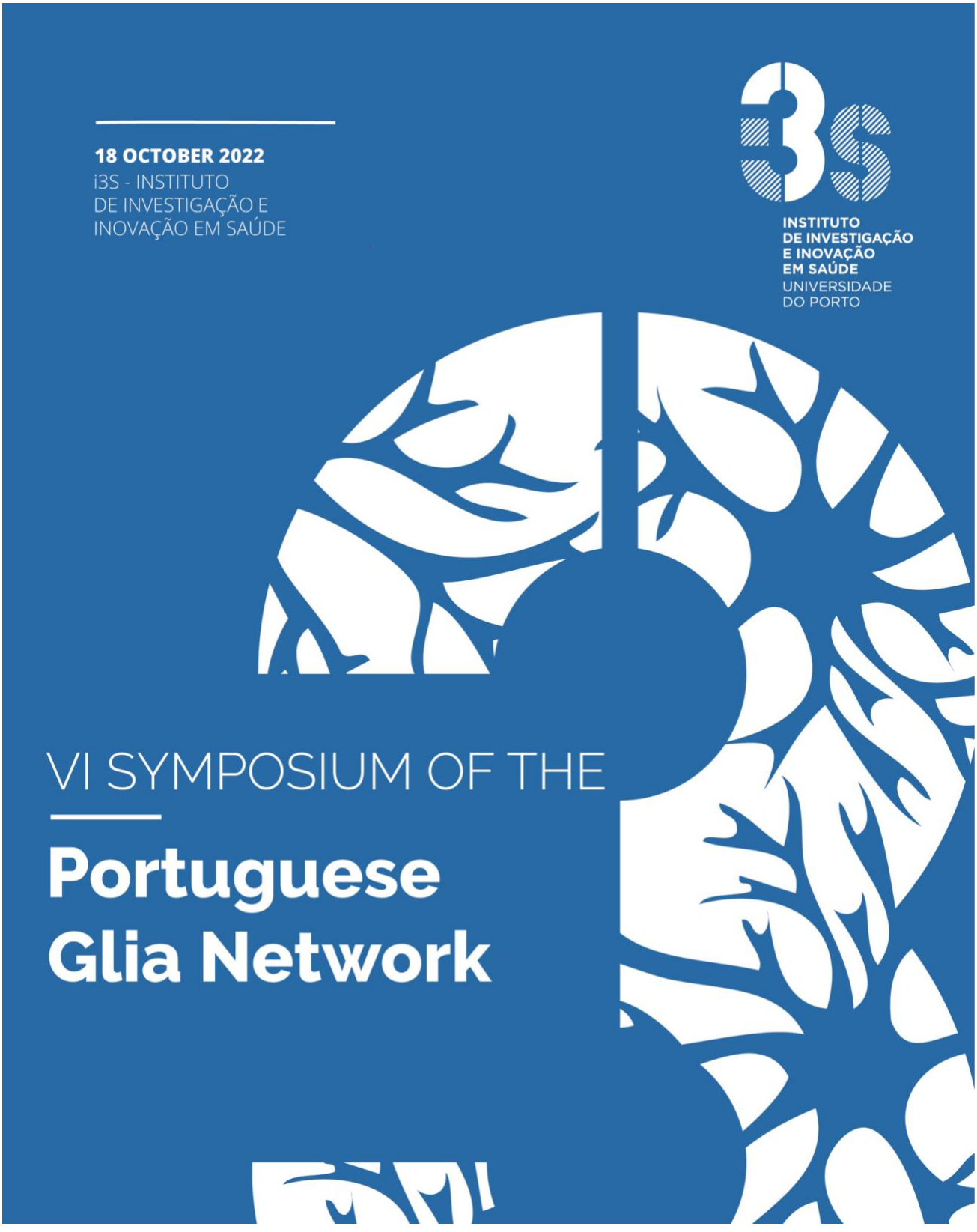
ABSTRACT BOOK



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PORTUGUESE GLIAL NETWORK

SPN SOCIEDADE PORTUGUESA DE NEUROCIÊNCIAS

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WELCOME

Dear Students and Colleagues,

This year's speakers have been chosen to cover a range of topics from the molecular determinants of glial identity and development, their physiological roles in controlling circuit function and, ultimately, behavior. We will showcase researchers from national research institutes, including João Oliveira and Luísa Pinto (ICVS, Universidade do Minho) and be joined by top European researchers, including Karine Loulier (Institut des Neurosciences de Montpellier, France), Alex Charlet (University of Strasbourg Institute for Advanced Study, France), Nathalie Rouach (Centre Interdisciplinaire de Recherche en Biologie, Paris, France), Mick Hastings (MRC Laboratory of Molecular Biology, Cambridge, UK) and Klaus Armin Nave (Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany), giving plenty of opportunities for discussion of cutting-edge glial research and networking.

This is an excellent opportunity for junior scientists to showcase their work to leaders in the field, which is why we selected four abstracts from junior scientists for oral presentations! Moreover, awards will be given to the best oral and poster presentations, so stay tuned!

We look forward to seeing you all in October at the i3S!

Matthew Holt and João Relvas
Co-Chairs

VENUE

Address
i3S – Instituto de Investigação e Inovação em Saúde
Universidade do Porto
Rua Alfredo Allen 208
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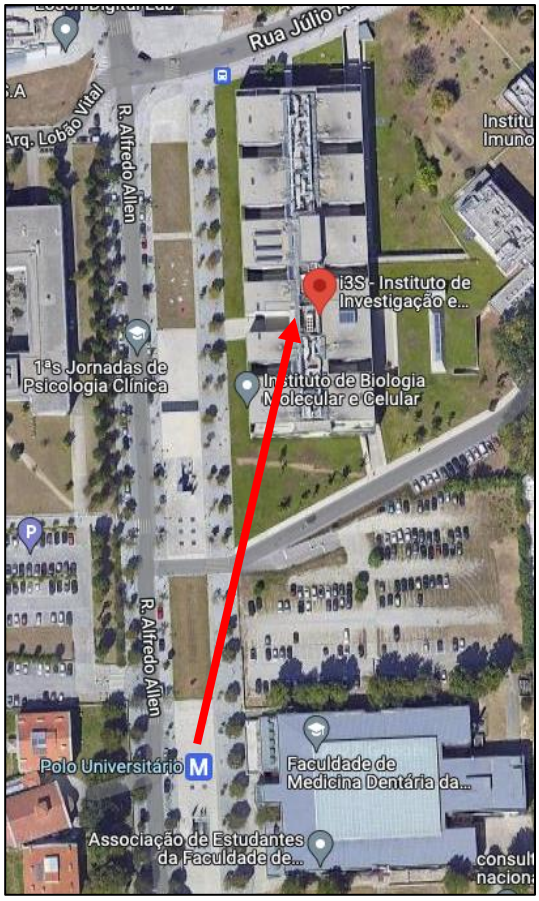

How to get to i3S
The subway (Ⓜ) is the easiest way to get to the i3S because there is a subway stop (Pólo Universitário) just next to it (<1 min walk).

From the Francisco Sá Carneiro airport

- Subway: take line E (direction: Estádio do Dragão) and get out at Trindade station. Change to line D (direction: Hospital São João) and get out at Pólo Universitário station.

From Campanhã train station

- Subway: all lines are possible (A, B, C, E and F). Get out at Trindade station and then change to line D (direction: Hospital São João) and get out at Pólo Universitário station.

From São Bento train station:

- Subway: take line D (direction: Hospital São João) and get out at Pólo Universitário station.

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PROGRAMME

8:30-9:00 REGISTRATION

9:00 WELCOME (Auditório Mariano Gago)

9:15-11:00 SESSION I
Chairs: Luísa Pinto (ICVS, University of Minho, Portugal) and Teresa Summavielle (i3S, Porto, Portugal)

9:15 1ST KEYNOTE TALK
Molecular Heterogeneity of Astrocytes: Potential Implications for Development and Function
Matthew Holt (i3S, Porto, Portugal)

10:00 Diving into the Diversity of Cortical Astrocytes through the Prism of the Unsuspected Complexity of their Developmental origin
Karine Loulier (Institut des Neurosciences de Montpellier, Montpellier, France)

10:30 Oxytocin Receptor Mediated Modulation of Amygdala Astro-Neuronal Circuits
Alex Charlet (University of Strasbourg Institute for Advanced Study, Strasbourg, France)

11:00 COFFEE BREAK

11:30-16:30 SESSION II
Chair: Matthew Holt (i3S, Porto, Portugal)

11:30 Astrocytes: Guardians of Critical Period Plasticity in the Visual Cortex
Nathalie Rouach (Centre Interdisciplinaire de Recherche en Biologie, Paris, France)

12:00 2ND KEYNOTE TALK
Astrocytes and Circadian Time-Keeping: Star Clocks
Mick Hastings (MRC Laboratory of Molecular Biology, Cambridge, UK)

12:45 Selected Talk 1: Adenosine Receptors: the On-and-Off Switch of Astrocytic Cannabinoid Signaling
Joana Gonçalves-Ribeiro (iMM João Lobo Antunes, Lisbon, Portugal)

PROGRAMME

13:00 LUNCH AND POSTER SESSION

14:30 3RD KEYNOTE TALK
Novel Functions of Oligodendrocytes in Axonal Energy Metabolism and Neurodegenerative Disease
Klaus Armin Nave (Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany)

15:15 The involvement of astrocyte calcium-dependent signaling in fear memory
João Oliveira (ICVS, University of Minho, Portugal)

15:45 Adult Astrogliogenesis: a Key Mechanism Underlying the Pathophysiology of Stress-Induced Depression
Luísa Pinto (ICVS, University of Minho, Portugal)

16:15 COFFE BREAK AND POSTER SESSION

17:15-18:15 SESSION III
Chair: João Relvas (i3S, Porto, Portugal)

17:15 Selected Talk 2: Age and Sex-specific Proteome Plasticity of Brain Microglia
Joana Moreira (i3S, Porto, Portugal)

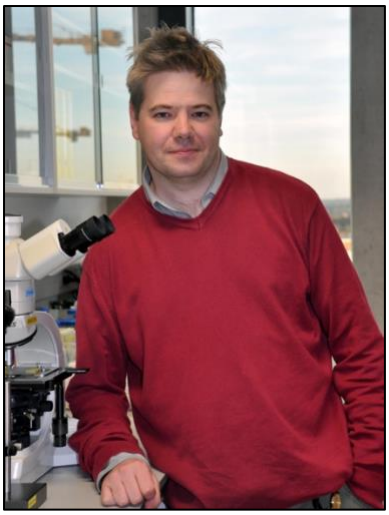
17:30 Selected Talk 3: Alterations in CNS Pathogenesis of the In Vivo Model of Multiple Sclerosis: Age Impact
Ana Rita Valente Ribeiro (iMed.ULisboa, University of Lisbon, Portugal)

17:45 Selected Talk 4: Intrathecal Application of miR-124-Based Secretome to Prevent Disease Progression in the ALS Mice
Marta Alexandra Santos (iMed.ULisboa, University of Lisbon, Portugal)

18:00 Selected Talk 5: RhoA Regulates the Onset of CNS Myelination
Raquel Vale-Silva (i3S, Porto, Portugal)

18:15 AWARDS FOR BEST ORAL AND POSTER PRESENTATIONS AND WRAP-UP

1st KEYNOTE TALK



Matthew Holt
i3S, Porto, Portugal

Matthew Holt is graduated in Applied Biochemistry (University of Liverpool) and completed his Ph.D. at the MRC Laboratory of Molecular Biology, working on the physiology of synaptic vesicle mobility and neurotransmission in retinal neurons. He went to the Max Planck Institute for Biophysical Chemistry for his post-doctoral work, which led to publication of a seminal paper on synaptic vesicle structure in Cell and is now routinely cited in neuroscience text books. He established his group at the KU Leuven, where he started looking into astrocyte-neuron interactions. As of February 2022, Matthew Holt is a group leader and ERA Chair Holder at i3S, where he runs the Synapse Biology group. A major translational spin-off from his work has been the design and exploitation of novel blood-brain barrier crossing viral vector systems for gene delivery, which allow sustained local production and secretion of therapeutics from CNS cells – the so-called 'biopharmacy' concept. He holds several patents from his work on AAV-based therapeutics and is co-founder of Aila Biotech.

SESSION I

Molecular Heterogeneity of Astrocytes: Potential Implications for Development and Function

Matthew G. Holt

Synapse Biology Group, i3S-Instituto de Investigação e Inovação em Saúde
Universidade do Porto, Portugal

Astrocytes are a numerous cell type in the central nervous system. They perform many important functions in synapse formation and maintenance, control of local homeostasis and modulation of synaptic transmission. However, the degree to which specialist astrocyte subtypes fulfil these specific tasks is currently unclear. In this talk, I will present our recent work using single cell transcriptomic and in situ hybridization approaches (1, 2), which demonstrates that astrocytes lying within the same region of adult mouse brain show distinct molecular and spatial profiles. Furthermore, I will present data showing that astrocyte layer identities are dependent on local neuronal cues and appear to be conserved in humans. Our findings are strong evidence for the existence of specialist astrocyte subtypes, which likely represent an evolutionary conserved mechanism to optimize local neural circuit formation and function (3).

1. Batiuk MY, Martirosyan A, Wahis J, de Vin F, Marneffe C, Kusserow C, et al. Identification of region-specific astrocyte subtypes at single cell resolution. Nat Commun. 2020;11(1):1220.
2. Bayraktar OA, Bartels T, Holmqvist S, Kleshchevnikov V, Martirosyan A, Polioudakis D, et al. Astrocyte layers in the mammalian cerebral cortex revealed by a single-cell in situ transcriptomic map. Nat Neurosci. 2020;23(4):500-9.
3. Pestana F, Edwards-Faret G, Belgard TG, Martirosyan A, Holt MG. No longer underappreciated: The emerging concept of astrocyte heterogeneity in neuroscience. Brain Sci. 2020;10(3).

SESSION I

Karine Loulier
Institut des Neurosciences
de Montpellier,
Montpellier, France

Throughout her career, Karine Loulier has developed and applied cutting-edge techniques (in utero electroporation, multiphoton time lapse imaging and transgenic strategies) to explore in vivo neural stem cell properties in embryonic and adult mouse brain. She contributed to highlight the role of several signaling pathways (Reelin[1], Sonic Hedgehog[2], Integrin beta1[3]) in the regulation of brain development. More recently, she implemented in J. Livet's lab MAGIC Markers multicolor genetic strategies that enable track multiple neighboring clones over long periods of time[4] and to study gliogenesis during cortical development[5]. After a PhD in Neuroscience from Paris-Sud University, she joined as a postdoctoral fellow the Center for Neuroscience Research in Washington DC and then the Vision Institute in Paris where she obtained an INSERM tenured researcher position. Karine is now leading an Atip-Avenir research group at the Institute for Neurosciences of Montpellier that probe neural cell diversity in brain development and pathologies.

Selected publications:

1. Hack I, et al (2002), *Nat Neurosci*
2. Loulier K, et al (2006), *J Neurochem*
3. Loulier K, et al (2009), *PLoS Biology*
4. Loulier K, et al (2014), *Neuron*
5. Clavreul S, et al (2019), *Nat Comms*

SESSION I

Diving Into the Diversity of Cortical Astrocytes Through the Prism of the Unsuspected Complexity of their Developmental Origin

Laura Dumas[1], Solène Clavreul[1,2], Edson Rodrigues[1], Alexandre Pattyn[1], Jason Durand[1], Hassan Boukhaddaoui[1], Jean Livet[2], Karine Loulier[1*]

[1]INSERM, Université de Montpellier, Institut des Neurosciences de Montpellier, France
[2] INSERM, Sorbonne Université, Paris, France

Introduction: Mammalian cerebral cortex functions rely on the cooperation of distinct cell types including neurons and glial cells that must be produced in defined proportions and whose imbalance can lead to severe neurodevelopmental disorders. During cerebral cortex development, neurons and glial cells, including astrocytes, are produced sequentially by neural progenitors and ensure together proper synaptic functions. While neurons have been extensively studied in the context of physiological and pathological development, cellular and molecular players responsible for the emergence of cortical diversity, and in particular astrocyte generation, remain poorly described. Astrocytes constitute an heterogeneous population in terms of morphology, molecular marker expression and function, within and among brain regions in mammals.

Materials and Methods: Using combinatorial genetic markers and multicolor imaging techniques, we marked adjacent cortical progenitors with rare color markers prior to gliogenesis and tracked their descent over long periods of time to study astrocyte development.

Results and Discussion: We showed that cortical astrocyte clones display extensive variability in terms of structural organization, location, number and subtype of generated cells. Furthermore, we demonstrated that cortical astrocyte network is generated through two developmental stages that comprise a dynamic phase of proliferation and spatial dispersion followed by a maturation phase where morphology complexity and volume increase at the single cell level. Besides, we determined that astrocyte network is supplied both pre- and perinatally by cortical progenitors. Although we did not identify dedicated subpopulations of cortical progenitors responsible for astrocyte diversity, we nevertheless found an alternative embryonic source for cortical astrocytes. In addition to characterizing the cellular and molecular properties of these progenitors located in a restricted area of the developing brain, we compared the characteristics of cortical astrocytes derived from these two distinct sources.

Conclusions: Altogether, our results highlighted the unsuspected complexity of cortical astrocyte genesis and their plasticity as they probably acquire their subtype features through interactions with their environment. Our work will allow to better understand cortical astrogliogenesis and its critical cellular and molecular components that could be altered in neurodevelopmental pathologies.

