a large number of patients. Novel and effective therapeutic strategies targeting RGC neuroprotection are needed to halt disease progression.

Müller cells, the main glial cell type of the retina, have important functions and have the ability to protect RGCs either by physical contact or by secreted factors. It is known that Müller cells release microvesicles (MVs). Due to the neuroprotective properties of Müller cells, we aimed to elucidate the role of the conditioned medium (CM) and MVs from Müller cells in the survival of RGCs in the context of elevated pressure.

Primary cultures of Müller cells and RGCs and MIO-M1 cells were exposed to elevated hydrostatic pressure (EHP) to mimic elevated IOP. Primary Müller cell cultures and MIO-M1 cell line were cultured in atmospheric (control) pressure and CM were collected. Primary RGC cultures and co-cultures of RGCs and Müller cells were exposed to EHP and incubated with CM. MVs from MIO-M1 cells (MVs MIO-M1) and from primary Müller cells were isolated from CM by low-speed ultracentrifugation and characterized. Primary cultures of RGCs were challenged with EHP in the presence of MVs to assess cell survival. Effect of MVs MIO-M1 in the retina of Wistar Han rats was determined 7 days after intravitreal injection.

RGC survival significantly decreased by EHP exposure for 24h. Müller cells exposure to EHP for 48h and 72h significantly decreased cell survival. However, when in co-culture, RGCs only presented a significant decrease in cell survival at 72h under EHP and Müller cells showed a tendency to a decrease in cell survival at 72h under EHP. MIO-M1 cells survival was not affected by EHP exposure for 24h or 48h. CM from both Müller cell cultures promoted RGCs survival when exposed to EHP. Both MVs increased RGC survival in EHP conditions. Intravitreal injection of MVs MIO-M1 increased the reactivity of Müller cells but did not change RGCs number.

Results demonstrate that CM from Müller cells can protect RGCs exposed to EHP. In vitro, MVs derived from Müller cells promoted RGCs survival, showing that MVs are an important component of Müller cells secretome for RGC survival. Intravitreal injection of MVs MIO-M1 do not seem noxious to RGCs since their survival was not altered. There was a slight increase in Müller cells reactivity. These results open new avenues in the field of neuroprotection for glaucoma treatment.

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POSTER 7

S100B DELETION PROTECTS FROM MULTIPLE SCLEROSIS-LIKE PATHOLOGY

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Introduction:

S100B, a small inflammatory molecule, has been described as a potential biomarker of disease pathology. In the autoimmune demyelinating disorder Multiple Sclerosis (MS), increased S100B levels in CSF and blood have been linked to disease severity and progression. Recently, we showed a correlation between increased S100B levels in human CSF/serum at the time of diagnosis and its high secretion by astrocytes in active and chronic-active lesions in post-mortem brain samples.

Aim:

Here, we aimed to study the relevance of S100B in MS development and further pathology, using the in vivo model of MS, the Experimental Autoimmune Encephalomyelitis (EAE).

Methods:

For initial studies, female S100B WT and S100B KO mice were EAE induced, and four groups were formed: CTRL and EAE S100B WT, and CTRL and EAE S100B KO animals. All animals were monitored daily according to a standardized clinical scale for motor symptoms. Spinal cord pathogenesis was characterized by immunohistochemistry at 30 days post-EAE induction. Inflammation was evaluated in demyelinating cerebellar organotypic slice cultures (COSC).

Results:

Our results demonstrated that S100B deletion delayed disease onset, reduced paralysis, and accelerated animal recovery, mostly in chronic-EAE stages, with no alterations in locomotor ability between WT and S100B KO animals prior to disease peak. Pathologically, lack of S100B significantly reduced the number of demyelinating lesions by preventing demyelination and cell infiltration, when compared to EAE WT animals. Decreased percentage of both astrocyte marker GFAP and microglia/macrophage marker Iba1 was observed in EAE KO mice, that was further confirmed in demyelinating COSC cultures. Additionally, no changes in iNOS expression and a slight increase in CX3CR1 were observed in EAE KO animals versus the respective control group. Demyelination of OCSC elicited significantly increased expression of TNF-α and IL-1β in S100B WT cultures, as well as the anti-inflammatory cytokine IL-10. Attractively, the opposite was observed in S100B KO animals.

Conclusion:

S100B deletion protects from chronic EAE pathology setting the basis for S100B modulation in order to reduce EAE-associated phenotype and ameliorate disease pathogenesis.

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POSTER 8

EXPLORING A HUMAN 3D STEM CELL-DERIVED MODEL TO STUDY ASTROCYTE ACTIVATION DURING NEUROINFLAMMATION

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Neuroinflammation is associated with pathogenic processes and disease states. Upon inflammatory stimuli, microglia and astrocyte activation contribute to tissue healing and restoration of CNS homeostasis. Nonetheless, excessive glial activation can cause neuronal death and chronic neuroinflammation. Much remains unknown in what concerns: i) the molecular mechanisms that trigger and sustain astrocyte activation; ii) the effector molecules of the downstream microenvironment remodeling. Experimental models in which the human neural cells and their microenvironment are represented will be key to study such processes. This work aims to establish and explore a human cell model of neuroinflammation to study astrocyte activation. We explored hiPSC-derived neurospheroids, a methodology pioneered by our team, in which. hiPSC-derived neural progenitor cells are cultured as spheroids in differentiated in perfusion stirred-tank bioreactors and differentiated into neurospheroids composed of neurons, astrocytes, and oligodendrocytes. We showed that neurospheroids recapitulate specific features of the brain microenvironment, such as human brain-like ECM deposition and neuron-glia interactions. Herein, we challenged neurospheroids with pro-inflammatory factors reported to induce activation of astrocytes in mice models (TNF-α, IL-α, and C1q). Upregulation of neuroinflammatory genes (e.g., SERPINA3 and C3), concomitant with the secretion of pro-inflammatory cytokines, such as MCP-1 and IL-6, along the 72h of insult, suggested astrocyte activation. Challenged astrocytes also displayed an impaired capacity to uptake glutamate and to secrete glutamine in comparison to the unstimulated control, implying functional impairment. We also observed increased neurite outgrowth of β-III tubulin-positive neurons on a laminin-rich substrate, suggesting ECM remodeling events. The results indicate that pro-inflammatory factors induce astrocyte activation in neurospheroids, recapitulating transcriptional changes and functional impairment, hallmarks of neuroinflammation. Ongoing work focuses on the co-culture of the neurospheroids with hiPSC-derived microglia, to further recapitulate to allow for further analysis of the neuron-glia crosstalk. Hence, we propose the human neurospheroid model can be a useful as a tool to dissect neuroinflammatory mechanisms associated with diseases.

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POSTER 9

DISTINCT MICROGLIA PHENOTYPES IN HIPO- AND HYPERFUNCTIONAL DOPAMINERGIC PATHWAYS FROM AN ANIMAL MODEL OF SCHIZOPHRENIA

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Microglia, immune cells responsive to neuronal signals, detect functional and dysfunctional neuronal contacts, strengthening or eliminating them. Microglia dysregulation during synapse establishment may disturb the proper selection of synapses and, consequently, lead to hyper- and hypofunctional pathways.

Schizophrenia (SCZ) is characterized by positive (e.g. hallucinations) and negative symptoms (e.g. social withdrawal) biologically associated with hyper- and hypofunctional dopaminergic pathways, respectively. This correlation between symptoms and abnormal pathways led us to hypothesize that microglia undergo dual morphological differentiation in dopaminergic pathways.

We used a model of SCZ consisting in the injection of methylazoxymethanol acetate (MAM) to pregnant Wistar rats at gestational day 17. The behavior of the progeny, males and females, was evaluated in the postnatal period and at postnatal day (PND) 30 (adolescence), in parallel with a morphometric study of microglia in the Nucleus accumbens (NAc) and prefrontal cortex (PFC), representative of hyper and hypofunctional pathways in the disease.

MAM males and females present a delay in maternal odor discrimination, taken as an indicator of emotional impairment. At adolescence, MAM males and females display social deficits without cognitive deficits, anxiety or anhedonia. Microglia morphology remain unaltered in PFC, while in the NAc, MAM males present less complex microglia.

These results show that, in this model, microglia morphological profiles vary according to the brain region/ pathway involved (unaltered in hypofunctional and atrophic in hyperfunctional pathways). If a correlation exists between morphology and function, these observations may help developing novel hypothesis to explain deviations from normal function of specific dopaminergic pathways.

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POSTER 10

MECHANISMS OF REINNERVATION DURING WOUND HEALING: EXPLORING GLIAL-EPITHELIAL CELL COMMUNICATION

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The skin is a complex organ where epithelial cells closely connect with neurons ensheathed with glia to ensure sensation. When injured, these elements work coordinately to reestablish tissue integrity. If the process becomes defective, due to ageing or disease, there can be a loss of sensory function and wounds may not resolve, evolving into severe skin conditions.

While wound healing has been extensively studied, research focuses mostly on the epidermis. Less is known concerning the sensory nervous system, namely, the mechanisms behind reinnervation after injury. With recent studies in vertebrates placing glia as potential modulators of skin repair, our aim is to uncover the specific mechanisms through which glia and epithelial cells interact to restore the skin, using the larval epidermis of Drosophila melanogaster, a genetically tractable model which displays conserved and well-characterized nervous and epithelial systems.

We are characterizing skin repair in vivo using spinning disk live imaging paired with confocal imaging, focusing on glia dynamics in relation to epithelial closure. We started by checking if glia would intervene in the healing process through morphological changes and found that there are no major structural alterations in glial cells upon a nearby epithelial injury.

The next step was to determine if glia could aid healing through signaling. To test if glia is activated upon skin wounding, we monitored glial calcium levels and found that injury triggers an immediate calcium burst in glia. In epithelial cells, calcium signaling induces the main events of the wounding response, thus we hypothesize that a similar signaling cascade might occur in glial cells. We performed a screen to find mediators of glial-epithelial communication by knocking down potential players of regeneration specifically in glia and observing how it affects epithelial closure. We are now characterizing the role of one of the strongest hits, Draper/MEGF10, a conserved regulator of phagocytosis in glia. Draper has been shown to be activated downstream of calcium and JNK signaling. Accordingly, our preliminary experiments reveal a pattern of JNK activation evolving throughout wound repair. We are still determining whether its origin is strictly glial or also neuronal, but this suggests that glia might play role in skin repair by phagocytosing cellular debris through JNK/Draper signaling

Our results hint at a complex yet essential glial contribution to epithelial closure, introducing a new perspective to wound healing. Besides the implication of calcium dynamics and the JNK pathway, we expect to uncover more regulators of skin repair and shed light on the pathways that mediate glial-epidermal interactions. Ultimately, the project will establish a blueprint for researching skin repair and reinnervation in Drosophila and provide a basis for investigation in vertebrate models.

Our research is supported by PTDC/BIA-BID/29709/2017."

POSTER 11

IMPACT OF BLUNTING ASTROCYTE ACTIVITY IN Aβ1-42-INDUCED DYSFUNCTION OF SYNAPTIC PLASTICITY

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Accumulation of amyloid-β (Aβ) peptides is an hallmark in the brain of Alzheimer's disease (AD) patients. Aβ accumulation precedes AD symptoms and is considered the main causative agent of synaptic dysfunction and loss, playing a key role in the onset and progression of AD. Hippocampal synaptic plasticity, involving patterns of long-term potentiation (LTP) and long-term depression (LTD), is crucial for information processing, learning, and memory. Since astrocytes can control synaptic plasticity, the present study aims to evaluate the impact of blunting astrocyte activity in synaptic dysfunction triggered by Aβ1-42. First, we tested the effects of Aβ1-42 on hippocampal synaptic plasticity of adult male C57BL/6 mice, under two different treatments: ex vivo (50 nM, 40 min) and in vivo (intracerebroventricular injection, 4 µl, 2.25 mg/ml). Both treatments significantly reduced hippocampal LTP amplitude, and impaired LTD causing a shift of LTD to LTP. To infer about astrocyte contribution in shaping synaptic plasticity, we superfused hippocampal slices with the gliotoxin L-α-aminoadipate (L-AA, 100 µM, 2h), previously validated to blunt astrocytes. Notably. L-AA worsened the reduction of LTP amplitude, but restored LTD impairment, caused by either ex vivo or in vivo Aβ exposure. These data suggest that hippocampal LTD impairment caused by Aβ1-42 exposure might be linked to astrocytes dysfunction, since blunting astrocytes restored synaptic LTD in AD-like conditions.

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POSTER 12

THE ROLE OF UNDERSTUDIED POST-TRANSLATIONAL MODIFICATIONS ON STAT3 BEHAVIOR AND FUNCTION

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Aims:

Signal Transducer and Activator of Transcription 3 (STAT3) is a pleiotropic transcription factor that plays key roles in development, immunity, response to stress/damage and cancer. These include several functions relevant to glia: developmental and adult (pathological) astrogliogenesis; reactive gliosis and neuroinflammation; and survival and malignancy of glioblastoma multiforme, the most common and deadly form of brain cancer. STAT3 activity is largely regulated by specific post-translational modifications (PTMs). Most studies focus only on the phosphorylation at Y705 and S727, neglecting almost 80 identified PTMs. One of the PTMs relevant to study is the phosphorylation at Y640 since a mutation that prevents this modification (Y640F) has been found in patients with cancer and inflammatory hepatocellular adenoma. We aimed at determining the influence of understudied PTMs on STAT3 behavior and function.

Methods:

We recently developed a Venus-STAT3 bimolecular fluorescence complementation (BiFC) assay to visualize and study STAT3 homo- and heterodimers in living cells. In BiFC assays, fluorescence is proportional to the dimerization of the proteins of interest. Venus-STAT3 BiFC constructs were modified by site-directed mutagenesis, mutating phosphorylatable residues (Y,S,T) to structurally similar, non-phosphorylatable residues (F,A). These constructs were transfected into STAT3-knockout HeLa cells to avoid the possible interference of endogenous STAT3.

Results:

STAT3 wild-type dimers and Y640F dimers accumulated rapidly in the nucleus in response to leukemia inhibitory factor (LIF) while Y705F mutant and Y705F/Y640F double mutant dimers did not. Interestingly, the accumulation was higher in the Y640F dimers suggesting that this mutation enhances the accumulation of STAT3 in the nucleus. We are using this experimental paradigm to analyze the role of other, less explored, PTMs on STAT3 behavior.

Conclusion:

Our results indicate that Y640 phosphorylation can impact the intracellular localization and activity of STAT3, and that there is interplay between this phosphorylation event and the rate-limiting Y705 phosphorylation. These observations could be relevant to understand better the behavior of STAT3 and to develop therapies for Y640F-associated tumors and inflammatory hepatocellular adenoma.

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POSTER 13

EFFECT OF ASTROCYTIC A2A RECEPTORS ON HIPPOCAMPAL SYNAPTIC PLASTICITY AND MEMORY

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Aims:

Characterize the impact of genetic silencing of astrocytic A2AR on hippocampal synaptic plasticity and memory.

Methods:

We bilaterally injected into the CA1 hippocampal area a viral construct that directed CRE recombinase expression for astrocytes (AVV5 -GFAP-GFP-CRE) in floxed-A2AR (A2ARflox/flox) male adult mice to silence astrocytic A2AR. As a control, A2ARflox/flox mice were injected with the viral construct AVV5-GFAP-eGFP. After 4 weeks, both animal groups underwent behavioural tests to evaluate hippocampal-dependent memory. Afterwards, electrophysiological recordings in the Schaffer collaterals-CA1 pyramidal synapses were performed in hippocampal slices by applying a high frequency stimulation (HFS) to induce LTP and low frequency stimulation (LFS) to induce LTD. Neurochemical studies were also performed to characterize this animal model.

Results:

Astrocytic A2AR inactivation significantly impaired performance in the object displacement test (CRE: 49.4±5.85% vs. control: 71.5±4.64%, $p<0.05$, n=8). Additionally, silencing astrocytic A2AR decreased hippocampal LTP amplitude (CRE: 44.0±16.10% vs. CTR: 104.7 ± 14.16%, $p<0.05$, n=4-5) and led to a shift from LTD to LTP (CRE: 16.4±9.63% vs. CTR: -21.8±6.7%, $p<0.05$) when a LFS was applied. Neurochemical analysis revealed an influence of A2AR in astrocytic morphology, as A2AR silencing led to an increase in astrocytic complexity.

Conclusion:

The data point out that the silencing of astrocytic A2AR negatively impacts on hippocampal synaptic plasticity and memory.

Fundings:

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This study was approved by the ORBEA_128_2015/04122015 and certified by Direção Geral de Alimentação e Veterinária (DGAV; 0421/000/000/2016 Ref 014420)

Competing interests:

Rodrigo A. Cunha is a scientific advisor of the Institute for Scientific Information on Coffee (ISIC).

POSTER 14

THE IMPACT OF SOCIAL ISOLATION ON BEHAVIOUR AND GLIAL CELLS – A STUDY IN YOUNG AND OLD MICE

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Introduction:

Social isolation is classified as a chronic mild stressor, which can lead to the development of neuropsychiatric disorders, including anxiety and depression. Chronic stress can induce an activation of neuroinflammatory mediators, being this molecular response also linked with depressive disorders. NLRP3 inflammasome activation integrates the stress-associated signals and the consequent release of cytokine IL-1B is associated with a higher risk of depression. Currently, there is a knowledge gap regarding glial cells and neuroinflammatory alterations induced by social isolation during both adolescence and old age periods.

Aims:

This study aims to explore the effects of isolation regarding neuroinflammation in a rodent model.

Methods:

Young and Aged C57BL7/J male mice were studied and divided into 2 groups: group housed (GH) and socially isolated (SI) for 3 weeks. Depression and anxiety were evaluated through Force Swim Test (FST) and Open Field (OF), respectively. Morris Water Maze (MWM) was performed to assess cognitive impairments. Following the behaviour tests, molecular approaches were performed, specifically ELISA and Western Blot, to evaluate glial cell and neuroinflammatory markers in hippocampus region, since they have a role in mental disorders.

Results:

Aged-SI mice shown a depressive and anxiety-like behaviour during FST and OFT, respectively. Moreover, social isolation induced spatial memory impairment in Aged mice. Microglia activation analyses demonstrated an increase of Iba-1 expression in Aged-SI group and a decrease of anti-inflammatory microglia marker (Ym1) in Young-SI. Also, socially isolated groups shown a higher NLRP3 protein expression. Pro-inflammatory cytokines levels were higher in Aged groups. In summary, depressive- and anxiety-like behaviour was observed as well as a neuroinflammatory response due to social isolation in Aged mice.

Conclusions:

Depressive- and anxiety-like behaviour and cognitive impairment was observed as well as a neuroinflammatory response due to social isolation in Aged mice.