SESSION I

Alexandre Charlet
University of Strasbourg Institute for Advanced Study
Strasbourg, France

Alexandre Charlet is a group leader in Strasbourg, CNRS, and his scientific focus is to better understand how neuropeptides shape brain cellular networks and emotional status governing our daily life. In the course of his career he received several scientific awards, among which Swiss Society of Biological Psychiatry, Young investigator award and recognitions from the French academies of medicine and pharmacy.

After the defence of his PhD with Prof. P. Poisbeau in Strasbourg in 2009, he joined the lab of Prof. R. Stoop (Lausanne, Switzerland) as a postdoc. Here, he demonstrated that hypothalamic oxytocinergic neurons send functional axonal projections to the central amygdala. He then obtained a position in Strasbourg , at the Institute for Cellular and Integrative Neuroscience, to study how neuropeptidergic systems, in first line of which oxytocin, tune cellular networks activity to modulate emotion-related behaviour: this resulted in milestone publications in the field in oxytocin in leading journals. Notably, his team identified a new key actor in the intermingle between neuropeptides and emotional processing: astrocytes, which is the topic of his present talk.

SESSION I

Oxytocin Receptor Mediated Modulation of Amygdala Astro-Neuronal Circuits

Alexandre Charlet

University of Strasbourg Institute for Advanced Study
Strasbourg, France

Oxytocin is a hypothalamic neuropeptide with a wide range of functions. The implication of this peptide have recently been highlighted in modulating neuronal networks responsible for complex emotional reactions, such as pain or anxiety. However, oxytocin effects on glia have long been left behind. Therefore, the actual dogma is that oxytocin, like other neuropeptides, acts exclusively via neuronal receptors in the central nervous system. However, we recently highlighted the key role of astrocytes in oxytocinergic modulation of neuronal networks in the central amygdala. During this presentation, I will recapitulate those findings and bring new insights into the complex regulatory functions of astrocytes, as driven by oxytocin.

SESSION II

Nathalie Rouach
Centre Interdisciplinaire de
Recherche en Biologie
Paris, France

Nathalie Rouach is a neurobiologist developing research on the role of glial cells in brain physiology and pathologies. She is a Director of Research at Collège de France, Paris. She received in 2002 her Ph.D. in Neuroscience, performed jointly at University Pierre and Marie Curie and the Weizmann Institute, where she studied the contribution of astrocytic gap junctional communication to neuroglial network interactions. She then joined the laboratory of Roger Nicoll at University of California San Francisco as a postdoc, where she worked on glutamate receptors trafficking and synaptic plasticity. She now runs the laboratory « Neuroglial Interactions in Cerebral Physiopathology and Pathologies » within the Interdisciplinary Center for Research in Biology at the Collège de France. Her research aims at determining whether and how astrocytes play a direct role in information processing. In particular, her team explores the molecular modalities and functional consequences of neuron-glia interactions in various physiological and pathological contexts, such as memory, social interactions, epilepsy or intellectual disability, with ex vivo and in vivo studies of neuronal excitability, synaptic transmission and plasticity, synchronization of neuronal networks, and cognitive functions in mouse models or human tissues. She is a member of several scientific councils and has received several awards including the Human Frontier Career Development award, Silver Medal of the City of Paris and ERC Consolidator grant. She is the author of 80 publications in peer-reviewed journals.

SESSION II

Astrocytes: Guardians of Critical Period Plasticity in the Visual Cortex

Nathalie Rouach

Centre Interdisciplinaire de Recherche en Biologie
Paris, France

Brain postnatal development is characterized by critical periods of experience-dependent remodeling. Termination of these periods of intense plasticity is associated with settling of neuronal circuits, allowing for efficient information processing. Failure to end critical periods thus results in neurodevelopmental disorders. Yet, the cellular processes defining the timing of these developmental periods remain unclear. Here I will present data showint in the mouse visual cortex that astrocytes control the closure of the critical period. We uncovered a novel underlying pathway involving regulation of the extracellular matrix that allows interneurons maturation via an unconventional astroglial connexin signaling. Our results thus demonstrate that astrocytes not only influence activity and plasticity of single synapses, but are also key elements in the experience-dependent wiring of brain developing circuits.

2nd KEYNOTE TALK

Mick Hastings
MRC Laboratory of Molecular Biology, Cambridge, UK

Michael Hastings is a Programme Leader and Head of the Division of Neurobiology at the MRC Laboratory of Molecular Biology, Cambridge, U.K. He graduated from the University of Liverpool (1977) with a BSc in Marine Biology. For his PhD in Marine Ecology, again at Liverpool, he studied tidal and lunar rhythms in intertidal crustaceans (1980). He then took a post-doc with Joe Herbert in the Department of Anatomy, University of Cambridge (Oct. 1981), to investigate the role of melatonin and circadian clocks in seasonal fertility of mammals.

Establishing his own laboratory in the Department of Anatomy, he was appointed to a Junior Lectureship in Anatomy in 1984 and became a Lecturer in 1988. He was elected a Fellow & College Lecturer at Queens' College, Cambridge (1987). His research interests moved towards the neurobiology of circadian clocks, with a focus on the suprachiasmatic nucleus (SCN), the brain's principal pacemaker, and the neurochemistry of its entrainment by environmental cues. He was appointed Reader in Neuroscience in 1998.

In 2001 he left the University to become a Programme Leader in Circadian Neurobiology at the MRC Laboratory of Molecular Biology, Cambridge, U.K. This enabled him to develop a molecular genetic approach to examine the core mechanisms of circadian time-keeping in the SCN, and its effects on behaviour and the temporal organisation of peripheral organs. Being an early adopter of transcriptomic and proteomic approaches, he has illuminated the inter-relationships between the SCN and subordinate tissue-specific clocks across the body, defining the impact of the circadian system on vital metabolic functions. His current areas of interest include elucidation of the molecular, genetic and cellular mechanisms that underpin cellular clocks, and definition of the mechanisms that weld the individual clock cells (both neurons and astroglia) of the SCN into a robust synchronised time-keeping circuit.

In 2008 he was elected to Fellowship of the Academy of Medical Sciences and was also elected as President of the Society for Research on Biological Rhythms. In 2010 he was elected to Fellowship of the Royal Society, London. In October 2013 he was appointed Joint Head of the Division of Neurobiology at the LMB and in May 2016 became sole Head of Division. Additional national roles include membership of the MRC Neurosciences and Mental Health Board, and Deputy Chair of the Royal Society University Research Fellowships Committee.

SESSION II

Astrocytes and Circadian Time-Keeping: Star Clocks

Michael H. Hastings, PhD, FMedSci, FRS

MRC Laboratory of Molecular Biology, Cambridge, UK

Circadian (*circa- -diem*) rhythms are the daily cycles of physiology and behaviour that adapt us to the solar cycle of day and night [1]. The most obvious is the cycle of sleep and wakefulness, but this is underpinned by myriad metabolic, neuroendocrine and autonomic cycles that sustain our 24-hour life. Disruption of these daily programmes, such as that arising from shift-work, ageing or neurodegeneration, carries a heavy cost for mental and physical health. These rhythms are generated endogenously, free-running in temporal isolation and co-ordinated by the central clock of the suprachiasmatic nucleus (SCN) of the hypothalamus[2]. The cellular clock mechanism pivots around a transcriptional/post-translational negative feedback loop (TTFL), in which *Period* (*Per*) and *Cryptochrome* (*Cry*) genes are trans-activated by heterodimers of CLOCK and BMAL, which is in turn inhibited by accumulating PER:CRY complexes, establishing an oscillation with a period of ~24 hours. Until recently attention has focused on SCN neurons as the primary time-keepers, but recent intersectional genetic approaches have revealed that SCN astrocytes also have active TTFLs and circadian cycles of cellular activity, as revealed by intracellular calcium rhythms ($[Ca^{2+}]_i$). Intriguingly, both TTFL and $[Ca^{2+}]_i$ rhythms are oppositely phased to those of SCN neurons, with peaks in circadian night whereas SCN neurons are active in circadian daytime [3]. This raises the possibility that astrocyte and neuronal clocks interact to control overall SCN function. Indeed, when the period of the cell-autonomous TTFL of SCN astrocytes is genetically modified in mice, the entire SCN adopts the new astrocyte-specific period and so do circadian rhythms of behavioural[3],[4],[5]. Furthermore when SCN astrocytes are the only circadian-competent cells in the mouse, they nevertheless sustain robust rhythms of rest/ activity [6]. The mechanism by which astrocytes communicate their circadian time to SCN neurons remains an open question. These findings demonstrate that astrocytes are capable of generating their own de novo information (circadian timing cues) and using it to control neuronal activity and thus whole-animal behaviour.

1. Dunlap, J. C., Loros, J. J. & Decoursey, P. J. *Chronobiology: biological timekeeping*. (Sinauer, 2004).

2. Hastings, M. H., Maywood, E. S. & Brancaccio, M. Generation of circadian rhythms in the suprachiasmatic nucleus. *Nat Rev Neurosci* 19, 453-469, doi:10.1038/s41583-018-0026-z (2018).

3. Brancaccio, M., Patton, A. P., Chesham, J. E., Maywood, E. S. & Hastings, M. H. Astrocytes Control Circadian Timekeeping in the Suprachiasmatic Nucleus via Glutamatergic Signaling. *Neuron* 93, 1420-1435 e1425, doi:10.1016/j.neuron.2017.02.030 (2017).

4. Tso, C. F. *et al.* Astrocytes Regulate Daily Rhythms in the Suprachiasmatic Nucleus and Behavior. *Curr Biol* 27, 1055-1061, doi:10.1016/j.cub.2017.02.037 (2017).

5. Patton, A. P., Smyllie, N. J., Chesham, J. E. & Hastings, M. H. Astrocytes sustain circadian oscillation and bidirectionally determine circadian period, but do not regulate circadian phase in the suprachiasmatic nucleus. *J Neurosci*, doi:10.1523/JNEUROSCI.2337-21.2022 (2022).

6. Brancaccio, M. *et al.* Cell-autonomous clock of astrocytes drives circadian behavior in mammals. *Science* 363, 187-192, doi:10.1126/science.aat4104 (2019).

SESSION II—SELECTED 1

Adenosine Receptors: the On-and-Off Switch of Astrocytic Cannabinoid Signaling

Joana Gonçalves-Ribeiro[1,2], Sara Pinto [1,2], Oksana. Savchak[1,2], Carmen Nanclares[5], Rafael Faria Lopes[3], Carlos Meneses[3], Rafael Santisteban[4], Alejandro Lillo[4], Gemma Navarro-Brugal[4], Rafael Franco[4,] Ana M. Sebastião[1,2], Alfonso Araque[5], Sandra H. Vaz[1,2]

[1]Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina da Universidade de Lisboa (FMUL), Lisboa, Portugal; [2]Instituto de Farmacologia e Neurociências, FMUL, Lisboa, Portugal; [3]Inst. Superior de Engenharia de Lisboa, Área departamental de Engenharia de electrónica e telecomunicações e de computadores, Lisboa, Portugal; [4] Molecular Neurobiology Laboratory, Department of Biochemistry and Molecular Biomedicine, Universitat de Barcelona, Barcelona, Spain; [5]Department of Neuroscience, University of Minnesota, Minneapolis, MN, USA

The medial prefrontal cortex (mPFC) is involved in high cognitive functions such as working memory, as well as emotional responses. Similarly to the rest of the cortex, it is organized into layers, in which neurons and astrocytes sample long-range inputs and interact via local connections (1). Astrocytes are electrically non-excitable cells that communicate with other cell types through different mechanisms that are largely regulated by intracellular Ca^{2+} levels. These Ca^{2+} fluctuations in astrocytes are then modulated by environmental conditions, such as the astrocytic CB1R activation, eliciting Ca^{2+} transients, that lead to the modulation of synaptic transmission(3). Astrocytes also express other GPCR receptors such as adenosine A_1 and A_{2A} receptors (A_1R, $A_{2A}R$), a Gi, and Gs GPCR respectively. Although not coupled to Gq receptors these receptors are able to modulate glial Ca^{2+} signaling by altering the activity of other GPCR receptors (4), however, nothing is known regarding adenosine receptors and CB1R crosstalk in astrocytes.

Thus, this work aims to fill the gap in the physical and functional crosstalk between CB1R and adenosine receptors specifically in astrocytes, and its role in synaptic activity in the mPFC.

In rat primary astrocytic cultures, it was observed a physical interaction between CB1R with both A1R and with A2AR by Proximation Ligation assay (PLA) and Bioluminescence Resonance Energy Transfer (BRET). CB1R-mediated Ca^{2+} transients (elicit by a puff with the synthetic cannabinoid ACEA) were positively modulated by the previous activation of A1R and inversely modulated by A2AR activation.

Field-excitatory post-synaptic potentials were recorded in mPFC of slices prepared from WT (IP3R2-WT) and knockout mouse of IP3R2 (which obliterates Ca^{2+} responses in astrocytes- IP3R2-KO). LTP was induced by delivering a train of 100 Hz (50 pulses, 0.5 s duration) for a priming effect, which was 15 min later followed by four trains of 100 Hz (50 pulses, 0.5 s duration, 1 every 10 s). The time course of the magnitude of long-term potentiation (LTP) was evaluated. CB1R activation lead to a significant increase of LTP magnitude in IP3R2WT,but surprising CB1R activation decreased significantly the LTP magnitude in the IP3R2KO mice. Moreover, our preliminary data indicated that in IP3R2-WT the blockade of A1R abolishes the CB1R effect on the mPFC LTP, however further experiments are needed.

Thus, it was shown that CB1R activation has an opposite effect on mPFC synaptic plasticity depending on the presence of astrocytic Ca^{2+} signaling. Regarding CB1R and adenosine receptors, both receptors crosstalk with CB1R and A1R increases CB1R-mediated Ca^{2+} signaling while A2AR decreases it, demonstrating direct crosstalk between cannabinoid and adenosine receptors in astrocytes.

Mitrić, Miodrag, et al. "Layer-and subregion-specific electrophysiological and morphological changes of the medial prefrontal cortex in a mouse model of neuropathic pain." Scientific reports 9.1 (2019): 1-13.

Kofuji, Paulo, and Alfonso Araque. "G-protein-coupled receptors in astrocyte–neuron communication." Neuroscience 456 (2021): 71-84.

Navarrete, Marta, and Alfonso Araque. "Endocannabinoids mediate neuron-astrocyte communication." Neuron 57.6 (2008): 883-893.

Porter, James T., and Ken D. McCarthy. "Adenosine receptors modulate [Ca2+] i in hippocampal astrocytes in situ." *Journal of neurochemistry* 65.4 (1995): 1515-1523.

SESSION II—SELECTED 2

Age and Sex-Specific Proteome Plasticity of Brain Microglia

Tedim-Moreira, J.[1,2], Relvas, J.B. [1,2], Socodato, R.[1]

[1]i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal
[2]Faculdade de Medicina, Universidade do Porto, Portugal

Corresponding author: jmoreira@ibmc.up.pt and renato.socodato@ibmc.up.pt

Microglia are myeloid cells that populate the nervous early during embryogenesis. They comprise approximately 5-10% of all cells in the adult brain. Under physiological conditions, microglia constantly extend and retract their many processes to scan the brain parenchyma. Detection of a pathogen, cell debris, or tissue injury triggers microglia activation, a process involving profound morphological and transcriptional changes. Activated microglia can re-establish tissue homeostasis, but exacerbated activation often contributes to tissue damage**.**

Here we aim to clarify how age and gender influence the overall proteome of brain microglia. To address this, we MAC-sorted microglia from male and female mice at two time points: young adult (P120) and old adult (P480). After that, we performed high-throughput quantitative label-free proteomic profiling to dissect age and gender changes in the amounts of proteins involved in specific microglial functions. Analyzing the comparison between young females and young males, we identified 4688 proteins, which is higher than what other datasets reported in the literature (1, 2). Regarding the same comparison for old adult mice, almost 5000 proteins were identified. Of the identified proteins, more than 400 were differentially expressed (DE) in young adults, and nearly 300 DE proteins were found in old adults. In parallel, we explored aging differences between old *vs.* young males and old *vs.* young females. More than 4500 and 5000 proteins were identified in these comparisons, respectively. Stringent statistical analyses identified more than 400 DE proteins in both comparisons. Subsequently, we used the DE proteins in downstream bioinformatics analyses to uncover the microglial signaling pathways explicitly influenced by gender and aging. Surprisingly, the identified pathways were related to different aspects of microglial immune signaling, with the most exacerbated differences being gender dependent.

We conclude that aging remodels the proteome of microglia independently of sex-dependent traits. Moreover, our data also predict that as aging progress, male and female microglia will trigger divergent intracellular transduction pathways when engaged by the same immune stressor.

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SESSION II—SELECTED 3

Alterations in CNS Pathogenesis of the In Vivo Model of Multiple Sclerosis: Age Impact

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Introduction: In Multiple Sclerosis (MS), a chronic autoimmune, inflammatory and neurodegenerative demyelinating disorder of the Central Nervous System (CNS), the age of onset has a tremendous impact on disease worsening along with poor response to treatments. 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. We recently describe that in an *in vivo* model of MS, age is positively correlated with worse disease phenotype[1]. However, less is known concerning the influence of age in the interplay between immune system and glial cell response in MS and how it may correlate with disease severity/progression along age. Therefore, we aimed to evaluate the impact of age in the response of glial cells and associated functional genes expression along disease progression 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. Mice were induced on day 0 and followed for 23 days. On sacrifice day, mice spinal cords were isolated for immunohistochemistry (Iba1 for microglia/macrophages, and GFAP for astrocytes; BASHY for myelin debris[2]; as well as CD4 and CD8 to effector and cytotoxic T cells) and for RealTime-qPCR gene expression.

Results: Our results demonstrated that 12-months-old EAE mice showed a significantly increase in the percentage of $Iba1^+$ and $GFAP^+$ cells in demyelinated lesion areas compared to 3- and 6-months-old EAE mice ($p<0.05$). Gene expression revealed decreased expression of complement genes (*C1qA*, *B* and *C*; $p<0.05$) in 12-month-old EAE mice as well as decreased expression of *TREM2* compared to 3- and 6-month-old EAE mice. Microglia phagocytic capacity, assessed by co-localization of myelin debris with Iba1, revealed altered function in 12-month-old EAE mice compared to 3- and 6-month-old EAE mice. Interestingly, 12-month-old EAE mice showed an upregulated T cell associated cytokines (*IFN-γ*, *IL-17* and *IL-10*; $p<0.05$) accompanied with increased number of infiltrating $CD4^+$ and $CD8^+$ T cells in spinal cord lesions assessed by immunostaining.

Conclusion: Our results demonstrated that age influences EAE course and is accompanied with increased gliosis in parallel with variable expression of inflammatory molecules. Most importantly, aging alters crucial functions of immune response and regenerative microglia phenotype potentially contributing to a more EAE aggressive disease phenotype.

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This work was supported by the following grants: Grant for Multiple Sclerosis Innovation – Merck Serono to AF, PhD grant SFRH/BD/138542/2018 to ARR from Fundação para a Ciência e Tecnologia, Portugal (FCT), and in part by UIDB/04138/2020 and UIDP/04138/2020 – from FCT to iMed.ULisboa. Nothing to disclose.

SESSION II

João Oliveira
Life and Health Sciences Research Institute, University of Minho, Portugal

João Filipe Oliveira (PhD) graduated in Pharmaceutical Sciences in 2004 at the Faculty of Pharmacy, University of Porto. He obtained his PhD degree in 2009 from the Medicine Faculty – University of Leipzig, in Germany under the supervision of Prof. Peter Illes.

João Filipe Oliveira received a Marie Curie Fellowship (FP7-PEOPLE-2010-IEF273936 STRESSEDASTROCYTES) to integrate the Institute of Health and Life Sciences Research, University of Minho, where he is currently Principal Investigator on the topic "Astrocyte involvement in behavior, in health and disease".

João Filipe Oliveira is last/corresponding author in several scientific reports. He has been invited to talk at national and international congresses. He is currently supervising post-doc and MSc and PhD students. He has been awarded research grants, fellowships, and contracts, namely the DAAD PhD Scholarship (2005) and Marie Curie Intra-European Fellowship (2011). He is frequently invited for reviewing grant and article proposals by international funding agencies and Scientific Journals.

João Filipe Oliveira is the coordinator of the Portuguese Glial Network and he is a member of the Portuguese Society of Neuroscience, FENS and SfN.

SESSION II

The Involvement of Astrocyte Calcium-Dependent Signaling in Fear Memory

João Filipe Oliveira[1,2,3]

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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 derived from different sources and displaying complex spatiotemporal properties. Calcium elevations appear spatially distributed in global (soma and main processes) and focal regions (microdomains). This astrocytic calcium activity is thought to underlie the involvement in synaptic transmission, metabolism, and brain homeostasis. In this work, we studied the IP3 receptor type 2 knockout (IP3R2 KO) mouse model that lacks global calcium elevations in astrocytes to disclose its implications for circuit structure and function. We found an influence of global astrocyte calcium on fear memory. We performed a structural and molecular analysis of cortico-limbic regions that revealed a shift to immature spines in pyramidal neurons of the dorsal hippocampus that could support the changes in synaptic plasticity underlying our behavioral observations. The detailed characterization of the IP3R2 KO mouse model revealed a calcium-dependent molecular pathway modulating hippocampal circuit function. These findings broaden the scope of astrocytic modulation of circuit function and behavior.

SESSION II

Luísa Pinto
Life and Health Sciences Research Institute, University of Minho, Portugal

Luísa Pinto graduated in Biochemistry from the University of Coimbra and performed an internship at the Karolinska Institute (Sweden) on therapeutic tools for Parkinson's Disease. She holds a PhD in Neurosciences by the Ludwig-Maximilians-Universität München (Germany). She is currently Assistant Researcher with tenure and team coordinator of the "Brain circuits and neuron-glia adaptations" thematic line at ICVS, focusing on novel therapeutics for Depression and Neurodegenerative Disorders. She is also Invited Assistant Professor at the School of Medicine, University of Minho, since 2009, CEO of a spin-off company "BNML–Behavioural & Molecular Lab", since 2012, and co-founder Member of the European College of NeuroPsychopharmacolgy (ECNP) Thematic Network on Resilience.

Luisa Pinto is currently supervising 5 PhD and 1 MSc students. Moreover, 1 Post-doc, 11 PhD and 16 MSc students already finished their degree under her supervision. With the goal of establishing a mechanistic link between brain neuro-glio plasticity and depression, research in her group led to i) demonstration that hippocampal cytogenesis is essential for the sustained remission from depression using animal models; ii) deeper understanding of processes implicated in the onset, treatment and recurrence of depression by characterizing the cellular signatures of antidepressants; iii) first proof that AP2y crucially modulates adult glutamatergic neurogenesis and cognition; iv) uncover a critical role of epigenetic mechanisms, namely TET3, in brain plasticity and behavior.

Luisa Pinto has 3 patents, published 70 scientific publications, many in high-profile journals such as *Neuron*, *Cell Stem Cell*, *Nature Neuroscience* (as 1st author) and *Molecular Psychiatry* (as senior author), with a total impact factor of 463 (average IF = 7) and cited above 2000 times (h index=28).

Luisa Pinto has received several distinctions for her work including the prestigious Doktorandenpreis des VdFF, 2009 in Germany, 1st Prize of the ANJE Young Entrepreneur Award in 2013 and the Nature Research Award for Driving Global Impact (runner up, 2019). She was granted various highly competitive positions, including an FCT Investigator position in 2014, and a CEEC Assistant Researcher position in 2020 (8% approval rate). Recognition of her scientific expertise and leadership qualities is reflected in invitations to serve as reviewer for the Postdoctoral IdEx Programme of Excellence, French National Research Agency, ERA-NET NEURON JTC2018-Mental Disorders, Research Foundation Flanders; Graduate Women in Science program and FCT. Her intra-curricular activities at ICVS include service as i) Coordinator and regular lecturer of post-graduate courses; ii) Coordinator of Bio-entreneurship (since 2017); iii) Coordinator of advanced post-graduate courses (since 2022). She gained scientific management experience by coordinating FCT-funded projects, a Bial-funded project and various pharma-sponsored projects. She also served as Local Coordinator of the EU-funded Horizon2020 ITN-Network on Metabolic Dysfunctions associated with Pharmacological Treatment of Schizophrenia (total 3.750,899€; 2017-2021).

SESSION II

Adult astrogliogenesis: a key mechanism underlying the pathophysiology of stress-induced depression

Luisa Alexandra Meireles Pinto

Life and Health Sciences Research Institute (ICVS),
School of Health Sciences, University of Minho, Braga, Portugal

Post-natal neuro and glio-plasticity is largely driven by the transduction of environmental stimuli into essential neuroadaptations. Neuro- glio-plastic maladaptations often result in the manifestation of pathological traits, from which depressive behavior is a paradigmatic example. We are investigating the pathological basis of both physiological and behavioral impairments and their potential epigenetic and molecular determinants. It is also our aim to study how depression and antidepressant drugs can modulate epigenetic patterns in key limbic areas and how this impact in the transduction of their effects.

In this talk I will focus on the mechanistic link between neuro- and glio-plasticity and depression, taking into account the dynamic spatio-temporal events that define plasticity and the dichotomy between dorsal and ventral hippocampus. Moreover, we will show how glial-restricted precursor cells (GRPs) are able to modulate neuronal networks and behavior in the adult brain hippocampus. Our results highlight GRPs as a promising therapeutic approach for specific behavioral domains known to be affected by mood disorders, such as depression. Altogether, we intend to dissect the molecular cascade leading to neuron-glia/behavioral dysfunction to gain insights into the underpinnings of susceptibility and resilience to depression.

SESSION II—SELECTED 4

Intrathecal Application of miR-124-Based Secretome to Prevent Disease Progression in the ALS Mice

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Introduction: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease with no effective cure, characterized by motor neuron (MN) loss, and glial dysfunction. We previously observed that inflammatory-associated microRNAs (miRNAs) were dysregulated in the spinal cord (SC) and cerebral cortex of SOD1^{G93A} (mSOD1) mice, as well as in the secretome from mSOD1 MNs and glial cells[1-3]. Our prior studies identified that upregulated levels of miR-124 in mSOD1 MNs led to their loss of function and microglia activation[4,5]. Interestingly, the transfection of mSOD1 MNs with anti-miR-124 prevented such impairment and their secretome (preconditioned secretome) counteracted pathological features and neuroimmune homeostatic imbalance in the SC organotypic cultures from 12-week-old early symptomatic mSOD1 mice[5]. Therapeutic effects of this preconditioned secretome were never explored in the mSOD1 mice, though recent evidence sustains that cell secretomes may have therapeutic effects[3]. In this study, we aimed to identify the ability of the secretome from anti-miR-124-treated MNs to halt/delay disease progression in the mSOD1 mice from the early to the symptomatic stage after its intrathecal injection.
Materials and Methods: mSOD1 NSC-34-MN-like cells were transfected with anti-miR-124, as usual in our lab[2]. The secretome from these treated cells was collected, concentrated, and administered intrathecally in the early symptomatic mSOD1 mice (12-week-old). Control groups were aged-matched WT and mSOD1 mice injected with vehicle (basal neuronal media). At 15-week-old (symptomatic stage), one week after the behavioral tests, the animals were sacrificed, and the SC was collected to assess neurodegeneration (Fluoro-Jade B staining) and gene (RT-qPCR) and protein (western blot and immunohistochemistry) profiles, through which we evaluated the inflamma-miRNA signature, glial phenotypes, and neuronal synaptic dynamics. GraphPad Prism 8.0.1 software was used for data analysis.
Results and Discussion: At the molecular level, the injection of this preconditioned secretome enhanced NeuN mRNA/protein expression levels, as well as the Dlg4/Mbp/Plp/Trem2/Arg1/Inos/Il-10 genes, thus precluding the neuronal/glial cell dysregulation that characterizes the ALS mice. The upregulated GFAP/Cx43/S100B/Iba-1 and the inflamma-associated miRNAs (miR-146a/miR-155/miR-21) displayed by the symptomatic mSOD1 mice were also prevented in mice injected with the preconditioned secretome. In summary, this innovative strategy prevented glial reactivity/dysfunction, neurodegeneration, motor deficits and the inflammatory-dynamic imbalance 3 weeks after the injection when the mSOD1 mice are at the symptomatic stage.
Conclusions: This study demonstrates that the pathological MNs can be engineered for miR-124 and their secretome used for autologous transplantation, once its intrathecal administration in the 12-week-old mSOD1 mice prevented pathological events at the symptomatic stage. We emphasize that this is a promising cell-free based therapeutic strategy with potential to be translated into clinics for patients stratified for miR-124 upregulated levels.

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1. Gomes *et al*. **2020**,*395*,112209; **2.** Cunha *et al*. **2018**,*55*,4207-4224; **3.** Barbosa *et al*. **2021**,*9*,634355; **4.**Pinto *et al*. **2017**,*11*,273; **5.** Vaz *et al*. **2021**,*22*

SESSION II—SELECTED 5

RhoA Regulates the Onset of CNS Myelination

Vale-Silva, R.[1,2], Seixas, A.[1], Paes de Faria, J.[1], Relvas, J.B.[1]

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The normal functioning of the central nervous system (CNS) greatly depends on myelination of axons by oligodendrocytes. Myelin ensheathment not only provides trophic support but also ensures a rapid propagation of signals by facilitating saltatory conduction of the nervous impulse (1, 2). Unsurprisingly, myelination is a highly controlled process, regulated by different intrinsic and extrinsic factors (reviewed in (3)). Among the multitude of known and putative regulators, RhoA has been described as a possible mediator of oligodendrocyte differentiation (ex: (4, 5)). Evidence of RhoA involvement in myelination was also provided by our lab, as RhoA activity is decreased in hypermyelinating *Pinch2* mutants (6).

With this work, we aim to clarify the role of RhoA in both oligodendrocyte biology and myelination. We are using conditional ablation of *Rhoa* in oligodendrocytes, together with electron microscopy, immunohistochemistry, western blot (WB) analyses and quantitative label-free proteomics in our studies. Results obtained so far showed that the ablation of RhoA leads to early onset of myelination and/or facilitation of myelin growth as supported by an increased number of myelinated axons in the optic nerve (ON) and spinal cord (SC) at P15. This results in increased myelin thickness, which can be observed in SCs at P15 and at P30 in both SCs and ONs. The analysis of canonical pathways associated with CNS myelination (reviewed in (3)) at P15 showed increased p-ERK1/2 levels, suggesting a regulation of protein biosynthesis. Also, levels of p-Cofilin were decreased, suggesting increased actin dynamics. At P30, WB results did not show significant alterations in myelination canonical pathways. However, proteomics data at P30 show downregulation of the majority of the identified proteins, including those associated with supramolecular organization, microtubule and actin cytoskeleton organization.

Overall, we hypothesize that RhoA may be important for the temporal regulation of the onset of myelination and possibly for the control of myelin wrapping. We are exploring whether changes in oligodendrocyte biomechanical properties resulting from the ablation of RhoA are involved in the phenotypes observed.

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3RD KEYNOTE TALK

Klaus Armin Nave
Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany

Klaus Armin Nave studied biology, chemistry and physics in Heidelberg (1977-1983), and obtained his PhD at the University of California San Diego in 1987. In 1988, he did a postdoc at The Salk Institute La Jolla. In 1991, he started as an independent group leader at the Centre for Molecular Biology Heidelberg (ZMBH), and obtained his German Habilitation in 1996. In 1998, he became Professor of Molecular Biology at the University of Heidelberg. Between 1999-2021, Klaus Armin Nave was the Director and Scientific Member at the Max Planck Institute of Experimental Medicine and, since 2022, he is now the director of the Max Planck Institute for Multidisciplinary Sciences.

SESSION III

Novel Functions of Oligodendrocytes in Axonal Energy Metabolism and Neurodegenerative Disease

Klaus Armin Nave

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Brain function and the integrity of white matter tracts depend on the continuous supply of glucose. We had previously shown that myelinating glial cells provide pyruvate/lactate to spiking axons. Since oligodendrocytes maintain myelin as a highly dynamic compartment with an active lipid metabolism, we hypothesized that fatty acids of the myelin compartment can also serve as an energy reserve when glucose is lacking. To model such white matter starvation in mice, we deleted the glucose transporter GLUT1/Slc2a1 gene from mature oligodendrocytes, which led to a gradual myelin sheath thinning in the absence of pathology. To obtain mechanistic insight, we studied acutely isolated optic nerves. Surprisingly, oligodendrocytes survive glucose deprivation better than astrocytes, but this requires both oxygen and fatty acid beta-oxidation. Fatty acid oxidation in white matter also contributes to axonal ATP generation and basic axonal conductivity. We suggest a model of myelin dynamics in which fatty acid turnover buffers energy metabolism under low glucose conditions. This may explain the gradual loss of white matter in a range of neurodegenerative diseases with underlying hypometabolism.

Using genetic models of premature white matter aging, such as Cnp1 mutant mice, we found that myelin abnormalities can be potent drivers of amyloid deposition in Alzheimer model mice, as quantified by whole brain light sheet microscopy. Underlying is an accumulation of the Aβ producing machinery within the axonal swellings, thereby increasing APP cleavage. Surprisingly, Alzheimer mice with perturbed myelin lack plaque-corralling microglia despite an overall increase in microglia numbers. Single-cell transcriptomics demonstrates the induction of disease-associated microglia (DAM) signatures that differ from known plaque-associated microglia. It appears that amyloid-DAM, which normally clear amyloid plaques, become distracted by nearby myelin-damage. Age-dependent structural defects of myelin may likewise promote plaque formation and constitute as an upstream AD risk factor.

This project is supported by an ERC Advanced Grant MyeliNANO.

POSTER 1

Overexpressing SVCT2 in Microglia to Target Learning and Memory Deficits in Parkinson's Disease

Ana M. Pacheco[1,2], Evelyn C. S. Santos[1,3], Márcia A. Liz[1,3], João B. Relvas[1,2,3] and Camila C. Portugal[1,3]

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Introduction: Parkinson's disease (PD) is a neurodegenerative disease that presents motor and non-motor symptoms such as deficits in learning and memory. Vitamin C is an essential antioxidant molecule in the central nervous system (CNS). Its reduced form, the ascorbate anion, uses the sodium vitamin C co-transporter 2 (SVCT2) to be transported into microglia and neurons. Microglia are the main immune resident cells in the CNS that respond to changes in the CNS milieu, such as neuronal hyperactivation or disease. These stimuli induce SVCT2 downregulation in microglia, leading to decreased amounts of ascorbate inside these cells, leading to microglial proinflammatory activation. Recently, studies suggested that microglial activation correlates with PD progression, including memory decline. This work aimed to investigate the role of SVCT2 overexpression in microglia from the hippocampus of the Thy1-αSyn mice and its relationship with some hippocampal-related deficits, such as hyposmia and learning and memory deficits presented by this model.