POSTER 15

MICROBIOTA DERIVED PHENOLIC METABOLITES AND THEIR CAPABILITY TO MODULATE MICROGLIA INFLAMMATORY RESPONSE

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Neurodegeneration reside on multifactorial changes with complex mechanisms and no existing cure. Nevertheless, evidences suggests that neuroinflammation is a key mechanism on the development of neurodegenerative diseases. Prevention and treatment will require multi-targeted therapeutics with a focus on their anti-inflammatory properties. Studies with (poly)phenols have proven their pleiotropic ability to modulate several cellular pathways important on inflammation and disease. However, for the most abundant (poly) phenol metabolites originated from our diet and detected circulating in humans, the low molecular weight polyphenol metabolites, much is still unknown. Absorption and blood concentrations of some of these low molecular weight (poly)phenol metabolites reach high blood concentrations and studies have shown their ability reach the brain. Yet, our understanding of their effects and mechanisms is still quite low. In this work we focus on the ability of a low molecular weight (poly)phenol metabolite, found in several human intervention studies at relative high blood concentrations to modulate the release of inflammatory cytokines in microglia cells. Furthermore, we are currently investigating the molecular mechanisms responsible for this modulation. Results have shown the ability of this molecule to impair NF-kB translocation and kinome analysis have shown the ability of this molecule to modulate several kinases important for cell signaling from NF-kB, MAPK and Jack-STAT pathways. In conclusion, we are deciphering the role of low molecular weight polyphenol metabolites at physiological relevant conditions, and exploring the mechanism for their anti-inflammatory properties in microglia cells. Altogether we hope to understand how these molecules could potentially be a useful tool to modulate neuroinflammation and limit neurodegeneration.

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POSTER 16

ACUTE METHYLMERCURY EXPOSURE INDUCES NEUROTOXICITY AND MICROGLIAL CELL DEATH

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This work aimed to clarify the acute toxic effects of MeHg in microglial cells and, in particular, pathways leading to cell death.

BV-2 mouse microglial cells were incubated with MeHg at different concentrations (0.01; 0.1; 1 and 10 mM) for 30 minutes or 1 hour prior to Lipopolysaccharide (LPS 0.5 mg/mL) exposure for 30 minutes, 6 or 24 hours. After cell exposure, the supernatants were harvested and IL-6 and TNF-α production and release were quantified by ELISA assays. Additionally, ROS production was evaluated using H2DCFDA probe, NO was quantified by Griess assay, iNOS by Western Blot, phagocytic activity was evaluated using fluorescent latex beads, metabolic activity was assessed by Resazurin Reduction Assay, and necrotic cell death was quantified by Propidium Iodide (PI) uptake staining.

BV-2 cells upon exposure to MeHg 10 mM showed a reduction in the production and secretion of pro-inflammatory proteins IL-6 ($n=5$, $p<0.05$), TNF-α ($n=4$, $p<0.01$), iNOS immunoreactivity ($n=3$, $p<0.05$), release of NO ($n=5$, $p<0.01$), as well as ROS formation ($n=4$, $p<0.0001$). Although MeHg treatment compromises the secretion of pro-inflammatory cytokines, MeHg did not affect the LPS-induced phagocytic activity of BV-2 cells. Furthermore, incubation of cells with the highest concentration of MeHg (10 mM), with and without LPS stimulation, induced a decrease in the metabolic activity of BV-2 cells ($n=4$, $p<0.01$) as seen in the Resazurin Reduction Assay and increased the number of PI-positive cells ($n=4$, $p<0.001$), when compared to the respective control groups.

Taken together, these results suggest that the short-term effects of a high concentration of MeHg have the potential to impair the production of various pro-inflammatory mediators, as well as to induce microglial cell death via necrosis, compromising their neuroinflammatory response. Clarifying the mechanisms underlying MeHg-induced neurotoxicity and neurodegeneration in brain cells is thus a critical step towards better understanding and preventing acute and long-term chronic effects of MeHg exposure.

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POSTER 17

OLIGODENDROCYTES HAVE FEELINGS TOO - TISSUE ENGINEERING MODELS TO UNCOVER THE MECHANOBIOLOGY OF MYELINATION

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Multiple sclerosis (MS) represents the most frequent demyelinating disease affecting young people. Although there are oligodendrocyte precursor cells (OPCs) capable of differentiating in oligodendrocytes (OLs) and producing myelin sheaths in denuded axons, the process of remyelination fails with disease progression leading to irreversible functional failure. Growing evidences suggest that matrix rigidity plays a crucial rule throughout OPC differentiation and OL myelination by unbalancing the intra/extracellular forces. We hypothesize that OLs respond to dynamic biomechanical changes occurring throughout demyelination, altering their differentiation capacity. By tuning mechanosensing mediated pathways one expects to be able to promote remyelination. Here we propose tissue engineered models to study the impact of mechanical properties changes of the OL environment on their differentiation. A polydimethylsiloxane (PDMS) micropillar array with biologically relevant dimensions and low stiffness was designed to act as surrogate axons and uncover the role of axonal diameter and stiffness on OPC differentiation. Additionally, OPCs were also embed within a modified alginate (ALG) matrix with tunable mechanical properties to fully recreate the 3D environment of the ECM. OPCs were shown to adhere, survive, differentiate and wrap around the PDMS micropillars. Automated image analysis suggested that the presence of the micropillars accelerate OPC differentiation. Interestingly, softer PDMS structures (Young's modulus of 1250 kPa vs 364 kPa) promoted higher expression levels of OL differentiation markers. Moreover, the wrapping ability was preferred in larger micropillars. ALG hydrogels were produced by combining modified ALG formulations containing the cell adhesive peptide RGD (GGGGRGDSP) or the matrix metalloproteinase sensitive peptide PVGLIG (GGYGPVG↓LIGGK). ALG hydrogels were non-toxic to OPCs and differentiation status was favored in matrices with high PVGLIG content. The impact of the mechanical properties' changes on OL differentiation was assessed by culturing OPCs in increased alginate content hydrogels (1%, 2% and 3% wt/v). OL metabolic activity and differentiation (assessed by the expression of the myelin basic protein, MBP) was favored in softer matrices (shear modulus, G* ~100 Pa) in comparison with low expression of MBP and decreased cell volumes for G* around 350 Pa and 1300 Pa. Additionally, impaired OL differentiation was verified in hydrogels with similar stiffness values but with increased stress-relaxation times, which indicates an enhanced cellular behavior in matrices with augmented capability of dissipating cell-induced forces. In the future, by embedding the micropillar array within the ALG hydrogels containing OPCs we are expecting to recreate, in a fully three-dimensional way, a model of the OL differentiation and myelination and dissect the role of biomechanical properties on OL biology.

POSTER 18

FAT-INDUCED ENERGY METABOLISM ALTERATIONS IN BV-2 MICROGLIA CELLS

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Microglia are the resident immune cells of the central nervous system (CNS) and in charge of maintaining a healthy microenvironment. Microglia carry out a non-stop patrol of the CNS, make contact with neurons and look for abnormalities, all of which requires a vast amount of energy. The energy demand increases after activation by pathophysiological conditions. High-fat diet (HFD) exposure rapidly triggers neuroinflammation with reactive microglia, and the lipid accumulation leads to insulin resistance, oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction, and cell death. Mitochondrial function is often a suggested target for lipotoxicity since these organelles are central in metabolic control and lipid homeostasis. Microglial metabolism might be associated with cell reactivity under stressor factors and a microglial metabolism switch may thus be the underlying cause of hypothalamic and hippocampal dysregulation, which is associated with obesity and dementia. We aimed at investigating microglial phenotype and metabolism under palmitate exposure, an abundant saturated fatty acid present in HFD. When in excess, palmitate causes lipotoxicity, and might affect mitochondrial dynamics with profound morphological and functional changes, thought to be less efficient at producing ATP. BV-2 microglial cells were plated with 30% of confluence and incubated either with palmitate (0.2 mM) or vehicle (0.25% BSA) for 24 hours. Cell viability and proliferation were quantified. mRNA expression measurement of proinflammatory cytokines (TNF-α, IL-6, IL-1β) and mitochondrial fusion and fission was performed. Mitochondrial respiration and glycolysis were determined by measuring oxygen consumption (OCR) and extracellular acidification (ECAR) rates in the SeaHorse 96XF. Metabolic substrates were analyzed by nuclear magnetic resonance (NMR). Morphologically, fatty acid incubation leads to a more rounded, less ramified microglia cell. Proliferation profile also increases with palmitate exposure for 24 hours, even in the absence of inflammatory pattern. Relative to controls, BV-2 cells exposed to palmitate showed impaired mitochondrial metabolism characterized by decreasing basal respiration, ATP production, maximal respiratory capacity and spare capacity. Palmitate exposure further promoted an increase of ECAR, suggesting exacerbated glycolysis. No differences in mitochondria content were found between the groups, although an increase in fission protein expression was evident under palmitate incubation. Results from NMR show a decreased production of glutamate with increased production of lactate in palmitate-treated cells, compared to vehicle. We conclude that palmitate exposure triggers a metabolic shift from oxidative to glycolytic alterations in BV-2 microglia cells in the absence of a pro-inflammatory phenotype.