Methods: We used the Thy1-αSyn mice model, which presents an increased expression of the human wild-type α-synuclein under the regulation of the Thy1 promoter. We injected three different mice groups: wild-type mice injected with the control vector (that express only mCherry); Thy1-αSyn mice injected with the control vector, and Thy1-αSyn mice injected with SVCT2 vector (that express mCherry and SVCT2). The vector delivery was done through stereotaxic administration of AAV particles, carrying these constructs into the hippocampus. The vector presents the CD68 promoter, which confines the mCherry and SVCT2 expression to microglial cells. This administration was performed at two months old and the evaluation four months later. After confirming the SVCT2 overexpression, these animals were evaluated in a behavior test battery composed of the grip strength, rotarod, olfactory, and the Moris Water Maze test.

Results/Discussion: Firstly, we observed in the Thy1-αSyn mice model, hippocampal hallmarks of microglial activation, such as changes in microglial morphology and increased microglial numbers compared to age-matched controls. To groundwork our hypothesis, we also evaluated SVCT2 mRNA expression in the hippocampal microglia of Thy1-αSyn mice. We observed lower SVCT2 mRNA levels in microglia from this group in comparison with littermate wild-type mice, concluding that the Thy1-αSyn mice present microglial activation and reduced levels of SVCT2 expression in microglia.
After that, we performed the vector delivery in hippocampal microglia (WT – Control Vector; Thy1-αSyn – Control Vector; Thy1-αSyn – SVCT2 Vector). Regarding the behavioral test battery, we observed minor changes in the olfactory test, translating low improvements in hyposmia in Thy1-αSyn injected with SVCT2 vector compared with Thy1-αSyn injected with the control vector. We also observed an extensive improvement in both learning and memory deficits in Thy1-αSyn injected with SVCT2 at the Moris Water Maze test.

Conclusions: Thy1-αSyn mice present microgliosis in the hippocampus and reduced levels of SVCT2 expression in microglia. After SVCT2 overexpression, we observed a tendency to ameliorate hyposmia, while learning and memory deficits were entirely recovered by SVCT2 overexpression in hippocampal microglia of Thy1-αSyn mice.

POSTER 2

Methamphetamine-Induced Remodelling of Hippocampal Neurons is Orchestrated via cdc42 Pathway

Ana Filipa Terceiro[1,2], Andrea Lobo[1], Lia Carvalhais[3,4], Miguel Aroso[5], Mafalda Sousa[6], Renato Socodato[7], Ana Magalhães[1,2], Paulo Aguiar[5], João Relvas[7,8], Teresa Summavielle[1,9†]

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The development of addiction is strongly influenced by synaptic and morphological adaptations in the brain provoked by drugs of abuse such as methamphetamine (Meth), a powerful psychostimulant. The hippocampus is one the regions more affected, contributing to the formation of drug-context associations and relapse. However, particularly in this region, the mechanisms regulating this complex process are not clear. We observed that in primary hippocampal neuronal cultures, exposure to Meth (24h) increased neurite outgrowth, dendritic spine density and impacted the expression of synaptic proteins. In vitro electrophysiology assays using multi-electrode arrays, have also demonstrated that Meth decreases spontaneous neuronal activity. RhoGTPases, as key regulators of the actin cytoskeleton, have been linked to drug-induced maladaptive neuronal restructuring. We demonstrate, using FRET assays, that cdc42 activity is increased at dendritic spines 5 min after drug exposure. Concurrently, the downstream pathway (N-Wasp, Arp3) is activated in synaptoneurosomes of hippocampal neuronal cultures, 15 min following Meth exposure. Inhibition of Intersectin1(Itsn1, a cdc42 specific activator)/cdc42 interaction, as well as Itsn1 knockdown prevented Meth effects on neuronal remodelling. Importantly, in WT mice exposed to a Meth binge regimen (5 mg/kg x4, 2h intervals), cdc42 activity was increased in hippocampal synaptoneurosomes 15 min after the last drug administration. Meth also increased neurite length, dendritic spines density and decreased PSD95 expression 24h following the last administration of Meth. Currently, we are assessing whether silencing cdc42 in vivo can prevent Meth-elicited neuronal remodelling. Collectively, our data demonstrates cdc42 as an important mediator of Meth-induced remodelling in the hippocampus and will elucidate if targeting Rho GTPases may contribute to improve treatment of addictive disorders.

POSTER 3

IL-10 Protects Female Mice from Methamphetamine-Induced Neuroinflammation

Ana Isabel Silva[1,2], Carolina Pinto[1,3], Elva B. Andrade[3], Ana Filipa Terceiro[1,2], Teresa Canedo[1,4], Ana Magalhães[1,2], João B. Relvas[4,5], Margarida Saraiva[6], Teresa Summavielle[1]

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Methamphetamine (Meth) is a highly addictive psychostimulant. The classic hallmarks of Meth-exposure include the disruption of the dopaminergic system, concomitant with terminal degeneration and eventual neuronal death. However, Meth is now also recognized for causing immune dysregulation and persistent neuroinflammation. Recently, our lab has demonstrated microglia expansion and activation under Meth-exposure in male animals. We also demonstrated that this activation is mediated by astrocytic release of glutamate and TNF [1]. Because other studies found that chronic administration of Meth decreases the circulating levels of IL-10, an anti-inflammatory cytokine, we hypothesized that IL-10 supplementation, could be protective against Meth-induced neuroinflammation. Here, we took advantage of a transgenic mouse model with controlled overexpression of IL-10 (pMT-10-IL10 inducible mice) [2] to investigate the mechanisms of immune regulation/protection elicited by IL-10 in the context of acute exposure to psychoactive drugs. In these animals, IL-10 overexpression happens when the animals are exposed to zinc (administered in the water, and using sucrose to increase its intake). Firstly, with this work we were able to demonstrate that, in female mice, Meth-exposure causes the same effects we had previously observed in males. In the pMT10 mutant females, we were able to recapitulate Meth-induced risk assessment impairment (evaluated in the elevated plus maze (EPM)), microglia proliferation and morphological alterations. Importantly, all observed Meth effects were reverted with overexpression of IL-10. However, in males, in pMT10 animals that did not receive zinc (control group), we were able to recapitulate Meth behavioral effects, but not microglia expansion and reactivity. In order to understand the lack of Meth effects in these animals, we are now conducting several experiments to understand the possible impact of the pMT10 genotype or sucrose administration. Because in clinical trials IL-10 was seen to be associated with increasing Interferon-gamma (IFNγ), a pro-inflammatory cytokine, we used also a transgenic mouse model with controlled overexpression of IL-10, but knock-out (KO) for IFNγ. We have already seen that, in the pMT10.IFNγ, IL-10 overexpression prevents Meth-induced changes in risk assesment behavior in the EPM. Importantly, this effect seems to be dependent on gammadelta (γδ) T cells. We predict that understanding how IL-10 and other related cytokines vary with Meth exposure will prove to be an innovative approach to devise treatment strategies for substance abuse disorders.

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POSTER 4

Engineering Small Extracellular Vesicles with miR-124-3p as a Future Therapeutic Strategy in Alzheimer's Disease

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Alzheimer's disease (AD) is one of the most common neurodegenerative disorders worldwide, and is characterized by an accumulation of amyloid-β (Aβ) and Tau protein which are cleared and disseminated throughout brain regions by small extracellular vesicles (sEVs), also known as exosomes, with 50-150 nm diameter size. By carrying microRNAs (miRNAs), sEVs influence recipient's cell function [1]. Using different human AD models, we identified that miR-124-3p can compromise neuronal function when downregulated and to be neuroprotective when at normal or slightly upregulated levels, conditions recapitulated by their derived sEVs [2]. It was also demonstrated that miR-124 expression by microglial cells is regulated by its content in neurons and their exosomes, when in coculture, influencing activated microglia phenotype [2], and brain regeneration [3].

Our aim was to find out the most optimal way to modulate miR-124 in microglial cells as a possible treatment for Alzheimer's disease, using neuronal exosomes. First, we isolated neuronal sEVs from SH-SY5Y cells transected with miR-124-3p mimic (sEV-SHT). On the other hand, we isolated sEVs from untransfected SH-SY5Y, and engineered them with miR-124-3p mimic, using Exo-Fect® (sEV-ExoFect). sEVs were isolated by differential centrifugation, characterized for their number and size, using Nanoparticle tracking analysis (NTA), and labeled with the fluorescent PKH67 to track their internalization by the microglial cells, using confocal microscopy. In the microglial recipient cells, quantification of the different miRNAs was carried out by RT-qPCR with specific predesigned primers.

Preliminary data showed that sEVs were successfully internalized by microglia, and that both approaches led to a microglial miR-124-3p increase comparing with mock-transfected sEVs. However, sEV-ExoFect was much more efficient in increasing microglial miR-124-3p, raising its levels more than 20 times, when compared to sEV-SHT. We also noticed that miR-21 and miR-155 levels were changed upon incubation with both types of sEVs. In the case of miR-21, we observed its increase in microglia incubated with sEVs from both methods, and even in microglia incubated with mock-transfected sEVs. Curiously, the opposite was observed for miR-155, since it was decreased regardless of the sEV-based approach used. Although miR-21 and miR-155 responded to the incubation with neuronal sEVs, we can state that such response was not intrinsically dependent on their miR-124 cargo.

In conclusion, our preliminary data support that the use of Exo-Fect® seems more efficient on the delivery of miR-124-3p from neuronal sEVs into microglia, and may be a tool to mediate sEV-based therapies in AD and other diseases. In the future, we aim to better characterize sEVs, produce them in large scale, evaluate their role in regulating neuroinflammation and inject them in animal models of AD to assess their effects on neuroprotection and cognitive recovery.

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POSTER 5

A RhoA-Mediated Biomechanical Response in Schwann Cells Modulates Peripheral Nerve Myelination

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Myelin improves axonal conduction velocity and is essential for nerve development and regeneration. In peripheral nerves, Schwann cells depend on bidirectional mechanical and biochemical signals to form the myelin sheath. The molecular mechanism that controls this biomechanical process is not fully understood. Rho GTPases are major integrators of “outside-in” signaling, linking cytoskeletal dynamics with cellular architecture to regulate adhesion and cell deformation. Using Schwann cell-specific gene inactivation in the mouse, we discovered that the GTPase RhoA promotes axonal sorting and the initiation of myelination, and is required to both drive and terminate myelin growth at different stages of peripheral myelination. RhoA targets the activity of specific cortical actin-binding proteins to modulate the organization, contractility and actin-membrane attachment of the SC cortex. This mechanism couples mechanical and biochemical properties of the cell surface with intracellular signaling to modulate axon-Schwann cell interaction and the growth of myelin membranes. We propose that RhoA participates in the biomechanical response that can independently control the switch of Schwann cells towards the myelinating and the homeostatic states.

POSTER 6

Inhibiting miR-125b Overexpression in ALS Spinal Microglia Restores Their Homeostatic Profile

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting motor neurons (MNs) localized in the cortex, brainstem and spinal cord (SC). After many years of being a neuron-centred disease, many studies have put forward the paramount role of glial cells, such as astrocytes and microglia, which contribute to the disease pathology by releasing inflammatory factors that will enhance the characteristic neuroinflammation. Recently, small non-coding RNAs, microRNAs (miRNAs), have been increasingly studied due to their potential capacity as biomarkers and therapeutic targets for an array of diseases, including ALS [1]. We have recently shown that miR-125b, an inflammatory-associated miRNA (inflamma-miRNA), is increased in NSC-34-MNs with the $SOD1^{G93A}$ mutation (mSOD1), as well as in the SC of symptomatic mSOD1 mice, pointing to its important role in this disease [2, 3]. Recently we observed that mSOD1 microglia when maintained in culture for 2 days *in vitro* (DIV) present a more pro-inflammatory phenotype, while those cultured for 16 DIV display instead a senescent-like profile, resembling what happens in ageing (Unpublished data), providing an interesting tool to dissect ALS progression.

In this study, our main goal was to understand whether the inhibition of miR-125b would be able to recover the profile of mSOD1 spinal aged-like microglia, and additionally if the secretome from anti-miR-125b modulated cells could correct the parenchymal pathogenicity observed in the early stages of the disease. To achieve this, we cultured mSOD1 spinal microglia from 8-days-old mice for 16 DIV after astrocytic layer removal [4], to mimic a senescent-like profile and modulated them or not with anti-miR-125b. We then analysed their inflamma-miRNAs (miR-124, miR-125b, miR-155) and inflammation-associated markers (IL-10, IL-1β, TNF-α, iNOS). Changes in their phagocytic capacity (engulfment of latex beads and MFG-E8 expression) and cell morphology were evaluated, as well. Additionally, the secretome from the modulated cells was incubated in the mSOD1 spinal organotypic cultures (SCOCs) from 10-12-week-old mice (early symptomatic) and the inflammatory markers (IL-10, IL-1β, iNOS) and the microglial phagocytosis assessed. WT samples were used as controls.

Our results show that spinal aged-like microglia present an upregulation of miR-125b/-124/155, decreased expression of inflammatory markers and of phagocytic ability (beads engulfment and MFG-E8), together with altered cell morphology. Cell modulation with anti-miR-125b prevented such changes, returning the cellular profile towards a more homeostatic phenotype and closer to that of WT microglia. The secretome from the anti-miR-125b modulated mSOD1 microglia was able to similarly recover parenchymal homeostasis in the SCOCs by abrogating inflammatory markers and recovering phagocytic ability.

To conclude, the inhibition of miR-125b poses a promising strategy to restore microglia neuroimmune properties in ALS.

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POSTER 7

Astrocytic Insulin Signaling Entrain Circadian Rhythms via Dopamine Signaling

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Introduction: The circadian system consists of a central clock in the hypothalamic suprachiasmatic nucleus (SCN) and peripheral clocks in tissues or organs throughout the body[1]. These clocks are synchronized daily with external environmental factors or zeitgebers (ZT). While the light-dark (LD) cycle is the ZT for SCN, the feeding pattern is the signal that synchronizes peripheral clocks[1]. The mechanism underlying circadian entrainment to feeding time is critical for understanding why mistimed feeding, as it occurs during shift work, disrupts circadian physiology, a state associated with chronic diseases such as metabolic disturbances and type 2 diabetes[1]. Remarkably, feeding-regulated hormone insulin (INS) reset peripheral clocks *in vivo* and *in vitro*[2]. Conversely, mistimed INS signaling disrupts the circadian organization of clock gene expression and mice behavior[2].

Recent findings showed the critical role of the astrocyte clock in regulating daily rhythms in physiology and behavior[3-7]. Moreover, astrocytes are at the interface between vessels and neurons to act as metabolic sensors of systemic cues[1]. Thus, we hypothesize that INS signaling might be crucial in communicating time-of-feeding to the astrocyte clock, coupling the central and peripheral tissues to control circadian physiology and behavior.

Materials and Methods: We used a tamoxifen (TM)-inducible Cre/lox approach to genetically delete INS receptors (IR) from GLAST-positive astrocytes[8]. At 8-10 weeks, control (IRflox/flox) and mutant (*IRcKO*) mice were treated with TM[8], and after 2 months, animals were subjected to an extensive circadian and metabolic phenotype characterization.

Results and Discussion: Our results indicate that INS signaling in astrocytes regulates body composition and energy expenditure in males and females both in standard and high-fat diets. Moreover, while astrocytic INS signaling does not play a major role in controlling the free-running period in male mice, it plays a crucial role in the entrainment of circadian rhythms with feeding time via dopamine signaling. On the other hand, we found that female *IRcKO* mice had increased periodicity in constant darkness and altered coupling of SCN oscillators that govern locomotor activities. Thus, besides its well-known homeostatic functions, we propose INS as a primary synchronizing cue for astrocyte clocks and circadian rhythms in mammals.

Conclusions: I. Astrocytic INS signaling controls energy homeostasis in male and female mice.
II. INS signaling in female astrocytes plays a crucial role in the light-entrainment of the SCN
III. Deletion of the INS receptor in male astrocytes alters the entrainment of circadian rhythms with feeding time via dopaminergic signaling.

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POSTER 8

Neuroinflammation Patterns in Alzheimer's Disease, Lewy Body Dementia and Cases with Mixed Pathology

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Introduction: Alzheimer's Disease (AD) and Lewy Body Dementia (Dementia with Lewy Bodies – DLB and Parkinson's Disease Dementia - PDD) are the two most common causes of neurodegenerative dementia.1 Neuropathology studies indicate that more than 60% of AD and DLB patients present co-pathology at autopsy, which is associated with worse phenotypes.2 This work aims to characterize neuroinflammation in patients with pure and overlapping Alzheimer and Lewy Body pathology, to contribute to more tailored future therapeutic approaches.

Materials and Methods: Neuroinflammation in human *post mortem* - frontal cortex gray and white matter was characterized in terms of microglial activation by CD68 immunohistochemistry in neuropathologically defined cases of AD, Lewy Body Dementia (either DLB or PDD) and co-pathology cases. A semi-automatic whole slide image digital neuropathology pipeline was established, optimized and validated.