POSTER 19

NEURONAL MIR-124 IS TRANSFERED TO MICROGLIA VIA PARACRINE SIGNALING AND PREVENTS THE ALZHEIME'S DISEASE ASSOCIATED PHENOTYPE

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Evidence indicates miRNA(miR)-124 as a player in Alzheimer's Disease (AD) [1]. We showed that miR-124 was upregulated in human SH-SY5Y-APP695 Swedish neuroblastoma cells (SWE) [2], and in neurons differentiated from iPSCs of a patient with the PSEN1dE9 mutation [3]. Secretome from both cells contained elevated levels of miR-124, either soluble or as part of exosome cargo. Lately, miR-124 -loaded exosomes revealed to attenuate microglia activation by cocaine [4]. Here, we transfected the SWE cells with miR-124 inhibitor/mimic to investigate effects by miR-124-depleted/enriched neuronal secretome on the dynamics of interferon-gamma(IFN-γ)-stimulated microglia, to mimic neuroinflammation in AD.

SWE cells were transfected with miR-124 inhibitor/mimic, human CHME-3 microglia were primed with IFN-γ for 24h and their co-cultures were established during 24 h. Assessments included cell viability by the Guava Nexin flow cytometry analysis, nitrite release by Griess method, gene expression by RT-qPCR, and proteomics by Mass Spectrometric with IsobarQuant analysis against human databases. Exosomes were isolated by sequential ultracentrifugation.

Upregulation of miR-124 levels were confirmed in the SWE cells, as compared with the naïve ones. Co-culture of SWE cells with IFN-γ-CHME-3 cells resulted in microglial miR-124 overexpression. Modulation of miR-124 levels in SWE cells with the inhibitor caused RAGE, HMGB1, iNOS and IL-1β overexpression, and IL-10 and Arginase-1 downregulation in the cocultured primed microglia. The mimic reduced the microglial production of nitrites and decreased TNF-α and iNOS gene expression. Such effects were supported by the proteomic analysis revealing a subset of 17 differentially expressed proteins involved in immune function/inflammation. Particularly, PRKC apoptosis WT1 regulator (PAWR) and EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1) were co-regulated accordingly with the SWE miR-124 levels. To further examine the cause of the microglial miR-124 boost, we silenced RNase III endonuclease Dicer1 in the microglia to block the production of new miRNAs. In such condition, we observed that miR-124 was still increased in the microglial cells upon incubation with the miR-124 modulated SWE cells or their derived exosomes, thus validating neuron-to-microglia transfer of miR-124.

In sum, neuronal miR-124 is delivered to microglia via secretome/exosomes where it regulates their neuro-immune functions, attesting that miR-124 regulation is key in preventing homeostatic imbalance and associated AD pathology. The axis between miR-124 and the AD-associated PAWR/EFEMP1 will be explored in future studies.

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POSTER 20

THE ROLE OF PADI2 ON OLIGODENDROCYTE DIFFERENTIATION ENHANCED BY MECHANOTRANSDUCTION

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Introduction:

Oligodendrocyte precursor cells (OPCs) are mechanosensitive cells, since their survival, proliferation, migration and differentiation are influenced by mechanical cues. We demonstrated that substrates mechanically compliant with the rat brain (6.5kPa) enhanced the differentiation of OPCs in vitro and this effect was potentiated in presence of laminin-2, an extracellular protein that promotes oligodendrocyte (OL) differentiation. Recently, using magnetic resonance elastography — a non-invasive imaging technique to assess the rheological properties of tissues —, it was shown that brain softening occurs with physiological ageing. Strikingly, individuals with multiple sclerosis (MS) present exacerbated softening when compared with age and gender-matched controls, presumably due to deregulated extracellular matrix, cell death and consequent loss of mechanostasis (mechanical homeostasis).

Taken together, data suggest that changes in mechanical properties of the brain might have an impact on OL cellular processes, with specific relevance in the context of MS. The mechanotransduction mechanisms involved in OL differentiation have not yet been fully elucidated, but it was reported that mechanical forces induce changes in chromatin structure, namely through mechanisms dependent on histones methylation. Gene expression and cell fate are controlled by epigenetic mechanisms, which play a key role on OL biology.

Aims:

We want to dissect the mechanisms underlying the enhancement of OL differentiation by mechanotransduction focusing on peptidyl-arginine deiminase 2 (PADI2).

Methods:

In this work, we used mouse primary OPCs or a cell line (CG4 cells) cultured on substrates with distinct rigidities and assessed PADI2 levels and its relevance for OL differentiation by qRT-PCR and western-blot analysis.

Results:

We observed changes on epigenetic markers through mechanisms dependent on histones citrullination and PADI2 protein level in OLs differentiated on substrates with distinct degrees of stiffness, suggesting a novel chromatin regulation mechanism on OLs in response to mechanical cues. Moreover, in the absence of PADI2, MBP expression decreased, hampering OL differentiation. We hypothesize that PADI2 acts through cytoskeleton changes, since PADI2 is a regulator of RhoA and Rac1, well known modulators of actin and myosin. Indeed, PADI2 inhibition leads to a decrease of RhoA during OL differentiation. Furthermore, we assessed RhoA activity by evaluating the phosphorylation level of cofilin-1, a proximal downstream target of the kinase cascade triggered by this GTPase. We observed a lower phosphorylation of cofilin-1 upon PADI2 inhibition, indicating reduced RhoA activity.

Conclusions:

Here, we dissect a possible mechanism of OL differentiation modulation by mechanotransduction. These results emphasize the significance of the work in the context of MS, and the importance of mimicking extracellular stiffness for OL differentiation in culture.

POSTER 21

NEUROINFLAMMATORY RESPONSE IN THE HIPPOCAMPAL FORMATION OF DAMS INDUCED BY GESTATIONAL DIABETES

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Gestational diabetes mellitus (GDM) is a frequent form of diabetes mellitus, characterized by insulin resistance or insulin deficiency, with onset during pregnancy. Despite studies with GDM offspring little is known about the effects of GDM on the mother. With this work we aimed to understand if GDM leads to impairment of the hippocampal formation of dams through an increased neuroinflammatory response.

Pregnant Wistar rats were either divided in control, diabetic (STZ) or insulin-treated diabetic (STZ+I) groups. GDM was induced through an intraperitoneal injection with Streptozotocin, and half of the STZ rats were surgically implanted with insulin pellets. One month after weaning period, behavioural tests were performed to evaluate anxiety behaviour and locomotor activity, learning and memory processes, and depression states. Brains were processed for quantification of GFAP- and Iba1-immunoreactive cells in hippocampal formation using stereological procedures. Finally, the expression levels of several pro-inflammatory cytokines were measured by qRT-PCR, such as, TNF-α, IL-6, IL-1β and COX-2.

STZ and STZ+I rats did not very much differ from controls with respect to anxiety, learning and memory functions, and locomotor activity. Our results showed that female rats exposed to untreated gestational diabetes do indeed have a heightened neuroinflammatory status. In fact, GDM induced a significant increase in the mRNA expression of both TNF-α and IL-6, and though there was an increase in the mRNA expression of COX-2 without significant changes, no changes were found in IL-1β levels.

POSTER 22

PSYCHOSTIMULANTS AND NEUROINFLAMMATION: FINDING CRITICAL PLAYERS IN THE CROSSTALK BETWEEN GLIAL CELLS AND NEURONS

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Introduction:

Exposure to psychostimulants has been classically associated with damage to neuronal terminals. However, it is now accepted that interaction between neuronal and glial cells also contributes to the addictive behavior. Contrary to the common held view, we have previously shown that methamphetamine (Meth), a potent psychostimulant frequently associated to neuroinflammation, cannot stimulate microglia in a cell-autonomous manner, supporting that this activation is likely mediated by other brain cells. Therefore, we are interested in clarifying the progression of neuroinflammation under chronic drug exposure and the role of different cells in this inflammatory process. We hypothesize that the long-term adverse consequences occurring within the brain's reward circuitry under psychostimulant exposure may result, at least in part, from the underlying neuroinflammatory process, and that limiting inflammation may be relevant to control the addictive behavior and reduce relapse rates.

To explore this issue we are evaluating the microglia reactivity state in different phases of the addictive process. We evaluated specific inflammatory microglia markers, microglia homeostatic signature genes and phagocytic markers and we have observed that chronic Meth administration results in unbalanced microglia homeostasis and that this effect persists for at least ten days after withdrawal. Specifically in the hippocampus, although the number of overall microglia cells is not significantly altered, we have observed that microglia presented a more ameboid-like shape after chronic Meth administration.

In addition, a crosstalk between neurons and microglia seems to be relevant for the behavioral expression of Meth. As such, we are dissecting the modulation of microglia by neurons under Meth exposure, evaluating neuroimmune regulatory ligand-receptor pairs that seem to impact on the neuron-microglia interaction. We have observed that the expression of some specific receptors in microglia is decreased after chronic Meth and also during abstinence, which may be associated with reduced neuronal ability to downregulate microglia reactivity, and lead to more neuronal damage.

Importantly, these type of receptors may act as interesting therapeutic targets for treatment of addiction, and therefore we will manipulated them to confirm their value in reducing relapse rates and improve addiction treatments.

POSTER 23

DUALITY OF CB1R EFFECT IS DEPENDENT ON ASTROCYTIC CALCIUM SIGNALING IN RODENT MPFC

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Aims:

The prefrontal cortex (PFC) is involved in cognitive and executive functions, such as decision making, working memory and behavioural flexibility (Dalley et al., 2004; Euston et al., 2013). The regulation of these types of behaviour is somewhat possible by the modulation of synaptic activity through several neuromodulators such as endocannabinoids. In numerous brain regions such as the hippocampus, suprachiasmatic nucleus (SCN) and the neocortex, astrocytic CB1R activation elicits Ca2+ transients, that lead to modulation of synaptic transmission (Navarrete and Araque, 2008; Halibitz et al., 2020; Durieux et al., 2021), however nothing is known regarding the role of astroglial CB1R in the mPFC. Thus, our work aims to fill the gap of the potential role of astroglial CB1R in PFC, namely upon synaptic plasticity as well as cognitive and depressive behavior.