Results and Discussion: Eighteen cases were selected (values expressed as mean [standard deviation]): AD (n=6; age at symptom onset (AASO), 62.7 [7.5] years; age at death (AAD), 69.8 [5.9] years), AD/LBD (n=4; AASO, 55.3 [3.1] years; AAD, 64.0 [6.5] years), LBD (n=5; AAOS, 62.8 [9.0] years; AAD, 72.8 [7.6] years), Control (n=3; AAD, 60.3 [12.0] years). Cases with AD and co-pathology cases were found to have increased CD68 protein load in the middle frontal gyrus (MFG) gray matter relative to control and Lewy body disease (LBD). No statistically significant difference was found between AD and co-pathology cases. LBD cases did not to differ from controls. No differences were found between study groups in MFG white matter.

Conclusions: Co-pathology cases were found to have a CD68 protein load suggestive of microglial activation in MFG gray matter that more closely resembled pure AD cases than pure LBD cases. Current results support a prevailing influence of AD pathology in co-pathology cases in terms of neuroinflammation at post mortem late stage disease. Pure AD cases and co-pathology cases did not differ for this feature which could suggest an absence of additive effect between AD and LBD pathology in terms of neuroinflammation in this context. A bigger sample size, considering other neuroinflammation markers and evaluating other brain regions will be required in order to infer if there is an additive effect of AD and LBD pathology in co-pathology cases in terms of neuroinflammation. A detailed characterization of clinicopathological correlations in cases with AD/LBD co-pathology will be important in order to inform current and future clinical trials targeting neuroinflammation in AD and LBD.

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POSTER 9

Unravelling Cell Type Specific Response to Parkinson's Disease at Single Cell Resolution

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Introduction: Parkinson's Disease (PD) is the second most common neurodegenerative disorder and is characterized by impaired motor functions[1]. The major pathological hallmark of PD is the abnormal aggregation of α-synuclein inside cells, primarily in the substantia nigra (SN). To uncover molecular mechanisms driving PD, we generated a large-scale single nuclei transcriptomics (snRNA-seq) dataset from post-mortem human SN, which allowed us to define the multi-cellular character of PD pathology and cell-type specific responses to the disease.

Materials and methods: SN samples of 29 individuals - 14 diagnosed with PD and 15 controls - were collected from the Oregon Brain Bank: all brains were subject to a full examination post-mortem and pathology reports were provided. Single nuclei were isolated and libraires prepared using a 10X Chromium system and v3 Reagents kits; sequencing was performed on a NovaSeq6000 system with ~40K average read depth and >40% saturation rate. After quality control steps, ~80K high quality nuclei were selected for downstream clustering analysis, which was performed using Seurat[2]. PD-related gene expression changes were assessed by fitting a linear mixed model using lme4[3]. Gene-enrichment and functional annotation analysis were performed using ClusterProfiler[4].

Results and discussion: Clustering analysis identified populations of neurons, oligodendrocytes, microglia, astrocytes, connective tissue cells, oligodendrocyte progenitor cells and T-cells. Our differential gene expression and pathway analysis allowed us to identify PD induced cell-type specific changes.

While we detected various classes of neurons, the major effect was on the dopaminergic system. Dopaminergic neurons, as well as populations of astrocytes, microglia and oligodendrocytes regulating dopamine metabolism, were lost in PD at statistically significant levels, consistent with the known role of glia cells in protecting neurons[5].

Amongst all glia populations quiescent and disease-responsive states were identified, with disease-responsive states enriched in PD samples.

A significant immune response to PD was observed, which is largely driven by activated microglia. In addition, we observed a significant increase in the total number of T-cells in the PD brain, which highlights the involvement of the immune system in PD.

A PD specific reactive astrocyte state was also detected, which appears largely driven by the **u**nfolded **p**rotein **r**esponse (UPR). This observation aligns with recent reports of a UPR induced response in astrocytes causing neurodegeneration[6].

Finally, oligodendrocytes show a massive response to PD with the highest number of significantly up-/down- regulated genes detected in differential gene expression analysis.

Conclusions: We provide a large resource for PD research: a snRNA-seq database of human post-mortem SN samples collected from diseased and control brains. Our analyses suggest a multi-cellular response to PD, with a previously unsuspected major involvement of glia cells in the pathology. Our data provide a valuable resource for future hypothesis-driven experiments, aimed at modifying PD prognosis.

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POSTER 10

Addressing the Role of Microglia in Multiple Sclerosis-Associated Cognitive Impairment

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Introduction: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system, affecting millions of people worldwide. Impaired cognition is emerging as one of the main clinical symptoms that enhances the risk of disease progression. Evidence demonstrated that MS patients have impaired structural network and that focal microglia may impact cognitive abilities. Moreover, we recently showed that microglia density/morphology is altered in cognitive-related brain regions as hippocampus in the MS *in vivo* model, the Experimental Autoimmune Encephalomyelitis, in parallel with decreased intact axons and emergence of cognitive deficits. Since cognitive impairment is present in more than 50% of MS patients, it becomes a high priority to understand the cellular/molecular mechanisms underlying this prevalent symptom. Here, we aimed to characterize microglia morphology in the cognitive-associated area, the hippocampus, of MS postmortem samples.

Materials and Methods: Hippocampal samples were obtained from the Netherlands Brain Bank considering information on MS patients' cognitive status (CDR score). CDR score provides information about the severity of the dementia by assessing six domains classified along a 5-point scale: 0 – no cognitive impairment; 0,5 – mild dementia; 1 – mild cognitive impairment; 2 – moderate cognitive impairment; and 3 – severe cognitive impairment. Based on this, cognitively preserved (MSCP, n=8) and impaired (MSCI, n=8) patients were included. Also, samples from healthy donors (n=8) without neuroinflammatory issues were used. Immunohistochemistry studies were performed to study myelin and microglia using specific markers (MBP and Iba1, respectively). Images were acquired using confocal microscopy and analyzed in different regions of myelin structures for microglial number and morphology using ImageJ plugins. Specifically, to study cell morphology, microglia skeleton was used with the MorphData plugin; and to study cell's surface and size, binary images were used with the FracLac plugin.

Results and Discussion*:* Based on MBP staining, we found six different patterns of myelination throughout the hippocampus, ranging from areas with low myelin density to fully myelinated areas. Notably, demyelinated areas were only found in MS patients. Interestingly, significantly increased microglia numbers were found in demyelinated areas of MSCI when compared to MSCP. Interestingly, microglia present in demyelinated areas show a reduced number of branches, in parallel with a decreased cell perimeter and roughness. Additionally, alterations in features associated with cell size were observed. Indeed, microglia area and the area surveilled by the cell were increased in MSCP while MSCI microglia presented a significantly reduced area.

Conclusions: Our data highlight the participation of microglia in cognitive impairment conditions and, particularly, in demyelinating conditions. Thus, these changes may play a critical role in the hippocampal cognitive-related functions, setting the basis for further studies.

POSTER 11

The Impact of Silencing Astrocytic A2A receptors on Hippocampal Synaptic Plasticity and Memory

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Introduction: Increasing evidence support the existence of a bidirectional communication between neurons and astrocytes in the control of brain function, giving rise to the concept of 'tripartite synapse', which postulates that astrocytes are the third active element of synapses [1]. Adenosine A_{2A} receptors ($A_{2A}R$) are known to facilitate long-term potentiation (LTP), being implicated in memory processes and $A_{2A}R$ activation was shown to impair memory [2,3]. However, little is known about the role of astrocytic $A_{2A}R$ in synaptic plasticity and memory. The present study aimed to evaluate the impact of astrocytic $A_{2A}R$ genetic silencing on hippocampal synaptic plasticity and memory.

Materials and Methods: We bilaterally injected into the CA1 hippocampal region a viral construct directing CRE recombinase expression to astrocytes (AVV5-GFAP-GFP-CRE) in floxed-$A_{2A}R$ ($A_{2A}R$flox/flox) male adult mice in order to silence astrocytic $A_{2A}R$ and AVV5-GFAP-eGFP in control mice. After four weeks, mice were behaviorally characterized using hippocampal-dependent tasks. 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 long-term depression (LTD). Furthermore, neurochemical studies were performed to evaluate the impact of $A_{2A}R$ silencing on astrocytic morphology through tri-dimensional reconstructions.

Results and Discussion: Astrocytic $A_{2A}R$ inactivation significantly impaired performance in the object displacement test (49.4±5.85% vs. control: 71.5±4.64%, $p<0.05$). These alterations were accompanied by a significant reduction on hippocampal LTP amplitude (44.0±16.10% vs. control: 104.7 ± 14.16%, $p<0.05$) and a shift from LTD to LTP (16.4±9.63% vs. control: -21.8±6.7%, $p<0.05$). Additionally, astrocytic $A_{2A}R$ silencing affected astrocytic complexity, since astrocytic tri-dimensional reconstructions showed an increase in the number of processes (98.9±5.5 vs control: 70.6±3.9, $p<0.001$) as well as total length (1515±82 µm vs. control: 1145±57 µm, $p<0.001$).

Conclusions: Data indicate that astrocytic $A_{2A}R$ silencing in mice hippocampus increments astrocytic complexity and negatively impacts hippocampal synaptic plasticity and memory.

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POSTER 12

ATP Release by Astrocytes Challenged with Aβ1-42 Peptides is Regulated by a Crosstalk Between Adenosine A2A Receptors and Connexin 43

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Introduction: Increasing evidence implicate astrocytes in the pathogenesis of Alzheimer's disease (AD), a neurodegenerative disorder characterized by a cognitive decline due to synaptic dysfunction and loss, which is linked with the accumulation of amyloid-β peptides (Aβ) [1]. Aβ can affect astrocytes function, mainly their ability to uptake and release neuroactive molecules. Astrocytes release ATP, which can be metabolized into adenosine by ecto-5'-nucleotidase, CD73, resulting in adenosine A_{2A} receptors ($A_{2A}R$) activation that in turn bolsters neurodegeneration. AD's brains exhibit increased levels of $A_{2A}R$ and connexins 43 (Cx43), which form astrocytic hemichannels (Cx43-HC) that mediate gliosignals release. Therefore, the present study aims to investigate a possible association between $A_{2A}R$ and Cx43 HC in astrocytes challenged with Aβ.

Materials and Methods: Primary cultures of astrocytes obtained from the cerebral cortex of Wistar rats (P1-P2) were used and exposed to $Aβ_{1-42}$ peptides (1μM) to mimic pathological conditions of AD. ATP extracellular levels were evaluated resorting to a bioluminescent assay and hemichannels activity through ethidium bromide uptake. Cx43 levels and association with $A_{2A}R$ were assessed by Western-blot, co-immunoprecipitation and proximity ligation assays.

Results and Discussion: Our results revealed an enhancement in ATP release (180.4±21.5%, $p<0.001$) mainly through Cx43-HC alongside an increase HC activity (129.7±4.4%, $p<0.001$) in astrocytes exposed to $Aβ_{1-42}$ relatively to control cells (100%). $Aβ_{1-42}$ was also shown to upregulate Cx43 total levels (148.9±14.0%, $p<0.05$) and phosphorylation (140.2±5.4%, $p<0.001$). These effects were mimicked by $A_{2A}R$ activation with the selective agonist and counteracted by $A_{2A}R$ selective antagonism and also by the blockade of adenosine formation through CD73 inhibition. Moreover, results also showed a physical association between $A_{2A}R$ and Cx43 through proximity ligation assay and co-immunoprecipitation.

Conclusions: Our data identified a feed-forward loop involving $A_{2A}R$ and Cx43-HC in Aβ-challenged astrocytes, whereby $A_{2A}R$ overfunction increases Cx43-HC activity leading to increased ATP release, which is converted into adenosine, by CD73, and sustains the increased $A_{2A}R$ activity.

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POSTER 13

The Involvement of Astrocyte Calcium-Dependent Signaling in Behavior

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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 derived from different sources and displaying complex spatiotemporal properties. Calcium elevations appear spatially distributed in global (soma and main processes) and focal regions (microdomains). Such astrocytic calcium activity is expected to underlie the astrocyte involvement in synaptic transmission, metabolism, and brain homeostasis. In this work, we studied the IP_3 receptor type 2 knockout (IP_3R2 KO) mouse model that lacks global calcium elevations in astrocytes to disclose its implications in cognitive function. We found an influence of global astrocyte calcium in fear memory. Thus, we performed a structural and molecular analysis of cortico-limbic regions that revealed a shift to immature spines in pyramidal neurons of the dorsal hippocampus. These structural alterations in dendritic spines may support the previously described synaptic plasticity changes that may underlie our behavioral observations. The characterization of the IP_3R2 KO mouse model provides new insights into the importance of astrocytic calcium-dependent signaling in the modulation of neural activity. These findings broaden the scope of astrocytic modulation of brain circuits.

POSTER 14

Targeting the Inhibitory Tripartite Synapse in the Context of Autism Spectrum Disorder: Astrocytes in the Spotlight

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Introduction: The neurobiology underlying Autism Spectrum Disorder (ASD) remains elusive. Results from large-scale genetic studies strongly implicate abnormal synaptic organization and inhibitory signaling in the pathophysiology of ASD[1][2]. To date, the research on ASD has been predominantly neuron-centric. However, astrocytes, a type of glial cell, closely interact with neurons at tripartite synapses, and recent studies strongly suggest that astrocytes differentially regulate excitatory and inhibitory tripartite synapse formation and function[3]. These results are particularly relevant for human diseases, such as ASD, schizophrenia, and epilepsy, which are characterized by impaired GABAergic signaling, E/I imbalance and circuit hyperexcitability[2]. Compared to our knowledge on the excitatory tripartite synapse, however, the composition of the inhibitory tripartite synapse and the mechanistic basis of its regulation remain poorly understood. Here, we hypothesize that specific defects in inhibitory tripartite synapse formation during early development may result in altered GABAergic signaling and disrupted inhibitory circuit maturation, leading to later manifestations of neuropsychiatric and neurodevelopmental disorders, such as ASD.

Materials and Methods: Our goal is to gain fundamental knowledge on the composition and function of the inhibitory tripartite synapse and the role this may play in ASD. For this, we use a novel proximity biotinylation-based approach called Split-TurboID[4], combined with brain-penetrating AAV-PHP.eB viral vectors, to identify astrocyte proteins, previously linked with ASD, which are present at inhibitory synapses in ASD-relevant brain areas. Then, we plan to assess the role of candidate proteins using a loss-of-function approach combined with testing for structural and functional effects on synapse formation, and *in vivo* behavioral phenotyping. Where possible, we will focus our efforts on synapses in the cerebellum, a brain area strongly linked to ASD pathophysiology.

Results and Discussion: Our preliminary data show cell type-specific Split-TurboID vector expression and robust biotinylation of proteins in the cerebellum. The successful implementation of our *in vivo* Split-TurboID strategy in this brain area reinforces the feasibility of our approach, and sets a relevant precedent for investigators interested in targeting the inhibitory tripartite synapse at this level.

Conclusions: By bringing astrocytes into the spotlight, our project has the potential to uncover the role of astrocyte-specific protein relevant for inhibitory synapse formation and function in ASD. Establishing a key role for astrocytes in controlling inhibitory synapse-specific wiring and function would profoundly shift our understanding of GABA-related disease mechanisms, potentially sparking intensive research in the ASD field, and opening up new avenues to explore for therapeutic interventions.

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POSTER 15

The *Soft* Side of the Brain: Bioengineered Platforms to Unveil the Role of Mechanobiology in Demyelinating Diseases

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In the central nervous system (CNS), myelination is initiated by oligodendrocyte precursor cells (OPCs), which differentiate into oligodendrocytes (OLs), enabling saltatory conduction of action potentials along axons. Destruction of myelin internodes, OL apoptosis and axonal degeneration are hallmarks of demyelinating diseases, such as multiple sclerosis (MS). With over 2.5 million people affected worldwide, MS represents a serious health, economic and social burden with no long-term suitable treatment. Although there are OLs in the adult CNS which can partially regenerate myelin sheaths in denuded axons, this remyelination process fails with disease progression, leading to irreversible functional failure. Growing evidence suggest that not only cell populations and soluble factors are altered in demyelinating conditions but also the extracellular (ECM) composition and mechanical properties are changed. Additionally, OPCs and OLs are proven mechanosensitive cells, so physical and mechanical cues play a role in oligodendroglial biology and, presumably, in the demyelination processes. The lack of platforms with biologically relevant features has hampered the study of these mechanobiology processes.

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

A new formulation of PDMS was explored to fabricate axon surrogates, corresponding to a significant advance in the production of structures with a great aspect-ratio and reduced stiffness. OPCs were found to adhere, survive, differentiate, and wrap around the PDMS micropillars. Interestingly, OPC antigenic and morphological differentiation is accelerated in softer PDMS structures (Young's modulus of 1250 kPa *vs* 364 kPa) while the wrapping capability is increased for larger diameter micropillars. Genetic analysis suggested that these events might be mediated by epigenetic alterations.

ALG hydrogels were produced by combining ALG formulations containing the cell adhesive peptide RGD or the matrix metalloproteinase sensitive peptide PVGLIG. When cultured in these hydrogels OPCs are viable, metabolically active and can differentiate in OLs. By embedding OPCs in increased ALG content hydrogels we were able to assess the impact of the mechanical properties' changes on OPC differentiation. Softer matrices (shear modulus, G*~100 Pa) favored OPC differentiation in comparison with stiffer matrices (G*~350 Pa and 1300 Pa.) Impaired OL differentiation was also 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.

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

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POSTER 16

Decoding the Mechanisms of Neuronal Wiring: a Lesson from Down Syndrome

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Introduction: The central nervous system (CNS) contains a diverse number of high-level cell types, including neurons and astrocytes. Neurons form chemical synapses, where information is transmitted from a pre-synaptic neuron to a post-synaptic neuron. The process of synapse formation and function is critically dependent on physical contact with astrocytes. This contact is (partially) maintained by the cell surface expression of multiple clustered protocadherins (cPcdhs), which are organized in α, β and γ gene clusters. Interestingly, only expression of γ-cPcdhs appear to be dysregulated in Down Syndrome (DS)[1]. However, the precise expression patterns of these genes and their contributions to local circuit formation remain unknown.

Materials and Methods: Non-spatial expression data was obtained from published single-cell RNA sequencing datasets enriched in neurons and astrocytes[2,3]. Spatial expression data was generated using Molecular Cartography spatial transcriptomics. Coronal sections were obtained from motor, somatosensory and visual cortices of P56 C57BL/6J wild-type mice. Fluorescently-tagged probes were generated against distinct cPcdh transcripts and cell-type specific transcripts. Regions of interest were imaged, and single RNA molecule signals identified. RNA counts were assigned to individual cells using DAPI-based segmentation in QuPath. After removal of low quality cells, the remaining high quality cells were clustered using Seurat[4]. The spatial dataset was integrated with a reference single cell (sc)RNA-seq dataset prepared from mouse cortex[5], and each cell in the spatial data was assigned to the closest reference cluster. Downstream analysis of cPcdh expression patterns across cell types and subregions was done on this merged dataset.

Results and Discussion: Initial analysis of published scRNA-seq datasets shows that mouse neurons are enriched in α-cPcdhs and to a lesser extent γ-cPcdh, whereas astrocytes are enriched in γ-cPcdhs and both cell types express β-cPcdhs at roughly equivalent levels. In tissue, major cell-types and subtypes identified in scRNA-seq datasets can be identified in the mouse brain using spatial transcriptomics using a standard panel of 44 probes. We also confirm that α and β-cPcdh isoforms are mainly expressed by both excitatory and inhibitory neurons, whereas γ isoforms appear to be expressed by both neurons and astrocytes. Additionally, spatial expression of isoforms across cortical layers and white matter is correlated with the spatial location of major CNS cell types. These results support the hypothesis that the specific dysregulation of γ-cPcdhs in Down Syndrome leads to impaired astrocytic function, contributing to aberrant neural network formation.

Conclusions: We believe that differential expression of cPcdh isoforms across cell-types and cell subtypes may be crucial for synapse formation and function in local circuits. Further spatial analysis will allow us to explore the basis of cell-cell interactions, dissect the potential role of individual cPcdh isoforms in the organization of specific cortical circuits and to gain insights into the contributions of astrocytes and neurons to aberrant neural network formation in Down Syndrome.

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POSTER 17

Modelling Astrocytic Response During Ischemic Conditions Using a 3D In Vitro Hydrogel Model

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Introduction: Neurological disorders are a major cause of death and disability worldwide [1]. The study of the underlying disease mechanisms is important for the identification of new therapeutics targeting the central nervous system (CNS). Astrocytes are emerging as crucial players in brain disease development and progression [2]. Their high heterogeneity and diverse morphologies present in the brain have been an active field of research. Different disease subtypes have been identified in previous work [3], induced upon different pathologies, specifically, neuroinflammation and ischemia. In our group, we are currently exploring a 3D *in vitro* model using alginate hydrogels as a biomimetic extracellular matrix (ECM) to model neuroinflammatory response and mimic glial scar formation. Here, we propose the development of a 3D *in vitro* platform to explore the astrocytic phenotype in ischemic conditions and further investigate in the future astrocyte heterogeneity in different pathological scenarios – neuroinflammation versus ischemia.
Materials and Methods: Primary rat astrocytes were cultured within high-molecular weight alginate hydrogels, modified with the cell adhesion peptide RGD and the matrix metalloproteinase sensitive peptide PVGLIG. To reproduce ischemic conditions, the cells seeded in the alginate hydrogels were subjected to a protocol of Oxygen and Glucose Deprivation/Reperfusion (OGD/R). The cell laden hydrogels were maintained in a medium lacking glucose in a hypoxic incubator for 6h and, subsequently, returned to normoxic conditions and cultured in normal culture media for additionally 24h and 48h for further analysis. Cellular response was assessed by measuring metabolic activity and by immunofluorescence for the following markers: Glial fibrillary acidic protein (GFAP), F-actin and Chondroitin sulfate proteoglycan (CSPGs). Image acquisition (confocal microscopy) and image analysis (Imaris) followed. Additionally, functional activity of embedded astrocytes was explored with the use of calcium imaging.
Results and Discussion: Astrocytes cultured in the formed alginate hydrogels remain viable, as determined by the live-dead assay. Calcium imaging indicated that embedded astrocytes in alginate hydrogels have calcium signals and respond to stimulation with potassium chloride (KCl). In contrast, the metabolic activity of the astrocytes cultured in alginate hydrogels appears to be decreased after 6h of OGD, although returning to control levels 24h after re-oxygenation. Additionally, from the image acquisition and analysis a trend was found for the increase of GFAP positive cells after 6h of OGD. Analysis is ongoing for the rest of the timepoints.
Conclusions: An oxygen and glucose deprivation protocol has been successfully implemented to assess astrocyte reactivity by markers expression and metabolic activity evaluation in a 3D *in vitro* hydrogel-based system. Our preliminary results indicate that 6h of OGD is contributing to an increase in the number of cells expressing GFAP possibly leading to altered phenotype and astrocytic reactivity.

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POSTER 18

Intracellular Astrocytic Calcium Signaling Controls Astrocyte Morphology and Synapse Coverage

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Astrocytes are the brain major glial type and play crucial roles in neuronal activity with numerous fine processes that contact thousands of synapses (1,2). Calcium signaling is one of the main feature of astrocytes regulating synaptic transmission and plasticity (3,4).

Since intracellular calcium can regulate cell cytoskeleton and adhesion (5, 6) and the morphology of astrocytes modulates neurotransmission (7, 8), we here investigated *in situ* whether altering astroglial calcium signaling modifies hippocampal astrocyte morphology and coverage of synapses.

We used molecular and pharmacological approaches to alter calcium signaling either chronically and locally in the soma, or acutely and throughout the whole astrocyte.

We here show that impairing hippocampal astroglial calcium signaling using molecular and pharmacological approaches alters astrocyte morphology and synapse coverage. These data, by identifying a calcium-dependent structural regulation of astrocytes, extend the repertoire of astroglial calcium signaling targets that could contribute to neurotransmission.

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POSTER 19

miR-21-5p: a New Possible Therapeutic Target in Alzheimer's Disease

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Alzheimer's disease (AD) is a devastating neurodegenerative disorder affecting over 50 million people worldwide. Besides neuronal dysfunction and glial activation, dysregulation of miRNAs is key in AD pathogenesis [1]. Even so, many immune-related miRNAs, as miRNA(miR)-21, remain poorly explored. We have previously identified increased miR-21 levels in AD neuroblastoma cell models, as well as in their secretome, either as free species or included in small extracellular vesicles (exosomes) [2]. We have also shown that coculture of AD neuroblastoma cells with microglia determine upregulation of miR-21 and its release via microglial exosomes [3].

In this work, we used a handful of promising AD models to explore the neuron-glia cross relevance of miR-21, among a select set of hit immune-related miRNAs. First, we established mouse organotypic hippocampal slice cultures (OHSCs) transplanted either with the neuroblastoma SH-SY5Y (SH) cells or with the SH cells expressing the human APP695 Swedish mutated form (SWE). Remarkably, the engraftment of SWE cells in hippocampal tissue prompted miR-21 expression and caused microglia activation together with astrocyte reactivity. Using the cerebrospinal fluid (CSF) samples from Mild Cognitive Impairment (MCI) patients who fulfilled criteria for MCI due to AD (MCI-AD), according to Albert et al., criteria [4], we validated the distinctive presence of miR-21 as a promising biomarker.

Wondering for the contribution of CNS cells in the unique miR-21 upregulation in OHSCs cultured with SWE cells and CSF from MCI-AD, we then explored miRNA dynamics in microglia, neurons and astrocytes differentiated from induced pluripotent stem cells (iPSCs) generated from AD patients. Our data showed that microglia, neurons, and astrocytes differentiated from AD-iPSCs, besides evidencing characteristic AD hallmarks, they all revealed a transversal and consistent upregulation of miR-21. Intriguingly, while the exosomes from AD neurons were also rich in miR-21, those from AD astrocytes required stressed conditions to reveal enriched miR-21 cargo. For astrocyte stimulation we used the C1q + IL-1α + TNFα cocktail identified as resulting from activating microglia and driving the neurotoxic reactive astrocyte phenotype [5]. Upon this stimulation, AD astrocytes revealed an altered phenotype including severe morphological alterations, increased S100B expression, high sAPPβ release, as well as marked inflammatory genes and release of cytokines. More interestingly, all these effects were concomitant with the exosomal enrichment in miR-21 and with the strong downregulation of its direct target peroxisome proliferator-activated receptor α (PPARα). Similar PPARα suppression was also found in the SWE-transplanted organotypic hippocampal slices, as well as in AD microglia, suggesting that its suppression by miR-21 may contribute for AD onset and progression.

Collectively, our tested experimental approaches validated miR-21 as a critical driver and promising therapeutic target in AD. Future studies may now focus on exploring key signaling mechanisms involving miR-21/PPARα crosstalk between neurons and glial cells. *In vivo* miR-21 modulation toward homeostatic levels, will be essential to test neuroprotective and cognitive benefits.

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POSTER 20

Umbilical Cord Blood Cells and Therapeutic Hypothermia – A Joint Action Against Glial Reactivity and Functional Deficits Triggered by Neonatal Hypoxic-Ischemic Encephalopathy

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Introduction: Neonatal Hypoxic-Ischemic Encephalopathy (HIE) is one of the leading causes of neonatal death and long-term disability, with an incidence of approximately 1 case per 1000 live-births in developed countries [1]. Therapeutic hypothermia (TH) is the standard of care for this condition but presents modest efficacy and must be initiated within 6 hours after birth, therefore being crucial to uncover effective therapies. Although the administration of umbilical cord blood cells (UCBC) has risen as a strong candidate, no preclinical studies assessed the effect of combining UCBC with TH [2]. Previous reports have shown that neuroinflammation strongly contributes to the neuropathological cascade associated with HIE. This complex response, which includes the activation of astrocytes and microglia as well as the infiltration of peripheral immune cells, is initiated immediately after the hypoxic-ischemic insult (HI) and can last for months or years after the injury [3]. Thus, the main objectives of this study were to evaluate if UCBC therapy could hamper glial reactivity after an HI in the developing brain and evaluate if the isolated and the combined therapies could result in the recovery of the functional deficits triggered by this injury.
Materials and Methods: To compare the joint action of these therapies with the action of each isolated therapy, TH alone or UCBC alone or combined with TH were applied to the Rice-Vannucci preclinical model for HIE. The impact of these treatments on cognitive and motor function was assessed at different neurodevelopmental stages through several behavioral tests. At P40, after completion of the behavioral tests, the animals were sacrificed, and brains were collected for histological analysis and immunohistochemistry.
Results and Discussion: Our results show that an HI to the developing brain increased GFAP and Iba-1 mean intensity and integrated density levels in the peri-infarct area, indicating astrocyte and microglia reactivity as well as higher migration/proliferation of these cells to the affected brain region. The intensity levels of GFAP and Iba-1 labeling were decreased in lesioned animals treated with UCBC, combined or not with TH, when compared to the HIE and HIE+TH groups, suggesting that the administration of UCBC lessened glial reactivity. Moreover, lesioned animals treated with UCBC recovered its sensorimotor and motor performance as well as fully regained its memory and learning capabilities, which was not observed in animals treated with TH alone. Importantly, the obtained results suggest that TH did not enhance the beneficial effects of UCBC, since HIE+TH+UCBC group had similar outcomes to the HIE+UCBC group.
Conclusions: Our data show that UCBC reduced glial reactivity, even when it was administered alone, which was translated into improved functional outcomes that were sustained on a long-term. Also, we demonstrated for the first time that therapy with UCBC alone was as effective as combining UCBC with TH in this model, suggesting that the administration of UCBC may also be a promising therapeutic approach for patients who are not eligible for TH.

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POSTER 21

Psychostimulants and Neuroinflammation: Finding Critical Players in the Crosstalk Between Glial Cells and Neurons

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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 commonly 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 microglia reactivity in different phases of the addictive process. We evaluated specific inflammatory and phagocytic microglia markers and microglia homeostatic signature genes, and 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, the number of microglia cells is significantly increased after chronic Meth administration and remains increased after two days of withdrawal. Regarding microglia morphology we have observed a more ameboid-like shape after chronic Meth administration, however its ramified morphology was recovered after two days of withdrawal.

In addition, a crosstalk between neurons and microglia seems to be relevant for the behavioral expression of Meth. As such, we are dissecting neuronal modulation of microglia 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, this type of receptors may serve as interesting therapeutic targets for treatment of addiction, and therefore we will manipulate them to confirm their value in reducing relapse rates and improve addiction treatments.

POSTER 22

Morphological Analysis of Astrocytes in Different Preparations

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Astrocytes are the most abundant glial cells in the brain, presenting a typical star-shaped morphology. Their extended morphology is pivotal to allowing 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. For that, we used the semi-automatic tool SNT to evaluate the morphological structure of astrocytes and quantify morphometric parameters such as total length, number of processes, and arbor complexity (Sholl analyses). In this work, we performed a 3D reconstruction of the backbone of hippocampal astrocytes in brain slices of C57BL6/J mice using immunohistochemistry staining of the Glial Fibrillary Acidic Protein (GFAP), the astrocyte-specific cytoskeleton protein.

Furthermore, we also studied brain slices from AstroTRAP mice expressing EGFP in astrocytic ribosomes, allowing an alternative identification of the astrocytic structure. Our results show that both preparations allow the detailed reconstruction of the astrocytic backbone, despite the considerable increase in detail provided by the AstroTRAP approach closer to the soma. Nevertheless, the more straightforward and flexible GFAP-immunostaining is enough to reveal intra-hippocampal heterogeneity, which could easily be applied to assess astrocyte structural alterations in rodent disease models.

POSTER 23

Astrocyte Structural Heterogeneity in the Mouse Hippocampus

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

POSTER 24

Microglial Ca2+ Levels: Crosstalk Between ATP-P2X7 and Adenosine A2A Receptors

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Introduction: Microglia-related neuroinflammation is associated with several brain disorders, and these glial cells are regulated by P_2X_7 (P_2X_7R) and adenosine A_{2A}($A_{2A}R$) receptors, which control intracellular Ca^{2+} signaling. However, it remains to be established whether P_2X_7R and $A_{2A}R$ are related functionally. This study aims to investigate the impact of P_2X_7R on microglial Ca^{2+} levels and test if this response is regulated by $A_{2A}R$, assessing a putative $P_2X_7R/A_{2A}R$ crosstalk in microglia.

Material & Methods: Intracellular Ca^{2+} concentration ($[Ca^{2+}]$) measurements using the fluorescent probe Fluo-4-AM (4 µM) were performed in a N9 murine microglial cell line stimulated with either BzATP (100 µM, P_2X_7R agonist), glutamate (100 µM) or CGS21680 (100 nM, $A_{2A}R$ agonist) and antagonists of $A_{2A}R$ (SCH58261, 50 nM) and of P_2X_7R (JNJ47965567, 1 µM) was also tested to study microglia stimulated Ca^{2+} responses ($\Delta[Ca^{2+}]$). Data were expressed as Ca^{2+} concentration (nM) and statistical analysis was performed using one-way ANOVA test.

Results: Microglial cells stimulated with P_2X_7R agonist had a $\Delta[Ca^{2+}]$ of 94.75 ± 14.50 nM, which was attenuated ($p<0.05$) by SCH58261 and potentiated ($p<0.05$) by CGS21680. Stimulation of N9 cells with CGS21680 also triggered a $\Delta[Ca^{2+}]$, which was significantly ($p<0.01$) inhibited by SCH58261 and JNJ47965567, suggesting a $P_2X_7R/A_{2A}R$ functional interaction. To evaluate if this crosstalk also occurs in conditions of excitotoxicity-induced microglial activation, the N9 cells were stimulated with glutamate, which triggered a $\Delta[Ca^{2+}]$ of 93.39±13.36 nM that was also inhibited by JNJ47965567 or SCH58261 ($p<0.05$).

Conclusions: Altogether the data unraveled a functional interaction between P_2X_7R and $A_{2A}R$ in controlling microglial Ca^{2+} dynamics, indicating a more complex involvement of the purinergic system in regulating microglia reactivity during neuroinflammatory processes.