Methods:

Field-excitatory post-synaptic potentials (fEPSP) were recorded in medial PFC (mPFC) of IP3R2-KO and IP3R2-WT mice (8-12 weeks old) by positioning of the stimulating eletrode in layer 2/3 of mPFC and the recording electrode in layer 5. LTP was triggered by a priming train of high-frequency stimulation (HFS), followed 15 minutes later by four HFS trains (50 pulses at 100 Hz, 0.5 s duration delivered every 10 s). Time course of the magnitude of LTP was evaluated. The CB1R effect was accessed by incubating slices with ACEA, a specific CB1R agonist. Behavioral tests were performed in of IP3R2-WT and IP3R2-KO mice from both genders intraperitoneally administered with vehicle or with the CB1R agonist, WIN-55,212, at a low dose (0.2mg/kg, i.p.). The vehicle or the WIN-55,212 were administered 23, 5, and 0.75 h before open field (OP) test, Y-maze test and forced swim test (FST). With this tests we accessed locomotion and anxiety-phenotype, percentage of correct choices and percentage of immobility, respectively.

Results:

mPFC slices superfusion with ACEA (1µM, 20 minutes before priming) lead to a significant increase of LTP magnitude in IP3R2WT while it had a significant decrease in IP3R2KO mice. In both cases, the effect was mediated by CB1R since its effect was abolished in the presence of CB1R selective antagonist, AM251 (1 µM). Regarding behavioral tests, no significant differences were observed, probably due to low n. However, there was a tendency of an increase on anti-depressant effect in IP3R2-WT like it is was previously described (Bambico et al., 2007), and the opposite effect was observed in IP3R2-KO while performing the FST. Similar results were observed regarding working memory by performing the y-maze test.

Conclusions: In the mPFC, CB1R activation has an opposite effect on synaptic transmission depending if there is a functional astrocytic Ca2+ signaling. Further experiments need to be done in order to confirm if such effect is due to CB1R expressed in astrocytes.

JGR is an FCT Fellow (PD/BD/ 150342/2019). The authors have no conflicts of interest to declare

POSTER 24

BDNF POTENTIATES OLIGODENDROGENESIS FROM SUBVENTRICULAR ZONE NEURAL STEM CELL CULTURES

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Oligodendrocytes (OLs) are the myelin-forming cells in the Central Nervous System of vertebrates. Under demyelinating conditions, oligodendrocyte precursor cells (OPCs) present in the brain parenchyma or derived from subventricular zone neural stem cells (SVZ-NSCs) can differentiate into oligodendrocytes (OLs), which migrate and partially remyelinate the lesioned areas. The role of modulators such as brain-derived neurotrophic factor (BDNF) and adenosine A2A receptors (A2ARs) on adult oligodendrogenesis from SVZ-NSCs remains unknown.

Hence, we aimed at studying how these modulators and the putative crosstalk between BDNF and A2ARs can influence OL differentiation from postnatal SVZ-NSCs.

The effects mediated by BDNF on postnatal oligodendrogenesis were evaluated in vitro, using C57BL6/J P1-3 mice SVZ neurospheres that were pharmacologically treated with BDNF (30 ng/mL), A2AR agonist (CGS21680, 30 nM) and A2AR antagonist (ZM 241385, 50 nM). Data were expressed as Mean±SEM. Statistical significance was determined using one-way ANOVA. All experiments were approved by ORBEA-iMM and DGAV.

Results obtained by immunocytochemistry 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+ZM 174.0±8.951; $p<0.01$), while the antagonist by itself had no effect when comparing with control (ZM 117.6±15.47; $p>0.05$). However, no changes were observed after treatment with the A2AR agonist at these timepoints in both OPC formation and OL maturation.

To date, this work outlined the role of BDNF in promoting the formation of OPCs derived from SVZ-NSCs. We are currently addressing if the effect of BDNF is dependent of A2ARs throughout OL maturation and also during proliferation (through BrdU staining protocol). Ultimately our work will contribute to the development of alternative therapeutic targets for OL formation and remyelination.

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The authors declare no conflicts of interests. "

POSTER 25

THE ROLE OF ASTROCYTIC CALCIUM-DEPENDENT SIGNALING IN COGNITIVE FUNCTION

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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). The modulation of astrocytic calcium signaling might have implications in neurotransmission, metabolism, and brain homeostasis. Here, we used the IP3 receptor type 2 knockout (IP3R2 KO) mouse model that lacks global calcium elevations in astrocytes to disclose its implications in cognitive function, namely in learning and memory. We found an influence of global astrocyte calcium in the cognitive tasks performed. Thus, we performed a structural and molecular analysis of cortico-limbic regions and identified relevant molecular signaling pathways to support our behavioral data. The characterization of the IP3R2 KO mouse model provided new information about the importance of astrocytic calcium signaling in the modulation of neural activity, allowing further studies exploring calcium dependent cell signaling in behavior.

POSTER 26

MORPHOLOGICAL ANALYSIS OF ASTROCYTES FROM THE HIPPOCAMPUS OF MICE

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Astrocytes are the most abundant glial cells in the brain, and they present a typical star-shaped morphology. Their extended morphology is pivotal to allow their dynamic interactions with neighboring neurons and glia. Therefore, assessing astrocytic morphology is crucial for further insights into their regional distribution and integration in neuronal networks. Here, we evaluated the morphological structure of astrocytes from several hippocampal sub-regions, namely CA1, CA3, and dentate gyrus of the dorsal and ventral hippocampi of wild-type mice. For that, we used a semi-automatic tool called Simple Neurite Tracer (SNT). The SNT is a free Fiji-ImageJ software plugin optimized and validated for 3D reconstructions of the primary structure of astrocytes. We gathered morphometric parameters such as total length, number of processes, and arbor complexity (Sholl analysis) of astrocytes. These analyses allowed the characterization of regional heterogeneity of astrocytic structure.

POSTER 27

MODULATION OF ASTROCYTIC CELL POPULATIONS BY HUMAN BONE MARROW MESENCHYMAL STEM CELL SECRETOME

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The central nervous system has a limited auto-regenerative capacity, imposing challenges for the development of new therapies. Previous studies from our lab have demonstrated the applicability of human bone marrow mesenchymal stem cells (hBM-MSCs) secretome, as a modulator of the hippocampal neurogenic niche. Astrocytes are glial cells that control the neurogenic niche through the secretion of trophic factors and neuromodulators. Thus, in the present work, we aimed to evaluate the modulatory impact of astrocyte signaling on the hippocampal neurogenic niche upon application of hBM-MSCs secretome, centering our analysis on niche-specific proliferative events and morphological responses of astrocytes. Hence, we employed both wild-type and dnSNARE mice, a transgenic model that presents a blockage of astrocytic exocytosis and function impairment. Animals received bilateral injections of hBM-MSCs secretome at 8 weeks of age and hippocampal slices were evaluated with immunohistochemistry assays. Results demonstrated increased hippocampal proliferation marked by the number of Ki-67 expressing cells in wild-type animals when treated with secretome. Additionally, we observed that dnSNARE animals injected with hBM-MSCs secretome disclosed increased proliferation of GFAP stained cells at the sub-granular zone which is indicative of a local proliferative response of radial glia and early neural progenitors. Morphometrical evaluation found increased process hypertrophy and branching of dnSNARE astrocytes when treated with secretome. These results are closely related with the trophic factors present in the secretome, but also demonstrate a mechanistic involvement of astrocyte exocytosis in the proliferative response conferred by the secretome, placing astrocytes as key cellular targets for CNS regenerative medicine approaches.

Authors declare no conflict of interest.

POSTER 28

DIETARY NITRATE, A METABOLIC PRECURSOR OF NITRIC OXIDE, MITIGATES DIABETIC RETINOPATHY IN A TYPE 2 DIABETES RODENT MODEL

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Diabetes is a chronic disease involving many overlapping and interrelated inflammatory events that result in complications like diabetic retinopathy, with potentially blinding consequences. The pathogenesis of diabetic retinopathy is driven by various pathophysiological pathways involving unbalanced oxidative stress, inflammation, and microvascular dysfunction, usually accompanied by impaired nitric oxide (●NO) bioavailability. A number of experimental studies have demonstrated the therapeutic value of nitrate supplementation in animal models of obesity, metabolic syndrome and type 2 diabetes (T2D). Recent evidence suggests that dietary nitrate, an in vivo metabolic precursor of ●NO via the nitrate-nitrite-nitric oxide pathway, is able to restore ●NO bioavailability and modulate oxidative stress in diabetic vasculature. Focusing on the involvement of inflammatory processes that contribute to the onset of neurovascular lesions of diabetic retinopathy, we aimed to address if dietary nitrate supplementation is able to influence the pathophysiologic progression of diabetic retinopathy of Goto-Kakisaki (GK) rats.

To accomplish our goal, we have used GK rats with 17 weeks of age. GK rats are a non-obese model of T2D, characterized by early and stable hyperglycemia, insulin resistance and hyperinsulinemia, associated to retinal alterations. A separate group of animals was supplemented with sodium nitrate in water (ad libitum) for 12 weeks before testing. Age-matched Wistar rats were used as controls. Retinal cryosections were prepared and used to evaluate total retinal thickness, number of microglia and retinal ganglion cells by (immuno)histochemical labeling.