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POSTER 25

Astrocytes Role in Protein Clearance Under Stress-Like Conditions: Modulation by Adenosine A2A Receptors

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Introduction: Stress-related disorders is a major social burden worldwide, being characterized by a dysfunction in hypothalamic-pituitary-adrenal axis that led to increased corticosteroid levels, which can exert their biological effects through glucocorticoid (GR) or mineralocorticoid (MR) receptors [1]. There are evidences that pathological stress impairs brain protein clearance, including glutamate that can lead to excitotoxicity. The clearance of extracellular proteins (oligomers and aggregates) is ensured by the glymphatic system (GS), a recently described system mainly composed by astrocytes. Thus, GS regulates extracellular protein accumulation and consequent neurotoxicity, however this system is dysregulated in stress-related disorders, including depression [2]. Previous studies showed that adenosine A_{2A} receptors ($A_{2A}R$) reverse mood and synaptic alterations in a stress mouse model and regulate GS activity [3,4] and that Dexamethasone (Dexa), a synthetic glucocorticoid used to mimic stress conditions, affects astrocytes function [3]. This study aims to study: i) the impact of Dexa on astrocytes protein and debris clearance ability and define whether its effects are mediated by GR or MR and ii) if $A_{2A}R$ blockade prevents Dexa effects

Material & Methods: Primary astrocytic cultures obtained from P2 Wistar rats were used to evaluate protein levels by Western blot and to access protein clearance using FITC-Dextran (8 mg/mL) or TRITC-Dextran (4 mg/mL), synthetic compounds that mimic proteins with 5 or 45 kDa, respectively. Cells were treated for 24h with Dexa (100 nM) to mimic stress-like conditions, and the antagonists of $A_{2A}R$ (SCH58261), GR (mifepristone) or MR (RU28318), to evaluate the contribution of these receptors to Dexa-induced effect on protein clearance. Data were expressed as percentage relatively to control and presented as mean ± SEM.

Results: Dexa exposure decreased the clearance of 5 kDa FITC-dextran ($p<0.01$) by 29%, an effect prevented by $A_{2A}R$ blockade. Likewise, the 45 kDa TRITC-dextran clearance were reduced by Dexa ($p<0.001$) and in the presence of SCH58261 protein clearance return to levels similar to control ($p<0.01$). Astrocytes treated with the selective GR antagonist mifepristone did not show significant alterations in non-pathologic conditions, whereas in the presence of Dexa increased the clearance of 5 and 45 kDa proteins ($p<0.05$), showing the involvement of MR in the decreased protein clearance by Dexa-treated astrocytes. The antagonist of MR, RU28318, only increased 45 kDa proteins clearance in astrocytes exposed to Dexa ($p<0.001$), suggesting the GR involvement in the uptake of these proteins from extracellular space. Moreover, Dexa significantly decreased GR density, which was not affected by $A_{2A}R$ blockade.

Conclusions: Overall, stress-like conditions affect astrocytes capacity to perform protein clearance, and effect mediated through glucocorticoid and mineralocorticoid receptors. Curiously, we the antagonism of $A_{2A}R$ prevented the effect of Dexa on astrocytes ability to perform protein clearance, suggesting a possible therapeutic target to prevent extracellular protein accumulation and neurodegeneration.

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POSTER 26

Star Power – Elucidating the Mechanisms by Which Astrocytes Promote Adult Brain Plasticity

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Astrocytes are star-shaped cells with extensive processes that ensheath neuronal synapses, forming the so-called 'tripartite' synapse. Astrocytes have been shown to play key roles in synapse formation and circuit refinement during development[1]. Additionally, in adulthood, their close interaction with neurons allows astrocytes to provide essential trophic support to neurons and places them in a privileged position to sense and respond to changes in synaptic activity, particularly in sensory stimulation[2]. Hence, astrocytes are potentially interesting players in modulating processes like neuroplasticity – the structural and functional adaptation of neuronal connections in response to changing stimuli. This process is particularly prominent during neurodevelopment, but persists, although to a much lesser extent, in adulthood, contributing to learning, memory, and recovery from injury and sensory loss[3].

Recently, using monocular enucleation (ME) of adult mice as a model to induce irreversible vision loss from one eye, we could demonstrate the important role of astrocytes in mediating adult cortical plasticity following vision loss and that astrocyte stimulation via G_i-DREADD activation boosted this phenomenon, while G_q-DREADD astrocyte activation hindered it[4]. We now aim at understanding the effect ME has on visual cortex astrocytes, neurons and microglia using single-nuclei RNA-seq. To specifically investigate transcriptomic adaptations taking place in astrocytes, particularly at the tripartite synapse, during ME-induced plasticity and following DREADD-based astrocyte activation, I use a combination of Translating Ribosome Affinity Purification (TRAP), which allows the isolation of translating mRNAs, DREADD, and synaptosome preparations. Astrocyte-specific immediate early genes[5] are validated as reporters of astrocytic activity in the visual cortex of ME mice in hybridization chain reaction experiments. *In vitro* astrocytic cultures are used to prepare secretome samples (conditioned media) for analysis by mass spectrometry to further identify putative synaptogenic factors which might be able to boost synapse assembly in cultured neurons.

Ultimately, unravelling astrocyte response to vision loss and the effect of DREADD activation could potentiate the development of novel therapeutical approaches for patients with late-onset sensory loss, and even combat neurodegeneration.

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POSTER 27

Hypothalamic Astrocytic-BMAL1 Regulates Energy Homeostasis and Circadian Behaviour in a Sex and Diet-Dependent Manner

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Introduction: The intimate interplay between nutrition, metabolism, exercise, and circadian physiology involves the molecular clock, a circuit present in virtually every cell and tissue of an organism, comprising rhythmic and self-sustained transcriptional–translational feedback loops[1]. The E-box-specific transcription BMAL1 and CLOCK heterodimerize to activate the transcription of many clock-controlled genes, which influence major components of energy homeostasis such as feeding behavior, thermogenesis, daily activity, sleep-wake cycle, and glucose metabolism[2]. In astrocytes, BMAL1 controls molecular, physiological, and behavioral circadian rhythms such as cognition, locomotor activity, glucose metabolism, and insulin sensitivity in male mice[3-8]

Studies in human and animal models indicate that sex influences circadian rhythms[9]. However, the regulatory circuits, signaling mechanisms, and how they impact metabolism are poorly understood. Here, we investigated whether gender influences astrocytes' actions on cyclic energy balance.

Material and Methods: We used a tamoxifen (TM)-inducible Cre/lox approach to genetically delete BMAL1 from GLAST-positive astrocytes[3,4]. At 8-10 weeks, control (*Bmal1flox/flox*) and mutants (*BmalcKO*) mice were treated with TM[3,4], and animals were subjected to extensive circadian and metabolic phenotype characterization in standard and high-fat diets. To ablate BMAL1 in astrocytes in the ventromedial nucleus of the hypothalamus, we stereotaxically injected adeno-associated virus (AAV) harboring GFAP-GFP (controls) and GFAP-Cre (mutants). The metabolic phenotypes were analyzed after one month of the AAV injections.

Results and Discussion: We previously reported that *Bmal1cKO* male mice had increased body weight with aging and altered glucose homeostasis[4]. Here we show that astrocytic BMAL1 contributes to the control of energy balance through the modulation of the metabolic rate, hepatic lipogenesis, and the activity of the brown adipose tissue in a diet and sex dimorphic manner. Specifically, the absence of astrocytic BMAL1 leads to negative energy balance in females and alters the basal metabolic cycles without affecting the central pacemaker in the hypothalamic suprachiasmatic nucleus. Notably, most of these alterations are specific to the ventromedial astrocyte clock. Remarkably, the metabolic phenotype upon astrocytic BMAL1 loss was reversed by a high-fat diet in females but blunted in males.

Conclusions:

I. Hypothalamic astrocyte clocks compute cyclic metabolic information to optimize energetic resources in a sexually dimorphic manner.

II. The consequences of BMAL1 loss selectively on astrocytes expand, rather than reduce, the overall metabolic differences between male and female mice. Thus, we propose astrocytic BMAL1 as a critical component of gender-specific circadian clockwork that links circadian timekeeping and metabolic outputs to regulate rhythms in physiology.

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POSTER 28

Energy Balance and Circadian Behavior in Mice are Regulated by Astrocytic-AMPK Signaling

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Introduction: Homeostasis in mammals is achieved through various complex mechanisms that control the balance between energy intake and expenditure[1]. A key feature of homeostatic regulation is its intrinsic rhythmic nature, which is largely controlled by circadian rhythms[2]. The circadian system ensures a temporal partitioning of catabolic and anabolic reactions synchronizing organism metabolism to the feeding-fasting cycle[2]. During fasting, AMP-activated protein kinase (AMPK) activation triggers catabolic processes and, in peripheral tissues, modulates clock-proteins rhythmic degradation[2]. However, the role of central AMPK signaling in controlling circadian entrainment to light and feeding time is largely unknown.

Astrocytes have been implicated in nutrient sensing and regulating molecular and behavioral circadian rhythms[3-7]. However, how the intrinsic astrocyte clock sense and respond to daily feeding and lighting by adjusting internal ~24 h rhythms to resonate with and anticipate external cycles of day and night is largely unexplored. Here, we investigate the role of astrocytic AMPK signaling in the control of circadian behavior and energy homeostasis in mice.

Materials and Methods: We employed a tamoxifen (TM)-inducible Cre/lox approach to genetically delete AMP-activated, gamma 2 non-catalytic subunit (Prkag2) in GLAST-positive astrocytes. At six weeks, control (AMPKγ2 flox/flox) and mutant (AMPKγ2KO) mice were treated with TM, and, after 20 weeks, mice were subjected to an extensive metabolic and circadian phenotype characterization.

On the other hand, we have generated mice with ventromedial hypothalamus targeted ablation of AMPKγ2 in adult astrocytes through stereotactic treatment with adenovirus harboring GFAP-GFP (control) or GFAP-Cre (mutants). After one month, the metabolic phenotype was characterized.

Results and Discussion: Our results indicate that AMPKγ2KO mice show higher body weight with aging and food intake on a standard diet. Moreover, they have increased periodicity, alterations in the coupling of the hypothalamic suprachiasmatic nucleus (SCN), and impairment of the anticipation of feeding time. Furthermore, our results reflect that AMPKγ2KO mice show decreased hypothalamic mTOR pathway, PER2, CRY1, and VIP. Remarkably, the ablation of AMPKγ2 in ventromedial astrocytes increases BAT thermogenesis.

Conclusions:

I. Deletion of AMPKγ2 in GLAST-positive astrocytes increases body weight with aging and food intake, a phenotype that AMPKγ2 does not mediate in ventromedial astrocytes.

II. Deletion of AMPKγ2 in GLAST astrocytes increases the period, alters the coupling of the SCN, and impairs the anticipation of feeding time, likely by suppressing the mTOR pathway, PER2, and VIP.

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POSTER 29

Development of an *in vitro* Model to Improve the Study, Characterization, and Modulation of Multiple Sclerosis-Like Foamy Microglia

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Introduction: Multiple Sclerosis (MS) is the most common debilitating autoimmune disorder of the Central Nervous System that is characterized by the destruction of myelin sheaths, inflammation/glial reactivity, and neurodegeneration. Microglia, the brain-resident macrophages, are commonly known for their wide variety of functions and, therefore, extensively described under several pathological conditions, including MS. Indeed, active MS lesions are characterized by the presence of highly phagocytic microglia containing abundant intracellular myelin debris (MD), known as foamy microglia, playing a key role for tissue repair, and remyelination in MS. However, upon intracellular processing, myelin lipids (ML) can induce critical physiological changes in foamy cells. While some studies describe the presence of lesion-associated foamy cells with an anti-inflammatory profile, further evidence correlates the continuous/excessive ML internalization with the appearance of a foamy microglia with decreased ability to effectively digest and efflux ML -mainly cholesterol. These cells will propagate a maladaptive pro-inflammatory response, and a vicious cycle of demyelination, meaning that (1)ML are potent inflammatory modulators and (2)myelin-carrying microglia are crucial players in MS. Thus, understanding foamy cell formation and related functional changes will be fundamental to better comprehend microglial role in MS.
Material & Methods: Here, we aim to implement an *in vitro* model to study foamy microglia by incubating human microglial cells with MD previously labeled with boronic acid salicylidenehydrazone-based dyes (BASHY), designed with increased specificity for nonpolar lipid structures like myelin aggregates [1]. Microglia were incubated with BASHY-MD using different concentrations (1mg/mL vs 10mg/mL), along different time-points (12h/24h/48h). As lesion-associated foamy microglia are known for their round/less ramified morphology, from a population of 294 individual cells, a total of 26 morphometric parameters were analyzed using the free software plugins Morphdata and FracLac, for ImageJ, as previously described [2, 3].
Results & Discussion: Following each incubation time-point, BASHY-MD was distinctly observed inside microglia, and mainly accumulated at 24h. Also after 24h of myelin internalization, microglial cells showed a decrease in the number of branches, ramifications per branch, cell perimeter, and cell roughness while displaying, on the other hand, an increase in cell circularity to a more amoeboid and foamy-like form, which is in line with previous data on foamy cell characterization.
Conclusion: Overall, with this study, we will develop and validate a new model of myelin-enriched microglia that will certainly create new opportunities for MS research, as we believe that targeting specific microglial subsets, the foamy cells in particular, will have profound implications on inflammation, lesion recovery, and remyelination in MS.

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POSTER 30

Duality of CB1R Effect in Depressive-Like Behaviour is Dependent on Astrocytic Calcium Signalling in Rodent mPFC

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Introduction: Prefrontal cortex (PFC) functions include decision making, working memory, and behavioral flexibility[1,2], can be regulated by neuromodulators such as endocannabinoids, that also modulate synaptic function. In regions such as the hippocampus, suprachiasmatic nucleus (SCN) and the neocortex, astrocytic CB1R activation evokes Ca^{2+} transients, modulating synaptic transmission[3,4] , however, little is known about the role of astroglial CB1R in the mPFC. It has been shown that low doses of CB1R agonist WIN55,212-2, exert potent antidepressant-like properties in forced-swim test (FST)[5]. Thus, we aim to fill a gap on the potential role of astroglial CB1R in PFC, specifically its effects on cognitive and depressive behaviour.

Materials and Methods: IP3R2-knockout mice and respective WT received 0.2mg/kg ip. injections of WIN55,212-2 (a non-selective CB1R agonist) or vehicle - 23 h, 5 h, 45 min before behavioural testing. The open field test was used to assess locomotor activity and anxiety-like behaviour. Working memory was assessed using Y-maze. FST explored the transition dynamics from active (swimming) to passive (immobility) modes of coping, testing depressive-like behaviour.

Results: No drug-treated group showed significantly different locomotor activity or anxiety-like behaviour from the vehicle-treated group, eliminating the possibility of a false positive in the FST. IP3R2-WT and IP3R2-KO did not differ in working memory, nor did WIN55,212-2 elicit differences in spontaneous alternation between the groups. When compared to vehicle, a low dose of CB1R agonist significantly decreased the total time spent in immobility for male IP3R2-WT; however, for female IP3R2-WT, no differences were found. WIN55,212-2 appears to increase immobility in FST in IP3R2-KO females.

Conclusions: A low dose of CB1R agonist WIN-55,212 appears to be anti-depressant in IP3R2-WT males while it seems to have the opposite effect in IP3R2-KO females, however further experiments are needed to understand sex-specific differences. No differences in the working memory were observed. CB1R activation in the mPFC affects synaptic transmission differently depending on whether astrocytic Ca^{2+} signaling is functional. Whether CB1R expression in astrocytes is responsible for such an effect requires further investigation.

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POSTER 31

Exploring the Role of Phospholipase D2 as a Neuroinflammation Mediator in Alzheimer's Disease

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Introduction: Alzheimer's Disease (AD) is a progressive neurodegenerative characterized by the brain accumulation of amyloid-β (Aβ) peptides, giving rise neuritic plaques and intracellular neurofibrillary tangles (NFTs), which are constituted by hyperphosphorylated tau. Additionally, neuroinflammation has been proposed as a third core feature of AD, with potential impact in exacerbating Aβ burden and NFTs. Phospholipase D (PLD) is a phosphodiesterase that hydrolyses phosphatidylcholine, in phosphatidic acid and free choline. Our team previously showed that Aβ increases total PLD activity in primary neuronal cultures and that the genetic ablation of the isoenzyme PLD2 ameliorates synaptic dysfunction and cognitive deficits in AD mice. Importantly, the PLD pathway has been implicated both in the normal functioning of the hippocampus and the inflammatory response. We then hypothesized that the genetic ablation of PLD2 could impact AD-phenotypes through neuroinflammation modulation. Therefore, the main goal of our study was to assess how PLD2 ablation affect the inflammatory reaction in the hippocampus in J20-line AD mice.

Materials and Methods: We used PLD2$^{-/-}$ and crossed them with J20 mice, which carry two mutations linked to familial AD, leading to high Aβ brain levels. We generated four different groups of mice of approximately 5-6 months old to conduct our experiments: PLD2 WT/nTg, PLD2 WT/J20, PLD2$^{-/-}$ / nTg and, PLD2$^{-/-}$/J20. We prepared coronal and horizonal sections of the dorsal (DH) and ventral (VH) hippocampus respectively and, immunohistochemistry staining was performed for Iba1 (1:1000, Wako) for microglia and GFAP (1:500, Dako) for astrocytes. Confocal images were acquired to quantify the percentage of area covered by microglia and astrocytes. To assess microglia morphology, a semi-automatic analysis was conducted to evaluate the number of endpoints/cell and process length/cell.

Results and Discussion: Regarding the percentage of area covered by Iba^{+} cells in the CA1, CA3 and DG regions, no statistical differences were founded between the four groups. Interestingly, we observed a trend for a decrease in the area covered by microglia in the DH and VH in transgenic mice lacking PLD2. Regarding the area occupied by GFAP^{+} cells, it was found to be decreased in PLD2$^{-/-}$/J20 mice compared to PLD2 WT/J20, both in the CA1 ($p= 0.0189$) and CA3 (p-value= 0.0038) VH subfields. No differences were founded in the DG or other subregions in the DH. We decided to focus on the CA1 region to perform the skeleton analysis of microglia, since the major output of the hippocampus are the pyramidal neurons in CA1 region. While no statistically significant changes were found, we observed a trend for the number of endpoints and process length/cell seems to be reduced in PLD2$^{-/-}$ / nonTg and, PLD2$^{-/-}$/J20 mice suggesting a higher activation state upon PLD2 ablation. Concerning biochemical analysis, the protein levels of Iba1 and GFAP in both the DH and VH were measured and no apparent differences were observed.