We have found that GK rats have diminished total retinal thickness compared with controls. This was accompanied by increased number of Iba1+ cells (particularly in the outer plexiform layer), and a decrease in the number of retinal ganglion cells in the inner plexiform layer as compared with the controls. These pathological features were absent in GK rats supplemented with dietary nitrate that exhibited closer characteristics to control animals in terms of increased total retinal thickness, decrease in the number if Iba1+ cells and a recovery in the number of retinal ganglion cells. These data suggest that nitrate supplementation was able to counteract the inflammatory chain that typically occurs in diabetic retinopathy and prevented retinal thinning.

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POSTER 29

CROSSTALK BETWEEN ATP-P2Y1 AND ADENOSINE A2A RECEPTORS IN THE MODULATION OF ASTROCYTIC Ca^{2+} DYNAMICS IN ALZHEIMER`S DISEASE CONDITIONS

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Aims:

Considering that astrocytes involvement in Alzheimer's disease (AD) remains to be clarified, the present work aims to investigate how Aβ peptides affect astrocytic Ca^{2+} dynamics and whether purinergic ATP receptors P2X7 (P2X7R) and P2Y1 (P2Y1R) and adenosine A2A (A2AR) receptors modulate Ca^{2+} responses in these glial cells.

Methods:

To achieve our aim, we performed live-cell Ca^{2+} imaging and intracellular [Ca^{2+}] ([Ca^{2+}]i) measurements, using the fluorescent probe Fluo-4-AM (4 μM), in cultured astrocytes stimulated with ATP (100 μM), BzATP (P2X7R agonist, 100 μM) or MRS2365 (P2Y1R agonist, 30 μM). Astrocytes were incubated with Aβ1-42 (1 μM, for 1 h) to mimic AD conditions, and further exposed to antagonists SCH58261 (A2AR, 50 nM), JNJ47965567 (P2X7R, 1 μM) and MRS2179 (P2Y1R, 30 μM) and PKA inhibitor H-89 (10 μM).

Results:

Aβ1-42 exposure significantly reduced astrocytic Ca^{2+} response amplitude to ATP stimulation, but increased its duration as compared with control astrocytes. Moreover, cells pre-incubated with Aβ1-42 and exposed to P2Y1R antagonist MRS2179 showed a decrease in the amplitude of Ca^{2+} response amplitude ($p<0.05$) but not in duration, whereas P2X7R antagonism was devoid of effects in ATP-evoked amplitude response and only slightly decreased the duration of Ca^{2+} response ($p>0.05$). Furthermore, the A2AR antagonist, SCH58261, prevented Aβ-induced duration decrease ($p<0.01$) without affecting amplitude response. Curiously, SCH58261 decreased [Ca^{2+}]i evoked by BzATP or MRS2365 in control cells ($p<0.05$), being devoid of effects in Aβ-exposed astrocytes. Stimulation with P2Y1R agonist significantly decreased ($p<0.01$) Ca^{2+} response in the presence of P2X7R antagonist, which was not observed in Aβ-treated cells. Moreover, PKA inhibitor reduced BzATP- and MRS2365-mediated Ca^{2+} responses in control astrocytes, but not in Aβ-treated astrocytes.

Conclusions:

Aβ1-42 affected the amplitude and duration of ATP-evoked Ca^{2+} response through a mechanism involving P2Y1R and A2AR, respectively. Moreover, our data suggest a crosstalk between A2AR and P2Y1R in controlling astrocytes that was absent in Aβ-treated cells. Thus, the disruption of P2Y1R/A2AR crosstalk might contribute to astrocytic Ca^{2+} deregulation under AD-like conditions.

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POSTER 30

COMPARISON OF THE MICROGLIAL CELL POPULATION BETWEEN RETINAL ORGANOTYPIC CULTURES AND AXOTOMIZED RETINAS IN VIVO

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Aims:

The objective of this study was to compare quantitative and qualitatively the population of microglial cells (MCs) in two models of neuronal degeneration in the mouse retina: organotypic retinal cultures (in vitro), an inherent model of axotomy and retinal detachment; and an optic nerve axotomy in vivo.

Methods:

For retinal organotypic cultures, retinas of adult C57Bl/6 mice were dissected and cultured in supplemented Neurobasal medium. For the in vivo model, the left optic nerve was crushed (ONC). In both models, the retinas were analyzed at intervals from 24 hours to 9 days (n= 6-8/group) and MC were immunodetected with anti-Iba1 antibody. MCs were photographed, the morphological changes were qualitatively analyzed and their mean density (MC/mm2) in the ganglion cell layer (GCL) of the central retina was assessed (n=4 micrographs/retina/time point).

Results:

After ONC, MCs are highly branched, with long radial extensions in the direction of the optic nerve. In contrast, the MCs observed in the in vitro model are rounder, barely branched, thicker and with fewer extensions. Quantitatively, there is a increase in MC density in the GCL that is progressive and follows the same trend in both models, although the density of MCs in vitro is, at all time points, significantly smaller than in vivo.

Conclusions:

The morphology of MCs differs in vivo and in vitro, suggesting a differential activation. In addition, the density of MCs in vitro is smaller than in vivo, indicating that in vivo MCs or circulating macrophages invade the retina to cope with RGC degeneration.

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POSTER 31

SACSIN DELETION DISRUPTS C6 RAT GLIOBLASTOMA CELLS TO INFLAMMATORY CUES

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Aim:

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a rare ataxia characterized by atrophy of the anterior cerebellar vermis associated with Purkinje cell death. This neurodegenerative disease is caused by mutations in the SACS gene that encodes for sacsin, a very large protein. Loss of sacsin causes alterations in the expression and distribution of intermediate filaments in neurons. Autophagy is elevated in ARSACS, but other histopathological features typical of neurodegenerative disorders, such as neuroinflammation and endoplasmic reticulum (ER) stress, have been barely studied for this disease. We aimed at analyzing the alterations in neuroinflammatory and ER stress pathways upon deletion of sacsin.

Methods:

We deleted sacsin from C6 rat glioblastoma cells by means of a CRISPR/Cas9 approach, isolated sacsin knockout cell lines by Flow Cytometry-assisted Cell Sorting (FACS) and and analyzed the effects of sacsin loss in the intermediate filaments network by means of immunocytochemistry and western blotting. Neuroinflammation and ER stress pathways were later analyzed upon stimulation by LIF, IL-6 and TNF pro-inflammatory cytokines for 20 min and 24h.

Results:

Wild type C6 cells expressed high levels of sacsin, as well the glial intermediate filaments glial fibrillary acidic protein (GFAP), vimentin and nestin. Removal of sacsin caused striking alterations in the expression and structure of intermediate filament networks, with juxtanuclear accumulation of all intermediate filaments. The inflammatory response of C6 KO cells to IL-6 and TNF caused a significant reduction in the active forms of the transcription factor STAT3 (i.e., phosphorylated and acetylated in key residues). No inflammatory response was registered upon LIF incubation. In terms of the ER stress, CHOP expression was highly reduced in C6 KO cells when compared to the C6 wild-type cells. There were no differences in the expression of other ER stress markers (e.g., calnexin, PERK) between the two cell lines either in the presence or absence of cytokines.

Conclusions:

Sacsin loss impairs glial intermediate filament assembly and intracellular distribution, response to inflammatory cytokines and ER stress pathways. These results point at a potential role for sacsin in glial cells, and could be relevant for the treatment of ARSACS, but also for a wide spectrum of human pathologies caused by disruption of the intermediate filaments network.

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POSTER 32

ASTROCYTE MORPHOLOGICAL CHANGES IN ABSENCE SEIZURES

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Introduction:

Typical absence seizures (ASs), characterized by 3-4 Hz spike-wave discharges in thalamocortical network, are the hallmark of Childhood Absence Epilepsy (CAE). In ASs animal models, dysfunction of thalamic GABA transporter-1 (GAT-1), exclusively expressed in thalamic astrocytes and responsible for GABA reuptake, enhances tonic inhibition. This enhancement is essential and sufficient for the generation of ASs. A neuropsychological impairment is observed in 60% of children with CAE.

Aims:

Using an ASs animal model, the Genetic Absence Epilepsy Rat from Strasbourg (GAERS), the respective Non-Epileptic control (NEC), and Wistar rats, we aimed to evaluate behavioral and cognitive comorbidities, as well as study cellular morphological differences between epileptic and non-epileptic animals.

Methods:

Behavior tests were carried out to assess learning and memory. Hippocampal synaptic plasticity was evaluated by performing long-term potentiation experiments where field excitatory postsynaptic potentials were recorded. Immunohistochemistry and Western blot assays were used to assess molecular and cellular morphological differences between epileptic and non-epileptic animals.

Results:

In Novel object recognition, Cross-modal object recognition, Y-Maze and Barnes Maze tests, we observed significant differences between GAERS and controls, suggesting an impairment in the prefrontal cortex, parahippocampal regions and hippocampal-dependent memory. LTP magnitude in GAERS was significantly lower than that from Wistar. GAERS display an increase in astrocytic morphological complexity, mainly in hippocampus, and increase GFAP expression levels, compared to Wistar and NEC.

Conclusions:

Our results suggest memory deficits in GAERS, especially in spatial working memory, object recognition and long-term memory, in line with the reduced LTP magnitude. Since GAT-1 is expressed in astrocytes and there is an increase in morphological complexity and GFAP expression in GAERS, the involvement of astrocytes in the pathology of ASs is reinforced.

Acknowledgements:

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POSTER 33

UNRAVEL THE CONTRIBUTION OF ASTROCYTES FOR THE NEUROINFLAMMATORY MILIEU IN ALZHEIMER'S DISEASE - AN INSIGHT INTO THE NLRP3 INFLAMMASOME CASCADE

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Introduction:

Alzheimer's disease (AD) is a chronic neurodegenerative disease caused by the accumulation of Amyloid-β (Aβ) peptides. AD has been associated with inflammatory events, in particular the activation of a multiprotein cytoplasmic complex, the NLRP3 inflammasome (NLRP3). This inflammasome leads to the processing of Interleukin (IL)-1β and IL-18 and can trigger pyroptosis, a form of cell death mediated by the N-terminal of Gasdermin D. In the brain regions affected by AD, astrocytes display the A1 neurotoxic phenotype and express high amounts of the Complement Component 3 (C3). It is known that this phenotype is triggered by activated microglia, through the release of IL-1α, TNF-α and C1q. However, in a context of Aβ-induced toxicity the role of NLRP3 is not yet fully understood in astrocytes. Therefore, this project aims to unravel the relevance of astrocytes for the neuroinflammatory milieu of AD, focusing on the NLRP3 cascade.

Material & Methods:

Primary cultures of astrocytes were prepared from P1-P3 Sprague Dawley rats as described by others. Astrocytes were stimulated for 24h with Aβ25-35 or with microglia-derived factors (F: IL-1α + TNF-α + C1q), or both (F/Aβ25-35), and analyzed by western blotting, immunocytochemistry and ELISA. One-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test, was used. Statistical significance was considered when $p<0.05$. Experimental approval was granted by the iMM's Institutional Animal Welfare Body (ORBEA-iMM) and the National competent authority (DGAV – Direção Geral de Alimentação e Veterinária).

Results:

Preliminary results obtained from primary cultures show a significant increase in NLRP3 domains (NLRP3, ASC and Caspase-1) with F and F/Aβ25-35 but not with Aβ25-35 alone. This was accompanied by a significant increase in IL-1β secretion as well as C3 and PTX3 upregulation, specific markers for A1-like and A2-like reactive astrocytes, respectively.

Conclusions:

These results point to an astrocytic NLRP3 activation, promoted by microglia-derived factors, that is accompanied by the formation of a heterozygous population of reactive astrocytes. Further experiments are needed to increase the number of samples studied, as well as to evaluate MCC950, a specific NLRP3 inhibitor, in astrocytic NLRP3 activation. In the future, results will be corroborated in a model of Aβ-induced toxicity in hippocampal acute slices, human tissue from AD patients and in an animal model of the disease.

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POSTER 34

INTRATHECAL INJECTION OF SECRETOME FROM ANTI-MIR-124-TREATED ALS MOTOR NEURONS IN MSOD1 MICE SHOWS PROMISE AS A THERAPEUTIC STRATEGY

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease with a fast progression and without an effective treatment. Modulation of deregulated inflammatory miRNAs in ALS [1,2,3] might be a very promising therapy. We observed that these miRNAs are part of the secretome, either as free species or cargo in small extracellular vesicles [1,4,5]. Lately, we showed that the secretome from SOD1-G93A (mSOD1) cortical astrocytes with downregulated miRNA(miR)-146a treated with its mimic recovered motor neuron (MN) and microglia steady-state functions [2]. In the same way, neurodegeneration was prevented in mSOD1 MNs with upregulated miR-124 after transfection with anti-miR-124, and their secretome counteracted microglia activation and cell spinal pathogenicity [3].

Here, we assessed the therapeutic potential of the secretome from anti-miR-124 treated mSOD1 MNs in recovering disabilities in the mSOD1 mice at early disease onset by assessing motor performance, inflamma-miRNA profile, astrocyte/microglia phenotypes and synaptic dynamics.

The modulated and concentrated secretome was injected intrathecally in the 12-week-old mSOD1 mice. Control groups were WT and mSOD1 mice, injected with the vehicle (NSC-34 basal media). We performed the limb clasping and grasping tests to evaluate the corticospinal function and footprint test to assess gait quality. The lumbar spinal cord (SC) was isolated one week after motor tests to evaluate inflammatory miRNAs (miR-124, miR-146a, miR-155), glial reactivity gene markers (GFAP, iNOS, arginase 1, IL-10 and TREM2) and synaptic-related genes (synaptophysin and PSD-95) by RT-qPCR.

The non-treated mSOD1 mice exhibited changes in hindlimb clasping and grasping and performed shorter strides. In addition, an upregulation of GFAP, miR-146a and miR-155 and downregulation of iNOS, arginase 1, IL-10, TREM2 and miR-124 were detected in mSOD1 lumbar SC. Moreover, a reduction of the post-synaptic PSD-95 with no changes in the pre-synaptic synaptophysin were also observed. However, the injected modulated secretome in mSOD1 mice recovered behaviour alterations, namely motor performance and corticospinal function, while also prevented the dysregulated miRNA profile and gene signature of neuronal/glial markers.

In sum, the secretome from the anti-miR-124 treated mSOD1 MNs prevented many disabilities associated to MN and glial pathological mechanisms in the mSOD1 mice showing promise as a novel therapeutic tool to be translated into ALS clinics if validated in future studies.

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POSTER 35

POST-TRANSLATIONAL MODIFICATIONS OF STAT3 MODULATE ITS CELLULAR DISTRIBUTION

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Aims:

Signal Transducer and Activator of Transcription 3 (STAT3) is a ubiquitous transcription factor involved in normal development, immunity and cancer. In particular, STAT3 controls GFAP gene expression for astrocyte differentiation. STAT3 activity is associated with its dimerization and subcellular localization, which are regulated by post-translational modifications (PTMs). Although STAT3 features more than 80 confirmed sites, most studies focus on the canonical tyrosine-phosphorylation at residue 705. We aim at identifying relevant PTMs involved in STAT3 subcellular localization and activity, with a focus on lesser studied residues.

Methods:

We developed a Venus-STAT3 bimolecular fluorescence complementation (BiFC) assay to visualize STAT3 homodimerization in living cells. Using this system as a template, we inactivated a series of residues that have been described to undergo PTMs. To rule out the interference of endogenous STAT3, we produced stable HeLa STAT3-Knockout (STAT3-KO) cell lines via CRISPR/Cas9 methodology. Cellular localization of STAT3 dimers was visualized in living cells by wide-field microscopy.

Results:

Resting wild-type STAT3 dimers exhibit a diffuse cytoplasmic localization, but rapidly translocate to the nucleus after incubation with Leukemia Inhibitory Factor (LIF). Taking advantage of the unique features of our Venus-STAT3 BiFC system, we showed that phosphorylation of one monomer at Y705 is enough for STAT3 nuclear translocation in response to LIF. Additional PTM-inactivating mutations on the Y705F monomer in asymmetric WT+Y705F dimers (i.e. K49R, K685R or S727A) modulate nuclear translocation. Finally, preliminary results revealed that the symmetry or asymmetry of PTM mutations have little impact on STAT3 transcriptional activity.

Conclusions:

Beyond the canonical Y705 phosphorylation, other residues susceptible to PTMs can influence nuclear translocation, such as K49, K685 and S727. We will continue to explore more symmetric and asymmetric pairs to identify relevant PTMs in STAT3 activity and biological functions. The outcomes will contribute to a better understanding of STAT3 regulation.

Conflicts of interest:

We declare no conflict of interest.

Funding and Acknowledgements:

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POSTER 36

PROTEIN COMPLEMENTATION ON NKX6-2 TRUNCATED PROTEINS ASSOCIATED WITH SPASTIC ATAXIA 8

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Aims:

NKX6-2 is a transcriptional repressor associated with cell fate of oligodendrocytes and neurons. Loss-of-function mutations cause Spastic Ataxia 8 (SPAX8), a rare recessive hereditary disease that causes hypomyelinating leukodystrophy. The most common NKX6-2 mutations involve premature termination codons (PTCs). Rendering the nascent mRNA a target for degradation via non-mediated mRNA decay. The surviving fraction will produce truncated, non-functional NKX6-2. We aim to overcome these two issues to lay the groundwork for the development of new therapies for SPAX8.

Methods:

Using site-directed mutagenesis, we recreated the 1-41 aa SPAX8-related mutation on a NKX6-2-Venus fusion protein, and the intracellular localization of wild-type and mutant forms was monitored by microscopy. We tried to recover NKX6-2 function by a protein complementation approach using 35-277 aa NKX6-2-Flagtag-P2A-T2A-mCherry fusion protein. The complementation effect on NKX6-2 localization was verified by microscopy and western blot. Additionally, we analyzed NKX6-2 activity as a transcriptional repressor through newly developed luciferase activity assays, where we inserted the NKX6-2 response element in a pRL miniTK-luciferase system.

Results:

NKX6-2-Venus full protein was observed to be fully localized in the nucleus, while SPAX8-causing mutation led to a homogeneous distribution on the cell, indicating loss of the nuclear localization signal (NLS). Bioinformatic analysis showed that the NLS is in or close to the homeobox domain (147-207 aa). Protein complementation induced a slight increase of truncated NKX6-2 in the nucleus. We are currently evaluating if the activity of NKX6-2 is recovered upon protein complementation employing our luciferase activity assay. Overexpression of wild-type NKX6-2 causes changes in nuclear morphology, and we are analyzing this effect on SPAX8-related mutation.

Conclusions:

We have confirmed some consequences cause by SPAX8-related mutation at the cellular level and tested the possibility of using protein complementation to prevent them. While our approach is currently restricted to NKX6-2/SPAX8, this proof-of-concept could be applied to other genetic disorders involving PTCs, which are associated to one-third of inherited human diseases.