Conclusions: Having in mind the roles that the PLD pathway plays in the immune system and the fact that the ablation of PLD2 appears to have a protective role in AD mice models, these results suggest that the genetic ablation of PLD2 could potentially affect chronic inflammation pathways in the context of AD.

POSTER 32

Do Astrocytes Respond to the Same Electrical Stimulus Used to Modulate Neuronal Activity?

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Introduction: The development of implantable medical devices to deliver electric signals to the nervous system created new opportunities to ameliorate the symptoms of neurodegenerative diseases. An excellent example is deep brain stimulation (DBS), a surgical technique used to modulate brain function with proven efficacy in movement disorders (e.g. Parkinson Disease)[1]. However, the mechanisms of action of DBS are still far from being understood. Neurons have been on the spotlight, but new findings point to the involvement of non-neuronal cells as well. Astrocytes, primarily viewed only as supportive units, are now emerging as active players in the information processing of the brain[2], and the dysregulation of astrocytes is directly related with several neurological disorders[3]. Here we sought to assess if astrocytes may have a role on DBS by being able to respond to the same electrical stimulus used to modulate neuronal activity.

Materials and Methods: Astrocytes were isolated from the cortex of Wistar Han rat pups 1 to 2 days after birth, and cultivated on microelectrode array (MEA) chips compatible with microscopy (ThinMEA). The MEA system was used to deliver voltage pulses (biphasic square stimulus from ±400mV up to ±800mV; 200µs/phase) to astrocytic cultures while their response was recorded by calcium imaging, and analysed via an algorithm developed in house.

Results and Discussion: Astrocytic activity can be observed via changes in intracellular calcium dynamics. Those calcium events have characteristic spatial and temporal properties, which can last for seconds and be confined to a single cell or propagate intercellularly, generating calcium waves that travel across several astrocytes[4]. Thus, astrocytic activity is generally assessed via calcium imaging. By applying an electrical stimulus to isolated astrocytes and monitoring their response via calcium imaging, we have demonstrated unequivocally that astrocytes respond to direct electrical stimulation on the same range as neurons do. Furthermore, we have shown that typical response of astrocytes to electrical stimulation evolves the generation of a calcium wave that travels across several astrocytes. This calcium waves are significantly different from spontaneous occurring calcium waves in terms of maximum amplitude, propagation velocity and disseminated area.

Conclusions: Here we demonstrate that astrocytes respond to the same electrical stimulus used to modulate neuronal activity, and that the dynamics of electrically evoked signals are different from the spontaneous Ca^{2+} waves. This is of particular relevance to better understand the effect of DBS on neural circuits. Therefore, a clear characterization of the effect of electrical stimulation on astrocytic activity and, subsequently, on neuronal modulation is urgently needed. We are now optimizing specialized platforms designed in our group, combining microfluidics with MEA, to better understand these mechanisms.

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POSTER 33

A Tissue Engineered In Vitro Model to Explore the Role of Mechanobiology on Astrocyte Behaviour in Health and Disease

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Introduction: One accepted reason for neuro-regeneration failure in central nervous system (CNS) neurodegenerative diseases is the formation of the scar tissue containing reactive astrocytes (astrogliosis). These cells change their phenotype from quiescent to reactive, overexpressing extracellular matrix (ECM) proteins and strongly modifying the biophysical and mechanical properties of the lesion environment. Although the process is inexorable, it is mutable in time. Our working hypothesis is that the alterations in the matrix caused by reactive astrocytes are a barrier to neuro-regeneration. Currently there is no suitable *in vitro* model that can recreate all features of astrogliosis. In this work we present a three-dimensional (3D) tissue-engineered model to study astrocyte reactivity, in health and disease contexts. In order to reconstruct a more physiologically relevant environment, astrocytes were embedded within modified alginate matrices. Alginate was chosen due to its compatibility with mammalian cell culturing, possibility of introducing modifications in its the structure to increase biological responses and tunable mechanical properties.

Materials and Methods: Alginate hydrogels were produced by mixing non-modified HMW alginate with alginate modified with cell adhesive peptide RGD and oxidized alginate with engrafted matrix metalloproteinase sensitive peptide PVGLIG (Alg Mod). Primary rat cortical astrocytes (P1-P2 pups) were embedded in hydrogels and maintained in culture for 7 days. A pro-inflammatory stimulus (LPS/IFNγ) for 72h was used to induce an astrogliosis-like phenotype. To induce alginate hydrogels mechanical properties changes, an external $BaCl_2$ bath was performed. Astrocytic phenotype was evaluated by qPCR and image analysis.

Results and Discussion: Astrocytes embedded in Alg Mod extend more and longer cellular processes and acquire an overall more complex morphology. Treatment with LPS/IFNγ did not alter metabolic activity or viability of cells and increased the expression of the *Lcn2, Il-1β and Il-6* genes. Mechanical properties of the hydrogel were not altered in the presence of the stimulus. After submitting cell-laden hydrogels to an external $BaCl_2$, matrices became stiffer (G* increased from 21.22 Pa to 144.03 Pa). Additionally, this treatment didn't affect cell viability and metabolic activity and seems to increase the expression of *Piezo1*, a mechanosensing gene.

Conclusions: Modifications introduced in alginate hydrogels allowed astrocytes to acquire a more complex morphology in line with what happens *in vivo*. Treatment with LPS/IFNγ did not alter metabolic activity nor viability and induced an increase in expression of genes related to astrogliosis. Using $BaCl_2$ we were able to increase astrocyte-laden alginate hydrogels' stiffness (G*) without compromising cell viability. In the future we will use this 3D culture system to combine these two types of stimuli (biochemical and mechanical) and characterize astrocytic response, shedding light into the complex world of mechanobiology of the glial scar and its impact on the healthy brain and demyelinating conditions.

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POSTER 34

Mechanisms Behind Synaptic and Astrocytic Dysfunction in SOD1G93A Mice

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Introduction: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease, affecting mainly motor function, as well as, in some cases, cognitive function. The relevance of astrocytes in excitotoxicity and neurodegeneration, involved in ALS, has been highly recognized[1]. Thus, we aimed to study the astrocyte contribution for synaptic activity and plasticity in the hippocampus and motor cortex of SOD1G93A (mSOD1) and wild-type (wt) mice.

Materials and Methods: Pre-symptomatic (4-6 weeks) and symptomatic (14-18 weeks) mSOD1 mice, as well as age-matched wt mice were used. Astrocytic metabolism was selectively reduced using fluorocitrate[2] (FC), and synaptic plasticity and transmission was assessed by eliciting long-term potentiation (LTP) protocols and recording input/output curves, respectively, while recording field excitatory postsynaptic potentials (fEPSPs) in the CA1 area of hippocampal slices and layer II/III of primary motor cortex slices[3,4].

Results and Discussion: In the presence of FC (200 μM), hippocampal synaptic responses were significantly lower in pre-symptomatic mSOD1 mice, when compared with wt mice. In the symptomatic phase, mSOD1 mice exhibited higher post-tetanic potentiation and LTP magnitudes when compared with wt mice. However, astrocytic inhibition impaired significantly LTP, as well as synaptic responses, in both wt and mSOD1 mice. Regarding the motor cortex, pre-symptomatic mSOD1 mice showed an impairment in LTP magnitude and basal synaptic transmission, as well as a reduced firing frequency of pyramidal cells in layer II/III. Interestingly, 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, suggesting an early dysfunction of these cells in the motor cortex.

Conclusions: Altogether, we further explored alterations in synaptic plasticity and transmission, as well as the role of astrocytes, in two 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, mSOD1 mice present early alterations in synaptic function and plasticity, and astrocytes seem to be impaired even before the onset of symptoms.

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POSTER 35

Electrical Stimulation of Astrocytes and its Impact on Astrocytic Calcium Signaling

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Astrocytes play a fundamental role in the nervous system, not only providing structural support to neurons but also participating in synaptic transmission (1). Perisynaptic astrocytic processes can be found in close apposition to the pre- and post-synaptic neuronal membranes (forming the so-called tripartite synapse) (2, 3) and have been described to 1) increase their cytosolic calcium (Ca^{2+}) levels in response to neuronal activity; and 2) be able to modulate synaptic transmission by releasing gliotransmitters in a Ca^{2+}-dependent manner (4, 5). Going beyond a neuro-centric view, astrocytes have a direct role in information transmission and processing in the nervous system.

Although, unlike neurons, astrocytes are unable to generate action potentials, they are excitable cells with Ca^{2+} fluctuations underlying their excitability (6). Astrocytic Ca^{2+} signals present characteristic spatial and temporal properties, which are dependent on several intra- and intercellular stimuli (7, 8). Calcium signals can last up to seconds and be limited to the cell or propagated throughout the astroglial syncytium in the form of Ca^{2+} waves (9). However, there are still a number of aspects of astrocytic Ca^{2+} activity left to unravel, namely in respect to their interaction with neuronal electric activity.

In order to uncover astrocytic Ca^{2+} dynamics in response to electrical activity, we performed Ca^{2+} imaging with simultaneous extracellular electrical stimulation of astrocytes using microelectrode arrays. Upon stimulation of astrocytes (within the same range of neuronal stimulation), we observed an increase in the intracellular Ca^{2+} levels in astrocytes adjacent to the stimulating microelectrode, which then propagated to neighboring cells. As far as we know, this has not been described before.

Although preliminary, our work demonstrates that understanding the complex astrocytic Ca^{2+} dynamics and its response to electrical activity is an essential matter. This information can provide further insight on the tripartite synapse function, namely on how astrocytes respond and contribute to neuronal synaptic transmission.

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POSTER 36

Untangling Plasticity Mechanisms Using Synaptically Patterned Networks in vitro

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INTRODUCTION: While changes to intrinsic excitability and interactions with glial cells play an important role in plasticity, mechanisms relating to synapse formation, structure, and dynamics have a more direct role in the modification of neural activity[1,2]. However, both *in* and *ex vivo* exploring the relationship between synaptic and neural activity is often confounded by the complexity of the networks involved. For instance, the temporal incoherence of the afferent signals combined with the heterogeneity of synapse type and location presents an obstacle in discovering the relative contribution of each synapse formed by a presynaptic neuron to the generation of an action potential of the post synaptic neuron. While unable to directly reflect physiological conditions, in vitro methods create a simpler environment where comparisons across individual synapses can be answered. In previous work we developed micro/nanofluidic structures leveraging the size difference between axons and dendritic spines to guide synapse formation and verified active synapses could be formed[3]. In this work, we are using this patterning method to vary the number of potential synapses between pre and post synaptic neurons in concert with optical and electrical stimulation/recordings to investigate the interplay of structural and synaptic plasticity in small-scale feed forward networks of primary neurons in vitro. We chose as our model network the classical Hebbian circuit where two presynaptic populations connect to a single postsynaptic. By asymmetrically varying the number of synapse locations for each presynaptic population we can evaluate the baseline correlation in activity between each node as well as any changes in correlation after inducing potentiation or depression.

METHODS: Master moulds were created using hybrid lithography where nanoscale features were generated through a combination of e-beam lithography and reactive ion etching while micron/millimetre scale features were made using photolithography. Soft replicates were made by pouring a thin layer of h-PDMS, allowing partial curing at 80˚C for 1 hour before addition of a thicker layer of Sylgard 184. After demoulding, structures are placed on coverslips or MEAs, plasma cleaned, and coated in PDL. Primary E18 neurons are seeded, AAVs transduced at about DIV4, and electrophysiology and imaging done at DIV12-18.

RESULTS: Successful patterning of synapses has been demonstrated in Mateus and Weaver et al.[3] We have demonstrated the ability to successfully generate small Hebbian networks, as shown in Figure 1. Further, though the results are preliminary, we have been able to successfully increase the correlation in activity between a presynaptic and the postsynaptic node after application of repeated 50Hz stimulation to each with a delay of ~10ms as is commonly done for inducing short term synaptic potentiation.

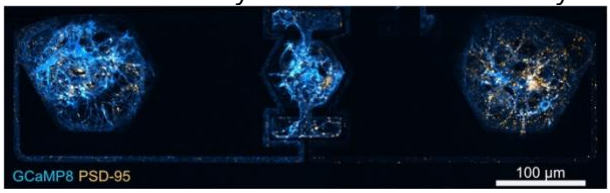

Fig. 1: Two presynaptic groups of neurons (right and left) make synaptic contacts with a single output group. Neurons are tagged with GCaMP8 for monitoring calcium dynamics and PSD95 to label putative synapses.

DISCUSSION & CONCLUSIONS: Having validated our protocols for inducing synaptic plasticity, we can soon begin to evaluate differences in structural plasticity simultaneously. In future works will gradually increase in complexity by including methods for modifying excitability or patterning glia interactions to discover the impact each has on neural communication.

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POSTER 37

Neurons Injured by Ischemia can be Rescued Through the Modulation of Astrocytes by HF-rMS

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Introduction: Ischemic stroke (IS) is caused by the reduction or blockage of blood flow to the brain and is the third most common cause of death in Portugal [1]. Due to its ability to improve the most frequent clinical sequelae left by brain ischemia, high-frequency repetitive transcranial magnetic stimulation (HF-rTMS) has been considered a promising therapeutic strategy for IS [2]. The observed improvements have been associated with changes in neurons and their synaptic liaisons [3]. However, the hypothesis that this technique modulates astrocytes, potentiating their neuroprotective capabilities, was also raised. With the present work, we aim to clarify which mechanisms triggered by high-frequency repetitive magnetic stimulation (HF-rMS) in astrocytes contribute to its neuroprotective effects.

Materials and Methods: Neuron-glia and astrocyte cortical cultures subject to oxygen and glucose deprivation were used as an *in vitro* model of ischemia. Neuroprotection promoted by HF-rMS was evaluated through the analysis of markers of neuronal activity and morphometric analysis of neurons. The levels of growth factors in the astrocyte-conditioned medium (CM) were assessed through a Growth Factor Array and glial-derived neurotrophic factor (GDNF) expression was analyzed by RT-PCR and Western blot.

Results and Discussion: Our results show that neurons injured by ischemia can be rescued through the modulation of astrocytes by HF-rMS. This modulation helps to maintain the number and length of neurites and increases the number of neurons expressing c-Fos. Quantification of glutamate transporters EAAT1 and EAAT2 in astrocyte extracts showed that EAAT2 levels were not affected by HF-rMS, however, EAAT1 levels were increased in cultures subjected to OGD and HF-rMS, suggesting that HF-rMS neuroprotection may involve a reduction of excitotoxicity. Furthermore, analysis of the astrocyte CM showed that HF-rMS stimulated the release of several trophic factors by astrocytes, namely GDNF. Neutralization of GDNF present in the CM impeded the recovery of neurite number and length induced by HF-rMS and blocked the increase of c-Fos$^+$ neurons, indicating that this neurotrophic factor plays a crucial role in the neuronal recovery induced by HF-rMS.

Conclusions: Our results show that modulation of astrocytes by HF-rMS effectively rescues neurons injured by ischemia by decreasing excitotoxicity and promoting neuroprotection through the release of GDNF by the astrocytes. This suggests that by targeting astrocytes, HF-rMS can be used to promote neuroprotection in other brain lesions.

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POSTER 38

Rac1 Mediates Microglial Response to External Environmental Cues

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Introduction: Microglia are highly ramified cells that are constantly extending and retracting their processes. By doing so, these cells are capable of scanning the central nervous system parenchyma (1, 2), searching for pathogens or cell debris, and checking the functionality of synapses. When triggered by a stimulus, microglia initiate a response, characterized by transcriptional and morphological changes (2). While this response is essential to reestablish brain homeostasis, excessive microglial response and exacerbated release of proinflammatory mediators can lead to tissue damage (3). Rac1 is a ubiquitously expressed RhoGTPase, classically associated with lamellipodia formation and membrane elongation for phagocytosis (4). Additionally, it is a component of the NADPH oxidase complex in monocytes (5) and it coordinates the activity of Nrf2 and NF-κB (6). Considering the abovementioned functions described for microglia and Rac1, we hypothesized that Rac1 could be an important player for microglia in homeostasis and in the context of neuroinflammation.

Materials and Methods: We combined cell-specific conditional gene ablation, flow cytometry, RNAseq profiling, immunofluorescence, single-cell live imaging and Förster resonance emission fluorescence to study the roles of Rac1 in microglia homeostasis.

Results and Discussion: Surprisingly, microglia-specific Rac1 ablation did not show an impact on phagocytosis and oxidative stress in RNAseq. However, we identified a subset of pathways altered by the absence of Rac1 in microglia associated with immune signaling. We functionally validated these results by treating microglia in vitro with LPS, to trigger a classical proinflammatory response, with phosphatidylcholine, to initiate the lipid sensing cascade, and with ATP, a danger-associated molecular pattern. We observed that microglia response to these stimuli was either reduced or completely absent when Rac1 was knocked down. To complement these observations, we also injected LPS in vivo to recapitulate a neuroinflammatory environment. While control mice displayed microgliosis and an increase in CD68 expression (characteristic of microglial activation), mice in which Rac1 was ablated did not present this response.

Conclusions: Here, we show that Rac1 ablation dampens microglia capacity to respond to different stimuli, while not showing a direct impact in steady state. This places Rac1 as a necessary element for microglia to sense and respond to external cues.

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