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POSTER 37

RHOA IS A PUTATIVE NEGATIVE REGULATOR OF CNS MYELINATION

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Myelin production by oligodendrocytes (OLs) is critical for the physiology of the central nervous system (CNS). Myelin promotes rapid saltatory conduction of the nervous impulse and provides trophic support to axons. Both OL differentiation and myelination are highly regulated processes. Based on previous work from our lab, RhoGTPase signaling arises as an essential requirement for OL differentiation and myelination. Here, using OL-specific conditional gene ablation in mice, we study the specific role of RhoA in CNS myelination. Ultrastructural, morphometric, immunochemical and proteomic data support an essential role for RhoA as a negative regulator of axon ensheathment and myelin growth during developmental myelination.

POSTER 38

MICROGLIA REMODEL THE SYNAPTIC SIGNALING REQUIRED FOR CONTEXT-DEPENDENT COGNITIVE PERFORMANCE

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Microglia modulate synaptic activity, essential for context-dependent cognitive performance, allowing organism-level adaptations to different environmental scenarios. Yet, the microglial molecular drivers required for synaptic remodeling related to cognitive performance remain largely elusive. Here, combining conditional gene targeting, single-cell live imaging, RNA-seq, high-throughput proteomics, systems biology, and animal behavior, we mapped a molecular nexus between microglia and synapses that instructs cognitive performance. Specifically, we found that microglia use the RhoGTPase Rac1 as a relay switch to sense the brain microenvironment and drive synaptic remodeling required for experience-dependent sociability and learning related to memory. Targeting this microglial relay modifies context-dependent cognitive performance.

POSTER 39

INVESTIGATING MITOCHONDRIAL DYNAMICS AS REGULATOR OF ADULT NEURAL STEM CELL DIFFERENTIATION

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Introduction:

Neural stem cells (NSCs) are found in discrete regions of the adult mammalian brain. During adulthood, NSCs can differentiate into neurons, astrocytes and oligodendrocytes, making them a powerful tool to treat disease-related neural loss. A major aspect that remains unclear is whether mitochondrial dynamics have a role in directing NSC fate.

Aim:

Our work aims to dissect how mitochondria biogenesis, morphology and bioenergetics can modulate NSC differentiation.

Methods:

NSCs were obtained by isolating subventricular zone (SVZ) and dentate gyrus (DG) cells from P1-3 C57Bl6 mice. The isolated cells were grown in neurospheres, and consequently passaged to guarantee higher yields of NSCs. Thereafter, neurospheres were plated under specific differentiation conditions giving raise to neurons, astrocytes and oligodendrocytes. Expression of proteins involved in mitochondrial biogenesis and fusion/fission was determined.

Results:

Primary and passaged neurospheres demonstrated to be mainly composed by undifferentiated cells (SOX2 and Nestin-positive cells). Moreover, NSC population shows a proliferative capacity, evaluated by ki67 expression. Additionally, and as expected, SVZ and DG-derived NSCs are multipotent. Overall, expression of mitochondrial biogenesis-related proteins did not significantly change with NSC differentiation, in both neurogenic niches. Importantly, the levels of proteins involved in mitochondrial fusion (Mfn1/Mfn2) significantly increased while proteins involved in fission (DRP1) significantly decreased along differentiation, only in SVZ cells. Furthermore, mitochondrial number, length and area was different in the different cell types (NSCs and differentiated cells). Indeed, mitochondrial number significantly increased during astroglial and neuronal differentiation. Moreover, both NSCs and oligodendrocyte precursor cells were the cells with more elongated mitochondria. Interestingly, mitochondrial area did not change in neuronal cells, while there were significant alterations along oligodendroglial differentiation.

Conclusions:

This work further elucidates the role of mitochondrial dynamics in directing the fate of postnatal NSCs, which in the future can lead to the development of therapeutic targets for the regeneration of the Central Nervous System.

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POSTER 40

ASTROCYTIC DYSFUNCTION IS A KEY PLAYER IN SYNAPTIC PLASTICITY AND TRANSMISSION DEFICITS IN SOD1G93A MICE

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Aims:

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease, characterized by the deterioration of both upper and lower motor neurons. Despite affecting mainly the motor cortex and spinal cord, ALS patients also show signs of cognitive impairment due to changes in the hippocampus, which has also already been described in SOD1G93A (mSOD1) mice, a mouse model used in ALS studies. Moreover, the relevance of astrocytes in ALS onset and progression has recently been highlighted and recognized, having an important role in excitotoxicity and neurodegeneration. Thus, we aimed to investigate the astrocytic contribution in both synaptic activity and plasticity in two different regions of the brain, the hippocampus and motor cortex of mSOD1 mice.

Methods:

Pre-symptomatic (4-6 weeks) and symptomatic (14-18 weeks) mSOD1 mice, as well as age-matched wild-type (wt) mice were used. Astrocytic metabolism was selectively reduced using fluorocitrate (FC), for at least 15 minutes prior to each experiment, and field excitatory postsynaptic potentials (fEPSP) were recorded from both the CA1 area of hippocampal slices and the layer II/III of primary motor cortex slices. Synaptic plasticity was assessed by eliciting long-term potentiation (LTP) protocols and synaptic transmission by recording input/output curves.

Results:

In the hippocampus, we observed that symptomatic mSOD1 mice exhibit alterations in synaptic plasticity, with a higher post-tetanic potentiation (PTP) and LTP magnitudes when compared with age-matched wt mice. However, astrocytic inhibition with FC (200 μM) impaired significantly LTP in both wt and mSOD1 mice. Moreover, in the presence of FC, synaptic responses in the hippocampus decreased in both pre-symptomatic mSOD1 and wt mice, although they were significantly lower in mSOD1 mice, when compared with wt mice in the same condition. In the symptomatic phase, FC led to a similar decrease in both wt and mSOD1 mice. Regarding the motor cortex, pre-symptomatic mSOD1 mice showed an impairment in LTP magnitude and basal synaptic transmission. Interestingly, the presence of FC (100 μM) led to an impairment of LTP only in wt mice, to similar levels that of mSOD1 mice, in both stages of disease.

Conclusions:

Altogether, we further explored alterations in synaptic plasticity and transmission, as well as the role of astrocytic dysfunction, in two different affected regions of the mSOD1 mice model. These findings suggest that, in the hippocampus, astrocytes are essential for the maintenance of LTP in healthy and ALS conditions. More importantly, in the motor cortex, one of the main affected regions of the disease, mSOD1 mice presented early alterations in synaptic plasticity mechanisms and basal synaptic function, and astrocytes seem to be impaired even before the onset of symptoms.

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POSTER 41

MATRIX STIFFNESS CONTRIBUTES TO EFFICIENT OLIGODENDROCYTE DIFFERENTIATION ALTERING CHROMATIN ACCESSIBILITY

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Introduction:

Oligodendrocyte precursor cells (OPCs) are mechanosensitive cells since their biology is influenced by mechanical cues. We demonstrated that differentiation of OPCs in vitro is enhanced by substrates compliant with the rat brain (6.5 kPa). Recently, it was demonstrated that multiple sclerosis (MS) patients present an exacerbated brain softening compared with age and gender-matched controls. Taken together, changes in mechanical properties might impact cellular processes of OLs, with relevance in the context of MS. Little is known about the molecular mechanisms underlying the modulation of OL differentiation by mechanotransduction. Yet, some studies suggest that mechanical forces impact chromatin state during OL differentiation. Compressive forces increase trimethylation of lysine-9 residues of histone-3 (H3K9me3) that is associated with OL differentiation and maturation. Conversely, tensile forces enhance histone deacetylation and increase OL differentiation.

Aims:

Assessment of the molecular mechanisms involved in OL differentiation modulated by substrate stiffness through chromatin changes and transcriptional regulation.

Methods:

In this work, we used the assay for transposase-accessible chromatin with sequencing (ATAC-seq) in mouse primary OLs differentiated on substrates with distinct rigidity to assess chromatin accessibility. We used de novo motif analysis on more accessible regions to identify candidate transcription factors (TF) that may bind in differentially accessible chromatin sites. Then, we assessed Sp1 activity regulated by mechanical forces and its role on OL differentiation.

Results:

Differential analysis of ATAC-seq peaks revealed a more compacted chromatin (higher heterochromatic content) in OL differentiated on compliant substrates (6.5 kPa) when comparing with stiffer (glass) or softer conditions (2.5 kPa). These results corroborate previous reports demonstrating the enhancement of heterochromatin on OL differentiation enhanced by compressive or tensile forces. HOMER motif-discovery tool identified Sp1 as the best match among known TF motifs in all conditions. Afterwards, we assessed Sp1 mechanosensitivity through its location. We verified a higher nuclear translocation on OLs cultured on compliant substrates when comparing with those on stiffer or softer conditions after 3 days of differentiation. Indeed, Sp1 activity was higher at day 3 of differentiation. Moreover, we inhibited Sp1 during OL differentiation and observed a decrease of Mbp and RhoA expression. Additionally, we assessed the role of Sp1 and stiffness on genes involved in OL differentiation which were upregulated on glass (Atg3 and Dmtf1) or on compliant substrates (Ehmt1) using qRT-PCR. We corroborate ATAC-seq results for these genes and Sp1 has a role in its expression.

Conclusion:

Our results emphasize the importance of culture conditions for epigenomic studies and reveal that chromatin state is a critical mediator of mechanotransduction.

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